Published in final edited form as:

Antiviral Res. 2015 September ; 121: 132–137. doi:10.1016/j.antiviral.2015.07.003.

Alterations in favipiravir (T-705) pharmacokinetics and biodistribution in a hamster model of viral hemorrhagic fever

Brian B. Gowen^{a,*}, Eric J. Sefing^a, Jonna B. Westover^a, Donald F. Smee^a, Joseph Hagloch^a, Yousuke Furuta^b, and Jeffery O. Hall^{a,*}

^aDepartment of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, Utah, USA

^bResearch Laboratories, Toyama Chemical Company, Ltd., Toyama, Japan

Abstract

Favipiravir (T-705) is a new anti-influenza drug approved for human use in Japan and progressing through Phase 3 clinical trials in the U.S. In addition to its potent inhibitory effects against influenza virus infection, the compound has been shown to be broadly active against RNA viruses from 9 different families, including the *Arenaviridae*. Several members of the *Arenaviridae* family of viruses are significant human pathogens that cause viral hemorrhagic fever, a severe systemic syndrome where vascular leak is a cardinal feature. Because arenaviral infections are unlikely to be diagnosed and treated until the illness has progressed to a more advanced state, it is important to understand the effects of the disease state on favipiravir pharmacokinetics (PK) and biodistribution to help guide therapeutic strategy. During acute arenavirus infection in hamsters, we found reduced plasma favipiravir concentrations and altered kinetics of absorption, elimination and time to maximum drug concentration. In addition, the amounts of the favipiravir M1 primary metabolite were higher in the infected animals, suggesting that favipiravir metabolism may favor the formation of this inactive metabolite during viral infection. We also discovered differences in favipiravir and M1 PK parameters associated with arenavirus infection in a number of hamster tissues. Finally, analysis at the individual animal level demonstrated a correlation between reduced plasma favipiravir concentration with increased disease burden as reflected by weight loss and viral load. Our study is the first to show the impact of active viral infection and disease on favipiravir PK and biodistribution, highlighting the need to consider alterations in these parameters when treating individuals with viral hemorrhagic fever of arenavirus or other etiology.

Keywords

Favipiravir; T-705; arenavirus; pharmacokinetics; infection

*Corresponding authors: brian.gowen@usu.edu, jeffery.hall@usu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

The *Arenaviridae* family of enveloped, single-stranded RNA viruses contains several significant emerging and re-emerging human pathogens. Infection by Lassa, Junín, Machupo, Guanarito, Lujo, Sabia and Chapare arenaviruses can cause viral hemorrhagic fever (VHF), a severe acute disease in humans characterized by intense fever, vascular leak and terminal shock with case fatality rates in the range of 10–80%, depending on the outbreak (Moraz and Kunz, 2011; Paweska et al., 2009). The pathogenic arenaviruses are harbored in a variety of rodent species with the transfer of viruses within and between the host populations contributing to the phylogenetic diversity in the *Arenaviridae* family (Buchmeier et al., 2001). Human infections typically occur by inhalation of, or direct contact with, infected rodent excreta, with human-to-human transmission occurring through contact with virus-containing fluids from infected individuals. These viruses pose a considerable public health risk in endemic regions of the world and several are classified as NIAID Category A priority pathogens because of the severity of the disease they cause, potential for intentional release and, outside of Argentina, the lack of safe and effective antivirals or approved vaccines (Borio et al., 2002; NIAID, 2015).

Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamine) is a potent antiinfluenza compound approved for use in Japan and presently in clinical development in the United States for the treatment of influenza. Favipiravir was initially reported as an inhibitor of influenza A, B, and C viruses in cell culture and against lethal influenza infections in mice (Furuta et al., 2002). Subsequent studies have demonstrated broad antiviral activity of favipiravir against more than 25 different viruses from 9 RNA virus families, including a number of VHF agents (Caroline et al., 2014; Furuta et al., 2013; Gowen et al., 2013; Oestereich et al., 2014a; Oestereich et al., 2014b; Scharton et al., 2014; Smither et al., 2014). The precise mechanism by which favipiravir interferes with viral replication is still a matter of debate with supporting evidence for both direct inhibition of the influenza viral polymerase by, and/or misincorporation of, the active ribofuranosyl triphosphate (T-705-RTP) form of the drug leading to chain termination or lethal mutagenesis, respectively (Baranovich et al., 2013; Jin et al., 2013; Sangawa et al., 2013). Clearly, the ability of host enzymes in various cell and tissue types to metabolize the parent compound to the active triphosphate is an important consideration concerning the antiviral efficacy of the compound. The RNA-dependent RNA polymerase is also the likely principal target of T-705-RTP against chikungunya virus, murine norovirus, and arenaviruses (Delang et al., 2014; Mendenhall et al., 2011; Rocha-Pereira et al., 2012).

Because arenaviral infections are unlikely to be diagnosed and treated with antiviral drugs until the illness has progressed to a more advanced state, understanding the effects of acute infection on the pharmacokinetics (PK) and biodistribution of favipiravir would be beneficial in terms of dosing patients with VHF. We previously showed in a small-scale study that acute Pichindé arenavirus (PICV) infection in hamsters altered the absorption kinetics and magnitude of plasma favipiravir concentrations following oral treatment (Gowen et al., 2008). In the present study, we investigated the effects of arenavirus infection on both PK and biodistribution. This more comprehensive study provides insights into alterations in absorption, distribution, and elimination of favipiravir in the context of treating

severe arenaviral infection, which may serve to guide dosing for preclinical studies in nonhuman primates and cases of human disease.

2. MATERIALS AND METHODS

2.1. Ethics statement

All animal procedures complied with USDA guidelines and were conducted at the AAALAC-accredited Laboratory Animal Research Center at Utah State University under protocol 2120, approved by the Utah State University Institutional Animal Care and Use Committee.

2.2. Animals and virus

Female 7–8 week-old Syrian golden hamsters (The Charles River Laboratory, Willimantic, CT) were used. They were quarantined for 6 days prior to challenge and fed Harlan Lab Block and tap water *ad libitum*.

2.3. Viruses

PICV, strain An 4763, was provided by Dr. David Gangemi (Clemson University, Clemson, SC). The virus was passaged once through hamsters and the stock (3.9×10^8 plaque-forming units (PFU)/ml) was prepared from pooled and clarified liver homogenates. Virus stock was diluted in sterile minimal essential medium (MEM; Hyclone, Logan, UT) and inoculated by intraperitoneal (i.p.) injections totaling 0.2 ml (2 injections of 0.1 ml each on the right side of the abdomen).

2.4. Compound

Favipiravir (T-705) was provided by the Toyama Chemical Co. (Toyama, Japan) and suspended in 0.4% carboxymethyl cellulose (CMC) (Sigma-Aldrich, St. Louis, MO) prior to administration.

2.5. Experimental design

Animals were weighed the day prior to challenge and grouped so that intergroup variability was 5 g or less. Hamsters were challenged by i.p. injection with 10 PFU of PICV (n=53) or MEM (n=53). A single, 0.2 ml oral (p.o.) treatment of 100 mg/kg favipiravir or placebo was administered on day 7 post-challenge and the percent weight change of each animal relative to its starting weight was determined as a measure of disease associated with PICV infection. Five animals from each challenge group were sacrificed at the following time points: 3, 6, 12, 24, or 40 min, 1, 3, 6, 12, or 24 h post-treatment. Three animals from each challenge group received the 0.4% CMC vehicle placebo and were sacrificed at 24 min, 1, or 6 h post-treatment. The study design is summarized in Table 1.

Whole brain, kidney, liver, lung, and spleen tissues were collected from each animal for analysis of favipiravir concentrations, the primary favipiravir metabolite (M1) concentrations, and viral titers. The M1 product (6-fluoro-3,5-dihydroxy-2-pyrazinecarboxamide) is an inactive dead-end metabolite. Tissues were weighed, packaged for different analytical testing, and then stored at -80°C until time of homogenization. Whole blood was

collected from each animal by cardiac stick and processed in plasma separation Microvette tubes (Sarstedt Inc., Newton, NC) according to the manufacturer's recommendations. Plasma samples were stored at -80°C .

2.6. Plasma and tissue virus titers

Virus titers were assayed using an infectious cell culture assay as previously described (Gowen et al., 2007). Briefly, a specific volume of clarified tissue homogenate or plasma was serially diluted and added in triplicate to wells of Vero (African green monkey kidney) cell monolayers in 96-well microtiter plates. The viral cytopathic effect (CPE) was determined 8 days after plating and the 50% endpoints were calculated as previously described (Reed and Muench, 1938).

2.7. High-pressure liquid chromatography (HPLC) analysis

Plasma and homogenized tissue samples were deproteinized by the addition of an equal volume of a 1:1 methanol:acetonitrile solution, clarified by centrifugation, and supernatants evaporated prior to reconstitution in the HPLC mobile phase as previously described (Gowen et al., 2008).

Both favipiravir and the M1 metabolite were analyzed by using an HPLC instrument (Waters Corp., Milford, MA) fitted with a Waters Symmetry $3.9 \times 22\text{ mm} - 5\text{ }\mu\text{m}$ C18 Guard column and a Thermo Scientific $4.6\text{ mm} \times 25\text{ cm} - 5\text{ }\mu\text{m}$ Hypersil ODS C-18 reverse phase separation column. Separation was achieved using an isocratic running buffer comprised of 1% acetonitrile in 100 mM triethylammonium phosphate (prepared with triethylamine and titrated to pH 6.5 with phosphoric acid) with the columns at a constant temperature of 30°C and a flow rate of 1.5 ml/min. Sample and standard injection volumes were 100 μl . Peak area was quantified at 360 nm using the Waters 2996 Photodiode Array Detector. Favipiravir plasma concentrations were quantified using a standard curve of known amounts of favipiravir (1, 4, 10, 40, and 100 $\mu\text{g/ml}$). Similarly, the M1 metabolite concentrations were quantified using a standard curve of known amounts of M1 (1, 4, 10, and 40 $\mu\text{g/ml}$).

Data analysis was performed using PK Solutions 2.0 software (Summit Research Services, Montrose, CO). To account for population variability and the fact that each animal only provided data for a single time point for each sample type analyzed, the five animal data values for each collection time were averaged to provide a single data point. For each sample type, the single data values for each of the collection time points were entered into the PK Solutions 2.0 software for kinetic analysis. A 2-component mathematical model that best fit and described the kinetics of sample type was derived using a curve stripping method. The bi-exponential equation representing the curve was represented by the following formula: $\text{Conc} = Ae^{-\alpha t} + Ee^{-\beta t}$. Conc was the concentration of the parent drug or metabolite in the matrix at a given time (t). The letters A and E designated the y-axis ($t = 0$) intercepts and α and β represented the rate constants for absorption and elimination, respectively. Curve stripping was performed to calculate the intercepts and rate constants. Each curve was stripped in the following order: 1) elimination phase and 2) absorption phase. Curve stripping involved mathematical subtraction of each term from the remaining expression to isolate and determine the individual linear constants associated with each

phase. The following kinetic parameters were determined and are reported: absorption half-life, elimination half-life, maximum concentration ($C_{\max}$), time to peak concentration ($t_{\max}$), and area under the curve ($AUC_{0-\infty}$). A trapezoidal method was used to determine the AUC of a log concentration vs. time graph. As individual sampling time points do not generally fall at the true maximums, the time to peak and maximum concentrations are reported based on calculations from the constants of the best-fit mathematic models.

2.8. Statistical analysis

The effect of PICV infection on the plasma concentration of favipiravir and the M1 metabolite was analyzed by two-way analysis of variance with a Bonferroni post test for multiple comparisons (Prism 5, GraphPad Software, La Jolla, CA). To analyze the correlation between plasma favipiravir and other disease parameters, animals in each of the PICV-challenged sacrifice groups were ranked based on their favipiravir plasma concentration ($\mu\text{g/ml}$) with 1 being the lowest score and 5 the highest. To determine the overall viral burden score, all PICV-infected animals were grouped from highest to lowest (a score of 4 being the highest to 1 being the lowest) based on the titers determined for plasma and each tissue analyzed. The collective average of the viral titer scores was used to rank the hamsters within each sacrifice group as done for the favipiravir score. This analysis was restricted to the time points within the first 60 min when significant concentrations of favipiravir could be readily measured. The correlation coefficient (Pearson's r) between favipiravir plasma concentration score, animal weight, and virus burden score was determined using Prism 5 (GraphPad Software).

3. RESULTS

3.1. PK and biodistribution profile of favipiravir

To assess the PK and biodistribution of favipiravir during acute arenavirus infection, hamsters infected with PICV or sham-infected were treated p.o. 7 days post-infection (dpi) with 100 mg/kg favipiravir and sacrificed at specific time points post-treatment for analysis of favipiravir and the M1 primary metabolite concentrations in plasma, brain, kidney, liver, lung, and spleen tissues. As shown in Table 2, it took 46.2 min for favipiravir to reach its maximum concentration of 40.9 $\mu\text{g/ml}$ in plasma of infected animals compared to only 28.5 min to reach 81.5 $\mu\text{g/ml}$ in plasma of sham-infected animals. As shown in Figure 1A and indicated in Table 2, the area under the plasma time curve was 4339.8 $\mu\text{g}\cdot\text{min/ml}$ in the PICV-infected animals compared to the markedly higher 7740.8 $\mu\text{g}\cdot\text{min/ml}$ in the sham animals. The virus-infected animals had a longer elimination half-life of favipiravir than sham-infected animals (Table 2), which could indicate a reduced metabolism or excretion of the parent drug. However, PICV-infected animals had greater concentrations of the inactive M1 metabolite, which was detectable out to 12 h post-treatment (Figure 1B). The metabolite reached peak concentrations faster and was eliminated more slowly from the bloodstream in the infected animals (Table 3).

In the brain, favipiravir reached similar peak concentrations in both PICV- and sham-infected animals, with peak values at approximately 20 min post-treatment (Table 2). However, the elimination half-life was twice as long in the sham-infected animals, which

also had an AUC that was 140% of the PICV infected animals. The M1 metabolite was below the level of detection in animals of both treatment groups (Table 3), indicating this metabolite does not cross the blood brain barrier and is not produced by brain tissues.

In the kidney, favipiravir reached similar peak concentrations (12.4 vs. 12.5 µg/ml) in the PICV-infected and sham-infected animals, with peak values in both groups occurring at approximately 25 min post-treatment (Table 2). The half-life of favipiravir uptake into the tissue was nearly identical for the two treatment groups at 6.3 and 6.7 minutes, but elimination half-life was longer (91.4 minutes vs. 72.6 minutes) and the AUC was greater (1992.1 vs. 1663.2 µg-min/ml) in the PICV-infected group. The increased AUC for the PICV group was primarily a result of longer elimination half-life of the drug. The M1 metabolite had similar time to peak and absorption half-life, but had lower maximum concentration (30.5 vs. 44.3 µg/ml) and a much longer elimination half-life (125.6 vs. 51.7 min) in the virus-infected group (Table 3). Overall, this resulted in a greater AUC (6437.0 vs. 4563.3 µg-min/ml) in the PICV-infected animals.

Parent favipiravir was not detected in the livers from animals of either treatment group (Table 2). The lack of parent favipiravir is likely due to rapid hepatic metabolism via the aldehyde oxidase enzyme pathway eliminating the parent drug during the short period of time during tissue processing, before the tissues were frozen. The M1 metabolite kinetics differed for the PICV-infected from the sham-infected animals with time to peak occurring much earlier (14.3 vs. 24.2 min), concentration maximum being lower (32.1 vs. 46.2 µg/ml), and accumulation half-life being shorter (2.8 vs. 7.4 min) (Table 3). And, the elimination half-life was also longer (86.9 vs. 51.9 min) for the PICV-infected animals. However, the overall AUC was similar between the treatments.

In the lung tissue, favipiravir reached lower peak concentrations in the PICV-infected animals than the sham-infected animals (11.9 vs. 15.0 µg/ml), with peak values occurring slightly faster (23.5 vs. 31.0 min) in the virus-infected animals (Table 2). The half-life of favipiravir uptake into the tissue was shorter in the infected animals (4.7 vs. 9.8 min) and elimination half-life was longer (131.4 vs. 62.0 min) which resulted in an AUC that was greater in the PICV-infected group (2546.7 vs. 1899.7 µg-min/ml). The M1 metabolite (Table 3) had similar time to peak, but had higher maximum concentration (17.2 vs. 11.3 µg/ml), smaller absorption half-life (5.5 vs. 10.2 min), and longer elimination half-life (116.3 vs. 44.5 min) in the PICV-infected animals compared to the sham-infected animals which resulted in a much greater AUC (3362.6 vs. 1123.9 µg-min/ml) in the virus-infected animals.

In the spleen tissue, favipiravir reached similar peak concentrations in the PICV-infected animals and the sham-infected animals (12.7 vs. 14.6 µg/ml), with peak values occurring at a similar time (24.4 and 23.7 min) (Table 2). The half-life of favipiravir uptake into the tissue was shorter in the infected animals (5.2 vs. 7.0 min) and the elimination half-life was much longer (119.37 vs. 54.1 min), resulting in an AUC was greater in the PICV-infected group (2529.0 vs. 1542.0 µg-min/ml). The M1 metabolite (Table 3) had longer time to peak (34.4 vs. 20.6 min), but had higher maximum concentration (10.4 vs. 8.8 µg/ml) in the infected animals. There was a longer accumulation half-life (8.1 vs. 6.1 min) and longer elimination half-life (128.1 vs. 47.0 min) in the PICV-infected animals compared to the sham-infected

animals, with an overall effect of a much greater AUC in the virus-infected animals (2311.5 vs. 807.4 $\mu\text{g}\cdot\text{min}/\text{ml}$).

3.2. Plasma favipiravir correlates with disease burden

In addition to the analysis of favipiravir PK and biodistribution, plasma and tissues were processed for viral titers and animal weights reflective of overall health status and body condition were measured for each animal. Because the viral loads, weight change, and plasma concentrations varied within the sacrifice groups, we further evaluated the impact of arenaviral disease at the individual hamster level. As described in the methods, a scoring system was used to measure favipiravir plasma level and viral burden with “1” being the lowest concentration of favipiravir or viral burden in a group of five animals. This allowed normalization across all time points in animals sacrificed between 3 and 60 min post-treatment for a more comprehensive analysis. As expected, a very strong inverse correlation was seen between overall viral burden and weight (Figure 2A). Consistent with the PK data wherein the healthy sham-infected animals had higher concentrations of plasma favipiravir, the animals that weighed the most, and thus were less ill from the PICV infection, had the highest plasma favipiravir scores (Figure 2B). An inverse correlation was also observed with virus burden and plasma favipiravir scores (Figure 2C).

Discussion

Severe arenaviral infections can lead to often-fatal VHF, a syndrome characterized by increased vascular permeability, coagulopathy, and hypovolemic shock (Bray, 2005). In addition to changes in vascular integrity that can ensue, the host response to arenaviral pantropic infection common with arenaviruses may affect kidney function which can lead to altered antiviral drug PK and biodistribution. Certainly, patients with renal insufficiency are at greater risk for adverse drug effects due to impairment in kidney function that can dramatically affect the course of drug and metabolite accumulation in the various tissues and fluids (Fabre and Balant, 1976). Our findings indicate that with PICV infection, increasing viral burden and associated arenaviral disease is linked to reduced favipiravir plasma levels and altered biodistribution in hamsters. Although we have not directly measured the effect of PICV infection on renal function, the virus does cause a substantial infection in hamster kidneys (Gowen et al., 2008). The infection also induces significant vascular leak starting on day 7 post-infection in some animals (Gowen et al., 2010), which coincides with the time at which we treated with favipiravir in the present study.

Our results suggest that advanced systemic PICV infection delays and reduces the overall absorption of favipiravir into the plasma central compartment; however, other factors including renal dysfunction and altered metabolic activity likely contribute to the combination of greater favipiravir M1 metabolite AUC, decreased parent drug AUC, and increased elimination half-life of the parent drug in the PICV-infected animals. For example, a greater first pass hepatic metabolism of favipiravir to M1 could be the result of higher body temperatures in the infected animals since the enzymatic activity of aldehyde oxidase increases with temperature (Gordon et al., 1940). Notably, the tissue accumulation of favipiravir was enhanced by the PICV infection, which could be a secondary effect of the

increased vascular leakage of drug into tissues. This was especially prominent in the lung and spleen, which are highly vascular tissues. If inflammatory effects that drive vascular leakage increase the tissue concentration of favipiravir, this could enhance drug delivery to sites of viral replication where it is needed for a beneficial therapeutic effect. Moreover, the prolonged elimination half-life of the parent drug in the PICV-infected hamsters could maintain therapeutic concentrations for longer periods in those animals.

Recent clinical investigations have shed light on the effects of severe viral infections on PK. Although PK parameters following zanamivir (neuraminidase inhibitor) administration in severely ill patients with influenza infection were generally consistent with studies conducted in healthy volunteers (Cass et al., 1999), dose adjustments were made for individuals with renal impairment to achieve the desired drug concentrations (Marty et al., 2013). Similarly, treatment of severe arenavirus infections with favipiravir would likely have to be managed based on clinical evidence of diminished kidney function. The longer elimination half-life of the parent drug can be explained by the expected impairment of renal function by the PICV infection. In humans, the principal elimination route of favipiravir is metabolism to M1, which is eliminated in the urine (Kobayashi et al., 2011).

In two independent open-label studies evaluating the PK of boceprevir (NS3 protease inhibitor approved for chronic hepatitis C infection) in patients with liver or renal insufficiency, the PK properties were not significantly altered in patients compared to respective healthy controls (Treitel et al., 2012). Notably, there was a trend towards increased mean peak plasma concentration in patients with increasing severity of liver impairment; however, the result was not sufficient to warrant adjustments to standard boceprevir dosing regimens. PICV infection in hamsters also reproduces the liver infection and varying degrees of hepatic degeneration and necrosis observed with human infection by prominent arenaviral hemorrhagic fever viruses (Grant et al., 2012; Yun and Walker, 2012), but higher favipiravir plasma concentrations in the PICV-infected animals or a correlation between liver viral loads and favipiravir was not observed.

Our findings provide insights into the challenges associated with dosing severely ill individuals with altered drug PK and biodistribution, support the need for further PK analysis in nonhuman primate models of severe arenaviral hemorrhagic fever that recapitulate the human disease, and may serve to guide dosing regimens to enhance the efficacy of favipiravir. It is also important to consider the impact of species differences in aldehyde oxidase (Pryde et al., 2010; Ueda et al., 2005), which can have a significant impact on the activity of favipiravir as the primary enzyme catalyzing the conversion of favipiravir to the inactive M1 metabolite (Kobayashi et al., 2011).

Acknowledgements

We are grateful to Makda Gebre, Luci Wandersee, Jacqueline LaRose, and Joseph Clyde for technical support.

This work was supported by a sub-award to BBG as part of National Institutes of Health grant U54 AI-065357 (Rocky Mountain RCE; J. Belisle, Principal Investigator).

References

- Baranovich T, Wong SS, Armstrong J, Marjuki H, Webby RJ, Webster RG, Govorkova EA. T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses in vitro. *J. Virol.* 2013; 87:3741–3751. [PubMed: 23325689]
- Borio L, Inglesby T, Peters CJ, Schmaljohn AL, Hughes JM, Jahrling PB, Ksiazek T, Johnson KM, Meyerhoff A, O'Toole T, Ascher MS, Bartlett J, Breman JG, Eitzen EM Jr, Hamburg M, Hauer J, Henderson DA, Johnson RT, Kwik G, Layton M, Lillibridge S, Nabel GJ, Osterholm MT, Perl TM, Russell P, Tonat K. Hemorrhagic fever viruses as biological weapons: medical and public health management. *JAMA.* 2002; 287:2391–2405. [PubMed: 11988060]
- Bray M. Pathogenesis of viral hemorrhagic fever. *Curr. Opin. Immunol.* 2005; 17:399–403. [PubMed: 15955687]
- Buchmeier, M.; Bowen, MD.; Peters, CJ. Arenaviridae: The viruses and their replication. In: Knipe, DM.; Howley, PM.; Griffin, DE.; Lamb, RA.; Martin, MA.; Roizman, B.; Straus, SE., editors. *Fields Virology*. 4th ed.. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1635-1638.
- Caroline AL, Powell DS, Bethel LM, Oury TD, Reed DS, Hartman AL. Broad spectrum antiviral activity of favipiravir (T-705): protection from highly lethal inhalational Rift Valley Fever. *PLoS Negl Trop Dis.* 2014; 8:e2790. [PubMed: 24722586]
- Cass LM, Efthymiopoulos C, Bye A. Pharmacokinetics of zanamivir after intravenous, oral, inhaled or intranasal administration to healthy volunteers. *Clinical Pharmacokinetics.* 1999; 36:1–11. [PubMed: 10429835]
- Delang L, Segura Guerrero N, Tas A, Querat G, Pastorino B, Froeyen M, Dallmeier K, Jochmans D, Herdewijn P, Bello F, Snijder EJ, de Lamballerie X, Martina B, Neyts J, van Hemert MJ, Leyssen P. Mutations in the chikungunya virus non-structural proteins cause resistance to favipiravir (T-705), a broad-spectrum antiviral. *J. Antimicrob. Chemother.* 2014; 69:2770–2784. [PubMed: 24951535]
- Fabre J, Balant L. Renal failure, drug pharmacokinetics and drug action. *Clinical Pharmacokinetics.* 1976; 1:99–120. [PubMed: 13956]
- Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. *Antiviral Res.* 2013; 100:446–454. [PubMed: 24084488]
- Furuta Y, Takahashi K, Fukuda Y, Kuno M, Kamiyama T, Kozaki K, Nomura N, Egawa H, Minami S, Watanabe Y, Narita H, Shiraki K. In vitro and in vivo activities of anti-influenza virus compound T-705. *Antimicrobial agents and chemotherapy.* 2002; 46:977–981. [PubMed: 11897578]
- Gowen BB, Juelich TL, Sefing EJ, Brasel T, Smith JK, Zhang L, Tigabu B, Hill TE, Yun T, Pietzsch C, Furuta Y, Freiberg AN. Favipiravir (T-705) inhibits Junin virus infection and reduces mortality in a guinea pig model of Argentine hemorrhagic fever. *PLoS Negl Trop Dis.* 2013; 7:e2614. [PubMed: 24386500]
- Gowen BB, Julander JG, London NR, Wong MH, Larson D, Morrey JD, Li DY, Bray M. Assessing changes in vascular permeability in a hamster model of viral hemorrhagic fever. *Virol J.* 2010; 7:240. [PubMed: 20846417]
- Gowen BB, Smee DF, Wong MH, Hall JO, Jung KH, Bailey KW, Stevens JR, Furuta Y, Morrey JD. 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.* 2008; 3:e3725. [PubMed: 19008960]
- Gowen BB, Wong MH, Jung KH, Sanders AB, Mendenhall M, Bailey KW, Furuta Y, Sidwell RW. In vitro and in vivo activities of T-705 against arenavirus and bunyavirus infections. *Antimicrob. Agents Chemother.* 2007; 51:3168–3176. [PubMed: 17606691]
- Grant A, Seregin A, Huang C, Kolokoltsova O, Brasier A, Peters C, Paessler S. Junin virus pathogenesis and virus replication. *Viruses.* 2012; 4:2317–2339. [PubMed: 23202466]
- Jin Z, Smith LK, Rajwanshi VK, Kim B, Deval J. The ambiguous basepairing and high substrate efficiency of T-705 (Favipiravir) Ribofuranosyl 5'-triphosphate towards influenza A virus polymerase. *PLoS One.* 2013; 8:e68347. [PubMed: 23874596]

- Kobayashi, O.; Noto, M.; Sakurai, T. Favipiravir, A Novel Anti-Influenza Virus Compound Inhibits Viral RNA Polymerase: the Safety, Tolerability and Pharmacokinetics of Higher Dose in Human. Interscience Conference of Antimicrobial Agents and Chemotherapy; Chicago, IL. 2011.
- Marty FM, Man CY, van der Horst C, Francois B, Garot D, Mánez R, Thamlikitkul V, Lorente JA, Alvarez-Lerma F, Brealey D, Zhao HH, Weller S, Yates PJ, Peppercorn AF. Safety and pharmacokinetics of intravenous zanamivir treatment in hospitalized adults with influenza: an open-label, multicenter, single-arm, phase II study. *Journal of Infectious Diseases*. 2013 Aug 27. Epub ahead of print.
- Mendenhall M, Russell A, Juelich T, Messina EL, Smee DF, Freiberg AN, Holbrook MR, Furuta Y, de la Torre JC, Nunberg JH, Gowen BB. T-705 (favipiravir) inhibition of arenavirus replication in cell culture. *Antimicrob. Agents Chemother*. 2011; 55:782–787. [PubMed: 21115797]
- Moraz ML, Kunz S. Pathogenesis of arenavirus hemorrhagic fevers. *Expert Rev Anti Infect Ther*. 2011; 9:49–59. [PubMed: 21171877]
- NIAID. Biodefense and Emerging Infectious Diseases. 2015. <http://www.niaid.nih.gov/topics/biodefense/biodefense/pages/cata.aspx>.
- Oestereich L, Ludtke A, Wurr S, Rieger T, Munoz-Fontela C, Gunther S. Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model. *Antiviral Res*. 2014a; 105:17–21. [PubMed: 24583123]
- Oestereich L, Rieger T, Neumann M, Bernreuther C, Lehmann M, Krasemann S, Wurr S, Emmerich P, de Lamballerie X, Olschlager S, Gunther S. Evaluation of antiviral efficacy of ribavirin, arbidol, and T-705 (favipiravir) in a mouse model for Crimean-Congo hemorrhagic fever. *PLoS Negl Trop Dis*. 2014b; 8:e2804. [PubMed: 24786461]
- Paweska JT, Sewlall NH, Ksiazek TG, Blumberg LH, Hale MJ, Lipkin WI, Weyer J, Nichol ST, Rollin PE, McMullan LK, Paddock CD, Briese T, Mnyaluza J, Dinh TH, Mukonka V, Ching P, Duse A, Richards G, de Jong G, Cohen C, Ikalafeng B, Mugero C, Asomugha C, Malotle MM, Nteo DM, Misiani E, Swanepoel R, Zaki SR. Nosocomial outbreak of novel arenavirus infection, southern Africa. *Emerg. Infect. Dis*. 2009; 15:1598–1602. [PubMed: 19861052]
- Pryde DC, Dalvie D, Hu Q, Jones P, Obach RS, Tran TD. Aldehyde oxidase: an enzyme of emerging importance in drug discovery. *J. Med. Chem*. 2010; 53:8441–8460. [PubMed: 20853847]
- Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. *Am. J. Hyg*. 1938; 27:493–497.
- Rocha-Pereira J, Jochmans D, Dallmeier K, Leyssen P, Nascimento MS, Neyts J. Favipiravir (T-705) inhibits in vitro norovirus replication. *Biochem. Biophys. Res. Commun*. 2012; 424:777–780. [PubMed: 22809499]
- Sangawa H, Komeno T, Nishikawa H, Yoshida A, Takahashi K, Nomura N, Furuta Y. Mechanism of Action of T-705 Ribosyl Triphosphate against Influenza Virus RNA Polymerase. *Antimicrob. Agents Chemother*. 2013; 57:5202–5208. [PubMed: 23917318]
- Scharton D, Bailey KW, Vest Z, Westover JB, Kumaki Y, Van Wettere A, Furuta Y, Gowen BB. Favipiravir (T-705) protects against peracute Rift Valley fever virus infection and reduces delayed-onset neurologic disease observed with ribavirin treatment. *Antiviral Res*. 2014; 104:84–92. [PubMed: 24486952]
- Smither SJ, Eastaugh LS, Steward JA, Nelson M, Lenk RP, Lever MS. Post-exposure efficacy of oral T-705 (Favipiravir) against inhalational Ebola virus infection in a mouse model. *Antiviral Res*. 2014; 104:153–155. [PubMed: 24462697]
- Treitel M, Marbury T, Preston RA, Triantafyllou I, Feely W, O'Mara E, Kasserra C, Gupta S, Hughes EA. Single-dose pharmacokinetics of boceprevir in subjects with impaired hepatic or renal function. *Clinical Pharmacokinetics*. 2012; 51:619–628. [PubMed: 22799589]
- Ueda O, Sugihara K, Ohta S, Kitamura S. Involvement of molybdenum hydroxylases in reductive metabolism of nitro polycyclic aromatic hydrocarbons in mammalian skin. *Drug Metab. Dispos*. 2005; 33:1312–1318. [PubMed: 15932950]
- Yun NE, Walker DH. Pathogenesis of Lassa fever. *Viruses*. 2012; 4:2031–2048. [PubMed: 23202452]

Highlights

- Arenavirus infection altered favipiravir plasma and tissue concentrations and kinetics in hamsters
- Favipiravir conversion to inactive M1 metabolite increased in infected animals
- Reduced plasma favipiravir concentrations correlated with increasing disease severity

Figure 1. Effect of PICV infection on plasma favipiravir and M1 metabolite concentrations

Animals ($n=5/\text{group}$) were treated p.o. with a single dose of 100 mg/kg favipiravir 7 days postchallenge. Deproteinized plasma samples were analyzed by HPLC for determination of A) favipiravir and B) M1 metabolite concentrations in PICV- or sham-infected hamsters, as described in the methods. For favipiravir, $P = 0.0198$ by two-way analysis of variance based on infection. * $P < 0.05$ by Bonferroni multiple comparison analysis.

Figure 2. Increased disease reflected by lower weight or viral burden correlates with plasma favipiravir levels

The weight of each animal is represented as a percentage of the initial day 0 weight for animals sacrificed from 3 to 60 min after favipiravir treatment. The A) virus burden score vs. the percent of initial weight, B) plasma favipiravir score vs. the percent of initial weight, and C) plasma favipiravir score vs. the virus burden score are shown. Significant correlation was observed in all 3 comparisons as determined by the Pearson's r test.

Table 1

Favipiravir PK and biodistribution during acute arenavirus infection study design.

Group	Treatment 7 dpi	No. of animals	Sacrifice time post- treatment
PICV-infected	Favipiravir 100 mg/kg	5 per time point	3, 6, 12, 24, & 40 min; 1, 3, 6, 12 & 24 h
	Placebo	3	24 min; 1 & 6 h
Sham-infected	Favipiravir 100 mg/kg	5 per time point	3, 6, 12, 24, & 40 min; 1, 3, 6, 12 & 24 h
	Placebo	3	24 min; 1 & 6 h

Days post-infection, dpi.

Table 2

Impact of PICV infection on favipiravir PK and biodistribution.

Groups ^a	Tissue	Time to Peak (min)	Absorption Half life (min)	Elimination Half life (min)	AUC ^b (µg-min/ml or /g)	Maximum Conc. (µg/ml or /g)
PICV-infected	Plasma	46.2	20.6	53.4	4339.8	40.9
	Brain	18.8	5.2	49.1	1053.8	11.4
	Kidney	26.2	6.3	91.4	1992.1	12.4
	Liver	ND	ND	ND	ND	ND
	Lung	23.5	4.7	131.4	2546.7	11.9
	Spleen	24.4	5.2	119.7	2529.0	12.7
Sham-infected	Plasma	28.5	11.1	40.3	7740.8	81.5
	Brain	22.3	4.9	97.2	1486.8	9.0
	Kidney	25.3	6.7	72.6	1663.2	12.5
	Liver	ND	ND	ND	ND	ND
	Lung	31.0	9.8	62.0	1899.7	15.0
	Spleen	23.7	7.0	54.1	1542.0	14.6

^a PICV-infected or sham-infected hamsters treated p.o. 7 dpi with 100 mg/kg of favipiravir and sacrificed at specified time points from 3 min to 24 h post-treatment (Table 1).^b Area under the curve, AUC; not detectable, ND.

Table 3

Effect of PICV infection on favipiravir M1 metabolite PK and biodistribution.

Groups ^a	Tissue	Time to Peak (min)	Absorption Half life (min)	Elimination Half life (min)	AUC ^b (µg-min/ml or /g)	Maximum Conc. (µg/ml or /g)
PICV-infected	Plasma	17.5	3.3	117.3	4812.0	25.6
	Brain	NA	NA	NA	NA	NA
	Kidney	27.5	6.0	125.6	6437.0	30.5
	Liver	14.3	2.8	86.9	4514.6	32.1
	Lung	25.5	5.5	116.3	3362.6	17.2
	Spleen	34.4	8.1	128.1	2311.5	10.4
Sham-infected	Plasma	30.9	9.8	61.9	3552.6	28.1
	Brain	NA	NA	NA	NA	NA
	Kidney	24.1	7.3	51.7	4563.3	44.3
	Liver	24.2	7.4	51.9	4777.7	46.2
	Lung	28.2	10.2	44.5	1123.9	11.3
	Spleen	20.6	6.1	47.0	807.4	8.8

^a PICV-infected or sham-infected hamsters treated p.o. 7 dpi with 100 mg/kg of favipiravir and sacrificed at specified time points from 3 min to 24 h post-treatment (Table 1).^b Area under the curve, AUC; not detectable, ND.