Published in final edited form as:

Antiviral Res. 2017 September ; 145: 131–135. doi:10.1016/j.antiviral.2017.07.019.

Enhanced protection against experimental Junin virus infection through the use of a modified favipiravir loading dose strategy

Brian B. Gowen^{1,*}, Jonna B. Westover¹, Eric J. Sefing¹, Arnaud J. Van Wettere^{1,2}, Kevin W. Bailey¹, Luci Wandersee¹, Takashi Komeno³, and Yousuke Furuta³

¹Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, USA

²Utah Veterinary Diagnostic Laboratory, Logan, Utah, USA

³Toyama Chemical Co., Ltd., Toyama, Japan

Abstract

A collection of Old and New World arenaviruses are etiologic agents of viral hemorrhagic fever, a syndrome that features hematologic abnormalities, vascular leak, hypovolemia, and multi-organ failure. Treatment is limited to ribavirin for Lassa fever and immune plasma for Argentine hemorrhagic fever. Improved therapeutic options that are safe, more effective and widely available are needed. Here, we show that modification of favipiravir treatment to include a high-dose loading period achieves complete protection in a guinea pig model of Argentine hemorrhagic fever when treatment was initiated two days following challenge with Junin virus (JUNV). This loading dose strategy also protected 50% of animals from lethal disease when treatment was delayed until 5 days post-infection and extended the survival time in those that succumbed. Consistent with the survival data, dramatic reductions in serum and tissue virus loads were observed in animals treated with favipiravir. This is the first report demonstrating complete protection against uniformly lethal JUNV infection in guinea pigs by administration of a small molecule antiviral drug.

Keywords

arenavirus; Junin virus; viral hemorrhagic fever; favipiravir; T-705

Junin virus (JUNV; family *Arenaviridae*, genus *Mammarenavirus*), the causative agent of Argentine hemorrhagic fever (AHF), is a single-stranded RNA virus with a trisegmented genome. The virus is maintained primarily in *Calomys musculinas* mice and is generally transmitted through exposure to infected excreta in the endemic fertile farming plains of central Argentina (Mills et al., 1994). Infection can lead to severe hemorrhagic and/or neurologic disease characterized by fever, vascular leak and shock, and with mortality rates as high as 30% (Gomez et al., 2011). Therapy is limited to immune plasma, but is associated with a late neurologic syndrome in approximately 10% of survivors and is not readily

*Corresponding author.

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.

available outside of Argentina (Enria et al., 2008). Favipiravir (T-705), a nucleobase analog approved as an anti-influenza drug in Japan and currently being reviewed by the US Food and Drug Administration for the same indication in the USA, has been found to be broadly active against a number of RNA viruses including arenaviruses (Furuta et al., 2013). Results from studies investigating the antiviral activity of favipiravir in a guinea pig model of AHF have been encouraging, but complete protection has not been achieved (Gowen et al., 2013; Westover et al., 2016),

To further assess the capacity of favipiravir as a candidate therapeutic for AHF, we designed a more aggressive treatment regimen that employed a 3-day loading dose strategy (Figure 1). Guinea pigs challenged with 300 PFU of JUNV (Romero strain) received a 400 mg/kg/day loading dose for the first 3 days, followed by a maintenance dose of the previously tested 300 mg/kg/day, by intraperitoneal (i.p.) injection. As shown in Figure 2A, the modified treatment proved to be 100% efficacious when initiated 2 days post-infection, and was able to protect half of the animals from lethal disease when delaying the treatment until day 5 after JUNV challenge. Notably, a remarkable delay in disease progression was observed in the three guinea pigs from the day-5 treatment initiation group which reached a moribund state on days 19–21 after virus challenge, whereas most of the animals (5 out of 6) in the placebo group required euthanasia 12–14 days post-infection.

Animal weights and temperatures were also recorded through the duration of the experiment as measures of morbidity. Weight gain in all groups plateaued from days 5–9, after which animals receiving placebo started losing weight (Figure 2B). Elevated body temperatures were noted in several animals in the placebo group on day 5 after JUNV challenge (Supplemental Figure 1), with peak mean temperature in this group approaching 41°C on day 11 post-infection (Figure 2C). Several animals in the day-5 favipiravir treatment group began losing weight 16–19 days post-infection, consistent with the delay in the time of animals becoming moribund and requiring euthanasia (Figure 2 and Supplemental Figure 2). In contrast, guinea pigs treated with favipiravir beginning 2 days after infection continued to gain weight at a rate similar to the sham-infected, normal control group after the plateau period and did not present with elevated body temperatures at any time during the study. Of note, only 1 of the 4 animals in the day-5 treatment group that became febrile recovered and survived the infection, (Supplemental Figure 1). It is likely that the elevated temperatures and weight loss in these animals, coinciding with the cessation of treatment, may have been due to virus rebound. Thus, extending the treatment an additional 4 to 7 days may have resulted in greater survival.

The effects of favipiravir on viral titers and histopathology were assessed in a subset of guinea pigs challenged with JUNV and treated in parallel to those observed for morbidity and mortality. Day 12 infectious viral loads were determined as previously described (Gowen et al., 2007) with no detectable viremia or liver, spleen or brain virus titers present in any animals treated with favipiravir beginning 2 days post-infection, and even when treatment was withheld until 5 days after JUNV infection only a single animal had viral titers in the liver just within the detectable range (Figure 3). Notably, one animal in the placebo group had lower than expected viral titers (Figure 3, represented as the triangle), and this finding was consistent with the lack of dramatic weight loss observed with the other two

placebo guinea pigs at the time of sacrifice on day 12 post-infection (data not shown). Further, this animal only had a mild neutrophilic splenitis noted by a board-certified veterinary pathologist (Utah Veterinary Diagnostic Laboratory, Logan, UT) during the analysis of liver, spleen, and brain tissues preserved in 10% neutral-buffered formalin (Supplemental Table 1). The other two animals receiving placebo presented with severe splenic necrosis, which was reduced in the favipiravir-treated animals to a minimal to mild neutrophilic splenitis with no or minimal tissue necrosis. The animals treated with favipiravir also did not develop hepatic lipidosis which was present in the guinea pigs that received placebo. JUNV titers were not detected in liver or spleen tissues in the favipiravir-treated animals despite the minimal to mild neutrophilic splenitis. Combined with the antiviral activity of favipiravir, a well-coordinated inflammatory response in the spleen likely aided in the control and elimination of the virus, as supported by the lack of or minimal cellular necrosis observed in the spleen despite the presence of neutrophils. No brain lesions were present in any of the animals analyzed, even with the detectable concentrations of JUNV in the brains of placebo-treated animals.

This study demonstrates for the first time that 100% protection against lethal JUNV infection in outbred guinea pigs can be achieved with a small molecule antiviral. We show that dosing with 400 mg/kg/day of favipiravir for 3 days followed by a 300 mg/kg/day maintenance dose for 7 days, is highly effective when initiated within 48 h of infection. Previously we had demonstrated up to 78% survival in JUNV-infected guinea pigs treated with a similar 14-day course of 300 mg/kg/day favipiravir, but without the loading dose (Gowen et al., 2013). Although the challenge dose (approximately 750 PFU) was slightly higher in that earlier study, the placebo treatment group survival curve from that experiment, which is representative of disease progression and severity, was very similar to that of the present study.

Albeit an aggressive dosing regimen which was employed, we have found that favipiravir is very well tolerated in guinea pigs when administered at doses as high as 500 mg/kg/day, twice daily, for 10 days (Mendenhall et al., 2011b). Moreover, the human equivalent dose based on body surface area dose translation is 86 and 65 mg/kg/day for the 400 and 300 mg/kg/day guinea pig loading and maintenance dosing, respectively (Reagan-Shaw et al., 2008). This regimen is not far off from the dosing used to treat patients in West Africa during the recent Ebola virus outbreak (Sissoko et al., 2016). In that setting, treatment consisted of a 1-day loading dose of approximately 100 mg/kg/day with a 40 mg/kg/day maintenance dose administered an additional 9 days.

The present study also investigated the ability of favipiravir to protect guinea pigs from lethal JUNV disease when delaying treatment until day 5 when many animals begin to show signs of illness. Although we were able to save 50% of the animals in this setting more representative of when a patient may seek medical attention, it is possible that extending the treatment could have improved the survival outcome. The 10-day favipiravir treatment regimen fell short of leading monoclonal antibody (mAb)-based therapeutics where uniform protection has been demonstrated when therapy was withheld until 6 days post-infection (Zeitlin et al., 2016). Despite the unparalleled level of protection observed with current anti-JUNV mAbs, they are highly specific and lack the broad-spectrum capacity necessary to

treat the expanding list of South American arenaviruses that cause hemorrhagic fever (Delgado et al., 2008). As a broadly active antiviral, favipiravir could also prove efficacious for the treatment of these emerging and re-emerging pathogenic New World arenaviruses (Mendenhall et al., 2011a).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Slobodan Paessler (University of Texas Medical Branch, Galveston, TX) for providing the molecular clone of the JUNV Romero strain and are grateful for the animal technical assistance provided by Brady Hickerson and Kie-Hoon Jung (Utah State University, Logan, UT).

Funding

This work was funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (HHSN272201000039I).

References

- Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albarino CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. *PLoS Pathog.* 2008; 4:e1000047. [PubMed: 18421377]
- Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. *Antiviral Res.* 2008; 78:132–139. [PubMed: 18054395]
- 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]
- Gomez RM, Jaquenod de Giusti C, Sanchez Vallduvi MM, Frik J, Ferrer MF, Schattner M. Junin virus. A XXI century update. *Microbes and infection/Institut Pasteur.* 2011; 13:303–311.
- 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, 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]
- 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.* 2011a; 55:782–787. [PubMed: 21115797]
- Mendenhall M, Russell A, Smee DF, Hall JO, Skirpstunas R, Furuta Y, Gowen BB. Effective oral favipiravir (T-705) therapy initiated after the onset of clinical disease in a model of arenavirus hemorrhagic fever. *PLoS neglected tropical diseases.* 2011b; 5:e1342. [PubMed: 22022624]
- Mills JN, Ellis BA, Childs JE, McKee KT Jr, Maiztegui JI, Peters CJ, Ksiazek TG, Jahrling PB. Prevalence of infection with Junin virus in rodent populations in the epidemic area of Argentine hemorrhagic fever. *The American journal of tropical medicine and hygiene.* 1994; 51:554–562. [PubMed: 7985747]
- Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J.* 2008; 22:659–661. [PubMed: 17942826]
- Sissoko D, Laouenan C, Folkesson E, M'Lebing AB, Beavogui AH, Baize S, Camara AM, Maes P, Shepherd S, Danel C, Carazo S, Conde MN, Gala JL, Colin G, Savini H, Bore JA, Le Marcis F, Koundouno FR, Petitjean F, Lamah MC, Diederich S, Tounkara A, Poelart G, Berbain E, Dindart JM, Duraffour S, Lefevre A, Leno T, Peyrouset O, Irengue L, Bangoura N, Palich R, Hinzmann J,

Kraus A, Barry TS, Berette S, Bongono A, Camara MS, Chanfreau Munoz V, Doumbouya L, Souley H, Kighoma PM, Koundouno FR, Rene L, Loua CM, Massala V, Moumouni K, Provost C, Samake N, Sekou C, Soumah A, Arnould I, Komano MS, Gustin L, Berutto C, Camara D, Camara FS, Colpaert J, Delamou L, Jansson L, Kourouma E, Loua M, Malme K, Manfrin E, Maomou A, Milinouno A, Ombelet S, Sidiboun AY, Verreckt I, Yombouno P, Bocquin A, Carbonnelle C, Carmoi T, Frange P, Mely S, Nguyen VK, Pannetier D, Taburet AM, Treluyer JM, Kolie J, Moh R, Gonzalez MC, Kuisma E, Liedigk B, Ngabo D, Rudolf M, Thom R, Kerber R, Gabriel M, Di Caro A, Wolfel R, Badir J, Bentahir M, Deccache Y, Dumont C, Durant JF, El Bakkouri K, Gasasira Uwamahoro M, Smits B, Toufik N, Van Cauwenberghe S, Ezzedine K, D'Ortenzio E, Pizarro L, Etienne A, Guedj J, Fizet A, Barte de Sainte Fare E, Murgue B, Tran-Minh T, Rapp C, Piguet P, Poncin M, Draguez B, Allaford Duverger T, Barbe S, Baret G, Defourny I, Carroll M, Raoul H, Augier A, Eholie SP, Yazdanpanah Y, Levy-Marchal C, Antierrens A, Van Herp M, Gunther S, de Lamballerie X, Keita S, Mentre F, Anglaret X, Malvy D, Group, J.S. Experimental Treatment with Favipiravir for Ebola Virus Disease (the JIKI Trial): A Historically Controlled, Single-Arm Proof-of-Concept Trial in Guinea. *PLoS Med.* 2016; 13:e1001967. [PubMed: 26930627]

Westover JB, Sefing EJ, Bailey KW, Van Wettere AJ, Jung KH, Dagley A, Wandersee L, Downs B, Smee DF, Furuta Y, Bray M, Gowen BB. Low-dose ribavirin potentiates the antiviral activity of favipiravir against hemorrhagic fever viruses. *Antiviral Res.* 2016; 126:62–68. [PubMed: 26711718]

Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. *Proc Natl Acad Sci U S A.* 2016; 113:4458–4463. [PubMed: 27044104]

Highlights

- A modified favipiravir loading dose treatment strategy was evaluated against lethal JUNV infection in guinea pigs
- Initiated 2 days post-JUNV challenge, the modified treatment regimen resulted in 100% survival
- Treatment initiated 5 days post-JUNV infection slowed disease progression and reduced mortality by 50%.
- Continued development of favipiravir as an antiviral therapy for the treatment of severe arenaviral infections is warranted

Figure 1.

Favipiravir loading dose strategy for treatment of lethal JUNV infection.

Figure 2.

Effect of a favipiravir (T-705) loading dose strategy on mortality and morbidity in guinea pigs challenged with JUNV. Animals in each group (n=6) were treated as described in Figure 1. A) Survival, B) body weight and C) temperature readings are shown. The percent changes in body weights are represented as the group mean and standard error of the mean (SEM) of the surviving animals relative to their starting weights on the day of challenge. Body temperature was measured using IPTT-300 electronic transponders subcutaneously implanted for identification and temperature measurement in conjunction with the DAS

6002 scanner (BMDS, Seaford, DE) and data are represented as the group mean and SEM. Sham-infected normal control animals (n=3) are included for comparison. *** $P < 0.001$ compared to animals receiving placebo by the Mantel-Cox log-rank test. Tx, treatment.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3.

Effect of favipiravir (T-705) treatment on serum and tissue viral titers. Three guinea pigs per treatment group were sacrificed on day 12 p.i. for determination of A) serum, B) liver, C) spleen and D) brain JUNV titers as previously described (Gowen et al., 2007). Unique symbols in each treatment group represent values for the same animal across all parameters. The starting point on the y-axis for each graph represents the respective assay detection limit. ** $P < 0.01$, *** $P < 0.001$ compared to animals receiving placebo by one-way analysis of variance with a Dunnett posttest to correct for multiple comparisons.