Antiviral Research 123 (2015) 70–77

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Ebola virus dynamics in mice treated with favipiravir

Vincent Madelain [a,b], Lisa Oestereich [c,d], Frederik Graw [e], Thi Huyen Tram Nguyen [a,b], Xavier de Lamballerie [f,g], France Mentré [a,b], Stephan Günther [c,d], Jeremie Guedj [a,b,*]

[a] INSERM, IAME, UMR 1137, F-75018 Paris, France
[b] Université Paris Diderot, IAME, UMR 1137, Sorbonne Paris Cité, F-75018 Paris, France
[c] Bernhard-Nocht-Institute for Tropical Medicine, 20359 Hamburg, Germany
[d] German Centre for Infection Research (DZIF), Partner Site Hamburg, Germany
[e] Center for Modeling and Simulation in the Biosciences, BioQuant-Center, Heidelberg University, 69120 Heidelberg, Germany
[f] Aix Marseille Université, IRD French Institute of Research for Development, EHESP French School of Public Health, EPV UMR_D 190 "Emergence des Pathologies Virales", F-13385 Marseille, France
[g] Institut Hospitalo-Universitaire Méditerranée Infection, F-13385 Marseille, France

ARTICLE INFO

Article history:
Received 21 April 2015
Revised 28 August 2015
Accepted 28 August 2015
Available online 2 September 2015

Keywords:
Ebola
Viral kinetics
Modeling
Favipiravir

ABSTRACT

The polymerase inhibitor favipiravir is a candidate for the treatment of Ebola virus disease. Here, we designed a mathematical model to characterize the viral dynamics in 20 mice experimentally infected with Ebola virus, which were either left untreated or treated with favipiravir at 6 or 8 days post infection. This approach provided estimates of kinetic parameters of Ebola virus reproduction, such as the half-life of productively infected cells, of about 6 h, and the basic reproductive number which indicates that virus produced by a single infected cell productively infects about 9 new cells. Furthermore, the model predicted that favipiravir efficiently blocks viral production, reaching an antiviral effectiveness of 95% and 99.6% at 2 and 6 days after initiation of treatment, respectively. The model could be particularly helpful to guide future studies evaluating favipiravir in larger animals.

1. Introduction

Ebola virus (EBOV) is among the deadliest pathogens currently known, leading to multiple organ failure and death in 40–90% of cases (Feldmann and Geisbert, 2011). Despite substantial and rapid progress (Geisbert, 2015), vaccines remain in early phases of clinical trials and potent antiviral drugs are urgently needed. Last year, two studies published in *Antiviral Research* showed that favipiravir, a pyrazinecarboxamide derivative approved for complicated influenza in Japan, had a large antiviral effectiveness against EBOV both *in vitro* and *in vivo* in infected mice (Oestereich et al., 2014; Smither et al., 2014). Moreover favipiravir can be given orally and has shown a good safety profile in over 2000 patients worldwide, leading the WHO to consider it as a potential antiviral candidate against EBOV. Indeed favipiravir, along with few other molecules, is currently tested in human clinical trials (Sissoko et al., 2015).

In order to support the development of new drugs, it is crucial to have a better quantitative understanding of EBOV dynamics *in vivo*. This can be achieved using mathematical models based on ordinary differential equations which characterize the nonlinear interactions between the pathogen, the host and the drug. This type of approach has provided many insights in the pathogenesis of several viruses, in particular influenza, HCV and HIV (Baccam et al., 2006; Neumann et al., 1998; Perelson, 2002). Further, by characterizing the effect of treatment on the viral load, these models have allowed to better understand the mechanisms of action and the antiviral effect of drugs *in vivo* (Perelson and Guedj, 2015).

Here we applied this approach to characterize the viral kinetics in mice treated with favipiravir or left untreated, using data previously published (Oestereich et al., 2014). Further, drug concentration data obtained in uninfected mice from different experiments were used to construct a pharmacokinetic model for favipiravir. By using a model combining both the viral kinetic and the pharmacokinetic data, we could provide an estimation of the infected cell half-life, the basic reproductive number of EBOV and the effectiveness of favipiravir in reducing viral production *in vivo*.

* Corresponding author at: INSERM, IAME, UMR 1137, F-75018 Paris, France.
E-mail address: jeremie.guedj@inserm.fr (J. Guedj).

http://dx.doi.org/10.1016/j.antiviral.2015.08.015
0166-3542/

2. Materials and methods

2.1. Data

2.1.1. Pharmacokinetic

The pharmacokinetic data were provided by Toyama Chemical (Tokyo, Japan) and came from two separate studies of $N = 54$ Crlj: CD1 female mice in total treated with favipiravir 150 mg/kg twice a day by oral route for 14 days. Nine sampling times were available on day 0 and on day 14. For each sampling time, three mice were sampled and then sacrificed. Plasma concentrations were quantified using high performance liquid chromatography, with a limit of quantitation of 0.1 μg/mL.

2.1.2. Viral kinetic

We reanalyzed viral load data from twenty C57BL/6 female mice lacking the type I interferon receptor that were infected with Zaire EBOV Mayinga 1976 strain (Oestereich et al., 2014). In brief, mice were either left untreated ($n = 10$) or were treated with oral favipiravir 150 mg/kg BID for 7 days, starting at 6 ($n = 5$) or 8 ($n = 5$) days post infection (dpi), respectively. All mice treated at 6 dpi survived while all mice left untreated or treated 8 dpi died within 15 dpi (Fig. 1). Viral load was quantified in immunofocus assay as focus forming units (FFU) with a limit of quantitation of 1.5 $\log_{10}$ FFU/mL.

2.2. Pharmacokinetic model of favipiravir

Favipiravir plasma concentration over time, $C(t)$, was characterized by a standard one compartment model with first-order absorption and elimination. In that model total plasma concentration, $C(t)$, is given by:

$$C(t) = \frac{D}{V_D}\frac{k_a}{k_a - k_e}(e^{-k_e t} - e^{-k_a t}) \quad (1)$$

where D is the dose, V_D is the apparent volume of distribution, k_a is the absorption rate and k_e is the elimination rate. Further a protein-binging rate, fu, of 0.90 (data from the manufacturer) was used, and therefore, the active free concentration of favipiravir was defined to $C_u(t) = C(t) \times fu$ where $C(t)$ is given by Eq. (1).

2.3. Viral kinetic model for EBOV

The change in viral load was characterized using a standard viral dynamic model during acute infection (Baccam et al., 2006):

$$\begin{aligned} \frac{dT}{dt} &= -\beta VT \\ \frac{dI_1}{dt} &= \beta VT - kI_1 \\ \frac{dI_2}{dt} &= kI_1 - \delta I_2 \\ \frac{dV}{dt} &= pI_2 - cV \end{aligned} \quad (2)$$

where T, I_1, I_2 and V denote uninfected target cells, non-productively infected cells, productively infected cells and free virions, respectively (Fig. 2). These four compartments are parameterized as concentrations related to plasma volume. Free virions infect target cells at rate β, and are cleared at rate c, i.e. $\ln(2)/c$ is plasma virion half-life. After infection, cells do not immediately produce virus and rather enter into an eclipse phase of half-life equal to $\ln(2)/k$. Lastly productively infected cells produce p virions per day and are lost with rate δ, i.e. $\ln(2)/\delta$ is the infected cell half-life. Therefore, the average total infected cell lifetime, t_{inf}, is given by $1/k + 1/\delta$.

The model predicts first an exponential growth of the viremia as long as there is a large number of available target cells for infection (up-slope). At the end of this first phase, the target cells are largely depleted, the number of new infections declines and therefore the loss of infected cells can no longer be compensated by new cell infections. Consequently, the number of infected cells, and hence the total viral production, rapidly declines (down-slope). Mathematical analysis shows that this decline is exponential with a rate proportional to the infected cell half-life. One can derive from this model the basic reproductive number, R_0, representing how many new productively infected cells would be generated by one infected cell, and is given by $R_0 = \frac{p\beta T_0}{\delta c}$. The model was reparametrized as a function of R_0, such as $\beta = 1/R_0$, and we constrained it to be larger than 1 to ensure productive infection. More details

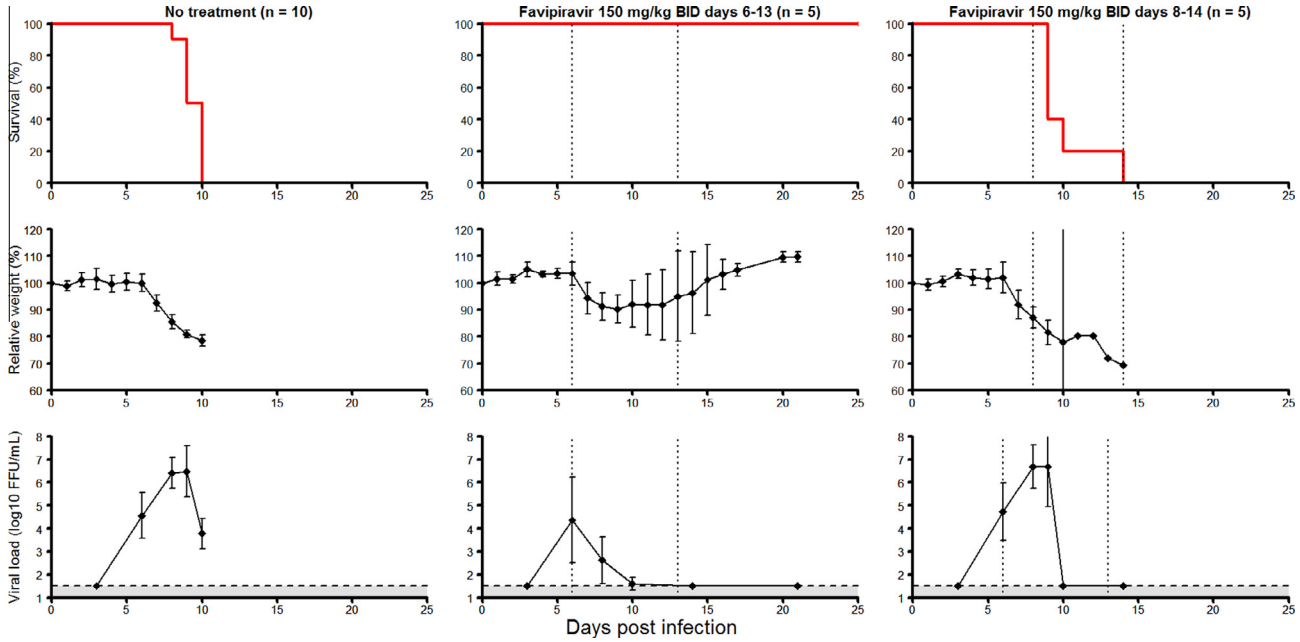

Fig. 1. Survival (top line), mean relative weight loss (middle line), and mean viremia (bottom line) in mice IFNαR−/− infected by 1000 FFU of Zaire Mayinga 1976 Ebola virus by nasal route, and receiving no treatment (left panels), favipiravir started on day 6 (middle panels), and on day 8 (right panels). Mice treated by favipiravir received dose of 150 mg/kg twice a day, by oral route. Error bars represent 95% confidence interval of the mean. Reproduced from Oestereich et al. (2014).