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A quantitative assessment of dynamical differences of RSV infections in vitro and in vivo

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ABSTRACT

Experimental results in vitro and in animal models are used to guide researchers in testing vaccines or treatment in humans. However, viral kinetics are different in vitro, in animals, and in humans, so it is sometimes difficult to translate results from one system to another. In this study, we use a mathematical model to fit experimental data from multiple cycle respiratory syncytial virus (RSV) infections in vitro, in african green monkey (AGM), and in humans in order to quantitatively compare viral kinetics in the different systems. We find that there are differences in viral clearance rate, productively infectious cell lifespan, and eclipse phase duration between in vitro and in vivo systems and among different in vivo systems. We show that these differences in viral kinetics lead to different estimates of drug effectiveness of fusion inhibitors in vitro and in AGM than in humans.

1. Introduction

Infants and the elderly are most likely to experience serious illness or death from respiratory syncytial virus (RSV) (Borchers et al., 2013). To alleviate this burden, researchers have long been searching for an antiviral or vaccine that would effectively treat or prevent RSV (Collins and Melero, 2011; Esposito and Pietro, 2016). Several fusion inhibitors have been tested in vitro and in animal models (Zheng et al., 2016; Perron et al., 2016; Bonfanti et al., 2008; Feng et al., 2015; Bond et al., 2015; Lundin et al., 2010; Andries et al., 2003; Cianci et al., 2005), but some have had difficulty making the transition from animals to humans. Likewise, many RSV vaccines have been tested in animal models, with the induced antibody neutralizing ability tested in vitro, but promising candidates have not been as effective in humans (Esposito and Pietro, 2016).

For RSV, as for many viral infections, in vitro and animal models are the primary systems of study used to understand the dynamics of the infection (Weiss et al., 2014). These same systems are also the primary test beds for new antivirals and vaccines. It is not clear, however, how experimental results translate from one system to another (Bem et al., 2011). This is particularly important when trying to extrapolate antiviral or vaccine studies from in vitro or animal models to humans (Jorquera et al., 2016). Differences in cell tropism (Jia et al., 2014; Taylor, 2017; Shakeri et al., 2015), the immune response (Taylor, 2017; Jorquera et al., 2016; Sacco et al., 2015), and other physiological interactions (Zanin et al., 2016) between these systems might lead to differences in infection dynamics and the efficacy of treatment. Our lack of understanding of how differences in preclinical systems and humans alter disease dynamics leads to a paltry ~ 12% success rate in moving treatments from preclinical through Phase III testing and an ~ 11% success rate for vaccines to move through the development pipeline (Davis et al., 2011).

In addition to differences between experimental systems and humans, there are also differences in infection dynamics in individual humans. Broadly, there are differences in the immune response to RSV between healthy adults, children, and the elderly (Walsh et al., 2013; Mcintosh et al., 1978; Chung et al., 2007). This leads to more serious infections, with higher mortality and hospitalization, in children and the elderly as compared to healthy adults (Borchers et al., 2013; Stein et al.,; Anderson et al., 2016). This also leads to differences in clinical manifestation of the disease with children and the elderly exhibiting more severe symptoms and showing a greater propensity for involvement of the lower respiratory tract (Dayar and Kocabas, 2016; Shi et al., 2015; Park et al., 2016).

While there are differences in host-cell interactions when RSV is introduced into different hosts, viral replication follows a similar basic process in all hosts. Respiratory syncytial virus is an enveloped virus containing negative-sense RNA that has 10 genes encoding 11 proteins (Lee et al., 2012). RSV binds to host cells through the G transmembrane protein (Teng et al., 2001) and fuses via the F transmembrane protein

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(Feldman et al., 2000). The F protein is also responsible for fusing membranes of neighboring cells giving rise to the syncytia that give the virus its name (Gonzalez-Reyes et al., 2001). Once internalized, the genome is released into the cell's cytoplasm where transcription and replication take place (Follett et al., 1975). Newly formed genomes and proteins migrate to the surface of the cell where they form filamentous viral particles before breaking through the cell membrane (Vanover et al., 2017; Shaikh et al., 2012). The final stages of filament maturation and budding of the virus from the cell are mediated by the matrix M protein (Shahriari et al., 2016; Foerster et al., 2015). These basic steps of replication can be captured and reproduced using mathematical equations to help us improve our understanding of RSV dynamics.

Mathematical models of the in-host dynamics of viral infections have been used to quantitatively describe the infection process for many different viral infections (Baccam et al., 2006; Nguyen et al., 2015; Perelson et al., 1996; Neumann et al., 1998; González-Parra and Dobrovolny, 2015; González-Parra et al.,). Specifically, mathematical models are now being used to quantitatively compare infections caused by different strains of virus (Pinilla et al., 2012; Paradis et al., 2015; Simon et al., 2016; Petrie et al., 2015) or by different infections (González-Parra et al., 2018). The simplest mathematical model captures the basic steps of virus entry, internal replication, and viral budding (Perelson et al., 1996; Baccam et al., 2006) and can be used to simulate a variety of scenarios including single-cycle infection and multiple-cycle infection by changing the initial conditions (Pinilla et al., 2012; Paradis et al., 2015; Beggs and Dobrovolny, 2015), or in vivo infections by changing the values of model parameters to reflect the effect of the immune response. Such studies lead to an understanding of which parts of the viral replication cycle are changed when moving from one virus-host system to another.

In this paper, we use a viral kinetics model to estimate parameters for RSV infection in five different systems: in vitro, African green monkey (AGM), elderly patients, pediatric patients, and healthy adults. This allows us to quantitatively compare the viral replication cycle in these different systems. We find differences in several viral kinetics parameters between the groups, including the viral clearance rate, the cell's productively infectious lifespan, and the duration of the eclipse phase. We show that these differences alter the effectiveness of drug treatment in the different systems such that EC_{50} measured in vitro or in animals does not reflect the EC_{50} needed for treatment of humans.

2. Material and methods

2.1. Models

We use two mathematical descriptions of viral dynamics. The first is an empirical description of the viral time course, first presented by Holder and Beauchemin (2011). While this model does not give insight into the underlying dynamics of the infection, it allows measurement of some of the important viral titer curve characteristics (González-Parra et al., 2016). The model is given by the equation

$$V(t) = \frac{2V_p}{\exp[-\lambda_g(t-t_p)] + \exp[\lambda_d(t-t_p)]}, \tag{1}$$

where λ_g and λ_d are the exponential growth and decay rates, respectively, V_p is the peak viral titer, and t_p is the time of viral titer peak. While this equation will not reproduce a single-cycle experiment well since the viral growth in this case is not exponential (Holder and Beauchemin, 2011), it can be used for multiple-cycle experiments where viral growth is exponential (González-Parra et al., 2016). The in vitro data used in this study were all from experiments with an MOI of less than 1 (González-Parra et al., 2018) with the exception of the data from Liesman et al. (2014), which has an MOI of 1. Note that this equation does not fully capture all the dynamics of a viral infection, as it notable neglects any initial transient changes in viral load, so is not meant to fully characterize viral kinetics, but it does estimate features that are traditionally used to characterize infections (González-Parra et al., 2016). This simple equation has only four independent parameters, making parameter estimation simpler than for a more complex, kinetic model of viral infection. We require at least four experimental data points, with at least two during the growth phase and two during the decay phase, to identify the parameters (González-Parra et al., 2016).

Our second model is a viral kinetics model that incorporates the basic biological processes that occur during the infection. The model is an extension of the basic viral infection model for influenza described in Baccam et al. (2006),

$$\begin{aligned}
\frac{dT}{dt} &= -\frac{\beta}{N}TV \\
\frac{dE_1}{dt} &= \frac{\beta}{N}TV - \frac{n_E}{\tau_E}E_1 \\
\frac{dE_j}{dt} &= \frac{n_E}{\tau_E}E_{j-1} - \frac{n_E}{\tau_E}E_j \quad \text{for } j = (2,\ldots,n_E) \\
\frac{dI_1}{dt} &= \frac{n_E}{\tau_E}E_{n_E} - \frac{n_I}{\tau_I}I_1 \\
\frac{dI_j}{dt} &= \frac{n_I}{\tau_I}I_{j-1} - \frac{n_I}{\tau_I}I_j \quad \text{for } j = (2,\ldots,n_I) \\
\frac{dV}{dt} &= p\sum_{j=1}^{n_I} I_j - cV.
\end{aligned} \tag{2}$$

In the model, virus V, infects target cells, T, at rate β. Once infected, the cells enter an eclipse state, E, during which they are producing viral proteins and RNA, but not yet releasing virus. After an average time τ_E, the cells transition to a productively infectious state, I, where they are producing virus at rate p. After an average time τ_I, the productively infectious cells die. Virus loses infectivity at a rate c.

This model captures the basic processes of viral entry, replication within the cell, and release of new virions, so can be applied to both in vitro and in vivo systems (Pinilla et al., 2012; Paradis et al., 2015; González-Parra and Dobrovolny, 2015; Petrie et al., 2015). While our model will be applied to patient data, we do not include an explicit immune response since there is not enough data to accurately determine the values of the extra parameters needed to describe the immune response. Instead, our estimates of the parameters in these different systems will reflect the effect of the immune response. For example, we expect the value of c, the viral clearance rate, to be larger in vivo than in vitro since in vivo systems include an antibody response that helps clear virus from the system.

This model assumes a gamma distribution, represented by the multiple compartments for E and I, for the transition times between the eclipse state and the productively infectious state, as well as for the transition times between the productively infectious and dead cells. The number of compartments in the eclipse state is given by n_E while the number of compartments in the productively infectious state is given by n_I. Models that include non-exponential transitions between cell states more accurately reproduce experimental viral kinetics (Holder and Beauchemin, 2011). This model has more parameters than the empirical model, some of which cannot be identified with viral titer data alone (Miao et al., 2011; Pinilla et al., 2012).

2.2. Experimental data

Our aim in this study is to compare the dynamics of RSV in several different experimental and clinical systems, thus we combined data from several different sources, summarized in Table 1 and briefly described below.

- Experimental data from in vitro RSV infections was collected from the literature as described in González-Parra et al. (2018). While we required that all in vitro data use the same strain of RSV (A2), the cell culture and experimental procedures for each data set varied since they were drawn from multiple sources. The sources and some details of the data sets are included in Table 2.