Eindopdracht

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Theory

Introduction

At the time of writing, there are no FDA-approved therapies available to fight the Rift Valley Fever Virus (RVFV). In an effort to find a good treatment for RVFV, a series of FDA-approved drugs have been screened to see their effect on RVFV. This article is about investigating the effect of different drugs on RVFV. The screened drugs have been evaluated for their potential for viral inhibition and cellular toxicity. Clusters of drugs with (partially) similar pathways or protein targets have been used.

The virus

The Rift Valley Fever Virus (RVFV) is a vector disease caused by the rift valley fever virus. This fever virus is a (-)ssRNA virus, which can occur in a large number of animal species, such as: Cattle, goats, sheep, and various types of rodents are susceptible to this disease. Rift valley fever is a zoonosis and can therefore also be transmitted to humans, making it a dangerous disease and high on the list of the OIE (World Organization for Animal Health).

The virus has the properties that it:

- Can survive several months at low temperatures ($< 6°C$)
- Can live up to 50°C
- Can withstand an alkaline environment, but does not function actively here.
- Can survive for a long time in dried manure and nasal discharge.

The virus is mainly found in Africa, where 19 more outbreaks were expected to follow since the first reported outbreak in 1931. At the moment (June 2021) there is an outbreak in Kenya, where of the 32 human infections, 11 people have already died.

Infections often take place via the vector. This is a mosquito or other winged insect that spreads the virus. Direct contamination can also be via animal body fluids. Or in humans cases, eating infected meat or drinking raw milk.

Rift valley fever has a short incubation period of 1 to 6 days. The symptoms very between species and are age dependent. In animals, the general symptoms are:

- Fever
- Vomit
- Abort pregnant animals

Some of the animals also die from this. The following symptoms occur in humans:

- Fever
- Headache
- Muscle strain
- Nausea

In minor cases, worse symptoms can also occur, such as:

- Blindness ((temporary) loss of sight)
- Meningitis
- Petechiae
- Bleeding (internal and external)
- Liver problems

People with internal bleeding have a mortality up to 50%.

Fighting the virus is difficult, because it is mainly spread through mosquitoes. Vaccination is a temporary solution because it only provides protection for 3 years. The disadvantage of this is that it leads to abortion in pregnant livestock. In Europe, the chance of infections occurring is very small, due to the lack of a suitable living environment for the vector. But with the changing climate, better environments are created in Europe and Asia for the vector. Making it a possible future threat for other continents.

The experiment

Different cell cultures were used for the experiment. Including human airway epithelial cells. Recombinants have been made for the virus with different plasmids. All tests of the experiment were performed *in vitro*. Some assays were infected with luciferase. SiRNA knockdowns have also been made for see the effect of gen knockdown.

A total of 420 approved drugs were tested for RVFV inhibition. These drugs are diverse and related to oncology, cardiology, anti-inflammatories, immune system and more, to test a wide variety of drugs. As a result, the drug Sorafenib showed the best desired effect in vitro. Sorafenib reduces RVFV replication in multiple cell types and has no or very few cell toxic processes. Sorafenib also affects different stages of the RVFV life course. Later, *in vito* experiments were also performed with Sorafenib. these also show positive results, although not statistically significant in every test.

Computer model

A computer model of the RVFV infection has been developed with the static software R. Virus behavior over time has been estimated with the package deSolve and the Isoda function. The aim here is to create a model for a natural virus infection, and then use the model to highlight the potential of the antiviral mechanism of Sorafenib.

Everything in the model is based on HSAECs cells.

The computer model is a 4-dimensional non-linear system. A simplified version of an existing model for the influenza virus. This model has 4 variables:

- U = Not infected
- E = Early infected cells that cannot yet produce viruses
- I = Virus producing cells (Infected)
- V = Virus

Normal cells replicate at a base rate g. The infection rate is indicated with b. When infected, an uninfected cell immediately becomes an infected cell. However, these infected cells cannot immediately produce virus particles, this delay is indicated by l. Infected cells, after the delay, produce viruses at a rate p, and eventually die at a rate d.

In the model it looks like this:

dU/dt = gU-bUV (1)
dE/dt = bUV-lE (2)
dI/dt = lE-dI (3)
dV/dt = pI-bUV (4)

Initial conditions were estimated by sampling 1h intubated assay wells. For the model parameters, groups of cells were quantified at 0h and 24h. and tested for infection rate, virus production rate ect.

Computer model results

To collect data for the computer model, the growth of RVFV *in vitro* with and without Sorafenib has been characterized. The cells were pre-treated with DSMO or Sorafenib and then infected with RVFV. At certain intervals, the cell cultures were washed and the free viruses were counted. in this way certain parameters and initial values could be established.

According to the article, the model is an excellent reflection of the real lab data. The model captures the natural infection dynamics well. This also makes it possible to see which part of the medicine would have an impact. What happens if the infection rate decreases? how effectively does the drug inhibit? All questions that can be answered on the basis of the dynamic model.

In the experiment it was asked which part of the virus life course can be disrupted by Sorafenib. as in, if Sorafenib blocks infection, then in the model this should be simulated as a lower b. The model simulates this well, the number of viruses and infections decreases both in the lab and in the model at approximately the same rate.

All in all, the model is a good and useful way to test hypotheses and run simulations, without the need for lab work.

Base Model

Below this text the results of the base model as described in Benedict et al. (2015) are displayed.

Table 1: Table1: standard model parameters/values.

Symbol	Value
g	$0.742 * 10^{-3}$ h^{-1}
b	0.195 h^{-1} U^{-1}
l	1/4 h^{-1}
d	0.0222 h^{-1}
p	0.531 h^{-1}
U(0)	$1.34 * 10^5$ HSAEC/ml
E(0)	$3.34 * 10^4$ HSAEC/ml
I(0)	0 HSAEC/ml
V(0)	0 PFU/ml

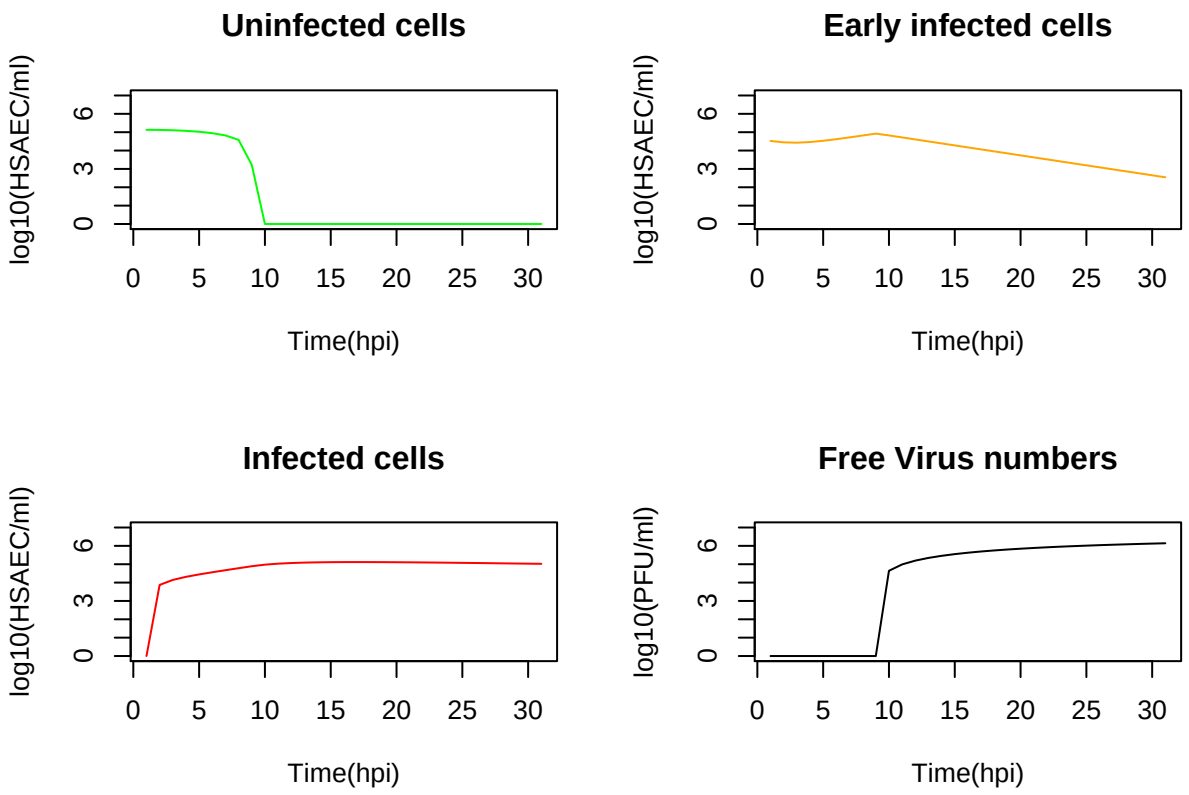

Figure 1: results of the standard model.

Different delays

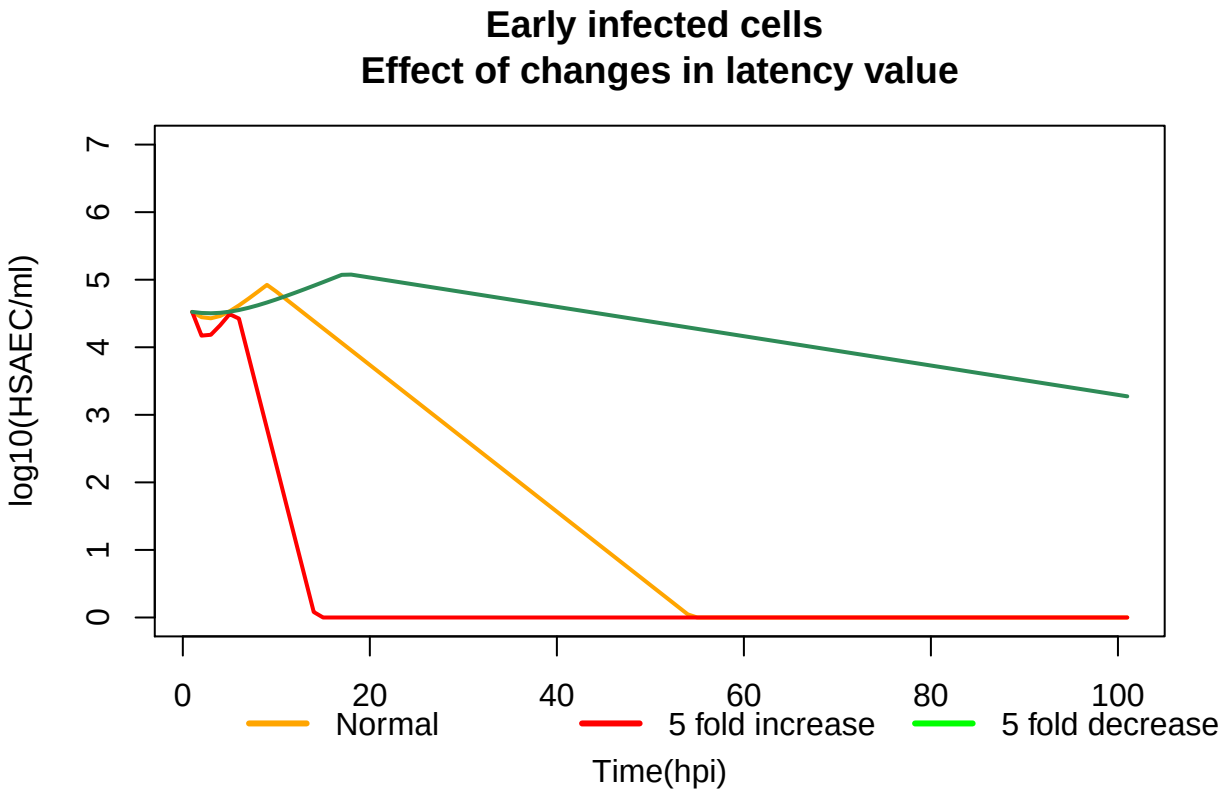

Figure 2: results of the standard model with altered delays.

The chart above shows the early infected cells, these infected cells cannot immediately produce virus particles. The delay on this was called *l*. In the graph, the values for the delay have been adjusted by a 5 fold increase and decrease. Where a decrease causes a longer delay and an increase of the delay value provides faster transition to infected state (All other parameters/values where unaltered as seen in table1).

With an increased delay value, all the early infected cells changed within 16 hours to the infected state. While with the normal rate this takes at least 45 hours.

Updated Model

Here is a model of the virus's interaction with Human Small Airway Epithelial Cells (HSAEC). Based upon a modified version of the base model Benedict et al. (2015) where we added a viral decay parameter to simulate it closer to reality based upon Handel's influenza model Handel, Longini, and Antia (2009) . Used here are the standard situation (no drugs) parameters and values, displayed in this table.

Table 2: Table2: standard parameters and values updated model.

Symbol	Value
g	$0.742 * 10^{-3}$ h^{-1}
b	0.195 h^{-1} U^{-1}
l	1/4 h^{-1}
d	0.0222 h^{-1}
p	0.531 h^{-1}
vd	2.4 h^{-1} (10/day) (under assumption that all viruses decay similarly Handel, Longini, and Antia (2009))
U(0)	$1.34 * 10^5$ HSAEC/ml
E(0)	$3.34 * 10^4$ HSAEC/ml
I(0)	0 HSAEC/ml
V(0)	0 PFU/ml
D(0)	0 HSAEC/ml

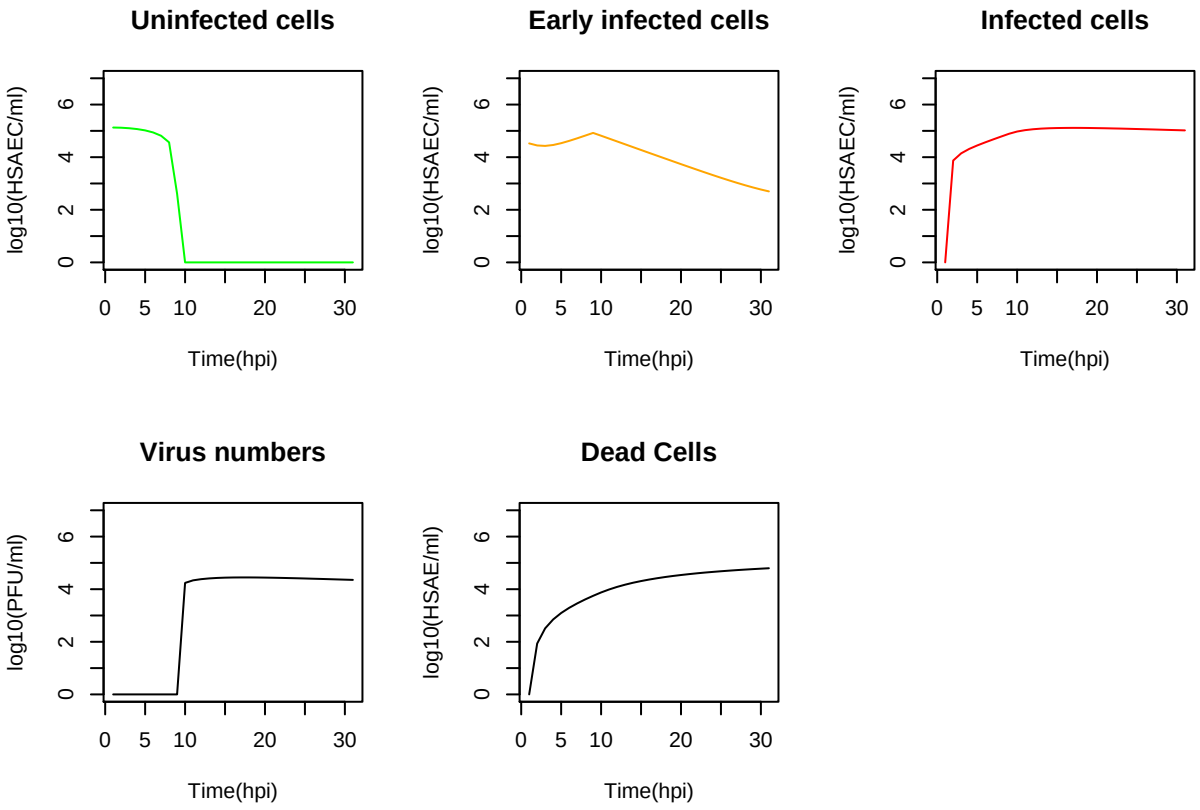

Figure 3: results of updated model using standard parameters/values.

Benedict, Ashwini, Neha Bansal, Svetlana Senina, Idris Hooper, Lindsay Lundberg, Cynthia de la Fuente, Aarthi Narayanan, Bradford Gutting, and Kylene Kehn-Hall. 2015. "Repurposing FDA-Approved Drugs as Therapeutics to Treat Rift Valley Fever Virus Infection." *Frontiers in Microbiology* 6 (July). https://doi.org/10.3389/fmicb.2015.00676.

Handel, Andreas, Ira M. Longini, and Rustom Antia. 2009. "Towards a Quantitative Understanding of the Within-Host Dynamics of Influenza A Infections." *Journal of The Royal Society Interface* 7 (42): 35–47. https://doi.org/10.1098/rsif.2009.0067.