Improving the experimental characterization of anti-influenza drug efficacy using mathematical models

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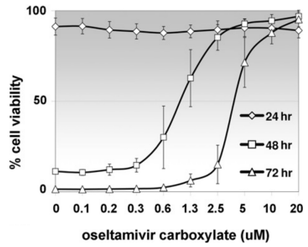
Background

- The degree of drug-resistance of an influenza virus strain can be obtained by determining an estimate of the 50% inhibitory concentration (IC_{50}); the IC_{50} is the drug concentration needed to inhibit half of a particular effect.
- There are problems with existing methods in estimating the IC_{50}, resulting in inconsistent estimates between laboratories.
- Discrepancies between the experimentalists' definition of the IC_{50} which is based on macroscopic quantities, and that of mathematical modellers' which is based on microscopic processes reduces the usefulness of these estimates.

Hypothesis



Inconsistent IC_{50} estimates could be the result of differences in the time at which experimentalists decide to take measurements to estimate the IC_{50}.

From Noah et al. 2007.

Objectives

- Confirm if changes in experimental measurement time affects IC_{50} estimates for any given drug-strain pair.
- Develop an experimental method where estimates of the IC_{50} will not be influenced by measurement time, and also minimize the difference between the experimentalists' and the modellers' definition of the IC_{50}.

A mathematical model for in vitro infection

The infection model is

$$\frac{dT}{dt} = -\beta TV$$
$$\frac{dL}{dt} = \beta TV - \frac{L}{\tau_L}$$
$$\frac{dI}{dt} = \frac{L}{\tau_L} - \frac{I}{\tau_I}$$
$$\frac{dV}{dt} = (1-\epsilon)pI - cV$$

where β is the rate at which target cells, T, are infected by virus, V, and enter the latently-infected state. After an average time, τ_L, the latently-infected cells, L, become productively-infected cells, I, and produce virus, V, continuously at rate p, and die from cytotoxic effects after producing virus for an average time, τ_I. c is the clearance rate of virus.

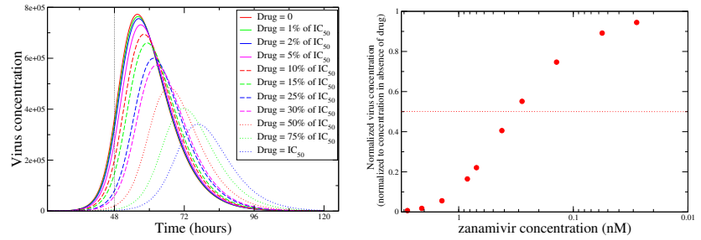
The drug (ϵ_{max}) model is

$$\epsilon = \frac{\epsilon_{max} D}{D + IC_{50}}$$

where D is the drug concentration, ϵ_{max} is its maximum effect, and IC_{50} is the input value (2.8 nM).

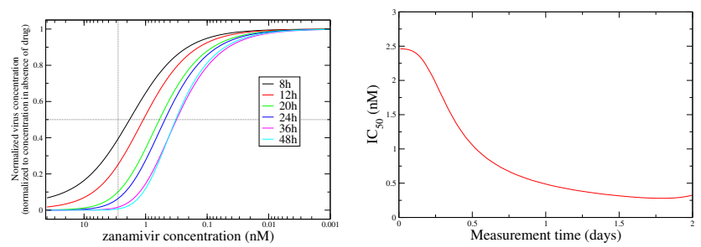
$$\text{fraction of dead cells} = 1 - \frac{T_t + L_t + I_t}{T_0}$$

Estimating the IC_{50} from virus produced



Left: As the drug concentration increases, the peak of the viral titer is reduced, and the growth of the virus concentration is delayed.

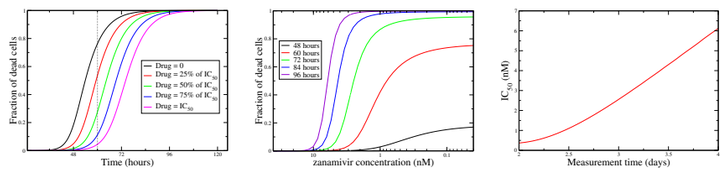
Right: Normalized virus concentrations (normalized to concentration in absence of drug) for different drug concentrations at 48 hours.



Left: Depending on the time of measurement, there can be a significant difference in the magnitude of the IC_{50} estimate.

Right: The estimate of the IC_{50} is neither independent of the measurement time, nor does it ever match the input value at any measurement time.

Estimating the IC_{50} from cell death



Left: As the drug concentration increases, a delay in cell death occurs.

Center: The IC_{50} estimate would not be the same if samples of dead cells were to be taken at different measurement times.

Right: When the samples of dead cells are taken at later measurement times, the estimate of the IC_{50} increases.

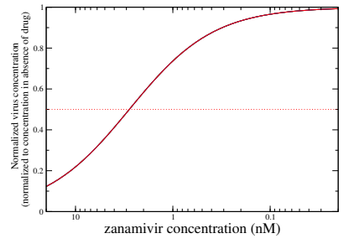
Simulating single cycle growth experiments

When productively-infected cell death, is neglected

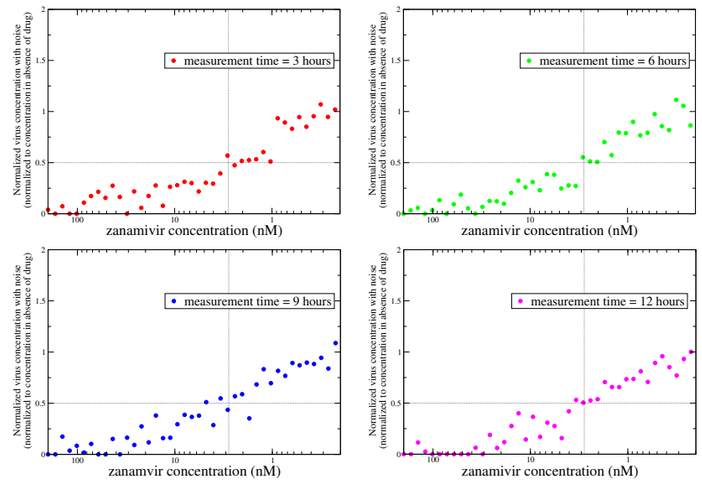
$$V(t) = \frac{\rho N}{c}(1 - e^{-ct})$$

where $\rho = (1-\epsilon)p$, and N is the number of productively-infected cells.

Estimating the IC_{50} from single cycle growth



The IC_{50} estimates extracted from the viral titer measurements at 3, 6, 9, 12, and 15 hours are independent of measurement time (superimposed).



Experimental variability (Gaussian noise of 10%) was added to the single cycle growth simulation to determine the robustness of using the single cycle growth method for estimating the IC_{50}.

Conclusions

- The estimates of the IC_{50} measured from the two experiments shown are susceptible to changes in measurement time.
- The IC_{50} estimates measured in the two common experimental methods differ from the IC_{50} value (2.8 nM) used as input in the simulations.
- The proposed single cycle growth experiment is one solution to the above problems. The theoretical IC_{50} used as an input value can be recovered reliably, and the extracted IC_{50} estimates are robust to changes in measurement time and variability in the measurements.

Future work

- IC_{50} estimates from single cycle growth experiments are limited to neuraminidase inhibitors. Other experimental methods should be explored that would yield consistent IC_{50} estimates for adamantanes.