Victoria 11/24/22

I’ve been working with Gib to simplify the monolayer ABM to parameterize the cellular uptake kinetics and metabolism of stirred cell suspensions under supraoxic and anoxic conditions. We did so by assuming no cell growth, proliferation or death during the experiment duration (3 hours). Cell uptake and metabolism is modelled in a single cell and scaled to the density of the cell suspension (typically 5 million cells per mL). The culture is continuously stirred so there is immediate equilibration of the drug volume upon addition.

I’ve been testing the model using our historical data for Tarloxotinib (Co of 0.5 to 50 uM) in FaDu single cell suspensions (5.4 million cells per mL), as we are intending on using the program to parameterize the cellular uptake and metabolism of several novel prodrugs that we believe will behave similarly (owing to having the same trigger moiety).

Firstly, I tried fitting CELL\_DIFF\_IN and CELL\_DIFF\_OUT to the Ct-profile of Tarloxotinib in supraoxic cultures (with Kmet = 0, C2 = 1, KO2 = 0.034 uM and T1/2 = 3000h). Model fits are pretty good and are shown below (experimental values are shown by the points, and lines show the model fits at the three different concentrations):-

Next, I tried parameterizing kmet0 by fitting to the Ct-profile of Tarloxotinib in anoxic cultures with the CELL\_DIFF\_IN (=3.7 min-1) and CELL\_DIFF\_OUT (=0.015 min-1) fixed to the values determined from supraoxic experiments. The fitted values for Kmet0 differed according to the initial concentration (Co), with the rate of intracellular metabolism significantly faster at a Co of 0.5 (kmet0 = 0.09 min-1) compared to a Co of 5 uM (kmet0 of 0.06 min-1) and slower still at a Co of 42 uM (Kmet = 0.01 min-1).

Co = 0.5, model fits shown are with Kmet = 0.09 min-1                                Co of 5 uM, model fits shown are with kmet = 0.06 min-1                   Co of 42 uM, model fits shown are with kmet = 0.01 min-1

It is thereby evident that unlike SN30000, PR-104A and CP-506, Tarloxotinib will require a concentration-dependent term to describe the rate of intracellular metabolism. The Km and Vmax were set to 0 for SN30000, PR-104A and CP-506.

I’m a bit confused as to how the kmet0 relates to the Vmax and Km, as I have never needed to fit all three simultaneously. In the past, we only parameterized the Vmax and Km to model the saturable kinetics of drug metabolism. We never integrated a term for kmet0 into the paradigm.

For reference, the Fortran code is as follows:

Any help would be much appreciated 😊

Kevin 11/24/22

There are 2 answers to the Kmet0 question. One is that it has no relationship. There is a concentration independent rate constant representing linear metabolism (kmet0) and a concentration dependent metabolism parameterized by Km and Vmax.

If you want to fit concentration dependent rates it would be easiest to try to fit Vmax and Km with Kmet0 =0.

So the second answer is kmet0 is equal rate/C so if you plot kmet0 vs concentration you should get a hyperbolic curve which will give you initial estimates of Km and Vmax. I suspect with n=3 this wont work but essentially the fitted Kmet0s give you a series of Vmax/(C +Km) to plot. If it really looks like this will occur with the new drugs you may want to plan an independent expt where you say use 8 -10 initial concentrations and just measure EC and IC at one time point - this looks good in papers anyway.

However looking at the graphs I suspect you will need an linear and non-linear component. This makes an initial estimate of Km more difficult

The fits look pretty good. I suspect the poorer fit under anoxia for 0.5uM is due to a very small EC or IC instability/metabolism that even shows up a bit under hyperoxia but is not big enough to visualise in the mass balance. Although LLQ will pay a part in that also?

The fits seem pretty good compared to the complexity we went through with Phoenix, so I would not trust our previous fits completely to calibrate what you are trying to do here.

Better to simulate some purely Michaelis-Menten metabolism and see how you get on.

Also remember that in all our work so far the intracellular metabolism function operates on total intracellular concentration making the kmets hard to compare across papers.

It would be interesting to simulate some (almost?) deterministic data (plenty of points) and see if Gib's fitting program can reproduce it. Hopefully you can then get a SSQ or other measure of goodness of fit, although some weighing micht be needed.

HTH but get back to me anytime