27/10

I’m interested in using the monolayer ABM to investigate the cellular uptake kinetics of a novel hypoxia-activated prodrug (i.e. parameterization of forward rate constant for cellular uptake, maximum rate of metabolic conversion of prodrug to effector under anoxic conditions, and reverse rate constant for cellular efflux). Would you have time to discuss the constraints of the model?

More specifically, I would like to use a different cell line to that originally used for parameterization of the oxygen/glucose consumption model (FaDu rather than HCT-116 cells). How much flexibility do we have in the input parameters? Am I right in thinking that this exchange would be relatively straight forward, in which, the oxygen consumption/transport parameters in the ‘nutrient’ tab are likely to remain constant. Differences in cell growth/proliferation would be accounted for by input of the measured cell volume of FaDu rather than HCT-116 (for ‘volume-dependent cell growth’).

We will not be monitoring glucose, lactate or a tracer in this instance, with oxygen (anoxia vs. normoxia (21%) or supraoxia (95%)) and the drug concentration being the only variables. In an ideal world, I would like to use the monolayer ABM to deduce the aforementioned cell uptake parameters in a stirred cell suspension format. In which, stirred vials of 50 million cells (5 million cells per mL in a 10-mL volume of culture medium without fetal calf serum) would be pre-equilibrated to strict anoxia (0% O2) or supraoxia (95% O2). A hypoxia-activated prodrug would be added to the vial in a 50-uL volume using a positive displacement pipettor, and samples would be attained for up to three hours thereafter. The concentration-time profile of the prodrug and effector in the intracellular and extracellular volumes would be determined by mass spectrometry. I’m not aware of the Monolayer ABM being used in this context – for a stirred cell suspension experiment – but assume that a simple ‘fix’ would be to set the medium diffusion coefficient to a high value so there is no constraint in drug diffusion.

Another key question that I have is in regards to protein binding. In monolayer based assays, fetal calf serum is used in the culture medium. From my experience, the fetal calf serum can act as a drug reservoir to slow cellular uptake. As far as I can see, protein binding isn’t formally included as an input parameter in the monolayer ABM so I shouldn’t have issue in the exclusion of fetal calf serum from my stirred cell suspension experiments.

Any thoughts would be much appreciated?

31/10

Hi Victoria,

I'm refreshing my familiarity with the monolayer model (it's been 2 years since I looked at it, and things slip away.)  I think what you want to do is feasible, but it might require some code changes.  What's really needed is a version of the model aimed at simulating stirred cell suspensions.

Before making any definite responses I'd like to get some clarification.  Since you are planning an experiment lasting only 3 hours, am I right in thinking that there will be no cell death?  In the normoxia and supraxia cases cells will be growing and some will be dividing, leading to some increase in the total cell count (I'm assuming that glucose is not constraining.)  Under anoxia growth will be suppressed.  Anoxic cells are fated to die, but death is delayed.  Can you explain to me how you pre-equilibrate to strict anoxia?  Naively, it seems to me that the duration of the equilibration phase will be an important factor, because if it is too long cells will start to die.  I have forgotten (or perhaps never knew) how anoxic cells behave with regard to uptake of a drug like your HAP.  We need to be sure that the model implements the assumptions that you require.

Let's start by helping me understand these issues.

31/10

Great questions, they have definitely got me thinking!

I wouldn’t anticipate drug-induced cytotoxicity during the experiment duration (~ 3 hours). Our released effectors (e.g. EGFR or FGFR inhibitors) are typically cytostatic with death resulting in the day or days thereafter. While we presume that there is no cell death during the experiment duration, the shear stress from continuous stirring can result in damage to cell membranes so there can be a degree of non-drug induced cell death (typically less than 5-10%).

I wouldn’t anticipate that an adherent cell line would continue to proliferate in suspension. Control cultures (oxygenated and anoxic) show a slight loss of cell density (5-10%) when counts are compared from the beginning to end of experiment. Glucose concentrations may be limiting, as cell densities are typically 3 - 5 million cells per mL. This hypothesis would be easy to interrogate though by measuring the cellular uptake of radiolabelled glucose (i.e. temporal decline in glucose concentration within the culture medium).

Pre-equilibration involves the continuous gassing of a stirred cell suspension with a supraoxic (95% O2, 5% CO2) or anoxic gas mixture (5% CO2, bal N2) for one hour prior to drug addition. Gassing is maintained for the duration of drug exposure (up to 3 hours thereafter). Total experiment duration is therefore about five hours.

I have investigated the cellular uptake of two novel prodrugs (CP-506 and Tarloxotinib) and plan to do so with another two more in the coming months. Cellular uptake data for CP-506 has been modelled using the monolayer ABM, as described in Figure 4 in <https://www.frontiersin.org/articles/10.3389/fphar.2022.803602/full>

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| [https://www.frontiersin.org/files/MyHome%20Article%20Library/803602/803602_Thumb_400.jpg](https://www.frontiersin.org/articles/10.3389/fphar.2022.803602/full) | [Frontiers | Tissue Pharmacokinetic Properties and Bystander Potential of Hypoxia-Activated Prodrug CP-506 by Agent-Based Modelling](https://www.frontiersin.org/articles/10.3389/fphar.2022.803602/full)  Hypoxia-activated prodrugs are bioactivated in oxygen-deficient tumour regions and represent a novel strategy to exploit this pharmacological sanctuary for therapeutic gain. The approach relies on the selective metabolism of the prodrug under pathological hypoxia to generate active metabolites with the potential to diffuse throughout the tumour microenvironment and potentiate cell killing by ...  www.frontiersin.org |

. The corresponding figures for Tarloxotinib are yet to be published, but do follow a similar trend (See below). Shown is the concentration-time profile of Tarloxotinib (prodrug, black circles) and its released effector TRLX-TKI (effector, white circles) in the extracellular medium (left) and cell extracts (right) after an anoxic culture (5 million cells per mL) was spiked with approximately 0.3 µM of Tarloxotinib (prodrug) at T = 0. Cellular uptake of the prodrug is rapid (occurs largely within the first 5 minutes of drug addition), with depletion from the extracellular medium by 1 hour after drug addition. The loss of prodrug mass balance is accompanied by a respective increase in effector mass balance (peaks at approx 1 hour after drug addition). The high concentrations of effector achieved within the intracellular compartment drive cellular efflux into the extracellular medium. The cellular uptake of CP-506 is not concentration-dependent (constant cellular uptake ratio of 50-fold irrespective of prodrug concentration), but is for Tarloxotinib (cellular uptake (Ci/Ce) ratios of 200 to 1500-fold across a concentration range of about 100-fold of the prodrug).

Thanks for your help,

Victoria

**Extracellular                                                  Intracellular**

31/10

Hi Victoria,

I wasn't thinking of killing by the drug, rather by lack of oxygen and/or glucose.  But in all these modes death is delayed for at least 12h, I think, therefore stirring damage seems to be the only cause of death within the 5h.  Not an issue.

I was not aware that proliferation was suppressed when the cells are in suspension.  That is useful, since it simplifies the model.  Some cells will grow to the volume at which cell division would normally occur.  I could either suppress further growth, or allow the cells to grow beyond that size.

Unless a very high initial glucose concentration is used I think glucose will be depleted and growth will be suppressed.

I need to study your CP-506 paper, which might change my thinking, but I am wondering if an agent-based model is really needed in this case.  Would it be feasible to simulate a single cell, with constant volume, extrapolating from its behaviour (i.e. multiplying by 50 million) to derive total uptake and release rates, and medium concentrations?  I could program something to do this.

31/10

The short duration of the experiment is very forgiving, so simplification in that manner sounds perfect.

31/10

Is the effector the only metabolite?

For the parent drug and each metabolite that is metabolised (i.e. all except the last one) there are 8 parameters:

CELL\_DIFF\_IN

CELL\_DIFF\_OUT

HALFLIFE

KMET0

C2

KO2

VMAX

KM

For the last metabolite, just the first 3.

How do you plan to provide values for the parameters beyond the 3 you mentioned in your initial email?

What are the names of the drug and effector?

31/10

Yes, there is only one cytotoxic metabolite for each prodrug (1:1 stoichiometry).

In the past, we used Phoenix WinNolin to model the cellular uptake and metabolism in stirred cell suspensions using a simple two compartment model with a non-saturable cellular uptake factor (rate constant q12par) from the extracellular medium (C1) into a central intracellular compartment (C2). The faculty no longer pays for the license hence my need to find an alternative means. Historically, I used the concentration-time profile of the prodrug in the extracellular medium and cell extracts of supraoxic cultures to fit the cellular uptake (rate constant q12par) and efflux parameters (rate constant q21par) for the prodrug. I fixed the CELL\_DIFF\_IN (Ci) and CELL\_DIFF\_OUT (Ce) of the effector to that the prodrug, given their similar physiochemical properties (if the trend differs significantly, a similar experiment would be performed with the effector under supraoxic conditions to get a better model fit). The half-life of the prodrug and metabolite were derived from their respective concentration-time profiles in stirred culture medium (without cells) by mass spectrometry. I then fixed the CELL\_DIFF\_IN and CELL\_DIFF\_OUT of anoxic cultures to the fitted values from supraoxic experiments to derive model estimates of the KMET0, Vmax and Km from the concentration-time profiles of the prodrug and effector in an iterative manner. I used the fitted values of PR-104A as a baseline for the iterations. Can you jog my memory as to what is meant by the C2? Initial concentrations were determined from the sum mass balance of the extracellular and intracellular compartments assuming no loss of mass balance from the system (with 1:1 stoichiometry of prodrug and effector). The KO2 is determined experimentally in stirred cell suspensions as the oxygen concentration required to inhibit prodrug metabolism by 50% of the anoxic culture (‘maximal rate’).

There are a few prodrugs we are interested in modelling, but the paradigm should be similar. It’s probably easier calling them generically ‘parent’ and ‘metabolite’.