Optimizing Time Delay for Cell Cycle Drug Response Modeling

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Abstract

Finding effective treatments through drug combinations can both be very effective but also very difficult. A previous linear chain trick (LCT) model was able to capture cancer cell population responses to drugs, and predict the effects of drug combinations. However, this LCT model had errors when predicting effects of drugs that affected S and G2 phases of the cell cycle in comparison to drugs that affected other phases. In order to improve upon this aspect of the model, the time delay from the LCT was altered by altering the number of sub-phases. As a result of this alteration, the model improved its ability to recreate the population response to these drugs and captured the dynamics of the effects more accurately. With improvements to the model, the error of the model is reduced, thus increasing the accuracy of quantifications of drug effects, allowing for better predictions for effective drug combinations to treat cancer.

Introduction

Using a combination of drugs often leads to more effective treatments for cancer [1]. By combining drugs, these treatments can improve response by affecting different pathways that impact cell division and survival [1] [2]. Thus, identifying drugs that work in an additive or synergistic manner is important for creating effective treatments. However, finding these combinations is difficult. Tumor development involves complex pathways and understanding how these pathways connect and how combinations might affect those pathways has limited the identification of effective treatments [2]. One approach involves testing doublet combinations using cell viability assays [2]. Additionally, there has been no agreed upon method with which to quantify the effectiveness of treatments [3].

One method to quantify the effectiveness of drugs involves tracking a cell population as it progresses through the cell cycle. The cell cycle is split up into four phases and each of these phases have corresponding checkpoints to assess any errors in the cell before it progresses on to the next phase [4]. These phases were found to be memoryless and thus, independent of one another [5]. Because cancer drugs target aspects of cell division that are tied to a specific phase [2], drugs that target different phases will have independent effects and thus, be more effective. Tracking the cell cycle as it responds to individual drugs could provide further insight into their effects and give rise to better predictions of effective combinations.

Using changes to cell growth rates can lead to better quantification of effects to the cell cycle [6]. However, while these growth rates might provide more insight into some effects, they have limitations. For example, this approach does not provide any insight into the changes to cell death rate, which could be similarly important in quantifying drug effects on the cell cycle. Thus, in an approach used by Gross SM et al., an ordinary differential equation (ODE) model explicitly incorporated both rates of progression through phases of the cell cycle as well as death rates [7]. One additional insight of their approach was the use of the linear chain trick (LCT) within this ODE model. LCT is a way for ODE models to simulate phase durations that follow a gamma distribution by adding more sub-phases [8]. Using the finding that phases follow a gamma distribution [5], the model simulates these durations through the use of LCT. By adding these sub-phases, the model is able to capture the dynamics of the drug responses, especially the oscillations that come as a result of the drug effects. When combining the effects of different drugs, the model predictions were congruent with experimental observations.

While the model created by Gross SM et al. performs well overall, it performs relatively worse when it comes to drugs that affect the G2 phases. This decrease in performance can cause the model to have worse predictions for combinations that involve these types of drugs, which limits the identification of effective combinations.. In this paper, attempts at optimizing the time delay in order to increase performance for these drugs were made. The number of sub-phases used in the original model was kept uniform across all drugs and was found using the gamma distribution of the control cells. These drugs affect different phases and could extend or decrease phase durations in varying degrees. Thus, keeping the same amount of time delay across all drugs could cause the model to perform poorly if the drug effects differ from the time delay used. In order to find out whether this is the reason for its poor performance over G2 drugs, the models with varying numbers of sub-phases to explore whether this change better fits the drug responses and better captures dynamics of the cell cycle.

Methods

Cell count data from Gross SM et al. were used for the model fitting and comparisons. To quantify drug responses, cell count tracking was done for 96 hours across six drugs. Varying concentrations of these six drugs were also tested and tracked [7]. For this study, data for three drugs were focused on: lapatinib, doxorubicin, and gemcitabine. Lapatinib was chosen as a baseline, since it is a G1 drug, to explore whether alterations in time delay can further improve the model. Doxorubicin and gemcitabine were the two S-G2 drugs that had the worst fit of the

original model. Thus, the main focus of the study is to improve the model through alterations of time delay for these two drugs.

The system of ODEs from Gross SM et al. had two states: G1 and S-G2. To incorporate the observation that phase durations follow a gamma distribution, the "linear chain trick" [8] was utilized. The model divides each state into 4 parts and then further divides each part into sub-phases. G1 has 2 sub-phases per part, so there are 8 total sub-phases within the G1 phase. Similarly, S-G2 has 5 sub-phases per part, so there are 20 total sub-phases within the S-G2 phase. These sub-phases were based on the shape of the distribution of the control durations, which was 6.6 and 22.6 for G1 and S-G2, respectively. The simplification was made to divide each state into 4 parts so that there are less parameters, even though it does not match the shape exactly.
The original model kept the number of sub-phases constant across all drugs, which might be the reason for the poor predictions for S-G2 drugs.

The original system of ODEs is:

(1) $$\frac{dG_{11,1}}{dt} = +2\beta_4 G_{24,5} - (\alpha_1 + \gamma_{1,1})G_{11,1}$$

(2) $$\frac{dG_{1k,1}}{dt} = +\alpha_{k-1} G_{1k-1,2} - (\alpha_k + \gamma_{1,k})G_{1k,1}$$

(3) $$\frac{dG_{1k,2}}{dt} = +\alpha_k G_{1k,1} - (\alpha_k + \gamma_{1,k})G_{1k,2}, 1 \leq k \leq 4$$

(4) $$\frac{dG_{21,1}}{dt} = +\alpha_4 G_{14,2} - (\beta_1 + \gamma_{2,1})G_{21,1}$$

(5) $$\frac{dG_{2i,j}}{dt} = +\beta_i G_{2i,j-1} - (\beta_i + \gamma_{2,1})G_{2i,j}, 2 \leq j \leq 5, 1 \leq i \leq 4$$

where α is the progression rate of G1, β is the progression rate of S-G2, and γ is the death rate.

To create model predictions, a Jacobian matrix was created from these parameters of size 28 by 28, coming from the sum of sub-phases of G1 and S-G2. This matrix was then solved by matrix exponential and model predictions were created for all time steps within 96 hours.

To fit this model onto the cell count data, the cell numbers predicted by the model were compared to the cell number across all time points and concentrations [7]. The cost function was the sum of squared error between the prediction and the actual data. To minimize this cost function, the parameters were optimized using the default optimizer from the BlackBoxOptim.jl Julia package. For the purpose of this study, the default adaptive differential evolution optimizer was used and an optimization maximum step count of 200,000 was set across all testing.

To alter the time delay within this system of ODEs, the optimizer was set to optimize both the parameters as well as the number of sub-phases. Thus, rather than having a fixed number of sub-phases and a fixed matrix size of 28, the optimizer will try to find the number of sub-phases that minimizes the cost function. Keeping the simplification of the original model, the number of parts in each phase will remain as 4 so the number of sub-phases will be a multiple of 4. This step is repeated 10 times and the number of sub-phases with the lowest cost function will be used for the next step.
After getting the most optimal number of sub-phases, this number will then be fixed and only parameters will be optimized. This step is to ensure that the optimizer can fully focus on optimizing the parameters and is run 10 times once again. With these optimized parameters, the final model predictions can be created and evaluated.
As a baseline comparison, the original model with 8 sub-phases for G1 and 20 sub-phases for S-G2 was also run 10 times.

Evaluation is done by calculating the fit by using the sum of squared errors (SSE) between model predictions and actual data for all concentrations and time steps. These predictions will also be plotted against the actual data to qualitatively evaluate the fit and compared to the original model. For example, a model might have higher SSE but follow the general trends of the actual data better. These SSEs along with the qualitative fit will then be compared across the other models to evaluate whether altering the time delay improved the fit for the three drugs.

Results

When comparing the SSEs for the three drugs tested, the models with an optimized number of sub-phases all decreased in error.

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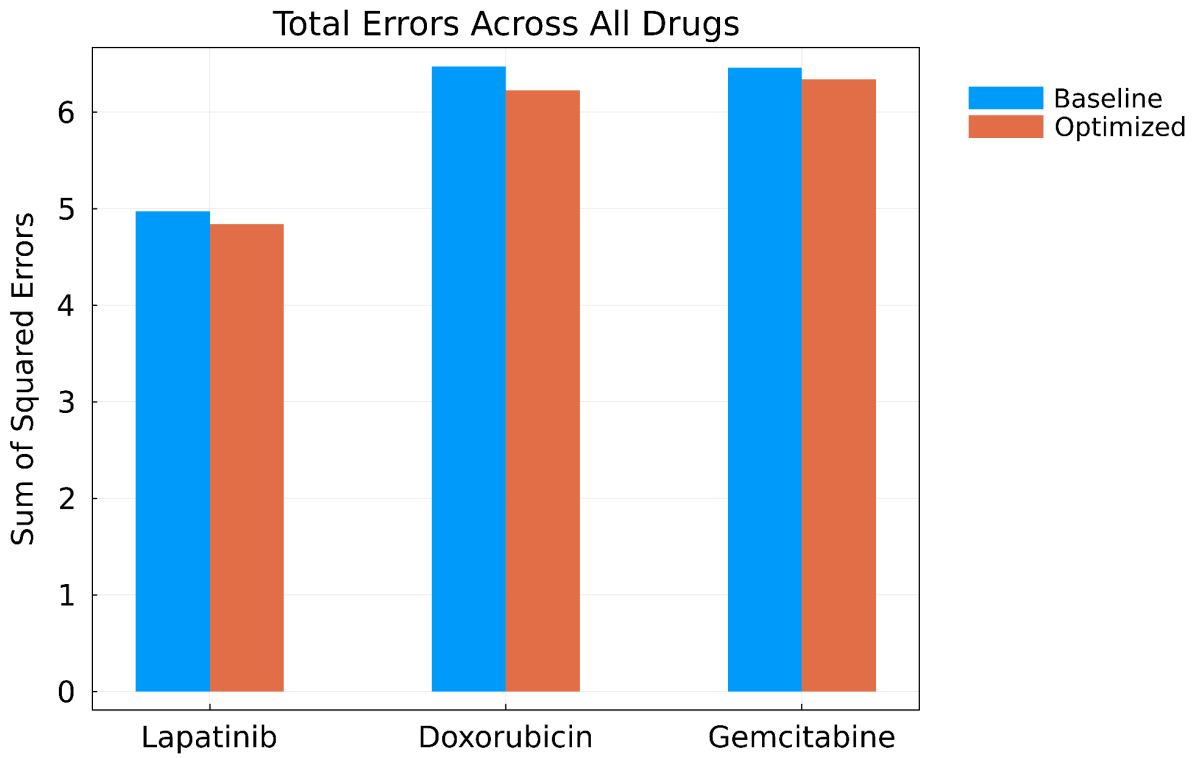

Fig 1. Comparisons of errors between the baseline model and the optimized model

Drug	**Baseline SSE**	**Optimized SSE**	**% Improvement**
Lapatinib	4.97	4.84	2.62%
Doxorubicin	6.47	6.23	3.80%
Gemcitabine	6.46	6.34	1.90%

Table 1. Improvement from the baseline model to the optimized model

However, as seen in Fig. 1 and Table 1, the improvements over the baseline model are minor, suggesting that the original model captured the dynamics of the population responses well already and optimizing time delay only improved that a small amount.
Additionally, when re-running the original model to get baseline comparisons, it appears that while the SSE for lapatinib are similar, the SSEs for doxorubicin and gemcitabine are

significantly reduced without any modifications to the model. This result suggests that the original SSEs for these drugs from Gross SM et al. are not as bad as originally suggested.

Drug	**Optimal G1 sub-phases**	**Optimal S-G2 sub-phases**
Lapatinib	4	20
Doxorubicin	4	28
Gemcitabine	4	24

Table 2. Optimized number of sub-phases for each drug

For all drugs, the optimized number of G1 sub-phases decreased to 4 from 8, suggesting that 4 sub-phases is better to capture the dynamics.
For doxorubicin and gemcitabine, this decrease in sub-phases aligns with what might be expected biologically as they should not be extending the duration of the G1 phase so the original number of 8 would be too high. This result is surprising for lapatinib, given that it is a drug that affects the G1 phase. It would be expected that the number of G1 sub-phases would increase since lapatinib should extend the duration of the G1 phase. However, this result might be due to the simplification of having 4 parts so the number is a multiple of 4 and the true optimized number might lie somewhere in between.

Additionally, the optimized number of S-G2 sub-phases is different across the three drugs. For lapatinib, it remains the same as the original model, supporting the idea that the original number of sub-phases for S-G2 was a good choice for G1 drugs. For doxorubicin and gemcitabine, both increased the number of S-G2 sub-phases, again aligning with biological expectations as S-G2 drugs should have some effect with extending the duration of the S-G2 phase.

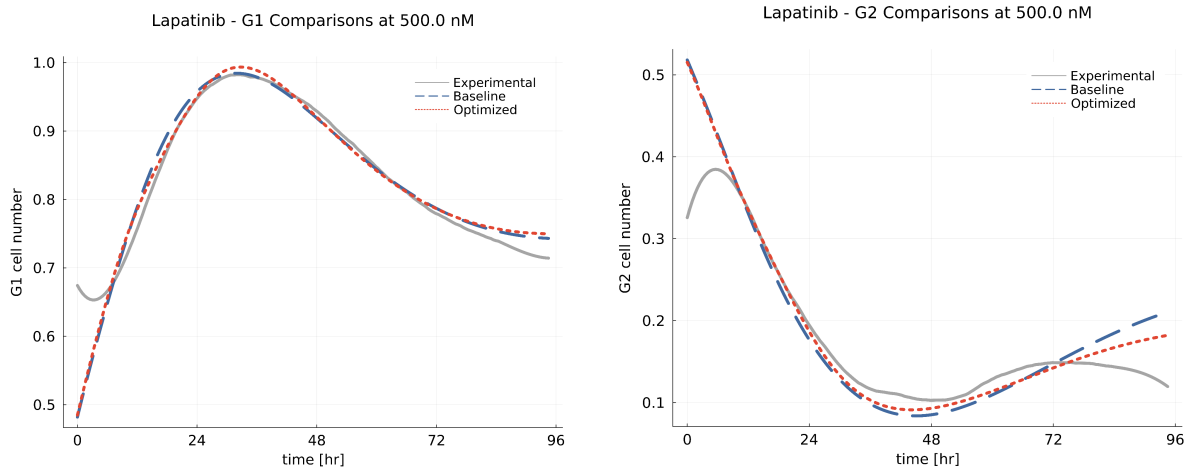

Fig. 2 Comparisons of baseline and optimized model predictions for lapatinib at its highest tested concentration

When comparing qualitative fits, for lapatinib in Fig. 2, both the baseline model and optimized model closely resemble both the experimental data and each other. The only visible difference is that for G2, the optimized model is slightly closer than the baseline model. These similarities suggest that while the optimized model made improvements, these improvements are very subtle.

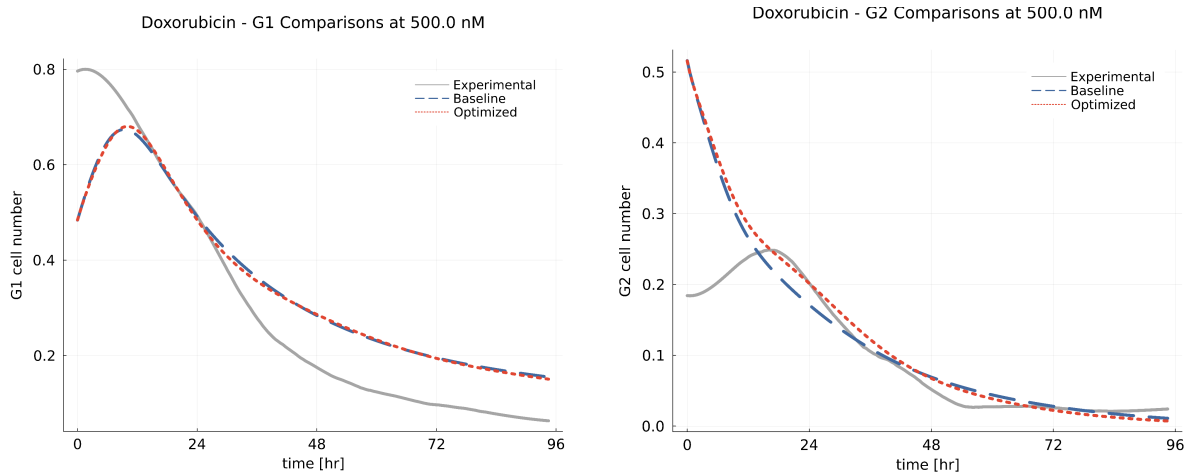

Fig. 3 Comparisons of baseline and optimized model predictions for doxorubicin at its highest tested concentration

A similar observation can be made for doxorubicin as the baseline and optimized predictions once again overlap with each other, with only a slight difference in fit for G2. However, the optimized model did not make any major improvements and both these models had difficulties

with following some dynamics as seen in G1 where they are unable to decrease to the same final cell numbers.

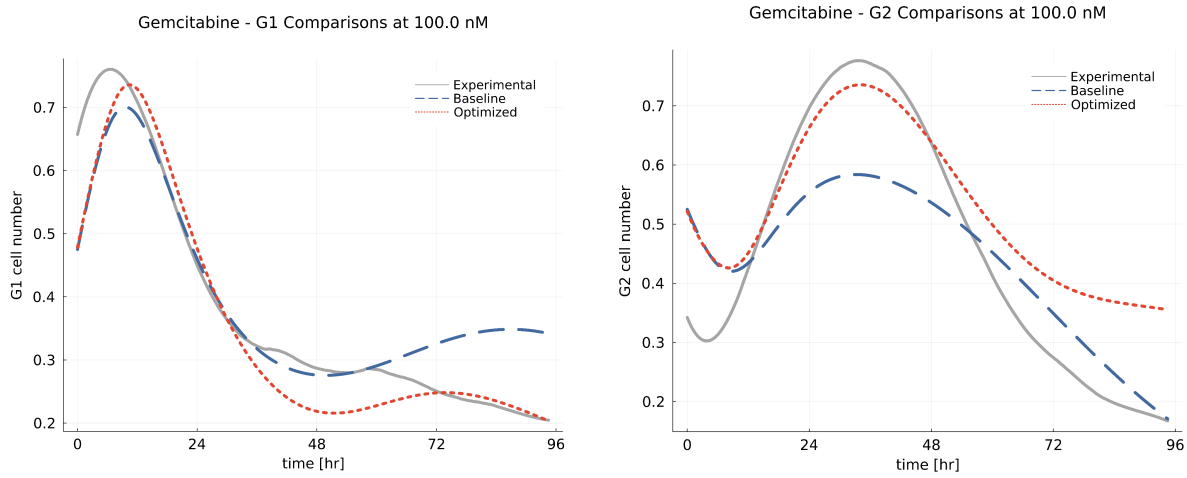

Fig. 4 Comparisons of baseline and optimized model predictions for gemcitabine at its highest tested concentration

Finally, with gemcitabine, there are some notable differences in the baseline model and the optimized model. For both G1 and G2, there is an oscillation where the cell numbers initially increases and then decreases and that oscillation is much better captured by the optimized model. There are some flaws with the optimized model as seen in G2 where it is unable to decrease to the same final cell number. However, due to capturing the initial oscillation, the optimized model for gemcitabine does seem to improve its ability to capture those population dynamics.

Discussion

For this model predicting cancer cell population dynamics in response to drug effects, altering time delay by changing the number of sub-phases did reduce the error across the three drugs tested. Additionally, it did show some qualitative improvements for the three drugs, especially in its ability to capture the dynamics of the drug gemcitabine. Overall, optimizing the number of sub-phases improved the model, especially for drugs affecting S and G2.

One limitation of this study was the stochastic nature of the optimization function with the lack of computational resources and time to properly explore the possibilities. While the optimization

function worked well, it is not perfect, due to the difficult nature of optimizing multiple variables. For example, the optimization function had some stochasticity in choosing initialization as well as what changes to the variables it would make next. That stochasticity might cause the variables and error to get stuck within a local minima when there could be changes made to variables to further reduce the error. Thus, multiple runs with the same settings could result in large differences in error. This study tried to mitigate that by running the settings ten times but that would not be enough to completely explore the possible results. Thus, while it does seem as though changing the sub-phases does improve the model, it might simply be due to the optimization function rather than the change itself.

Additionally, further increasing the number of iterations past 200,000 might further improve the optimization and reduce the error but it was once again limited by computational resources and time.

Larger scale studies could explore this method with more trials in order to verify the result found with this study. Additionally, as this is a simple change to time delay and time delay seems worthwhile to explore, other studies could look to change the model in more complex ways. For example, having a new phase representing mitosis might improve the model by introducing biologically accurate time delay between S-G2 and G1. Removing the simplification of having four parts within each phase and instead allowing each equation to have unique parameters might also show promise in improving the model but this change would also greatly increase computational costs.

While changing the number of sub-phases did not result in major improvements to the model, the model was still improved overall, even for lapatinib, the drug that performed the best with the original model. Even though the reduction in error was minor, this change is simple to implement and the optimized number of sub-phases allow for more insight and biological interpretability. Thus, implementing this change seems worthwhile to improve the model. By improving the model, the errors of model predictions decrease, allowing for more accurate quantifications of drug effects. By making these quantifications more accurate, better insights can be found which improves the model's overall ability to predict effective drug treatments and combinations for cancer.

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