Analysis of resonance chemotherapy in leukemia treatment via multi-staged population balance models

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Abstract

An age-structured population balance model that explicitly models cell cycle phases is developed to investigate the effects of cell cycle specific (CCS) drugs. In particular, the benefits of timing CCS drug treatments in resonance chemotherapy are predicted and measured directly in vitro before evaluating likely in vivo scenarios. The phase transition rates are measured in vitro for the HL60 leukemia cell line and are used to predict the transient phase dynamics after exposure to the S phase specific drug, camptothecin. The phase oscillations predicted by the model are observed experimentally and the timing of a second camptothecin pulse is shown to significantly alter the overall treatment effectiveness. To explore the feasibility of designing resonance chemotherapeutic treatments to preferentially eliminate one cell type over another, Jurkat and HL60 leukemia cells are exposed to the same dual-pulse camptothecin treatment regimen. With the model framework validated for simplified cases, the model is used to extrapolate the effectiveness of resonance chemotherapy considering in vivo effects such as quiescence, drug metabolism, drug properties, and transport considerations that were not included in the in vitro experiments. While resonance chemotherapy is intuitive and looks promising in vitro, when in vivo considerations are included in the model, the phenomenon is dampened and the window of applicability becomes narrower.

Keywords: Mathematical modeling; Age-structure; Cell cycle

1. Introduction

For chemotherapeutic drugs to be effective, they should discriminate between normal and cancerous tissue. Because cancerous cells are normal cells that have lost functionality and growth control, these differences are often subtle and variable between patients. Many drugs utilize the increased growth rate of cancerous tissue by killing only cells that are proliferating. The overall growth of a tumor is known to follow Gompertzian-like growth kinetics. A small tumor will exhibit exponential growth, but the growth decays as the tumor expands. Overall growth expressions such as this have been used in various analyses such as determining the growth stability of a tumor (Adam, 1986), predicting the effectiveness of treatments (Fister and Panetta, 2000; Murray, 1990), or outlining treatment strategies for various growth conditions (Gardner, 2002; Costa et al., 1995; Swan and Vincent, 1977).

Using drugs that are selective for proliferating cells provides some selectivity which may be enhanced with more advanced consideration given to the drug or cellular actions (Darzynkiewicz, 1995; Blagosklonny and Pardee, 2001). Cell death by apoptosis occurs when a drug disrupts an essential cellular process by interacting with the appropriate molecules. Drugs can be broadly classified as cell cycle phase-specific (CCS) or non-specific (nCCS) depending on whether the drug activity is highly dependant upon the cell cycle phase. When a CCS drug is applied, a void in the cell cycle will be created in the susceptible phase. As cells continue to cycle, the void traverses the cell cycle but eventually will disappear due to the natural asynchrony of the cell cycle. Thus, the effectiveness of subsequent CCS
doses will depend on the location and size of this void in the cell cycle as pointed out by Agur (1985).

This idea can be taken a step further by acknowledging that a CCS drug will affect many, different cell types that traverse the cell cycle at different rates. In leukemia, the strength of chemotherapy doses is constrained by the potential collateral damage to be inflicted on the immune system via the death of bone marrow tissue. For optimal resonance chemotherapy, after an initial bolus of a CCS drug, second and third administrations can be timed to maximize or minimize damage to a particular cell type. Since bone marrow cells are known to traverse the cell cycle more quickly than most leukemia cells (Andreeff, 1992), the ability to correctly time drug administrations would be beneficial. We show that resonance chemotherapy works in vitro and that a model based on the cell cycle properties can be used to design selective treatments.

Mathematical models that distinguish between cells in the population based on their properties (Frederickson and Tsuchiya, 1963; Frederickson et al., 1967) are required to deal with the non-uniform drug effects. In 1959 Von Foerster, with analysis by Trucco (1965), introduced age as a structure variable and showed how the use of an age-density yields exponential growth (Von Foerster, 1959). Webb has used this model to analyse resonance chemotherapy by giving an age range over which CCS drugs are effective (Webb, 1990). Chuang and Lloyd (1975) furthered the analysis by dividing the cell cycle into discrete phases by coupling the Von Foerster equations for each phase. Age-densities are written for viable phases as well as a quiescent and death phase based on a diagram proposed by Skipper (1969). This system provides the advantage that each phase is modeled explicitly. However, a solution could not be achieved at the time due to limited computational capabilities.

In these systems, cell age is continuous with time and the phase transition and death rates are functions of age. However, models that have been used to analyse resonance chemotherapy and cell cycle movement (Agur et al., 1988; Cojocaru and Agur, 1992; Dibrov et al., 1985; Tyrcha, 2001; Ledzewicz and Schättler, 2002; Basse et al., 2003) have made various simplifications that increase the tractability of the problem but with a loss of resolution of system features. A general multi-staged population balance model is presented here as a means to capture the dynamics of cell cycle specific problems as fully as possible. This model expands the scope of theoretical modeling work published by Gaffney (2004) by addressing concerns that have been raised regarding the practical or clinical nature of resonance chemotherapy. In particular, in vitro results are subjected to hypothetical in vivo considerations to evaluate the likelihood that resonance will be beneficial in vivo.

2. Model structure

This model lumps the hierarchal group of cell types, from stem cells to fully differentiated ones appearing in the periphery, into a single diagram. Drug resistance via mutation is currently neglected as the object at hand is to model the bone marrow in order to get the greatest treatment benefit with the least damage to the patient. However, for a tumor to be completely eradicated, these subpopulations play a crucial role and could be added in future modifications to the model. Resistance studies are available (Goldie and Coldman, 1979; Costa et al., 1995; Gardner, 2002).

The cell cycle model is discretized into six phases: a quiescent, G0 phase; three stages for proliferating cells, the G1, S, and G2/M phases; dead cells; and cells in the periphery. Fig. 1 shows the six phases of the cell cycle and the transition rates between the phases. Once a cell begins proliferating, it proceeds through the sequence of proliferation phases before dividing. The age density function describing the cells proceeding through the cell cycle can be affected by the presence of chemotherapeutic agents (Pallavicini et al., 1985; Sena et al., 1999) but we found experimentally that these effects were minimal when the drug pulse is sufficiently short. Thus, each of the phases can be described transiently by an age-density function, \( n_i(\tau, t) \) where \( \tau \) is the time that a cell has spent in the \( i \)th phase and \( t \) is time. Integrating over all ages in a phase gives the total number of cells in a phase, \( N_i(t) \), with the total number of proliferating cells being \( N(t) = \sum_i N_i(t) \). The age density function changes as cells transition between phases, die, and age.

The death rate in a proliferation phase, \( k_i(C) \), is a function of the drug concentration to which a cell is

![Fig. 1. Six phases of the cell cycle model. Arrows indicate the potential transitions between phases and their transition rates. Actively proliferating cells in the G1, S, and G2 phases may be susceptible to chemo drugs while feedback with and maturation of cells in the quiescent, G0, regulates the total cell population.](image-url)
exposed. Bone marrow is a highly vascularized tissue, so an assumption is made that all cells within the marrow are exposed to the same drug environment. Also, the cellular response to a drug is assumed to be homogeneous within a phase with no resistance development. Since the drug concentration is time dependent, the death rate is implicitly a function of time.

For proliferating cells, the rate of transition out of a phase is given by the age-dependent function, $\Gamma(t, C)$. The age variable is used to capture the net behavior of the numerous checkpoints and steps before transition. A time dependency arises due to the presence of a drug. In addition, feedback between the quiescent and proliferating phases is considered as quiescent cells are recruited at a rate $p(N_{G0})$ and $G_1$ cells become inactive at a rate $q(N_{G0}, t)$. In the quiescent phase, cells may differentiate at a rate, $m$, and leave the bone marrow to enter the periphery where they are removed at a rate $r(t)$.

The proliferating phases of the cell cycle are each governed by a number density while an age-independent equation is applied to the quiescent phase. Thus, the growth dynamics are governed by

$$\frac{\partial n(t, t)}{\partial t} + \frac{\partial n(t, t)}{\partial t} = -D_{\text{out}}(t, C, N_{G0}(t))n(t, t),$$

(2.1)

where

$$n(t, t) = \begin{bmatrix} n_{G0}(t) \\ n_{G1}(t) \\ n_{S}(t, t) \\ n_{G2}(t, t) \\ n_{per}(t, t) \end{bmatrix}$$

(2.2)

and

$$D_{\text{out}}(t, C, N_{G0}(t)) = \text{Diag} \begin{bmatrix} p(N_{G0}(t)) + m \\ q(N_{G0}(t)) + \Gamma_{G1}(t, C) + k_{G1}(C) \\ \Gamma_{S}(t, C) + k_{S}(C) \\ \Gamma_{G2}(t, C) + k_{G2}(C) \\ r(t) \end{bmatrix}$$

(2.3)

are the age density vector and the transition matrix. The age densities are subject to the initial condition

$$n(t, 0) = n_0(t)$$

(2.4)

and the boundary condition

$$n(0, t) = \int_0^{\infty} D_{\text{in}}(t, C, N_{G0}(t))n(t, t) \, dt,$$

(2.5)

where

$$D_{\text{in}}(t, C, N_{G0}(t)) = \begin{bmatrix} 0 & q(N_{G0}(t)) & 0 & 0 & 0 \\ p(N_{G0}(t)) & 0 & 0 & 2\Gamma_{G2}(t, C) & 0 \\ 0 & \Gamma_{G1}(t, C) & 0 & 0 & 0 \\ 0 & 0 & \Gamma_S(t, C) & 0 & 0 \\ m & 0 & 0 & 0 & 0 \end{bmatrix}.$$

(2.6)

A solution to the above equations is obtained using the method of characteristics (Ramkrishna, 2000). Analytical expressions were used to compute the integrals of $D_{\text{out}}$, but numerical methods were required for both the outer integrals. Adaptive steps are not applicable due to the nature of the iteration, so a combination of higher order Newton–Cotes and Gaussian techniques were applied. Convergence was verified solving the problem with the largest gradients to be tested: multiple applications of the drug with the smallest half-life for the longest time period to be simulated (10 days). The time step was varied until it was found that time steps of 0.005 days or smaller gave the same results.

3. Model rate terms

3.1. Phase transition rates

In each phase of the cell cycle, a cell must complete certain tasks or pass checkpoints before it can proceed to the next phase. Cells that have just entered a phase are highly unlikely to transition and most cells will take around the same amount of time to complete a phase. Experimental phase residence time distributions confirm the need for an age-structure, but also show that there is a positive bias to the distribution (Steel, 1977). Random delays in progression cause some cells to take longer and shift the distribution. A log-normal phase residence time distribution whose corresponding normal distribution has a mean, $\mu_i$, and standard deviation, $\sigma_i$, is used to describe the transition rate for each of the $i$ phases.

$$\Gamma_i(t) = \sqrt{\frac{2}{\pi} \frac{1}{\sigma_i^2}} \exp(-1/2((\log \tau - \mu_i)/\sigma_i)^2)\left(1 + \text{erf}((\mu_i - \log \tau)/\sqrt{2\sigma_i})\right).$$

(3.1)

To regulate the number of quiescent cells in the bone marrow, a negative feedback mechanism proposed by Andersen and Mackey (2001) is used. The maximum transition rates between proliferation and quiescence, $\lambda_P$ or $\lambda_Q$, are scaled using a proliferation limit, $N_P$ or $N_Q$, and the total number of cells. The transition from quiescent to proliferation can occur at any age,

$$p(N_{G0}(t)) = \frac{\lambda_P}{1 + (N_{G0}(t)/N_P)^\gamma},$$

(3.2)
but it is assumed that the proliferation to quiescent transition is most likely to occur within a specific age window in the G1 phase governed by a normal distribution with mean, \( \mu_{P,Q} \), and standard deviation, \( \sigma_{P,Q} \):

\[
q(N_{G0}(t)) = \frac{\hat{\lambda}_Q}{1 + \left( \frac{N_0}{N_{G0}(t)} \right)} \frac{1}{\sqrt{2\pi\sigma_{P,Q}}} \exp \left[ -\left( \frac{\tau - \mu_{P,Q}}{2\sigma_{P,Q}} \right)^2 \right].
\]  

(3.3)

Using these expressions the system will attain a steady-state set-point and the quiescent transition rates will adjust to maintain that set-point. For example, after application of a chemotherapeutic drug, \( N_{G0} \) will decrease and more quiescent cells will begin to proliferate with fewer proliferating cells becoming quiescent in an attempt to rejuvenate the population. A similar situation has been noted clinically: normally around 80\% of the bone marrow is quiescent but this percentage can drop to nearly zero after application of a chemotherapeutic agent (Andersen and Mackey, 2001).

3.2. Active drug concentrations

The exponential kill model of Gardner (2000), which yields sigmoid dose–response curves, is used to find the instantaneous death rate as a function of the drug concentration in the bone marrow, \( C(t) \) (Gardner, 2002). Various pharmacokinetic models can be incorporated to govern the concentration of active drug. A drug may be administered as a pill, intravenously, or as a bolus injection. The administration technique is reflected in the dose administration rate \( y_{ij} \text{ mg/m}^2/\text{h} \) which is given for a duration \( t_{ij} \) h during the \( j \)th dose of the \( i \)th drug. The total concentration of each drug will consist of the current application plus the residual amount, \( C_{i,\text{residual}}(t) \), from the previous applications, \( PA \), which were begun \( t_{AD} \) hrs ago. The drugs are each assumed to be metabolized or filtered or removed from the periphery according to first order kinetics with half-lives from the literature (Gardner, 2002) used to find the decay constants, \( \lambda_i \):

\[
C_i(t) = \begin{cases} 
\frac{y_i}{\tau} \left( 1 - e^{-\lambda_i(t-t_{AD})} \right) + C_{i,\text{residual}}(t), & \text{during } j\text{th application}, \\
C_{i,\text{residual}}(t) = \sum_{j=1}^{PA} \frac{y_i}{\tau} e^{-\lambda_i(t_t-t_{AD})}(e^{\lambda_i t_{ij}} - 1), & \text{between applications}. 
\end{cases}
\]  

(3.4)

3.3. Drug metabolism and transport

When specifically stated as being included, first order transition rates are used to model the kinetics of transport of each drug from the periphery to the bone marrow or metabolism from an inactive form to an active metabolite (see Fig. 2). Separate first order decay constants are again used to describe the decay in the bone marrow.

Fig. 2. Drug transport from blood stream to bone marrow and drug activation are lumped into a two-compartment, pharmacokinetic model.

3.4. Cell death

Just as the concentration of drug to which the cells are exposed varies with time, the death rate will also vary with time. The level of survival for cells susceptible to a given drug decreases exponentially with concentration (Gardner, 2002). The parameter, \( a_i \), scales the effectiveness of concentration of a drug and \( k_{i,max} \) scales the death response. If multiple drugs are given, the drug effects are assumed to be independent of each other. The death rate is given by

\[
k_d(C) = k_{i,max} \left( 1 - \prod_{i=1}^{drugs} SF_i(C) \right) \text{[1/day]},
\]  

(3.5)

where the survival fraction,

\[
SF_i(C) = \begin{cases} \exp(a_iC(t)), & \text{susceptible phase}, \\
1, & \text{otherwise} 
\end{cases}
\]  

(3.6)

depends on which phase a cell is in as well as the concentration profile of the drug over that interval.

4. Model parameters

4.1. Determining the residence time distributions in each phase

The age of a cell since making its last phase transition is difficult to measure directly. However, using a pulse–chase system these residence times have been approximated and have been shown to take on a log-normal positive bias (Steel, 1977). The pulse–chase cannot measure the distribution directly because the ages of the cells at the initial time is not known, but distributions can be fitted to match the observations under exponential growth conditions. Under exponential growth conditions, a pulse of BrdU is added to the cells. The BrdU positively labels cells that are synthesizing their DNA at that time and has been shown not to affect the growth characteristics of the cells. Collecting samples with cell counts at fixed intervals and labeling for BrdU incorporation and DNA content allows plugs of labeled and unlabeled cells to be followed around the cell cycle:

G2: Initially, the G2 phase will be mostly BrdU negative with a few S labeled cells entering. But as time passes...
more of the unlabeled cells will divide and more labeled cells will enter and take their place. The G₂ transition should fit the number of unlabeled cells leaving and the S transition should fit the number of labeled cells entering.

S: The S phase cells are initially BrdU positive. Labeled cells will leave the S phase and unlabeled G₁ cells will enter the S phase with time. The G₁ transition should fit the number of G₁ cells entering the S phase.

G₁: The G₁ cells start as unlabeled. Unlabeled G₂ cells immediately begin to divide into the G₁ phase, but labeled cells will eventually enter the G₁ phase after moving from S through G₂.

The traditional pulse–chase fit for transition rates involves following the percentage of labeled cells in each phase for several generations and using the width and frequency of the distribution to extract the mean and standard deviation (Steel, 1977; Yanagisawa et al., 1985). This procedure was modified slightly as each proliferation phases’ mean and standard deviation, for the assumed lognormal distribution, was least-squares fitted to the labeled and unlabelled percentages using the model (Baisch et al., 1995) (see Table 1). Leukemia HL60 cells in exponential growth (as described in Section 5.1) were sampled every 2 h for 8 h after the addition of a BrdU pulse following the protocol set forth by FITC BrdU flow kit (BD Biosciences Pharmingen 557891).

4.2. Drug parameters

To evaluate potential clinical treatments, the drug application properties and half-life of existing drugs are required. The drug parameters are taken from the Gardner model (Gardner, 2002), from which the cell death kinetics are derived, for two CCS and two nCCS drugs and presented in Table 2.

5. Experimental verification of model

The first stage of model validation neglects the quiescent phase, pharmacokinetics, drug transport, and assumes the transition rates are independent of any drug treatment. This simplified model approximates an in vitro leukemia cell culture system and is used to check whether the model framework describes the dynamics of cells in response to a drug. This simplified system also represents the ideal case for resonance chemotherapy. Addition of a quiescent phase, pharmacokineti, or broadening transition rates will all disrupt the resonance as shown in the following sections. Hence, the cell culture system is used to determine if the model framework adequately describes the observed cell cycle dynamics. In particular, the importance of modeling the cell cycle phases explicitly and using the transition rate distributions is to be gauged.

5.1. Materials and methods

The leukemia HL60 and Jurkat cell lines were cultured in a 75 cm² flask with a 80/20 mixture of Dulbecco Iscove's modified media and fetal bovine serum supplemented with 1mLL-glutamine. HL60 is a suspended cell line with very little clumping which lends itself well to cytometry studies. Jurkat cells are also a suspended cell line and their tendency to clump can be circumvented by pipetting 2–3 times before taking a sample or cell count. Also, there is no need to detach the cells from the flask wall to take a sample and the flask can be swirled for mixing. Concentrations were maintained in exponential growth between 10⁵ and 10⁶ cells/mL by passing every 2 days. One day before an experiment, the cells are passed to a concentration of 50–60/10⁵ cells/mL.

When media are replaced, a flask of fresh media is placed in the incubator 24 h before the transfer. The contents of the flask with cells are centrifuged such that the old media can be removed before the cells are resuspended in the fresh media. Control experiments confirm that neither multiple centrifugations nor multiple media replacement significantly affect cells during balanced growth.

The CCS drug, camptothecin, is used to induce S-phase specific death (Gorczyca et al., 1993). A 10μL pulse of

Table 1

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean, μ (days)</th>
<th>Standard deviation, σ (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>0.43</td>
<td>0.11</td>
</tr>
<tr>
<td>S</td>
<td>0.32</td>
<td>0.05</td>
</tr>
<tr>
<td>G₂</td>
<td>0.23</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Abbreviation</th>
<th>Plasma half-life (h)</th>
<th>Scaled dose intensity, base (mg/m²/(21 days))</th>
<th>Administration time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Flourouracil (CCS)</td>
<td>5FU or F</td>
<td>0.3</td>
<td>5</td>
<td>0.1</td>
</tr>
<tr>
<td>Methotrexate (CCS)</td>
<td>MTX or M</td>
<td>8</td>
<td>0.005</td>
<td>0.1</td>
</tr>
<tr>
<td>Cyclophosphamide (nCCS)</td>
<td>C</td>
<td>4</td>
<td>0.0009</td>
<td>0.1</td>
</tr>
<tr>
<td>Adriamycin (nCCS)</td>
<td>A</td>
<td>25</td>
<td>0.00012</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Note: The base concentrations are chosen such that a single administration of each drug results in identical populations after 30 days. Adapted from Gardner (2002).
1 mM camptothecin is given for every 10 mL of media. To minimize the drug and cell death effect on the non-susceptible cells, the media is replaced 2, 4, and 8 h after the administration of a drug. Appreciable levels of dead cells do not begin to accumulate until roughly 4 h after the addition of camptothecin as indicated by trypan blue and Vindelov’s propidium iodide (PI) sub-G1 viability assays. DNA histograms are prepared by taking a sample of media containing roughly $10^6$ cells and, after two PBS washes, resuspending the cells in 0.5 mL of PBS before fixation by pipetting into 4 mL of EtOH. For analysis, after two PBS washes, the cells are resuspended in 1 mL of a PI solution prepared by mixing 10 mL PBS, 100 µL Triton-X, 2 mg RNase, and 200 µL of a stock PI solution. When the Jurkat and HL60 cells are mixed in the same flask, the DNA histograms are distinguished by labeling the Jurkat cells with a FITC conjugated CD3 antibody for 30 min prior to the addition of the PI solution. Two PBS washes precede the PI staining. All cell samples are analysed on a multichannel BD-Elite flow cytometer using a 488 nm argon laser with FITC emission collected at 530 nm and PI emission at 620 nm. FITC+/PI− and FITC−/PI+ controls are used to calculate the compensation levels.

The number of cells in a phase is the product of the phase percentage from DNA histograms and the total number of cells from hemacytometer cell counts at the time of sampling. On the DNA histogram, dividing the count of cells in each phase by the total count of cells will give the phase percentages.

5.2. HL60 response to one camptothecin pulse

Simulations applying exponential growth transition parameters to cell growth during and after the administration of a theoretical camptothecin pulse were used to verify the phase oscillation essential for resonant chemotherapy. In the experiment, a pulse of camptothecin remains in the flask for 2 h before being removed. It is assumed that the effectiveness of the drug does not diminish over this time frame, so the simulations use a step change between constant drug concentration and no drug. The effective camptothecin concentration used in the simulations is fit to match the initial decline in the S phase resulting from this drug pulse, see Fig. 3 for $t < 5$ h.

By examining the percentage of cells in each phase (see Fig. 3), sustained, significant phase oscillations do result. If a second pulse of drug is given when the S phase is greater (10–16 h) than the balanced growth S phase percentage of 31%, it is anticipated that the treatment will be more effective than the initial treatment while if the S percentage is less than the base percentage it will be less effective.

The removal of the drug after a short drug pulse also seems not to have affected the transition rates over a small time frame (32 h) as the timings and magnitudes of the oscillations observed experimentally match very well with those predicted by the model assuming exponential transition parameters, stated in Table 1, from the BrdU experiments. The matching of the phase percentages indicates that the cell cycle-specific death and relative transitions rates are being accounted for adequately in the mathematical model.

However, the predictions for the number of cells in each phase are significantly higher than what is observed, see Fig. 4A. Potential explanations were investigated including: (i) drug activity in other phases; (ii) residual drug exposure after the washes which continues to have lingering effects; (iii) cells stop cycling; or (iv) the resulting cell death instigates further cell death. The first three factors can be eliminated as potential causes of the mismatch between predicted and observed levels by the following arguments. If the drug were effective in other phases, there would be a decline in these phases just like the S phase. However, the decline is not seen until the S void reaches those phases. If the drug is not removed, then we would see a residual amount of cell death in the S phase. This sink would disrupt the magnitudes in the phase percentage oscillations: which is not seen. Formation of a quiescent phase or a slowing of the cycling at such a level to explain the data would also disrupt magnitudes and timing of the phase oscillation, respectively.

The most likely explanation is that a level of lingering nearly non-cell cycle specific death occurs even after the camptothecin has been removed. This could be due to extraneous nCCS drug effects or system stress due to the surrounding death. Non-cell cycle specific death would not affect the timing of the oscillations nor the magnitudes while lowering the number of cells in each phase. The model was modified to incorporate a low-level nCCS death rate whose magnitudes decreases step-wise after each media
replacement. It was found that including a nCCS death rate of 0.8 deaths/day after drug application, which is decreased by a factor of two after each media replacement, fit the one pulse experimental, cell populations (see Fig. 4B).

5.3. HL60 response to two camptothecin pulses

The model describes the response of cells in culture to a single pulse of drug, but the goal is to describe the transient effects of multiple, timed pulses with flexible scheduling. The implication of the oscillations observed after a single pulse is that properly timed pulses will increase effectiveness while poorly timed treatments would be detrimental. The model’s applicability for multiple treatments was tested experimentally by applying a second pulse of camptothecin 12, 20, 24, or 32 h after an initial pulse and taking samples at least every 4 h for 56 h after the initial pulse. This timing was selected to administer the 2nd pulse as the S phase was at the first maximum, entering the first minimum, first minimum, and entering the second maximum as seen in Fig. 3. The second pulse treatment is represented in the model nearly identically to the first: S phase specific death occurs for 2 h followed by a decaying nCCS death rate that is reduced each time that the media is switched. For the second drug pulse, it was assumed that the maximum nCCS death rate is proportional to the relative level of dead cells induced by the second application versus the dead cells from the first drug application.

The experimental results follow the qualitative trends expected from the timed pulses (see Fig. 5). When the second pulse is given at or near the minimum observed with the single dose shown in Fig. 4, the treatment is less effective than when given at or near the maxima. Giving the second pulse near the minimum (20 or 24 h) versus at 32 h shows a roughly 33% increase in the population at 56 h. If a pulse were given at the second maximum (36 h) the benefits of the timed treatment would be even greater. The agreement between the model predictions and experimental results are quite good with the exception of the results for the second pulse delivered 12 h after the first. The 12 h pulse may be inaccurate because the cells did not have time to recover from the first pulse before administration of the second pulse. When observing the flasks, the cells become smaller after application of the
drug and return to a normal size or look more normal after roughly 16 h. The 12 h flask never returned to the “normal” state and the cells looked like they never recovered from the second application of the drug. As such, this data series will not be useful for verification of the model.

6. Co-cultured cell response to one camptothecin pulse

6.1. HL60 and Jurkat co-culture experiment

For a single cell line, experiments were performed to show that phase oscillations are observed and that timing treatments will vary the degree of treatment effectiveness. An analogous experiment is performed that follows the phase oscillations of two cell types, HL60 and Jurkat, with slightly different growth characteristics to show how the selectivity of a CCS changes after an initial application. As a single cell-type suspension, Jurkat cells exhibit a higher G1 phase percentage and longer doubling time, ~24 h, than HL60 cells, ~20 h; and it is anticipated that these differences will lead to observable differences in post-application phase transients. A bolus of camptothecin is applied and the phase percentages of each cell line are tracked in anticipation that multiple downstream treatment timings favor HL60 cells or Jurkat cells (see Fig. 6).

In order to ensure that the environment of both cell types is identical, the cell types are combined and co-cultured for 3 days before the start of an experiment. A FITC conjugated CD3 antibody was used to distinguish the two cell types so a DNA histogram of each could be produced using a flow cytometer. The co-cultured DNA histograms show that the phase percentages are slightly different from those during single cell cultures. This implies that the cell cycle kinetics have been slightly altered and simulation of the co-culture system with model parameters collected from single cell experiments are not as predictive in the timing and amplitude of the oscillations. There is a possible interaction between cell lines that need be accounted for and model parameters should be measured for the cell lines under co-culture conditions in order to produce a predictive co-culture model. However, the phase percentage and cytometry data illustrate the differences in the cycling behavior that could be utilized in timing treatments shown in Fig. 6.

The HL60 cells have a higher initial S fraction, so the first application of a CCS treatment will favor the Jurkat cells. The camptothecin induced HL60 and Jurkat oscillations are not synchronized, so the implication is that it is possible to deliver a second pulse to select one cell line over the other. For example, with a higher background G1 population, after the camptothecin bolus there are more Jurkats that enter the S phase and for a longer period of time. Thus, if the next application is between 4 and 24 h after the first application of camptothecin, there is a relative advantage to the HL60 cells. However, a delay of at least 12 h is advisable as we assume the return to “normal” after the 1st bolus should be similar to that observed for the HL60. Once the G1 and G2 phases have fluxed through the S phase, the HL60 cells are re-entering the S phase. Applying the drug between 26 and 36 h will be beneficial for Jurkat cells. However, the S phase percentage difference between 26 and 36 h is roughly the S phase difference before treatment, so this difference is of little significance. Then for a few hours past 36 h, the advantage returns to the HL60 cells. Again, with highly variable cell counts, the timed treatment comparison is difficult to do.

Both the Jurkat and HL60 cell lines exhibit phase oscillations in response to a CCS drug. As shown previously, these oscillations can be employed to manipulate the effectiveness of subsequent CCS drug treatments of the individual cell lines. However, many times selective cell type death is more important than the quantity. This experiment illustrates that selective treatments can be designed when the drug effects on multiple cell lines are under consideration.

6.2. Bone marrow and cancer dynamics

Analogous to the HL60 and Jurkat co-culture experiment, the bone marrow consists of several different cell types with individual response behaviors. The treatment of leukemia is concerned primarily with the relative effects of a treatment on the bone marrow and cancerous cells. The objective is to kill as many cancerous cells while maintaining a bone marrow population above an acceptable threshold. Thus, it is of interest to compare the dynamics of bone marrow cells to cancerous cells when optimizing a treatment protocol.

Also analogous to the HL60 and Jurkat experiments is that one of the cell types, Jurkats and cancerous tissue, has a longer, more variable G1 phase than the other cell type. Thus, the bone marrow is naturally more susceptible to a CCS drug, so resonance chemotherapy must be utilized if the CCS treatment is to have the potential to be more effective than an nCCS drug treatment. Effective resonant chemotherapy could overcome the natural S phase bias by using the first bolus of drug to induce phase oscillations while the second bolus would be applied when the S phase percentage is higher in the cancerous tissue than in the bone marrow.

Simulations of various camptothecin scheduled administrations, using the simplified model system, were conducted assuming that the bone marrow tissue parameters are similar to those of the isolated HL60 cells while cancerous cells have a 20% longer cell cycle length with an increase in duration and variability only in the G1 phase. To compare the various schedule combinations, a relative effectiveness, E, is defined in Eq. (6.1). The relative effectiveness compares the ratio of the number of cancerous to bone marrow cells at the end of the 10 days with treatment against the ratio of the number of cancerous to bone marrow cells with no treatment. A value of one is subtracted from this ratio such that a positive value indicates the resonance chemotherapy is beneficial while a
Fig. 6. Experimental results on co-cultured HL60 and Jurkat cells showing they have asynchronous responses to a 0.2 μM camptothecin pulse given at $t = 0$ h to purely proliferating cells. (A) Percentages of co-cultured HL60 and Jurkat cells in phases of cell cycle obtained from flow cytometry data. The frequency of oscillations is roughly 24 h for Jurkat cells and 20 h for HL60 cells. (B) The DNA histogram sequence for Jurkat cell progression through cell cycle after application of camptothecin at $t = 0$ h is an excellent illustration of how the phase oscillations occur. Note: The y-axis height is scaled by the height of the G1 peak as the number of cells (10,000–30,000) in each DNA histogram is non-uniform. The relative number of cells in each phase will determine how effective a CCS treatment will be.
negative number implies that a non-cell cycle-specific treatment would be preferable.

$$E = \frac{N_{\text{marrow,treatment}}/N_{\text{cancer,treatment}} - 1}{N_{\text{marrow,nothing}}/N_{\text{cancer,nothing}}}$$

(6.1)

For such a biased system with a substantial difference in initial S phase percentages, we find that only negligibly small windows of timing exist where timed treatments show benefits over a nCCS or continuous CCS treatment (see Fig. 7A). Proper timing does help increase the effectiveness, but it is not enough to compensate for the deficit created by the first pulse. However, if the system can be modified to ensure the bone marrow and cancerous S phase fractions initially match resonance treatment concentrations and timings exists where timed treatments are beneficial (see Fig. 7B) as there is no initial barrier to overcome. Even in this case, if the concentration is too high the oscillations are lost or if the concentration is too low, the oscillations cannot develop. Within the useful concentration window that exhibits oscillations that could be capitalized on by resonance chemotherapy, timings exist such that the treatment has the potential to be either beneficial or detrimental depending on when the second bolus is applied. Thus, the cell cycle parameters of the bone marrow and cancerous tissue are of paramount importance in determining whether resonant chemotherapy will be of benefit as well as identifying the ideal drug timing and concentrations.

7. Considerations in modeling the in vivo case

The previous results are based on an experimental system intended to emulate an ideal in vivo system. But, there will obviously be several differences between an in vitro and in vivo system. Since potential clinical applications of resonance chemotherapy are of interest, it is necessary to evaluate the sensitivity of resonance to other factors. These factors could include properties of the drug as well as the cells such as: the half-life of the drug, drug concentration, drug activation, drug diffusion to the site, the residence time distributions of the cell cycle phases, and a quiescent cell cycle phase.

7.1. Drug parameters—half-life, concentration, and pharmacokinetics

The efficacy of resonance chemotherapy depends upon the existence of oscillations in the concentrations of cells in the various phases of the cell cycle after the first application of a CCS drug. Simulations of the model shown in Fig. 8 demonstrate that CCS drugs with smaller half-lives at larger concentrations are most relevant for timing of resonance chemotherapy treatments. For example, 5-flourouracil (5FU, plasma $t_{1/2} = 2.4$ h) induces oscillations in simulations even at dilute concentrations while methotrexate (MTX, plasma $t_{1/2} = 8$ h) requires several times the base concentration to induce significant oscillations (see Fig. 8).

However, the half-life of a drug is not always indicative of its effective active time period; often the activity is determined by the properties of its metabolic products. Depending upon the active mechanism of the drug, the half-life and concentration profile of the metabolite may ultimately determine the effectiveness of the resonance treatments. This dependency on metabolites may greatly distort the resonance possibilities to the extent that it may be irrelevant as many intracellular metabolites remain for days rather than hours. For example, even though 5FU may have a very short plasma lifetime, the drug is metabolized within the cells such that its effects linger (or may not even begin until) long after 5FU has disappeared from the periphery. If the active metabolite for 5FU had a half-life equivalent to that of MTX, assuming instantaneous metabolization and adjusted concentrations, the
resonance profile would be that of MTX rather than 5FU. This is consistent with the observation that a bolus and a continuous infusion of 5FU are indistinguishable clinically (Dorr and Fritz, 1980).

Just as the half-life of the metabolite will affect resonance, so will the drug metabolism or transport rates by broadening the concentration profile. These concerns are relevant as: (i) many drugs are tabulates following the kinetics of absorption; (ii) all chemotherapeutic drugs work intracellularly and must enter the cell; and (iii) the area of interest is a specific physical region so transport is important. To incorporate these effects into the model, first order kinetics are used for both transport rates and metabolism (see Fig. 2). Assuming that all forms of 5FU have the same half-life and scaling the bolus concentration for comparison, Fig. 9 shows how a single transport rate can reduce the magnitude of oscillations by several factors even if the half-life of the metabolite and drug are short.

In the previously described (Sections 4–6) in vitro camptothecin experiments, the camptothecin is the active metabolite and transport effects are eliminated by dissolving the drug in DMSO and briefly stirring the flask. The half-life was manipulated artificially by replacing the media at intervals to eliminate and dilute the camptothecin. The intention of these experiments was to discern whether resonance is achievable experimentally by mimicking the ideal in vivo case where metabolites and transport are not a concern. The results verified the efficacy of resonance chemotherapy for simplistic situations and verified the modeling approach for these cases. As described in this section, this work extends the model to consider other factors not tested experimentally using what was learned from the experiments to apply that knowledge to broader, more realistic, scenarios.

7.2. Cell parameters—residence time distributions and quiescent phase

The duration of the oscillations as well as their magnitudes depends on the residence time distributions within the various phases of the cell cycle. While a sharp distribution will create sustained oscillations that support resonance chemotherapy, broader distributions will limit the effectiveness of timing the treatments. Most of the variability in the cell cycle is thought to come from the G1 to S transition. This may be due to the more mechanical nature of the S and G2 phases while the G1 phase has more feedback and signals controlling progression which will vary from cell to cell. This has led modelers to describe the G1 transition with an age-averaged or transition probability and the S and G2 transition functions implying a unique age of transition (Basse et al., 2003, 2004).

Since log-normal distributions are assumed, the width of the distribution is governed by the standard deviation. The standard deviations extracted from the HL60 cells imply that the oscillations will sustain themselves for several days (see Fig. 10A). This is an ideal growth condition and any additional sources of non-homogeneity will increase the variability between cells. Assuming that the S and G2 phases are not affected and retain the measured standard deviations, the effects of variability in the G1 phase are shown to slightly reduce the initial oscillations while having a dramatic effect on later oscillations (see Fig. 10B).

Increasing the standard deviation by a factor of 3 or more
allows only the first oscillation to persist before dampening occurs.

Some of the variability associated with the G1 phase may be attributed to the inability to resolve the G0 and G1 phases easily. A quiescent phase that includes the richness of feedback control is impossible to achieve in vitro or in silico, but an attempt is made to capture the behavior qualitatively. In the model, the proliferation and quiescent phases are coupled by G0 to G1 rate terms. These rates have feedback terms such that a pseudo-steady state is reached. Once this state is reached, a reduction in the G0 phase increases the proliferation level to replace lost cells while an increase in the G0 phase reduces the proliferation level to curb cellular production. Thus, when a void created by CCS chemotherapy reaches the G1 phases, there will be fewer cells to enter the G0 phase and the size of the G0 phase will decrease. These result in an increased flux from quiescence to proliferation to fill the void created by the CCS chemotherapy (see Fig. 11). Thus the feedback mechanisms between G0 and G1 phases will serve to dampen the effectiveness of resonance chemotherapy.

To mimic the level of cell loss and feedback control anticipated in a patient, the CCS drug FU was supplemented with the nCCS drugs cyclophosphamide, C, and adriamycin, A, with concentrations of the CAF treatment given in Table 2.

8. Conclusions

Various approaches have been taken in attempt to design better chemotherapy treatments. The particular case of
timing the application of cell cycle specific drugs to select for cancerous cells in leukemia is dealt with in detail herein. A general model is presented that uses age distributions to describe the transitions between cell cycle phases. For a simplified model, these distributions are measured for an in vitro cell leukemia culture HL60 cell line and these parameters are used in simulations to predict the optimal timing for a second bolus of drug. The oscillations observed in the in vitro system very closely resemble the model predictions and the model quantitatively predicts response of the cell system to several schedules of timed camptothecin treatment.

The relative effects of a treatment protocol on multiple cell lines can be analysed by applying a treatment to cell lines with different properties. An in vitro setting using HL60 and Jurkat cells was used to illustrate how cells with different properties develop regions of selectivity following the application of the CCS drug, camptothecin. The analysis of the model showed that the potential for resonance chemotherapy will depend on the properties of the specific cell lines. When considering scenarios resembling those of purely proliferating bone marrow and cancerous cells, resonance chemotherapy was shown to have minimal effect.

Additional factors and processes present in the human body will disrupt the resonance phenomenon further. In particular, if (i) intracellular metabolites rather than the parent drug are the active ingredient, (ii) transport effects are significant, or (iii) a quiescent phase is included, these factors dampen the effectiveness of resonance considerably in the model simulations. While resonance chemotherapy works in a controlled and simplified laboratory setting, the applicability to an in vivo setting is still unproven. These analytical studies suggest that resonance chemotherapy may not be beneficial and one clinical attempt at interval treatments for a single CCS drug did not show significant differences (Hainsworth and Greco, 2004).

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References


