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A MATHEMATICAL ANALYSIS OF A MODEL OF DRUG ACTION ON INTRACELLULAR CALCIUM DYNAMICS

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A MATHEMATICAL ANALYSIS OF A MODEL OF DRUG ACTION ON INTRACELLULAR CALCIUM DYNAMICS

by

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ABSTRACT

A MATHEMATICAL ANALYSIS OF A MODEL OF
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Faculty Mentor: Hristo Kojouharov

The dysregulation of intracellular Ca^{2+} dynamics is a hallmark feature of several types of cancer. Administering a combination of a drug that blocks store-operated Ca^{2+} entry and a drug that inhibits tyrosine kinases is a novel chemotherapeutic approach currently being explored. Using data from experimental trials of these drugs, an existing model of Ca^{2+} dynamics was modified via the Michaelis-Menten theory of enzyme kinetics to reflect the behavior of the cell upon administration of the drugs. Mathematical analyses were performed to determine the overall repercussions of this modification. This included sensitivity analysis to reveal the model's sensitivity to changes to particular parameters and stability analysis to find if the model will approach equilibrium. With the goal of predicting drug action in mind, this expanded model is a step in the right direction but still needs refinement to increase accuracy and have real-world applications.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

BIOLOGICAL BACKGROUND

Intracellular Ca^{2+} functions as an important second messenger system that regulates gene expression, proliferation, apoptosis, and migration. Tumor cells disrupt normal Ca^{2+} signaling pathways in order to more efficiently utilize its benefits. That is, they can proliferate faster, avoid apoptosis, and migrate in order to metastasize throughout the body. This fact can be exploited by administering drugs that target specific Ca^{2+} pathways in order to treat various types of cancer. In this case, the focus is on the drugs Afatinib and RP4010 (Cui et al. 2017).

Afatinib blocks epidermal growth factor receptors (EGFRs) by inhibiting the ATP binding site on the tyrosine kinase (Spicer et al. 2017, Bryant et al. 2004). After EGF binds to an EGFR, the extracellular domain of the EGFR experiences a conformational change and the tyrosine kinases in the intracellular domain experience dimerization and transphosphorylation (Schlessinger 2002). These events are then followed by the phosphorylation of phospholipase C-γ (PLC-γ) (Meisenhelder et al. 1989). The activation of PLC-γ causes the hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) into inositol 1,4,5-triphosphate (IP_3) and diaglycerol. This IP_3 then activates the IP_3 receptors (IP_3Rs) on the ER membrane, which opens the associated channel and allows Ca^{2+} to flow out of the ER and into the cytoplasm (Rhee et al. 1992, Furuichi et al. 1989). Afatinib disrupts this process, keeping the Ca^{2+} in the ER so it can't participate intracellular signaling.

The drug RP4010 disrupts store-operated Ca^{2+} entry (SOCE). A decrease in the concentration of Ca^{2+} in the ER will trigger the activation of stromal interaction molecule 1 (STIM1). This molecule binds to the termini of the ORAI1 subunit that forms the ion pore of Ca^{2+} release-activated Ca^{2+} (CRAC) channels. STIM1 binding to this subunit causes the CRAC channel to open and therefore allows Ca^{2+} to flow into the cell (Bergemeier et al. 2013). The exact mechanism(s) of inhibition that RP4010 employs has yet to be determined, but it does prevent the opening of these channels and thus prevents Ca^{2+} entry.

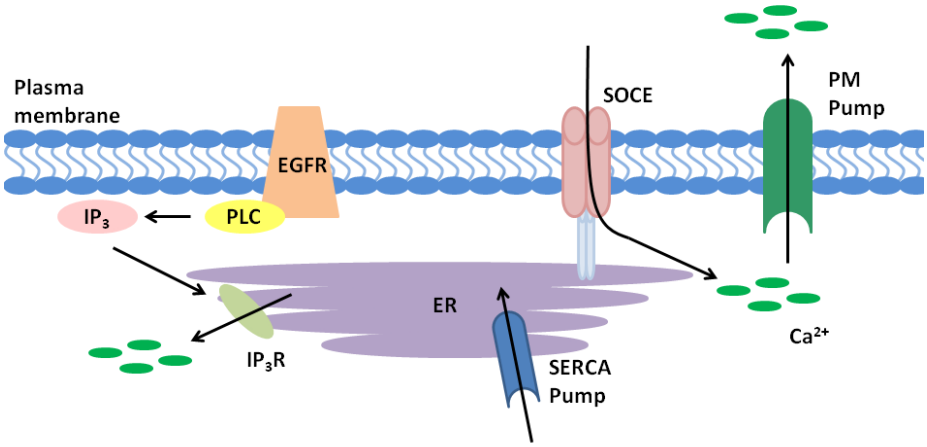

Figure 1.1: Diagram of Ca^{2+} Dynamics

Utilizing both of these means of inhibiting Ca^{2+} entry into the cytoplasm provides a novel treatment for cancer. The actions of these drugs were incorporated into an existing model of calcium dynamics, with the ultimate goal being to use this model to predict the optimal treatment method.

CHAPTER 2

ENZYME KINETICS

The theories of mass action kinetics and Michaelis-Menten kinetics are described in more detail by Keener and Sneyd (2009) but are briefly summarized here.

2.1 Mass Action Kinetics

Mass action kinetics are used to describe reaction kinetics in terms of how fast the molecules collide. The law of mass action states that for two reactants, R_1 and R_2, that react to form a product P as in the reaction below:

$$R_1 + R_2 \xrightarrow{k} P$$

The rate at which P is produced can be written in the form of the following differential equation:

$$\frac{dP}{dt} = k[R_1][R_2]$$

The parameter k is a rate constant for the forward direction of this reaction. However, it is incorrect to say that all reactions are one way. In thermodynamics, all reactions are considered two way unless the reverse reaction is excessively slow compared to the forward reaction. In the case of a two way reaction, the forward rate constant is called k_+ and the reverse called k_-. From here the rate constants can determine the equilibrium constant K_{eq} as in the equation shown below, which can then reveal whether the reaction favors the reactants or the products. A large K_{eq} would mean that the reaction favors the reactants, while a small K_{eq} would indicate that the products are favored.

$$\frac{k_-}{k_+} = K_{eq} = \frac{[R_1]_{eq}[R_2]_{eq}}{[P]_{eq}}$$

2.2 Michaelis-Menten Kinetics

When studying an enzyme-catalyzed reaction, the reaction kinetics deviate from the law of mass action. A catalyst will not change throughout the course of a reaction and unlike what the law of mass action states, the rate of an enzyme-catalyzed reaction will not increase linearly with the concentration of substrate. Instead, the rate will reach a maximum velocity after which an increase in substrate will have no effect. Michaelis and Menten proposed the following model to circumvent this issue.

A substrate S will bind to an enzyme E to form a complex SE, which then dissociates into the product P and the conserved enzyme E. The reaction can be seen below. The forward rate constant of the first step is denoted k_1 and the reverse is denoted k_{-1}. Since the conditions following the second step are such that the product is continuously removed, only one rate constant is needed for that reaction, k_2.

$$S + E \rightleftharpoons SE \rightarrow P + E$$

Let s be the substrate concentration, e be the enzyme concentration, c be the concentration of the complex SE, and p be the concentration of the product. The rate at which the concentrations of these molecules change can be modeled with the following differential equations:

$$\frac{ds}{dt} = k_{-1}c - k_1 se$$

$$\frac{de}{dt} = (k_{-1} + k_2)c - k_1 se$$

$$\frac{dc}{dt} = k_1 se - (k_2 + k_{-1})c$$

$$\frac{dp}{dt} = k_2 c$$

Noting that the total amount of enzyme $e_0 = e + c$ and assuming at equilibrium $k_1 se = k_{-1} c$, the variable *c* can then be written as:

$$c = \frac{e_0 s}{K_1 + s}$$

Here, $K_1 = \frac{k_{-1}}{k_1}$. Finally, the overall velocity of the reaction *V* can be expressed in the following way:

$$V = \frac{dp}{dt} = k_2 c = \frac{k_2 e_0 s}{K_1 + s}$$

By letting $V_{max} = k_2 e_0$, the equation can be simplified to:

$$V = \frac{V_{max} s}{K_1 + s}$$

V_{max} is the maximum velocity the reaction can attain and K_1 is the concentration of substrate at which the reaction rate is half V_{max}. Below, Figure 2.1 depicts what the relationship between substrate concentration and reaction velocity looks like. In this case, V_{max} was set at 1 and K_1 was set at 0.25. The function increases quite fast at first but eventually slows down as it approaches V_{max}=1 and begins to plateau. The reaction velocity will never reach its maximum because in order to do so, K_1 would have to equal zero.

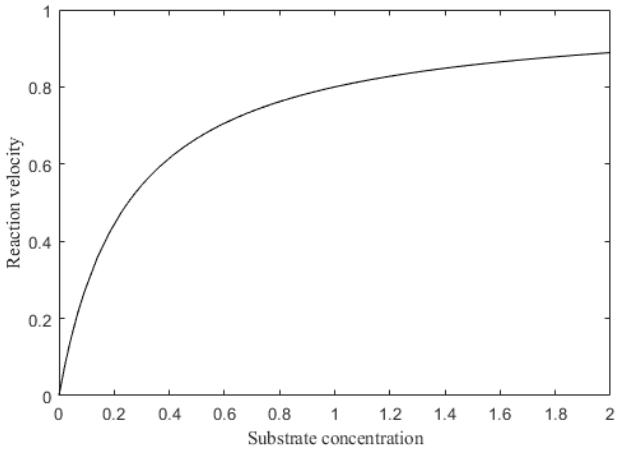

Figure 2.1: Example of Michaelis-Menten Kinetics

CHAPTER 3

MATHEMATICAL MODEL

The model used has gone through several iterations over the past 20 to 30 years and recently was used by Sneyd and colleagues in a study of various cell types, including epithelial cells similar to those used in the experiments to which this paper refers (Sneyd et al. 2017, Dupont et al. 2016). This base model was modified to include the effects of Afatinib and RP4010 on Ca^{2+} oscillations. Table 3.2 contains descriptions and values for all of the parameters seen in the equations in the following sections.

3.1 The Base Model

For variable notation, let c be the intracellular calcium concentration ($[Ca^{2+}]$), c_e be the calcium concentration in the ER ($[Ca^{2+}]_{ER}$), p be the IP_3 concentration ($[IP_3]$), and h be the IP_3R activation rate via calcium. The model equations are below:

$$\frac{dc}{dt} = J_{IPR} - J_{serca} + \delta(J_{in} - J_{pm})$$

$$\frac{dc_e}{dt} = \gamma(J_{serca} - J_{IPR})$$

$$\frac{dh}{dt} = (h_\infty(c) - h)\frac{1}{\tau_h(c)}$$

$$\frac{dp}{dt} = \nu - \beta_p p$$

And the flux equations are as follows:

$$J_{IPR} = k_f P_O (c_e - c)$$

$$J_{serca} = \frac{V_p(c^2 - \bar{K}c_e^2)}{c^2 + k_p^2}$$

$$J_{in} = \alpha_0 + \alpha_1 \frac{K_e^4}{K_e^4 + c_e^4}$$

$$J_{pm} = \frac{V_{pm}c^2}{K_{pm}^2 + c^2}$$

The J terms are flux equations that represent the flow of Ca^{2+} through a specific channel in either the plasma membrane or ER membrane. The J_{IPR} equation models the flow of Ca^{2+} through the IP_3Rs and directly depends on both c and c_e. If c_e is greater, then Ca^{2+} will enter the cytoplasm at a greater rate and if c is greater, then Ca^{2+} will not flow as fast into the cytoplasm. The parameter k_f can be viewed as a measure of IP_3R density or simply as a scaling factor. P_O is the open probability of IP_3Rs and is further expanded upon below. The J_{serca} equation models the flow of Ca^{2+}: from the cytoplasm to the ER through the SERCA pump. As c grows significantly bigger compared to c_e, the flux term grows larger to represent the increased amount of Ca^{2+} flowing into the ER and the opposite is true when c grows smaller. The ER acts as a Ca^{2+} storage center, so if the difference between c and c_e is too great the cell will adjust accordingly using either IP_3Rs or the SERCA pump. To express Ca^{2+} flowing into the cell from the extracellular space, leak channels (α_0) and SOCE (the entire second term) are both lumped into the J_{in} equation. Keeping c_e confined to the denominator ensures that as c_e increases, the model will respond by reducing the Ca^{2+} flow into the cell. J_{pm} is solely concerned with the plasma membrane pump, which actively moves Ca^{2+} out of the cell. It is a standard Hill equation with V_{pm} as the maximum rate at which Ca^{2+} can flow through the pump and K_{pm} as the amount of Ca^{2+} that flows through when half of the binding sites on the pump are occupied. The parameter

δ is a scale factor relating the plasma and ER membrane fluxes and γ is the ratio of cytoplasm and ER volumes. Regarding the p equation, β_p is the rate at which p decays to its steady state, p_s, and $v=\beta_p p_s$.

The following equations expand upon the P_O term and the $\frac{dh}{dt}$ equation:

$$P_O = \frac{\beta}{\beta + k_\beta(\beta + \alpha)}$$

$$\alpha = A(p)(1 - \bar{m}_\alpha(c)\bar{h}_\alpha(c))$$

$$\beta = B(p)\bar{m}_b(c)h(c,t)$$

$$\bar{m}_\alpha(c) = \bar{m}_\beta(c) = \frac{c^4}{K_c^4 + c^4}$$

$$\bar{h}_\alpha = h_\infty(c) = \frac{K_h^4}{K_h^4 + c^4}$$

$$1 - A(p) = B(p) = \frac{p^2}{K_p^2 + p^2}$$

$$\tau_h = \tau_{max}\frac{K_\tau^4}{K_\tau^4 + c^4}$$

The equations $\alpha(c,p)$ and $\beta(c,p,t)$ model the rates at which Ca^{2+} inactivates and activates the IP_3Rs, respectively.

3.2 The Addition of Drugs

The two drugs are incorporated into the model as seen below, where D_1 represents the drug effect on the plasma membrane pump and D_2 represents the drug effect on the IP_3Rs.

$$\frac{dp}{dt} = D_1 v - \beta_p p$$

$$J_{in} = \alpha_0 + D_2\alpha_1\frac{K_e^4}{K_e^4 + c_e^4}$$

Afatinib reduces Ca^{2+} flow out of the ER by inhibiting tyrosine kinases and therefore negatively impacting the cascade that activates IP_3Rs. In terms of the model, it exerts its effects through the $\frac{dp}{dt}$ equation. Since RP4010 is a SOCE inhibitor, the drug is added to the model in such a way that increased amounts of the drug decrease the inward Ca^{2+} flow through SOCE-associated channels. To do this, D_2 is placed against the term in the J_{in} equation associated with SOCE. Experimental data suggests that Afatinib also has a negative impact on SOCE as well, so the D_2 equation contains terms modeling both drug effects.

$$D_1 = 1 - f_1(S_1)$$

$$D_2 = 1 - (f_2(S_2) - f_3(S_1))$$

The functions f_i are Michaelis-Menten equations as described earlier where S_1 and S_2 are the doses (μM) of Afatinib and RP4010, respectively. To make a one-to-one correspondence to the equation given in section 2.2: the substrate concentration s is the amount of drug, V_{max} is c_i, K_1 is k_i, and V is the overall drug effect.

$$f_i(S) = \frac{c_i S}{k_i + S}, \qquad i = 1, 2, 3$$

Under the assumption that c oscillates in a tumor cell at a period of about 32 seconds and in a healthy cell at a period of about 600 seconds, the parameters k_f, α_1, and τ_{max} were adjusted from the values in the original model (Sneyd et al. 2017). They were adjusted so that when k_f is divided by 5 and α_1 is divided by 3 the healthy period is achieved, to represent the physiological differences between healthy and tumor cells. With the values seen in Table 1, the model has the tumor cell oscillating at 32.66 seconds and the healthy cell at 600.89 seconds. The parameters c_i and k_i were determined by fitting to experimental data relating the period of Ca^{2+} oscillations to a given dose of one or both drugs.

In order to carry out the numerical simulations, Mathworks' MATLAB software was used. Within that, a system of differential equations was solved with the "ode23s" function and the "lsqcurvefit" function was used to fit the model to the experimental data. Computer simulations of the oscillations can be seen in Figure 3.1. The top left chart displays the oscillations of healthy cells (black) and tumor cells (pink). The top right and bottom left show the results of administering Afatinib and RP4010 at doses of 1.5 μM, respectively. The final graph in the bottom right depicts the oscillations after adding Afatinib at a dose of 0.5 μM and RP4010 at a dose of 1 μM.

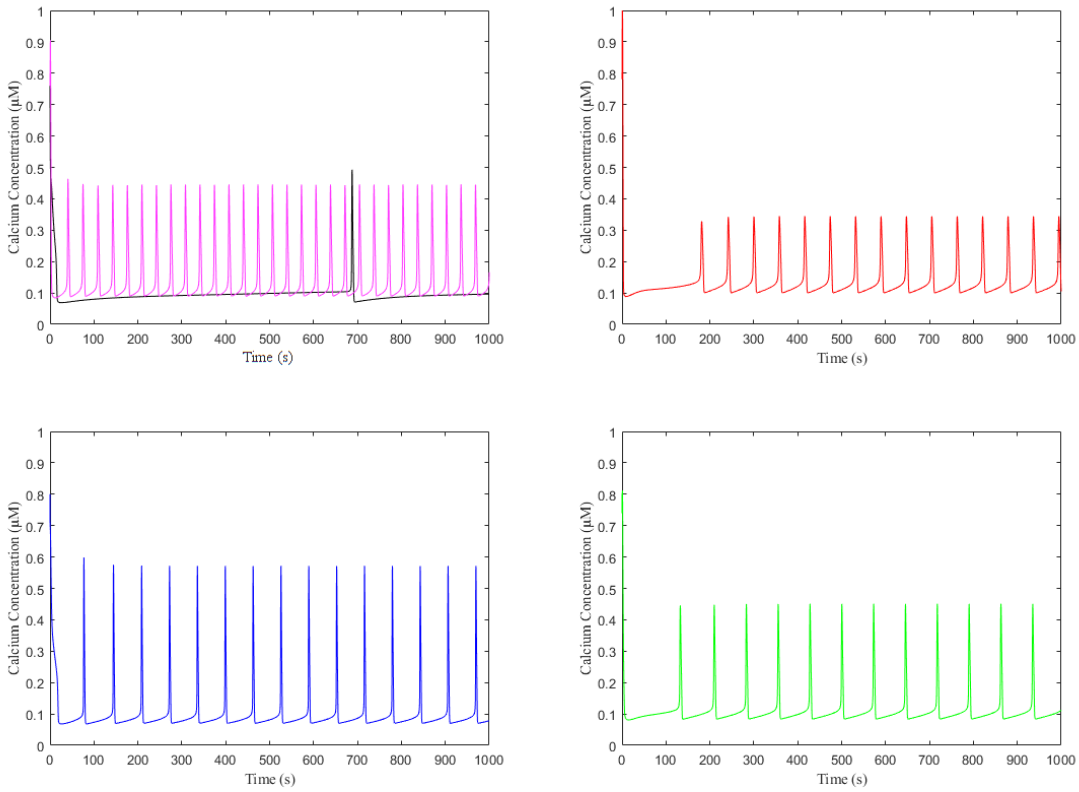

Figure 3.1: Simulations of Ca^{2+} Oscillations

The experimental results were obtained from Dr. Zui Pan's lab (Chang et al. 2018). The drugs Afatinib and RP4010 were administered to esophageal cells afflicted with esophageal squamous cell carcinoma at various doses separately and combined in a ratio

of 2:1 RP4010:Afatinib. After drug administration, the cells were given a fluorescent dye that marked the changes in Ca^{2+} concentration. The fluorescent intensities were recorded and the times between peaks in oscillations were calculated. Figure 3.2 depicts these experimental results plotted with simulated curves of the drug dose versus the period of Ca^{2+} oscillations. This model leaves room for accuracy improvements since it is clear that the simulated curve for the combined drug treatment does not fit well for very low doses or very high doses.

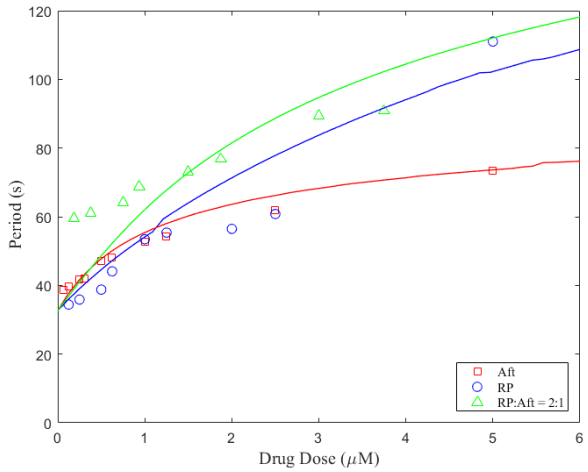

Figure 3.2: Experimental Data and Simulated Curves

The parameter values in Table 3.1 were taken from the model presented by Sneyd and colleagues in 2017 with the exceptions of k_f, α_1, and τ_{max}. These were the parameters changed to adjust the model to fit the type of cells used in the experiments, as mentioned earlier.

Table 3.1: Parameter Descriptions and Values

Parameter	Description	Value	Units
δ	Adjusts the ratio of c across the plasma membrane to ER membrane	1.5	N/A
γ	Ratio of cytoplasm volume to ER volume	5.5	N/A
k_f	Scaling factor controlling c flux through IPR	3.9	s^{-1}
α_0	Calcium flow through unspecified leak channels	0.0027	$\mu M s^{-1}$
α_1	Rate constant for SOCE channels	0.385	$\mu M s^{-1}$
τ_{max}	Controls rate at which β responds to changes in c	1,420	s^{-1}
β_p	Rate at which p decays to ps	0.027	s^{-1}
p_s	Steady state of p	0.1	μM
V_p	Maximum capacity of SERCA pump	0.9	$\mu M s^{-1}$
V_{pm}	Maximum capacity of PM pump	0.11	$\mu M s^{-1}$
$\bar{K}$	Adjusts c in ER	1.9×10^{-5}	N/A
K_e	Half-maximal ce for SOCE channels	8	μM
K_{pm}	Half-maximal c for PM pump	0.3	μM
K_c	Half-maximal c for IP3Rs	0.2	μM
K_h	c activating IP3Rs	0.08	μM
K_p	Half-maximal p for IP3Rs	0.2	μM
K_τ	c in response to β	0.1	μM

CHAPTER 4

MATHEMATICAL ANALYSIS

Sensitivity analysis was performed to determine how the model responds to changes in particular parameters without drugs and in the presence of one or both drugs. This is also an important tool to use to determine how drastic of an effect any error would have on the results.

4.1 Sensitivity Analysis

To carry out sensitivity analysis, the parameters to analyze were chosen. In this case, the parameters were: k_f, α_1, all c_i, and all k_i. These specific parameters were the ones modified to fit the experimental data and the ones introduced in order to model the drug effect. Determining the consequences of changing and adding these parameters will provide insight regarding how the cell responds to the drug. After choosing the parameters, they were increased and decreased by 1%, 5%, 10%, and 20%. These changed parameters were then implemented one at a time under the following conditions: no drug, 1.5 μM Afatinib, 1.5 μM RP4010, and 1.5 μM Afatinib and RP4010 in a 2:1 ratio. The resulting periods were compared to the periods when the parameters are at baseline values to calculate the percentage that the periods changed.

In the figures below the black lines represent no drug, the red lines represent Afatinib at a dose of 1.5 μM, the blue lines represent RP4010 at a dose of 1.5 μM, and the green lines represent Afatinib at a dose of 0.5 μM and RP4010 at a dose of 1 μM combined.

The parameter α_1 has an inverse relationship with the period of Ca^{2+} oscillations, meaning that increasing α_1 causes the period to decrease and decreasing α_1 causes the period to increase. These results are visualized in the right side of Figure 4.1. This makes sense because an increase in α_1 will increase the J_{in} term which raises the rate of change of c compared to that of c_e. The addition of RP4010 does not change this relationship. In the presence of Afatinib, the change in period is more exaggerated and in the presence of both drugs the change in period is only slightly more exaggerated. The results from comparing the k_f simulations, as seen in the left side of Figure 4.1, are similar to the α_1 results. The relationship between k_f and period is inverse and administering Afatinib only causes this relationship to be more extreme.

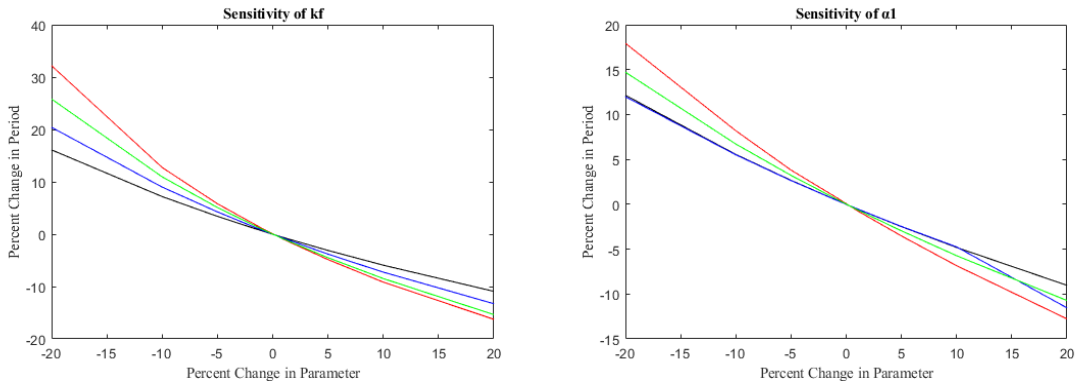

Figure 4.1: Sensitivity of the Parameters Adjusted to Fit the Model to Esophageal Cells

The parameters c_1 and c_2 both have a direct relationship with the period, as seen in the top left of Figures 4.2 and 4.3. In addition to this, the presence of just one drug causes changes in c_1 and c_2 to evoke greater changes in the period than with a combination of both drugs. The period responds almost equally to changes in c_3 (bottom left of Figure 4.2) with just Afatinib as it does with both drugs. Opposite to c_1 and c_2, there is an inverse relationship between c_3 and period. According to the top right of Figures 4.2 and 4.3,

parameters k_1 and k_2 are also inversely related to the period. Only k_2 exhibits a greater change in period with just one drug (RP4010) than both drugs; for k_1 the change in period is slightly less with just one drug (Afatinib) than with both drugs. Finally, the parameter k_3 (bottom right of Figure 4.2) is directly related to the period and the magnitude the period changes is greater with both drugs than with just Afatinib.

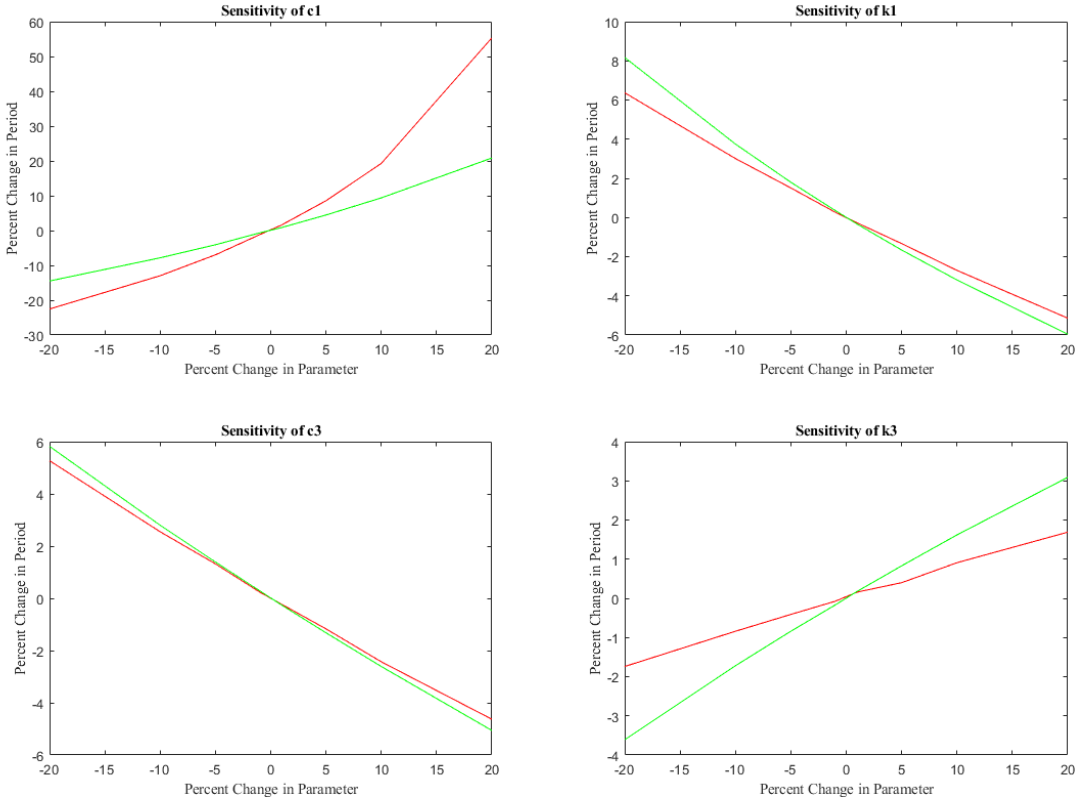

Figure 4.2: Sensitivity Analysis Results for the Effect of Afatinib

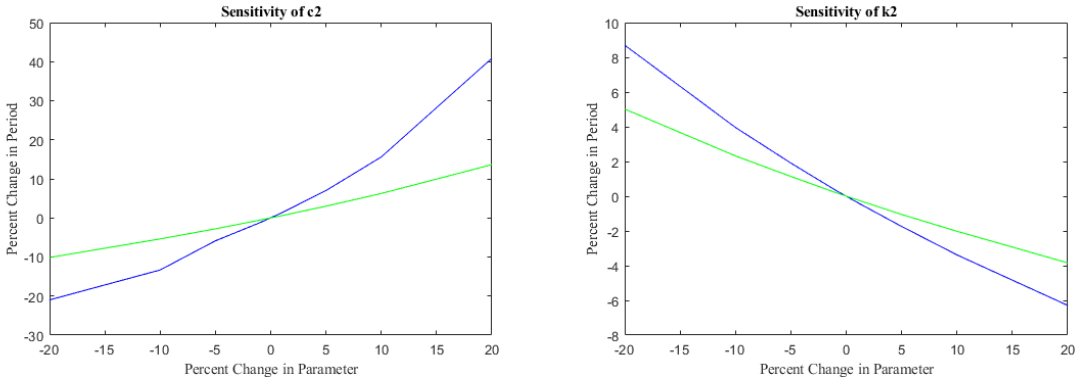

Figure 4.3: Sensitivity Analysis Results for the Effect of RP4010

Overall, c_1 causes the most change in the period of Ca^{2+} oscillations, with c_2 following closely behind it. At the end of the spectrum, k_3 and c_3 have the smallest effect on Ca^{2+} oscillations. These two parameters control the effect that Afatinib has on SOCE and the results of the sensitivity analysis suggest that the action of Afatinib on SOCE is small. Therefore, c_1 and c_2 would be the parameters to worry most about when it comes to reducing the amount of error in the model. They cause a change in period much greater than the change in parameter implemented, so finding well-fitting candidates for c_1 and c_2 is a must.

Most of the sensitivity plots appear linear. Although the relationship between parameter change and change in period is not one-to-one, where a 5% change in parameter would cause a 5% change in period, the change is still consistent. These plots each could be fitted to a linear function. For the nonlinear plots, they could be fitted to exponential or rational functions. Theoretically, these functions could then help find a parameter value for a desired period. This would make fitting the model to different types of cells easier.

Table 5.1 contains a portion of the data generated from the sensitivity analysis of the 8 parameters. In it are the baseline values for all the parameters analyzed, the values of the parameters when increased and decreased by 10%, and the percentage that the period

changed as a result of the parameter change. This table demonstrates where the data plotted in the figures above came from and provides more detail regarding the nature of the results of the sensitivity analysis.

Table 4.1: Sample Sensitivity Analysis

Dose (μM)	Parameter	Baseline	New Value		% Change in Period	
			10%	-10%	10%	-10%
Aft: 0 RP: 0	k_f	3.9	4.29	3.51	-5.92	7.18
	α_1	0.385	0.4235	0.3465	-4.81	5.55
Aft: 1.5 RP: 0	k_f	3.9	4.29	3.51	-9.16	12.74
	α_1	0.385	0.4235	0.3465	-6.84	8.19
	c_1	0.51	0.561	0.459	19.29	-12.97
	c_3	0.76	0.836	0.684	-2.43	2.55
	k_1	0.34	0.374	0.306	-2.7	3.01
	k_3	0.8	0.88	0.72	0.91	-0.84
Aft: 0 RP: 1.5	k_f	3.9	4.29	3.51	-7.25	8.99
	α_1	0.385	0.4235	0.3465	-4.75	5.51
	c_2	1	1.1	0.9	15.61	-13.32
	k_2	0.6	0.66	0.54	-3.38	3.96
Aft: 0.5 RP: 1.0	k_f	3.9	4.29	3.51	-8.48	10.95
	α_1	0.385	0.4235	0.3465	-5.73	6.71
	c_1	0.51	0.561	0.459	9.39	-7.83
	c_2	1	1.1	0.9	6.26	-5.38
	c_3	0.76	0.836	0.684	-2.61	2.8
	k_1	0.34	0.374	0.306	-3.19	3.75
	k_2	0.6	0.66	0.54	-2.02	2.32
	k_3	0.8	0.88	0.72	1.62	-1.72

4.2 Stability Analysis

Stability analysis was attempted using the method described by Otto and Day (2007). The steps for this are as follows:

1. Find equilibrium points by setting each differential equation from the model equal to zero and solving for the corresponding variable.

2. Find the Jacobian matrix for the system by taking the derivative of each differential equation with respect to each variable.
3. Substitute the equilibrium points into the Jacobian matrix.
4. Use the characteristic polynomial calculated from solving $\det(A - \lambda I) = 0$ to find the eigenvalues λ_i of the matrix.
5. Interpret the eigenvalues to determine the stability of the model.

The equilibrium points for p and h were trivial to find, however finding those for c and c_e were not so. Searching for these points yielded only negative values or complex values for c and c_e. Since negative and complex values have no biological relevance, stability analysis could not be performed.

CHAPTER 5

CONCLUSION

The mathematical model that was presented in this paper has its limitations, but still presents promising results. This model was developed by taking an existing model of intracellular Ca^{2+} dynamics and using the theory of Michelis-Menten kinetics to incorporate the drugs Afatinib and RP4010.

Sensitivity analysis was performed to determine how the model responds to the parameters changed from the original model and the parameters introduced to model the drug action. The model is most sensitive to changes in c_1, so Afatinib may be used to evoke the fastest and most efficient changes in Ca^{2+} oscillations. Although in practice, RP4010 has a greater effect on Ca^{2+} dynamics (Figure 3.2). The sensitivity analysis also revealed that the effect Afatinib has on SOCE, while present, is small. Since the model shows the greatest sensitivity to c_1 and c_2, they are the most likely culprit for the error seen in the model and must be carefully fitted.

Due to the model's complexity, stability analysis was unable to be performed. To combat this, simplification of the model or use of an older, simpler iteration may be necessary. However, a simpler model may respond differently to the addition of drugs and therefore might not return results that reflect the behavior of the current model.

To improve the model's accuracy, perhaps the Hill equation may be useful. This equation is very similar to the Michaelis-Menten model, but incorporates multiple substrates or binding sites. The equation for this is as follows:

$$V = \frac{V_{max} s^n}{K_m^n + s^n}$$

In this equation, *n* can be viewed as the number of substrate molecules binding to the given enzyme or as the number of binding sites. In theory, this number would have an integer value. However, this is often not the case since there may be negative or positive cooperativity between the enzyme and substrate (Keener and Sneyd 2009).

The model presented can be useful in predicting the results of treatments using doses of the drugs at mid-range values, but not values at either extreme. At these mid-range values using a combination of drugs produces a greater period, so it is more effective at reigning in the cell's use of Ca^{2+}. Therefore, a combination of both Afatinib and RP4010 is more efficient at treating esophageal cancer than either drug alone because for a smaller total dose the same period can be achieved. Overall however, the model isn't as sensitive to parameter changes in the presence of both drugs. This suggests that the drugs interact in some way that the model does not account for. It seems as if the model is most dependent on c_1 and c_2, which is consistent with their role as maximal reaction velocities. Further refinement of this model may lead to more accurate predictions and therefore greater therapeutic benefit. This model works for Afatinib and RP4010 combined in a ratio of 1:2, but it could be adapted to different combinations of those two drugs. This would be beneficial in determining the optimal ratio of drugs in addition to finding the optimal amount of drug. Even different drugs could be added to the model, either in concert with Afatinib and RP4010 to treat cancer or completely independent of them in order to treat other illnesses involving Ca^{2+} dynamics.

REFERENCES

Bergmeier, W., Weidinger, C., Zee, I. & Feske, S. Emerging roles of store-operated Ca^{2+} entry through STIM and ORAI proteins in immunity, hemostasis and cancer. *Channels* 7, 379–391 (2013).

Bryant, J. et al. EGF activates intracellular and intercellular calcium signaling by distinct pathways in tumor cells. *Cancer biology & therapy* 3, 1243–1249 (2004).

Chang, Y. et al. Experimental and mathematical models of intracellular calcium dynamics for evaluating combined chemotherapy effects. Submitted 2018.

Cui, C., Merritt, R., Fu, L. & Pan, Z. Targeting calcium signaling in cancer therapy. *Acta pharmaceutica sinica B* 7, 3–17(2017).

Cui, C.et al. Targeting ORAI1-mediated store-operated calcium entry by RP4010 for anti-tumor activity in esophagus squamous cell carcinoma. *Cancer letters* (2018).

Dupont, G., Falcke, M., Kirk, V. & Sneyd, J. *Models of Calcium Signaling* (Kindle Edition, 2016).

Furuichi, T.et al. Primary structure and functional expression of the inositol 1, 4, 5-trisphosphate-binding protein P400. *Nature* 342, 32 (1989).

Keener, J. and Sneyd, J. *Mathematical Physiology*. Springer Science+Business Media, LLC, New York, NY, 2009.

Meisenhelder, J., Suh, P. G., Rhee, S. G. & Hunter, T. Phospholipase c-γ is a substrate for the PDGF and EGF receptor protein-tyrosine kinases in vivo and in vitro. *Cell* 57, 1109–1122 (1989).

Otto S. and Day T. *A Biologist's Guide to Mathematical Modeling in Ecology and Evolution*. Princeton University Press, Oxfordshire, UK, 2007. Hardback.

Rhee, S. G.et al. Regulation of inositol phospholipid-specific phospholipase c isozymes. *Regulation* 267, 12393–12396(1992).

Schlessinger, J. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. *Cell* 110, 669–672 (2002).

Sneyd, J.et al. On the dynamical structure of calcium oscillations. *Proc. Natl. Acad. Sci.*114, 1456–1461 (2017). DOI10.1073/pnas.1614613114.

Spicer, J.et al. A phase I study of afatinib combined with paclitaxel and bevacizumab in patients with advanced solid tumors. *Cancer chemotherapy pharmacology* 79, 17–27 (2017).

Zhu, H.et al. Elevated ORAI1 expression mediates tumor-promoting intracellular Ca^{2+} oscillations in human esophageal squamous cell carcinoma. *Oncotarget* 5, 3455 (2014).

BIOGRAPHICAL INFORMATION

Marah Funk began her undergraduate degree at Texas Tech University, but transferred to the University of Texas at Arlington to complete a Bachelor of Science in Mathematics and a Bachelor of Science in Biology. She has been working with Dr. Hristo Kojouharov and Dr. Benito Chen on a research project on which this thesis is based since September 2017. This project is a joint effort between individuals in the Math and Nursing Departments to explore novel chemotherapeutic treatments for cancer that target Ca^{2+} dynamics However, her main area of interest is Neuroscience and she plans to use her knowledge of this field to research and design prosthetics. After she graduates in December 2019, she will pursue a graduate degree in Bioengineering in order to facilitate this desire.