Pharmacokinetic Modelling of PBE309 Drug Delivery

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ABSTRACT: Mutation of the p53 gene, among other genetic mutations, is a common target for anti-cancer drugs and therapeutics. Mathematical pharmacokinetic models have become increasingly important in determining dosage and efficacy of these drugs. The motivation behind this study was to consider two different PBE309 drug delivery models, A and B. Model A was a simple closed loop model between the arteries and veins while Model B added the effect of drug diffusion into the capillaries/tissue. Mathematical models were generated for A and B to predict injection protocols necessary to maintain an equilibrium arterial drug concentration of 0.01 to 0.015 M (Model A) and a capillary drug concentration of 0.018 to 0.022 M for 1 hour (Model B). To reach these targets, in model A, a single 1 mL injection of 0.55 M was used, while in Model B an initial injection of 0.65 M was used followed by subsequent injections of 0.1667 M whenever capillary concentration reached 0.018 M. There were multiple sources of error affecting the volumes measured in this study and the experimentally determined mass transfer and flow rate constants (k and Q)*;* however, by applying superposition of error, 95% confidence intervals for the theoretical models A and B were created that were able to respectively predict 99.29% and 96.45% of the experimental data points.

INTRODUCTION:

Mutated p53 accounts for 50% of all human cancers. The function of the wildtype p53 protein is to eliminate abnormal cell proliferation. While p53 is minimally present in a normal cell, cellular stress causes the activation and upregulation of the p53 pathway. This leads to either cell cycle arrest until the stress is corrected or eventual apoptosis. Absent or mutated p53 causes uncontrollable cell division, leading to tumor propagation (1). Thus, p53 is a large drug target.

Pharmacokinetics can be used to model the mass transfer of these drugs through the systems of the body. By modeling the tissues and vasculature of the body, generalized equations of drug propagation can be constructed (2). Hundreds of pharmacokinetic models exist in the literature, of which recent studies stretch from the compartmental modelling of arterial and venous recirculation of ketamine to the infant exposure to the HIV antiviral efavirenz via breastfeeding (3,4). These numerous models involve the representation of various delivery methods such as injection or oral ingestion into various targets such as the bloodstream or GI tract (3,4). More specifically, pharmacokinetic studies have been done on several other drugs targeting p53 including in-vivo PK of p53-stabilizing CP-31398 after IV or oral dosage and in-silico dosage PK of the p53 pathway inhibitor Nutlin (5,6).

Pharmacokinetic models can be constructed with varying levels of complexity. Two models are discussed in this paper to represent the transfer of the proposed p53 targeting drug, PBE309, into the body via injection. One is a simple closed loop model (Model A), which involves injection into the arterial system, transfer to the venous system and recirculation into the arterial system. This model was used to determine the injection concentration that would lead to an arterial equilibrium drug concentration of between 0.01 and 0.015 M, which was proven to cause apoptosis in-vitro. The other model (Model B) is a more complex representation which additionally involves modeling the metabolism of the drug in the capillaries and tissue. Mathematical modeling was used to create a drug injection protocol necessary to keep the patient's drug concentration in the capillaries between 0.018 and 0.022 M for one hour in Model B. We hypothesized that the implementation of these protocols could be modelled by equations involving the factors k, Q, vasculature volumes and drug concentration.

MATERIALS AND METHODS:

Calibration: For this experiment,calibration curves were constructed each session to relate concentration of PBE309 to voltage. First, PBE309 solutions of 0, 0.02, 0.04, 0.06, and 0.08 M were made. Each solution (60 mL each) was then measured with a conductivity meter (Model 1056, Amber Science, Eugene, OR) that was connected to a Biopac Data acquisition system (MP35, Biopac, Goleta, CA). ANOVA and ANCOVA tests were used to respectively ensure that the means of the three replicates were not different from each other ($P>0.05$) and that the resulting slopes of the regression lines from each replicate did not differ ($P>0.05$). The average voltage at each concentration was then calculated, and a linear relationship between concentration and voltage was fit using MATLAB (R2016B). T-tests of zero slope and intercept were run, and the data was refit if, for either slope or intercept, $P>0.05$. Representative calibration results are provided in the results section.

Experimental Setup: Figure 1 shows the setup for both Models A and B. Arterial and Venous solutions were kept spinning through the duration of each experiment to ensure homogenous distribution of solutions. The pumps used in Models A and B were Variable Flow Peristaltic Pumps (Fisher Scientific, Hampton, NH) set to fast and approximately 1.5 to 1.6 rounds per second. Dialysis tubing with a membrane area of 73 cm^2 and a molecular weight cut-off of 10 kDa was used in Model B to simulate the capillaries. Also unique to Model B, all tubing was filled with water prior to each drug injection experiment. In order to measure the volume of the model capillaries, 3 mL syringes were used to fill the dialysis tubing with water until the point of overflow. The volume of water in the connective tubes was subtracted, so the volume of water injected by the syringes was taken to be the

volume of the fluid held in the model capillaries. This volume was found to be 2.51 mL. The volume of the arteries was given to be 24 mL and the volume of the veins was given to be 26 mL.

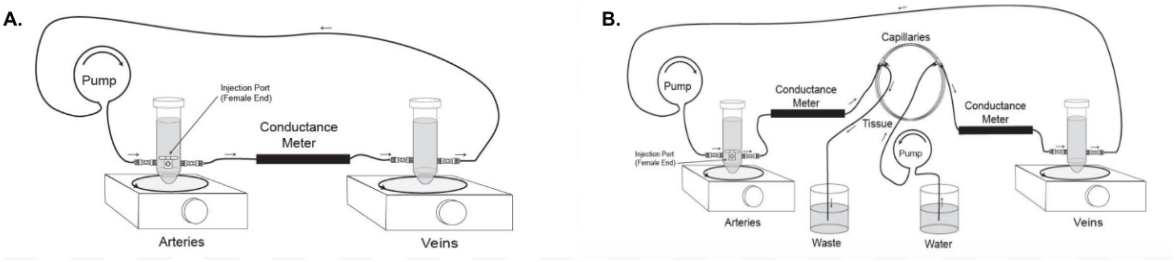

Figure 1: Experimental Setup. A. Shown is the Model A experimental setup. Arterial concentration is read (1). **B.** Shown is the Model B experimental setup. Arterial and Capillary concentrations are read (1).

PBE309 Injection Trials: In both models, 1 mL of the desired drug concentration was injected into the arterial compartment and allowed to stir for 30 seconds. Fluid flow valves in the arteries and veins were then opened and pumps were turned to forward. In Model A the following mathematical equations were derived from the differential equations for material flow:

$C_A = \alpha - \beta(\frac{V_v}{V_A} e^{\frac{-Q(V_A+V_V)t}{V_A V_V}})$ (eq. 1) and $C_V = \alpha + \beta(e^{\frac{-Q(V_A+V_V)t}{V_A V_V}})$ (eq. 2) where $\alpha = \frac{C_{A,0}}{1+\frac{V_V}{V_A}}$ and $\beta = \frac{-C_{A,0}}{1+\frac{V_A}{V_V}}$

Here, C_A is the concentration in the arterial system (M), C_v is the concentration in the venular system (M), V_a is the volume of the arterial system (mL), V_v is the volume of the venular system (mL), Q is the flow rate (mL/sec) and $C_{A,0}$ is the initial arterial concentration. $C_{A,0}$ can be defined as $\frac{C_I}{V_A+V_I}$, where C_I and V_I are the concentration and volume of injection, respectively. Trials were were run until C_a reached equilibrium (± 5% its theoretical value) to account for extra volume left in the tubing and uncertainty in the calibration curve (quantified by 95% prediction intervals). In Model B the following differential equations were used:

$\frac{dC_c}{dt} = \frac{-kA(C_C - C_T) + Q(C_A - C_C)}{V_C}$ (eq. 3), $\frac{dC_A}{dt} = \frac{Q(C_V - C_A)}{V_A}$ (eq. 4) and $\frac{dC_v}{dt} = \frac{Q(C_C - C_v)}{V_v}$ (eq. 5)

The variables represent the same as above, with the addition of k (cm/sec) as the mass transfer coefficient of the capillaries, C_c as concentration in the capillary system (M) and V_c as the volume of the capillary system (mL). Trials were run for 10 min. to procure adequate data to find k and Q.

Data Analysis: Data from the Biopac was first converted from voltage to concentration using the calibration curve found for each session. The Q value for Model A was found by running 5 trials using a 1 mL injection of 1M PBE309. Q was estimated for each trial by fitting an exponential function (of the form of eq. 1) to the experimental data for C_A. A Shapiro Wilks test of normality was then run on the 5 values for Q (P>.05) and the average was taken for the model and a 95% confidence interval was constructed. 5 trials were run using a 1 mL injection of 1M PBE309 to determine Model B values of k and Q. Optimal Q and k values for each trial of Model B were found by looping through possible Q and k values and minimizing the RMSE between the experimental concentrations and theoretical C_A, C_c, and C_V determined by MATLAB simulation of eqs 3, 4, 5. A Shapiro Wilk's test of normality was conducted on the Q and k values of Model B, finding P>0.05 for both. The average k and Q were then used in Model B and 95% confidence intervals were constructed.

Model Construction: Model A was constructed by plugging the average value for Q into eqs 1 and 2. Model B was constructed by plugging the average values of Q and k into eqs 3,4,5. Eqs 3,4,5 were then simulated in MATLAB. A MATLAB model was created to develop a multi-injection protocol of PBE309 to keep C_c between 0.018 and 0.022 M over the period of an hour. 95% confidence bounds were constructed using superposition of error theorem for both models A and B. In model A, $C_{A,0}$, V_A, V_v, and Q were allowed to vary in accordance to a normal distribution of their mean and standard

deviation. MATLAB code was written to compute $C_A(t)$ 10,000 times for values of time ranging from 0 to 3 mins. The resulting 2.5th and 97.5th percentile for $C_A(t)$ was then taken at every point in time to construct 95% confidence bands. The same procedure was used to construct 95% confidence bands for Model B; however, k and V_c were additionally allowed to vary in accordance to a normal distribution of their mean and standard deviation with a simulation time of 3600 secs. However, in this case $C_c(t)$ rather than $C_A(t)$ was computed. In both models, the contribution of each parameter to total error was estimated using multiple regression on the total variance of $C_A(t)$ or $C_c(t)$. A 1 mL injection of 0.55 M PBE was picked for Model A to reach a target equilibrium concentration of 0.01 to 0.015 M in the arteries. In Model B the following 1 mL multiple injection protocol was followed. Ordered pairs give the concentration injected followed by the time of injection (C_I (M), t (s)): (0.65, 0), (0.1667, 30), (0.1667,64), (0.1667,131), (0.1667, 915), (.1667,2000), (0.1667, 3051).

RESULTS:

Table 1: Respective Q Values Determined in Model A Trials

Model	Trial Number	Q (mL/s)
A	1	0.8007
A	2	0.8060
A	3	0.3141
A	4	0.7854
A	5	0.3548
A	6	0.3910
A	7	0.7854

Table 2: Respective Q and k Values Determined in Model B Trials

Model	Trial Number	Q (mL/s)	k (cm/s)
B	1	0.32	1.13×10^{-4}
B	2	0.69	3.44×10^{-4}
B	3	0.49	$.55 \times 10^{-4}$
B	4	0.28	1.05×10^{-4}
B	5	0.41	0.79×10^{-4}

A calibration curve relating concentration to voltage readout was calculated for each trial session. The intercept of each was forced through 0 after a t-test for regression intercept was found to have P>0.05. A representative calibration curve equation is Volts=10029.1(mv/M)*Conc. with R^2 of 0.9968. 95% prediction intervals on the calibration curve were also calculated each session.Table 1 shows the calculated Q for the 7 Model A trials. A Shapiro-Wilk test of normality was run on the Q values of the trials, resulting in no evidence to reject the fact that Q follows a normal distribution (P>.05). A 95% confidence interval for Q was constructed from the 7 trials, giving Q= 0.6053 ± 0.2309 mL/s. Figure 2.A shows the results of using the model to equilibrate C_A between 0.01 and 0.015 M using a C_I of 0.55 M. When these parameters are plugged into eqs 1 and 2 the model becomes: $C_A = \alpha - \beta(\frac{26}{24}\, e^{\frac{-Q(24+26)t}{24*26}})$ and $C_V = \alpha + \beta(e^{\frac{-Q(24+26)t}{24*26}})$ where $\alpha = \frac{C_{A,0}}{1+\frac{26}{24}}$ and $\beta = \frac{-C_{A,0}}{1+\frac{24}{26}}$ with $C_{A,0} = \frac{.55}{24+1}$, where C_A is in M.

Trial 1 and 2 reached an equilibrium of 0.0114 M and 0.0106 M, respectively, while the model predicted an equilibrium of 0.0106 M (2.A). While equilibrium concentrations of Trial 2 and the model were the same, there was some variability between the 2 trials run (2. A). However, the model was able to capture this variability between its 95% confidence limits as 99.29% of the points across the two trials are within the 95% confidence limit of the model (2.A,B). Furthermore, trials 1 and 2 had low root mean square errors of 0.1055 M and 0.3125 M, indicating a strong fit.

Table 2 shows the calculated Q and k for the 5 Model B trials. A Shapiro-Wilk test of normality was run on the Q and k values, resulting in no evidence to reject the fact that either Q or k follows a normal distribution (P>.05). With this knowledge, 95% confidence intervals for Q and k were constructed from the 5 trials, yielding Q=0.4380 ± 0.2317 mL/s and k=1.39 x 10^{-4} ± 1.0238 x 10^{-4} cm/s. When these average values for Q and k are plugged into equations 3, 4 and 5 the model becomes: $\frac{dC_c}{dt} = \frac{-(1.39\, x\, 10^{-4})(.73)(C_C - C_T) + (.438)(C_A - C_C)}{2.51}$, $\frac{dC_A}{dt} = \frac{(.438)(C_V - C_A)}{24}$ and $\frac{dC_v}{dt} = \frac{(.438)(C_C - C_v)}{26}$.

Figure 2C,D shows the representative results of running two trials, attempting to follow the theoretical multiple drug injection protocol of Model B mentioned in the methods. The experimental trials differed from the model in the timing of injections. In Trial 1, the following injection sequence was

used (C_I (M), t (s)): (0.65,0), (0.1667,80), (0.1667,143), (0.1667,661), (0.1667,1992), and (0.1667,3080). In Trial 2: (0.65, 0), (0.1667, 41), (0.1667, 76), (0.1667,535), (0.1667,1900), and (0.1667,2989) were used. This timing error is explained by the model in that at all points of injection in Trials 1 and 2, 0.018M is included in the model 95% CI. Since the injection plan involved injecting 0.1667 M drug whenever the concentration in the capillaries reached approximately 0.018M, the 95% CI of the model can explain the difference in injection timing between the trials and the theoretical model. (2.C,D). Data from the two trials varied; however, 96.45% of the points across the two trials are included in the 95% CI of the model. Furthermore, trials 1 and 2 had low respective root mean square errors of 0.0311 M and 0.0492 M, which indicates a strong model fit. As predicted by Model B, a multi-injection protocol involving injection sequences given above was able to maintain a capillary concentration between 0.018 and 0.022 M for 1 hour (2.C).

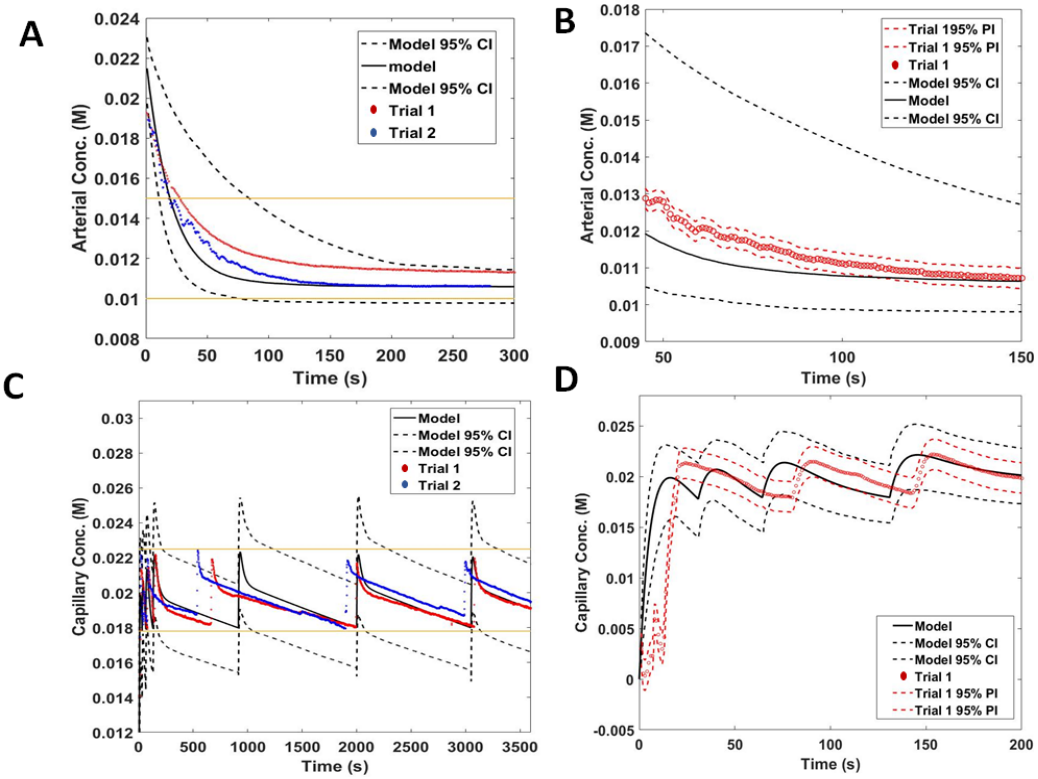

Figure 2: Application of Models. A. Shown are duplicates of a 1 mL 0.55 M PBE309 injection in Model A. along with the model with 95% CI. The horizontal lines represent the target conc. range (0.01 to 0.015 M). **B.** A zoomed in image of the Model A Trial 1 along with its 95% prediction interval is shown. The model is able to capture the variance of the experimental data . **C.** Shown are duplicates of the multi-injection trial (outlined in methods) with horizontal lines representing the target range (0.018- 0.022 M). 95% Confidence bounds of the model are given to evaluate its success in predicting trial data. **D.** Shown is a close up of the first 200s of Model B Trial 1. The model captures a majority of the prediction interval for Trial 1.

DISCUSSION:

The superposition of error theorem was used to quantitatively determine how sources of error affected our results. . Error was introduced in the measurement of C_I, V_C, V_A, V_V, *k,* and Q. Error in V_A, V_C, V_V, C_I stemmed from imprecision in pipetting ±0.25 mL (SD) listed by manufacturer. Error in k and Q for both models was captured by their respective 95% confidence intervals. By superposition of error (multiple regression), in Model A, error in Q accounted for 96.46% of the total error, error in V_V accounted for 0.4917% of the total error, error in V_A accounted for 0.6949% of the total error, and error in C_I accounted for 2.3504% of the total error. Similarly, in Model B, error in Q accounted for 40.3% of the total error, error in k accounted for 10.6%, and error in V_C accounted for 48.8% In order to reduce error, more accurate volume measuring equipment should be used and

more trials should be run in order to garner more accurate values for k and Q. More accurate pumps should also be used to minimize error in Q and allow for precise rounds per second. To discuss broader implications of the work done in this study, the work presented in this paper reaffirms the notion that mathematical drug modeling techniques can be used to significantly optimize existing drug products and even facilitate the development of new pharmaceuticals (2).

There are technical constraints inherent in the models used in this study. For instance, while Model B is slightly more accurate than Model A since it models capillary uptake, both models unrealistically approximate no loss of drug as it moves through the vascular system. Food, for example, can seriously affect drug absorption, with the time between food and medication intake determining the extent of the hindrance (7). In order to account for food-drug interactions or other systemic drug losses, a drug decay term could be introduced into the models.

This experiment is just a preliminary analysis of how drug delivery models can be used to effectively treat patients. Further work can be done with more accurate representations of the body. For example, peristaltic pumps were used to represent the heart, however these pump at a fixed rate. A patient's heart rate will vary and thus a model that has a variable flow rate as function of time, Q(t), would be more accurate. In addition, composition was vastly oversimplified by the use of water rather than an ionic solute to model blood. Lastly, the chosen model tubing could be selectively permeable to model the variable permeability of the body's vasculature; permeability is crucial to maintain homeostasis in the body and even increases during conditions like wound healing (8).

CONCLUSION:

The experiment demonstrated that a simplified model could be developed to equilibrate arterial concentration between 0.01 and 0.015 M. This was done by determining the intrinsic flow rate, Q, of the model. 95% confidence bounds for the theoretical model were able to capture 99.26% of the experimental data points with 1mL injection parameter 0.55 M. A more complex model was also developed to determine a multi injection protocol to keep capillary concentration between 0.018 and 0.022 M for one hour by determining Q and the mass transfer coefficient, k. 95% confidence bounds for the theoretical model were able to capture 96.45% of the experimental data points with a 1mL injection protocol of a 0.65 M injection followed by multiple 0.1667 M injections. It is important to note that further work must be done to expand on this study, such using an ionic solute to model blood and using a variable flow rate, Q(time).

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