PHARMACOKINETIC/PHARMACODYNAMIC MODELING CAN GUIDE DRUG CANDIDATE OPTIMIZATION

by

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Abstract

Optimization of drug candidates involves modification of chemical structures in order to improve a compound's desirable properties. We aim to use pharmacokinetic/pharmacodynamics (PK/PD) modelling to prioritize alterations in drug properties such as potency, clearance, and free drug concentrations in order to reduce the predicted efficacious dose. We have used simple differential equations and simulation software to build simple direct effect models to explore how changes in these factors affects a) maintenance of drug levels above 50% inhibition of a biological target or b) maintenance a certain efficacious area under the curve (AUC). We have demonstrated that when the pharmacodynamic target was to maintain >50% inhibition of a biological target, decreasing clearance led to greater decreases in predicted efficacious dose compared to improvements in potency. Changes in free drug levels affects both potency and clearance. The overall effect on dose was dependent on whether the drug was a high, moderate, or low clearance drug. When the pharmacodynamics target was efficacious AUC, improvements in predicted efficacious dose changed linearly with improvements in clearance or potency. These results indicate that the choice of property to optimize depends on the pharmacodynamic endpoint. When target is an efficacious AUC, one can choose to improve either clearance or potency with similar effects in steady-state dose improvements. However, when the target is maintenance of a specific level of target inhibition, better gains in dose reduction can be made by improvement of clearance rather than potency. Our study also demonstrates that application of PK/PD modelling can guide compound optimization in a more rational manner.

Lay Summary

Creation and development of new medicines is a complex process. Drugs are discovered through many iterations of chemical modifications in order to identify a safe and effective drug. Chemical modifications are made to increase drug potency, so that drugs will work at lower doses. Further, modifications are made to ensure the body will not eliminate the drug before it has a chance to produce a beneficial effect. However, we do not always know which modifications will be the most important to focus on. My research goal was to build a computer mathematical model that could help simulate and predict how changing characteristics of drugs will improve its dose. This model can help guide decision making in terms of which properties should be prioritized and may help improve efficiency in the drug discovery and development process.

Preface

This thesis is original and unpublished work based on experiments I performed under the supervision of Dr. Harvey Wong.

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List of Abbreviations

ADME	Absorption, distribution, metabolism, elimination
Apparent V_d	Apparent Volume of Distribution
AUC	Area under the curve
CL	Clearance
CL_H	Hepatic Clearance
CL_{int}	Intrinsic Clearance
E	Extraction Ratio
EC_{50}	Concentration that produces 50% of maximum effect
E_{max}	Maximum effect
F	Bioavailability
f_u	Free/unbound fraction of drug
IC_{50}	Concentration that produces 50% of maximum inhibition
I_{max}	Maximum inhibition
PK	Pharmacokinetics
PD	Pharmacodynamics
Q	Hepatic Blood Flow rate
V	Volume
V_d	Volume of Distribution

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I also would like to give special thanks to my parents and sister, who have supported me throughout all my years at UBC, financially and emotionally. I owe it to them for always pushing to push for higher aspirations and goals.

Chapter 1: General Introduction

Successful drug discovery is often a long and arduous process. Modern drug discovery involves screening chemical libraries to identify compounds with promising properties, followed by making chemical modifications to these chemical starting points, or 'hits' to make them more 'drug like' [16, 37]. For a compound to be a good drug, it must be able to reach and interact with its target *in vivo* for a reasonable amount of time, and at a reasonable concentration in the body, reached with an acceptable dose [26]. Thus, it is of paramount importance to correctly identify properties that can be improved and optimize them in order to create a successful drug.

1.1 Drug-Like Properties

While initial screening involves looking for hits that will interact with a target of interest to produce a pharmacological effect, further chemical modifications are often geared toward giving the drug candidate acceptable pharmacokinetic and safety profiles, so it is appropriate for use in humans [35]. Drug-like properties are intrinsic properties of the molecules, and can be chemically altered to become more favourable [26]. Some properties of interest include the following:

Structural properties: hydrogen bonding, molecular weight.

Physiochemical properties: solubility, chemical stability, permeability, lipophilicity

Biochemical properties: protein and tissue binding, metabolism, drug potency

Pharmacokinetic (PK) and toxicity: clearance, half-life, drug-drug interaction potential

The optimization process often involves balancing these properties to produce a clinical candidate that will be safe and efficacious [16]. Most of these properties can be altered by modifying the structure of a chemical hit, and oftentimes large batches of modifications are synthesized from the lead compound and comprehensively tested for each property in an effort to find compounds that have the best combination of all the properties [37].

1.1.1 Potency and Clearance

Though several properties have been introduced above, the two properties of the greatest interest for this thesis are potency and clearance. The formal definitions for both terms are as follows:

Potency: the concentration of drug necessary to produce an effect of a certain intensity. This is related to its minimal therapeutic concentration, and often expressed in terms of the concentration that gives the half-maximal response (EC_{50}), or in the case of an inhibitor, the concentration that gives half-maximal inhibition (IC_{50}).

Clearance: the volume of blood that can be cleared of drug in a given time. This is often related to the drug's elimination (metabolism, and excretion). The term is often abbreviated as CL.

Going back to the earlier definition of what makes a good drug, it is clear that both of these properties have an important role. A drug must be able to interact with its biological targets at an acceptable concentration, which relates to its potency. A drug must also be able to stay in the body long enough to produce an effect, which is related to clearance. Of the two, chemical modifications that can enhance potency has been traditionally more focused on, with chemical

libraries often opting to include smaller molecules of more potent hits, and modifications to improve affinity of a molecule for its biological target [52, 12], and the multitude of techniques geared toward assessing potency as opposed to clearance [29]. On an intuitive level, this makes sense, as a highly potent drug would naturally require lower doses to achieve a satisfactory effect. However, improved clearance would also logically lower dose, since a drug that is cleared more slowly would have more time to interact with the target receptor, thus also requiring a smaller amount or at the very least less frequent dosing to achieve a desired effect. Thus far, there has not been a direct evaluation of which property has a more dramatic effect on the predicted efficacious dose.

1.2 Issues with Traditional Methods of Drug Discovery

As described above, methods of drug discovery and optimization makes use of screening chemical libraries for hits, followed by creating further chemical modifications to try and optimize compound properties. Taking all that in as a whole, we can quickly see that this is a time and resource consuming process. Historically, optimization has been a sequential process, with only one issue addressed at one time. In addition, affinity for the biological target was most commonly seen as the most important factor, and was often optimized at the expensive of other facets such as solubility, permeability, or metabolic stability [37]. This was of course inefficient, and often costly [30, 37]. In later years, high-throughput screening became more prevalent and became the major way of identifying leads for pharmaceutical companies [30]. Use of high throughput screening created a new issue of the need to be able to test a vast number of compounds. Libraries in the industry are often comprised of compounds representing millions of chemical variations [37]. There are theoretically infinite number of modifications that could be

made to hits identified from high throughput screens. Therefore, screening entire compound collections is neither feasible nor meaningful [37]. Despite this, in the past it was thought that the screening of thousands to millions of compounds increases the probability of a hit, and thus large and unwieldy libraries were often generated with the hope to cover all possibilities, without consideration of the targets tested [24, 30, 37]. Newer research has found that this premise is often not the case, as the hit rate remained unsatisfactorily low despite the use of large libraries of compounds for screening [30, 25]. As well, there is the ever-present issue of false positives or false negatives that can mislead synthesis efforts [29]. In other words, brute testing of giant libraries, no matter how fast the technology, is still inefficient and resource intensive.

Several attempts to combat this issue have emerged over the years. One change is a shift from activity-based screens, which often rely on chromophores or radioactive labels to measure the activity of a target, to affinity-based screens, which examines the direct binding of a molecule to a drug target without using labels that may create artifacts, as well as allowing for parallel processing of multiple variations of the target [3, 12]. Other examples include creation of more focused drug-like subsets based on structure-activity relationships [29, 37, 16], and using computer-based filtering tools to guide chemical modifications [47]. However, these methods just generate more information on compounds without providing any means to prioritize the importance of each property.

One of the biggest reasons for failure of leads becoming successful drugs is arguably poor pharmacokinetics. As mentioned before, past thinking put focus mostly on affinity and binding to the molecular target. However, Teague et al (2000) showed that many low-affinity leads have

led to successful drugs, often through optimization not just potency but also the pharmacokinetic profile [52]. These include well known examples such as progesterone to mifepristone and histamine to cimetidine [52]. Furthermore, poor pharmacokinetics has been linked to discovery inefficiencies and failures during clinical trials [16, 33]. Failure in the late stage of discovery and development has led much more significant losses in terms of time and resources [16, 37]. Since 1991, recognition of poor pharmacokinetics being a major cause of attrition has led to pharmacokinetic assessment being implemented much earlier in the discovery and development pipeline, and has led to a dramatic decrease in this being a cause of attrition [16, 33].

Poor pharmacokinetic profiles often result in much higher doses. Acetaminophen is an older drug with a poor PK profile, having a half-life of around 2 hours [20]. Thus, for effective treatment, typical dosing is around 325-500mg/pill, 2 doses every 4-6 hours [1]. This drug was developed before the importance of PK optimization was understood. Since then, most drug development processes incorporated optimization for PK parameters, and modern drugs typically have better profiles and lower doses [33]. For example, if we compare acetaminophen with rosuvastatin, a newer compound with a better PK profile and a half-life of 19 hours, the dosing regimen is markedly simpler, being 5-40mg/day, usually given as one dose per day [46, 14]. From these examples, we can see that drugs with better PK profiles lead to lower doses, which are generally easier to administer as they come in smaller or fewer pills. Furthermore, simpler and less frequent dosing regimens are typically associated with better adherence and compliance [50, 11]. Better compliance generally leads to better health outcomes, which would have both social and economic benefits.

An obvious conclusion from these examples, is that we need to optimize for both compound potency and PK in order to have a drug with desirable properties. Unfortunately, it is not always possible to optimize certain properties without compromising the others [52, 37]. Oftentimes, there is a focus on improving only drug potency, which can result in poor pharmacokinetic attributes such as absorption, distribution, metabolism, and/or elimination (ADME), preventing the candidate from progressing through clinical trials [37]. Furthermore, it is not always clear whether these properties would affect the final dose of drug candidates in a linear fashion, and there is a lack of direct comparisons of the contributions of each property to determining final dose. Since optimization of chemical leads is complex process and can lead to large many iterations of chemical modification before a drug candidate can be identified, there is an argument to be made for a more rational approach of prioritizing optimization of certain compound properties over others.

1.3 Pharmacokinetic-Pharmacodynamic (PK/PD) Modeling

With the advent of advanced computing power, pharmacokinetic-pharmacodynamic (PK/PD) modelling and simulation has become an important tool in the drug discovery process. It involves using mathematical equations to represent a biological system and is able to account for its dynamic nature [57]. These mathematical models allow for the quantitative assessment of relationships between drug exposure to effect and can account for the interplay between drug properties and biological systems in order to gain better insight into these relationships [57]. Further, these models offer the ability to predict drug concentration-time, drug effect-time, and drug concentration-effect relationships based on dose, drug specific parameters, and biological

parameters [15]. As such, PK/PD models can be used to simulate and predict the influence of changes on drug dose when one or more drug properties are altered.

These advantages have made PK/PD modeling an indispensable aid in preclinical drug development in terms of understanding efficacy, downstream decision making, and translation of pre-clinical data to the clinic [57]. Thus, it is no surprise that a majority of pharmaceutical companies are now incorporating such PK/PD analysis in their drug discovery and development process, with many planning to expend more resources in this direction [48].

1.3.1 Direct-Effect Model

Direct effect models are a type of PK/PD model that can be used to relate drug concentration in the body to its biological effect. In this type of model, there is an assumption that rapid equilibrium is achieved between the drug concentration in the blood and relevant target site in the body. In this situation, a change in concentration in the blood results in a simultaneous and direct pharmacodynamic response [57].

A commonly used direct effect model is the E_{max} model, described by the Hill Equation:

$$E = \frac{E_{max} \times C^n}{EC_{50}^n + C^n}$$

where E_{max} is the maximum effect, EC_{50} is the concentration producing the half-maximal effect, C is the concentration of drug, and n is the Hill coefficient [32]. Another form of the equation

exists when the drug in question is an inhibitor and wishes to represent effect as inhibition instead. The equation is as follows:

$$I = 100 - \frac{I_{max} \times C^n}{IC_{50}^n + C^n}$$

In this case, I represent inhibitory effect, I_{max} is the maximum inhibitory effect, and IC_{50} is the concentration which produces 50% inhibitory effect. When there is no inhibition, the normal effect is at 100%, which decreases as concentration of inhibitor increases.

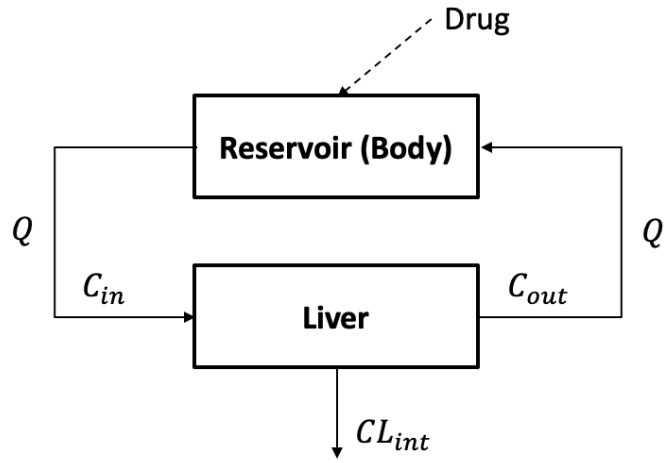
The E_{max} or I_{max} model provides a simple yet effective framework for assessing the impact of not only changes in concentration on effect, but also changes in potency (as measured by EC_{50} or IC_{50}) on effect. Since potency is one of the properties of interest to us, we will be using this framework to explore how alterations in potency can affect predicted dose.

1.3.2 Hepatic Clearance Model

It is well established that the liver is the major source of metabolism and clearance for drugs. This has led to the proposal of various hepatic clearance models that provide a mathematical framework to understand how the liver processes drug. One example is the 'parallel tube' model, which describes the liver as a series of parallel tubes that represent sinusoids through which drug travels. Enzymes are distributed evenly in each cross section of the sinusoid, and drug levels decrease as drug moves along the tube [39, 56]. Another is the 'Dispersion' model, in which drug movement in the liver can be described as being analogous to non-ideal flow in a packed-bed chemical reactor [57]. Perhaps the most well-known hepatic clearance model, however, is

the well-stirred model, which we will be using as a framework to describe hepatic clearance in our studies [22, 45, 56].

The well-stirred model is relatively simple and can be represented by Figure 1. This model assumes that the liver is a single tank in which the inputted drug is well-mixed. It also assumes that only free or unbound drug (f_u) is available for elimination. Drug is shuttled between the liver and rest of the body reservoir by the hepatic blood flow, termed Q. Each pass through the liver removes a certain amount of drug. The ratio of the amount of drug removed and the amount of drug inputted is termed the extraction ratio, represented as *E*. Extraction ratio is determined by several factors, including hepatic blood flow, the intrinsic clearance (CL_{int}), and the level of free drug, and can also be expressed in those terms (Figure 1).



$$CL_{hepatic} = QE$$

$$E = \frac{C_{in} - C_{out}}{C_{in}}$$

$$E = \frac{f_u * CL_{int}}{f_u * CL_{int} + Q}$$

Figure 1: Schematic and equations representing the well-stirred model, a simple physiological model of hepatic clearance

While hepatic blood flow (Q) is a physiological parameter and is not readily optimized, it is possible to chemically modify hits to improve intrinsic clearance, and/or improve free drug

concentrations. An example of improving intrinsic clearance in literature is the described chemical modification of the Bruton's tyrosine kinase inhibitor GDC-0834, which was naturally highly metabolized in human studies, to be more metabolically stable (thus having a lower intrinsic clearance) [61]. In a second example, Fauber et al (2014) reported reducing lipophilicity of tertiary sulfonamide retinoic acid receptor-related orphan receptor gamma (RORc) inverse agonists in order to improve free drug concentrations and potency [19]. It is of interest for us to explore how improvements in intrinsic clearance and free drug concentrations may impact predicted efficacious dose, using the well-stirred model as a framework.

1.4 Objective

We have identified several problems with traditional drug discovery methods in terms of inefficient compound optimization, and potential attrition due to poor drug properties. We proposed that PK/PD models may be used to provide a more rational approach to compound selection and prioritization of drug properties to optimize in the drug discovery phase, specifically in the lead optimization/candidate selection phase of the drug discovery and development pipline [37]. The overarching goal of this thesis is to build PK/PD models that can predict changes in predicted effective dose when certain drug properties are changed for a given pharmacodynamic target. We hypothesize that we can use PK/PD models to prioritize drug properties and guide decisions in the drug optimization process.

To test our hypothesis, we built models to explore two pharmacodynamic endpoints. The first pharmacodynamics endpoint was to maintain >50% inhibition of a biological target for 24 hours

following daily dosing. The second pharmacodynamic endpoint was to target a specific efficacious area under the curve (AUC).

We constructed two different PK/PD models. Our first PK/PD model aimed to simply examine the effect of improvements in drug potency and clearance on the predicted efficacious dose, Our second more complex PK/PD model aimed to study clearance more in depth, and specifically examined how improvements in intrinsic CL and unbound drug levels effects the predicted efficacious dose.

Chapter 2: Methods

Construction of pharmacokinetic/pharmacodynamic models and subsequent simulations were performed using SAAM II (The Episilon Group, Charlottesville, VA).

Area under the concentration time curve (AUC) and AUC of the effect curve were calculated using the trapezoid rule [21].

2.1 Study 1: Influence of Changes in Drug Candidate Potency and Clearance on Predicted Dose

We aimed to study the effect of altering potency or clearance on the predicted dose required to maintain >50% inhibition at steady state for 24 hours, with once daily dosing. An improvement in potency is defined as a decrease in IC_{50}, whereas improvement in clearance is defined as a decrease in total clearance (CL).

2.1.1 Drug Starting Properties

Our simulation starting point is a hypothetical drug compound with properties listed in Table 1. Our compound is an inhibitor with non-ideal properties, being high clearance and non-ideal potency. The predicted dose to achieve our PD target for this drug is 2910 mg.

Table 1: Starting properties of hypothetical test compound for study 1

Property	Value
Apparent V_d	5 L/kg
Starting Clearance	16 mL/min/kg
Starting Potency	IC_{50} = 250 nM
k_a	1 hr^{-1}

Values were chosen to create a starting compound with non-ideal properties (high clearance, low potency). Apparent V_d and k_a were arbitrarily selected.

For this hypothetical compound, we assume that the compound is metabolized by the liver such that hepatic clearance approximates total clearance, and as such the maximal clearance is capped at 20 mL/min/kg, which is the liver blood flow in humans.

2.1.2 Model 1

The first model that we used is represented by Figure 2 and described by equations 1 to 3. This is a one-compartment, direct effect model with oral dosing. X_0 represents the amount of drug in the oral compartment. We assume that drug absorption follows first order kinetics, and that elimination occurs only in the central compartment. Thus, the change in drug amount in the oral compartment is described by the following differential equation:

$$\frac{dX_0}{dt} = -k_a X_0 \qquad \textit{Equation 1}$$

Where k_a represents the first order oral absorption rate constant, which was arbitrarily assigned a value of 1 h^{-1}.

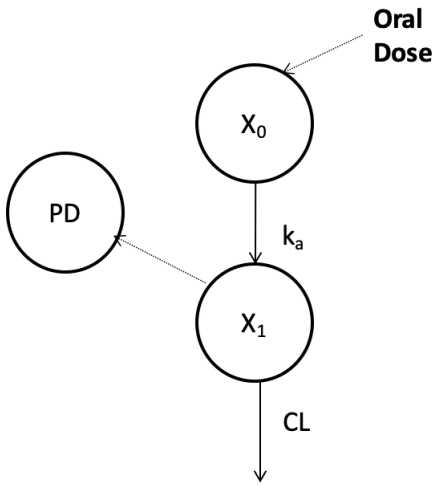


Figure 2: Schematic of a one-compartment, direct-effect model. X_0 and X_1 represent the amount of compound in the oral and central compartment and PD represents the pharmacodynamics effect.

After drug enters the central compartment, it is represented as X_1, and is subject to elimination at a rate dependent on the drug's clearance. We assume elimination from this compartment is first-order, and that the first order elimination constant, k_e, can be described as:

$$k_e = {}^{CL}/_{V} \quad \textit{Equation 2}$$

Where CL represents clearance (L/hr), and V represents the apparent volume of distribution (L), defined as V_d/F, where F refers to bioavailability after oral dosing. Thus, the change in drug amount in the central compartment (X_1) can be described by the following differential equation:

$$\frac{dX_1}{dt} = k_a X_0 - \boldsymbol{CL} * \left(\frac{X_1}{V}\right) \qquad \textit{Equation 3}$$

Where $k_a X_0$ describes the rate that the drug enters the central compartment via oral absorption, and $\boldsymbol{CL} * \left(\frac{X_1}{V}\right)$ represents the rate of elimination of the drug from the central compartment.

PD in the figure represents the pharmacodynamic effect of our drug. It is directly related to the drug concentration in the central compartment (X_1/V) *via* the Hill equation. Since we are using an inhibitor, we used an Inhibitory I_{max} model where I_{max} is the maximal inhibition (%) and IC_{50} is the concentration required for 50% inhibition. The change in PD effect with time is described by the following differential equation:

$$\frac{dPD}{dt} = 100 - \frac{I_{max} \times \left(\frac{X_1}{V}\right)^n}{\boldsymbol{IC_{50}^n} + \left(\frac{X_1}{V}\right)^n} \qquad \textit{Equation 4}$$

Where n is the Hill coefficient, which was assigned a value of 1 for our simulations.

2.1.3 Testing Change in Potency and Clearance on Predicted Dose

Model 1 was used to perform simulations in order to identify the dose that could achieve our PD target of maintaining >50% inhibition for 24 hours at steady-state following once daily dosing for the hypothetical drug describe in Table 1.

Additional simulations were performed in order to identify doses that achieve the defined PD target when potency was increased (250, 200, 150, 100, 75, 50, 25, 10, 4.3, 2.5, 1, 0.25 nM) or clearance was reduced (20, 18, 16, 14, 10, 6, 2 ml/min/kg). Both potency and clearance were

adjusted in order to achieve a predicted target dose of 50 mg in a 70 kg human (about 0.71 mg/kg).

2.2 Study 2: Effect of Altering Unbound Drug Concentrations and Hepatic Intrinsic Clearance on Dose

Our second study aimed to examine the effect of altering levels of free/unbound drug or hepatic intrinsic clearance on the predicted active dose. The PD endpoint of this study was to maintain >50% inhibition at steady state for 24 hours, with once a day dosing as described previously. An improvement in hepatic intrinsic clearance is defined as a decrease in CL_{int}, and an improvement in unbound fraction is defined as an increase in f_u.

2.2.1 Drug Starting Properties

Additional simulations were performed with a hypothetical drug with properties listed in Table 2. The difference in this study is that we assumed that the hypothetical drug had an unbound fraction of 0.05 making the total IC_{50} of 250 nM correspond to an unbound IC_{50} of 12.5 nM.

Table 2: Starting properties of hypothetical test compound for study 2

Property	Value
Apparent Volume of Distribution (V/f)	5 L/kg
k_a	1 hr^{-1}
Starting Fraction unbound (f_u)	5%
Starting Potency (Total Drug)	IC_{50} = 250 nM
Unbound IC_{50}	12.5 nM
Starting Extraction Ratio (E)	0.75
Starting Intrinsic Clearance	1242 ml/min/kg

Values were chosen to create a starting compound with non-ideal properties (high extraction ratio, low unbound fraction, and low potency). Intrinsic clearance of 1242 ml/min/kg was back calculated from E=0.75, which was set to be the threshold for a high extraction ratio. Unbound IC_{50} of 12.5 nM was calculated from the starting unbound fraction multiplied by the starting IC_{50} based on total drug (5% x 250nM). Apparent V_d and k_a were arbitrarily selected.

2.2.2 Model 2

A second model (Model 2) was constructed that allowed for investigation of changes in free/unbound drug concentrations and hepatic intrinsic clearance. Briefly, the second model expanded on Model 1 (Figure 2) by replacing the *CL* term in Equation 3 with the following equation:

$$\boldsymbol{CL} = Q\frac{f_u * CL_{int}}{f_u * CL_{int} + Q} \qquad \textit{Equation 5}$$

Where Q refers to the hepatic blood flow (20.7 ml/min/kg), CL_{int} refers to hepatic intrinsic clearance (L/hr), and f_u refers to the unbound fraction of the drug.

2.2.3 Alterations to Intrinsic Clearance

Simulations were performed over a range of hepatic intrinsic clearances (CL_{int}) (1242, 621, 414, 310.5, 207, 138, 103.5, 69, 34.5 ml/min/kg) in order to identify doses that achieved the stated PD target.

We began with a CL_{int} of 1242 ml/min/kg because this was the CL_{int} associated with a hypothetical drug with a high extraction ratio (E = 0.75) and a free fraction (f_u) of 0.05. Total clearances were back-calculated from each associated intrinsic clearance using Equation 5.

2.2.4 Alterations to Free Concentrations

Simulations were performed in order to assess the influence of changes in free/unbound drug concentrations on the dose required to achieve the stated PD target (i.e. maintain >50% inhibition at steady state for 24 hours, with once a day dosing). Specifically, unbound fractions of 5%, 10%, and 20% were used in simulations. Table 2 shows, the corresponding CL (for a high, low and moderate CL compound) and IC_{50} total associated with unbound fractions (Table 3).

In Table 3, a high clearance was defined as a drug with an initial extraction ratio of 0.75, a moderate clearance was defined as having an initial extraction ratio of 0.5, and low clearance was defined as having an initial extraction ratio of 0.25. Intrinsic clearances for each category was calculated using the initial conditions (f_u of 5%, Q of 20.7 ml/min/kg) and the extraction ratio equation (Figure 1). The values were as follows: CL_{int} = 1242 ml/min/kg for E=0.75, CL_{int} = 414 ml/min/kg for E = 0.50, and CL_{int} = 138 ml/min/kg for E = 0.25. Total clearances were back-calculated using Equation 5, using the respective CL_{int} for each extraction ratio category, and the f_u being tested, and are shown in Table 3. The total IC_{50}'s in Table 3 reflect how total IC_{50} would change with changed in f_u in order to maintain an unbound IC_{50} of 12.5 nM.

Table 3: Total clearance and IC_{50} at various levels of free/unbound drug

High Clearance (Initial E=0.75)			Moderate Clearance (Initial E=0.5)			Low Clearance (Initial E=0.25)		
f_u	**Total Clearance (ml/min/kg)**	**IC_{50} of total drug (nM)**	**f_u**	**Total Clearance (ml/min/kg)**	**IC_{50} of total drug (nM)**	**f_u**	**Total Clearance (ml/min/kg)**	**IC_{50} of total drug (nM)**
5%	15.5	250	**5%**	10.4	250	**5%**	5.2	250
10%	17.7	125	**10%**	13.8	125	**10%**	8.3	125
20%	19.1	62.5	**20%**	16.6	62.5	**20%**	11.8	62.5

Starting f_u was set at 5%, and improvement considered an increase in f_u. Total clearances were back-calculated using an initial CL_{int} = 1242 ml/min/kg for E=0.75, CL_{int} = 414 ml/min/kg for E = 0.50, and CL_{int} = 138 ml/min/kg for E = 0.25. IC_{50}'s reflect how total IC_{50} changes in accordance to changes in f_u.

2.3 Study 3: Effect of Potency and Clearance on Dose When PD Target is Efficacious Exposure (AUC)

A third simulation study was performed in a similar manner to study 1 with the exception that the PD target was modified. Rather than maintaining >50% inhibition for 24 hours at steady-state following once-daily dosing, the new PD target was to maintain a certain efficacious AUC. For changes in clearance, we targeted the AUC of the concentration-time curve, which was arbitrarily set to 8 mg*h/L. For changes in potency, we targeted the AUC of the effect-time curve, which was arbitrarily set as 1200 %*h. The drug starting properties followed that of Table 1, and the model was based on the Model 1, represented by equations 1 to 3. As before, potency and clearance are altered independently and predicted active doses associated with these alterations are identified.

Chapter 3: Results

3.1 Results Where Pharmacodynamic Target is to Maintain >50% Inhibition of the Biological Target

The following describes results where our PD target was maintenance of > 50% inhibition. The properties tested include potency, total clearance, intrinsic clearance, and unbound fraction.

3.1.1 Influence of Potency and Clearance on Predicted Efficacious Dose in Study 1

PK/PD simulations were performed using Model 1 to determine the predicted efficacious dose required to maintain >50% inhibition for 24 hours with once daily dosing for our hypothetical starting compound with the following properties: IC_{50} of 250 nM, clearance of 16 ml/min/kg, and apparent V_d of 5L/kg. The predicted efficacious dose was for the hypothetical starting compound was determined to be 2910 mg in a 70 kg human. The concentration versus time (PK profile) and effect versus time (PD profile) graphs for the hypothetical starting compound at this dose is shown in Figure 3.

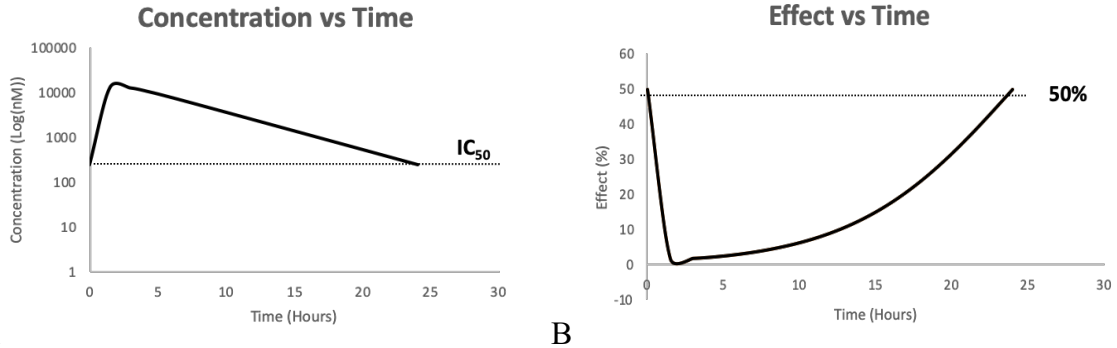


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Figure 3: **Concentration vs time and effect vs time profiles of hypothetical drug**. Graphs show the simulation output of our starting compound, given orally at a dose that inhibits biological target for >50% at steady state for 24 hours following once daily dosing. Starting conditions for the hypothetical starting compound has the following properties: IC_{50} = 250 nM, CL = 16 ml/min/kg, apparent Vd = 5 L/kg in a 70 kg patient. A: Log(concentration) versus time curve. B: Effect (% inhibition) vs time curve.

3.1.2 Improvements in Potency

PK/PD simulations were performed using Model 1 in order to identify predicted efficacious doses associated with improvements in compound potency. Our goal was to reduce the dose of our hypothetical compound from 2910 mg to 50 mg in a 70 kg human. We tested a range of IC_{50}'s from a starting value of 250 nM to 0.25 nM and identified an IC_{50} of 4.3 nM that was required to have a 50 mg dose achieve the PD target.

The PK and PD profiles of the initial and final conditions are shown in Figure 4. As shown in Figure 4A, the PK profile for the post-optimized compound (50 mg) retains the same shape as the pre-optimized compound (2910 mg) but is proportionally lower. The PD profile for the pre and post optimization overlap (Figure 4B).

The relationship between predicted efficacious dose over a range of potencies is summarized in Figure 5. Overall, improvements in potency resulted in linear and proportional reductions in predicted efficacious doses (i.e. a 2x improvement in potency resulted in a 2x reduction in dose).

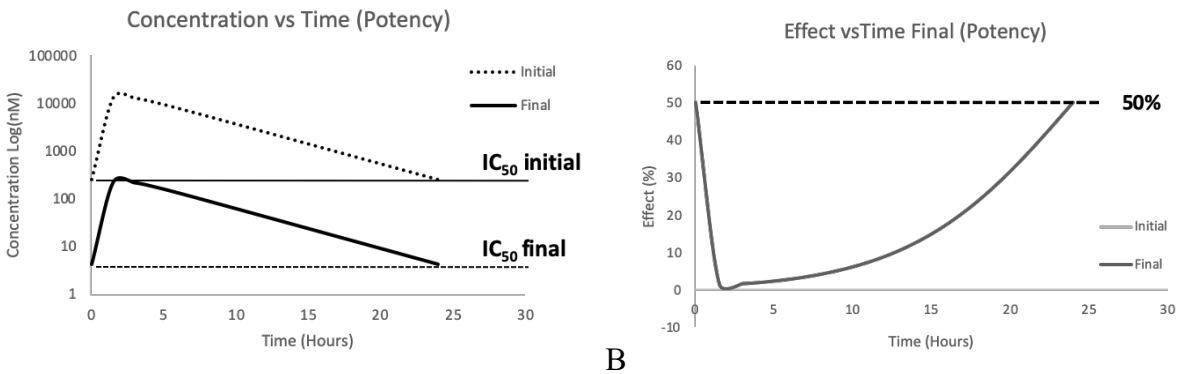


Figure 4: Concentration vs time and effect vs time profiles of hypothetical drugs at the starting dose of 2910 mg and the target dose of 50 mg after changes in potency. Graphs show the simulation output of our pre and post optimized compound given orally at a dose that inhibits biological target for >50% for 24 hours when given once daily. Starting conditions for the hypothetical compound has the following properties: IC_{50} = 250 nM, CL=16 ml/min/kg and Vd = 5 L/kg in a 70kg patient. Final compound properties: IC_{50} = 4.3 nM, CL=16 ml/min/kg and apparent Vd = 5 L/kg. A: Log(concentration) versus time curve. B: Effect (% inhibition) vs time curve.

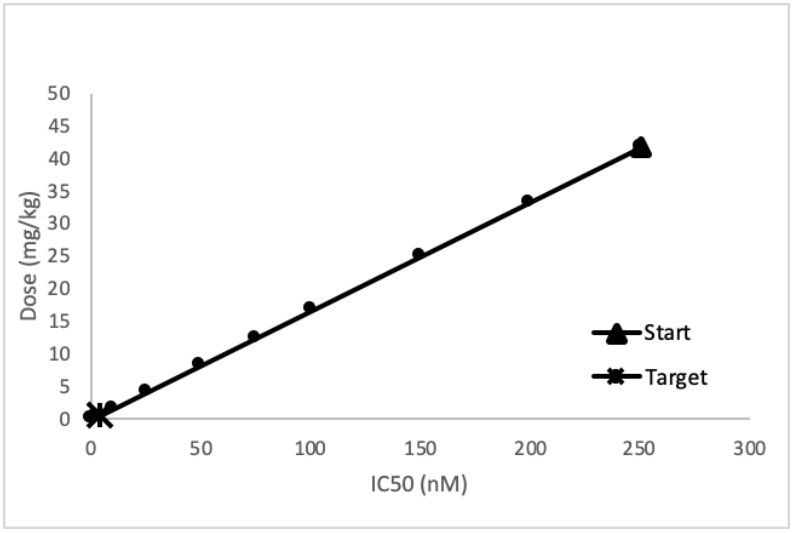


Figure 5: Effect of improvements in potency on predicted active dose with PD target being >50% inhibition of target for 24 hours with once daily dosing. The start represents the starting conditions for a hypothetical starting compound with the following properties: IC_{50}=250 nM, CL=16 ml/min/kg, and apparent Vd=5 L/kg in a 70kg patient. The target represents conditions where the dose has been improved to 50 mg in a 70 kg patient.

3.1.3 Improvements in Clearance

Simulations were performed using Model 1 in order to identify predicted efficacious doses associated with improvements in compound clearance. As before, our goal was to reduce the dose of our hypothetical compound from 2910 mg to 50 mg in a 70 kg human. We tested a range of clearances from a starting value of 16 ml/min/kg to 2 ml/min/kg and identified a clearance of 3.1 mL/min/kg that was required to have a 50 mg dose achieve the PD target.

The PK and PD profiles of the initial and final conditions are shown in Figure 6. As shown in Figure 6A, the PK profile for the post-optimized compound (50 mg) has a much flatter profile compared to the pre-optimized compound (2910 mg) due to the reduction in clearance. Similarly, the PD profile for the post optimized compound is much more shallow. (Figure 6B).

The relationship between predicted efficacious dose over a range of clearances is summarized in Figure 7. Overall, improvements in clearance resulted in non-linear reductions in predicted efficacious dose, with the change being especially dramatic at higher clearances.

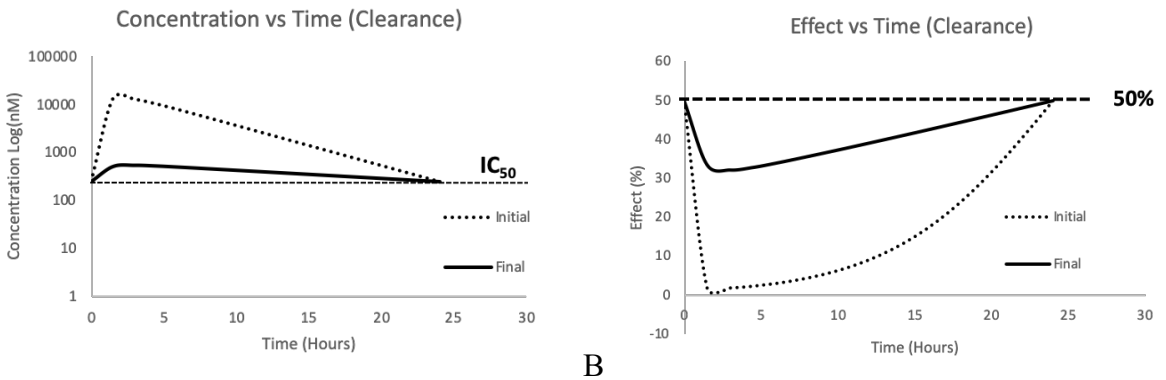


Figure 6: Concentration vs time and effect vs time profiles of hypothetical drugs at the starting dose of 2910 mg and the target dose of 50 mg after changes in clearance. Graphs show the simulation output of our initial and final compounds given orally at a dose that inhibits biological target for >50% for 24 hours when given once daily. Starting conditions for the compound has the following properties: IC_{50}=250 nM, CL=16 ml/min/kg, and apparent Vd=5L/kg in a 70kg patient. Final compound properties: IC_{50}=250 nM, CL=3.1ml/min/kg. A: Log(concentration) versus time curves. B: Effect (% inhibition) vs time curves.

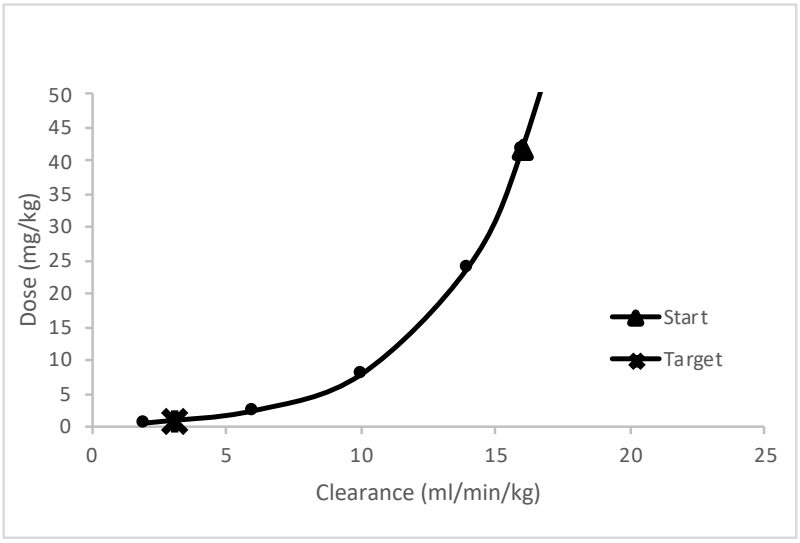


Figure 7: Effect of improvements in clearance on predicted active dose with PD target being >50% inhibition of target for 24 hours with once daily dosing. The start represents the starting conditions for a hypothetical compound with the following properties: IC_{50}=250 nM, CL=16 ml/min/kg, and apparent Vd=5 L/kg in a 70 kg patient. The target represents conditions where the dose has been improved to 50 mg in a 70 kg patient.

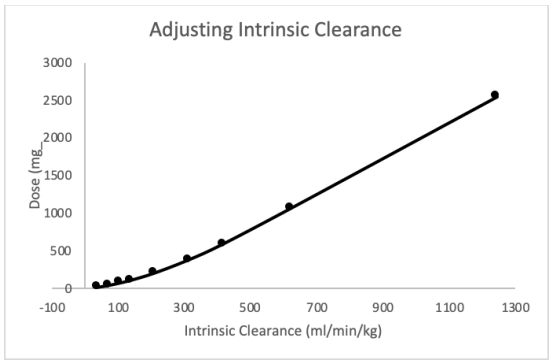
Overall, when comparing the effect of improvements in clearance versus improvements of potency on predicted efficacious dose, our simulations suggest that more gains (decrease to dose) can be made by improving clearance rather than potency.

3.1.4 Influence of Changes in Intrinsic Clearance and Unbound Drug on Predicted Efficacious Dose in Study 2

As improvements in clearance provided greater improvements in predicted efficacious dose, we wished to explore the effect of alterations in intrinsic clearance and free drug levels, two factors that influence clearance, on predicted efficacious dose. The next set of simulations used Model 2 to explore the effect of these changes on dose. In these simulations, the properties of the hypothetical starting compound are shown in Table 2 of the Methods section. As with the previous section, our pharmacodynamic target remains inhibition of >50% of the biological target for 24 hours with once daily dosing.

3.1.4.1 Improvements in Intrinsic Clearance

Figure 8 and Table 4 show changes in clearance and predicted efficacious dose that occur with improvements in intrinsic clearance. A reduction in intrinsic clearance results in a reduction of total clearance (Table 4). As intrinsic clearance is related to total clearance via the well-stirred model, the relationship between changes in intrinsic clearance and total CL is nonlinear in nature. The reductions in intrinsic clearance results in slight non-linear improvements in predicted efficacious dose (Figure 8A). As observed in the previous section, the relationship between CL (corresponding to the changes in intrinsic clearance) and predicted efficacious dose is very non-linear in nature with greater than proportional reductions in predicted efficacious dose with reductions in clearance (Figure 8B).

A: 

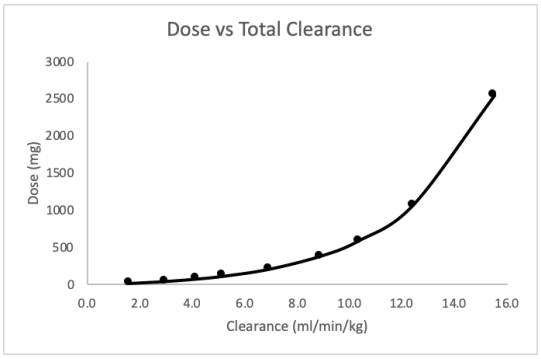
B: 

Figure 8: Characterization of the effect of changes in intrinsic clearance on predicted active dose with PD target being >50% inhibition of target for 24 hours with once daily dosing. Baseline hypothetical compound assumes 5% unbound drug and an IC_{50} of 12.5 nM free/unbound drug (calculated using 5% free and an IC_{50} of 250 nM total drug) and apparent Vd=5 L/kg in a 70kg patient. A) effect of changes in intrinsic clearance on dose. B) effect of corresponding total clearances on dose.

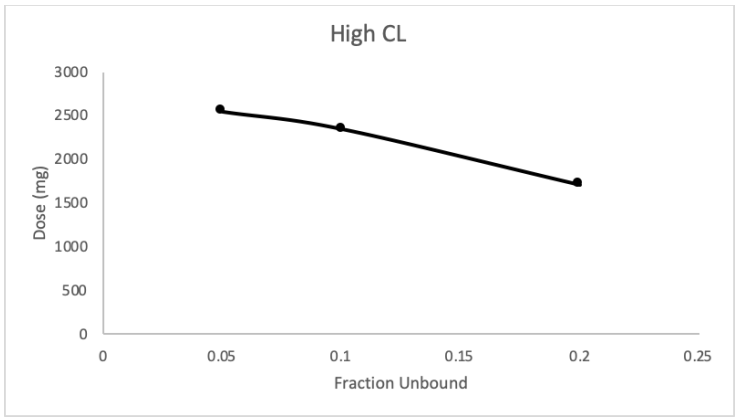
Table 4: Changes in CL_{int} and its resultant changes in total clearance and dose required to inhibit biological target for >50% for 24 hours following once a day dosing.

CL_{int} (ml/min/kg)	Dose (mg)	Total Clearance (ml/min/kg)
1242	2550	15.5
621	1073	12.4
414	594	10.4
311	385	8.9
207	209	6.9
138	117	5.2
104	79	4.1
69	47	3.0
35	21	1.6

3.1.4.2 Improvements in Free Drug Concentrations

Improvements in free concentrations (i.e, increase in unbound fraction (f_u) has different effects on dose depending on whether the hypothetical starting compound is a high, moderate, or low clearance compound. For a high clearance compound, improvements in f_u resulted in decreases in dose (Figure 9A). However, for both moderate and low clearance compounds, improvements in f_u resulted in increases in dose (Figure 9B and 9C).

A:



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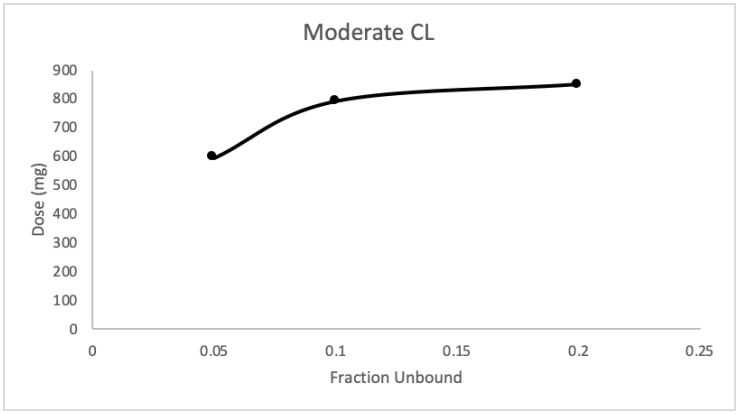

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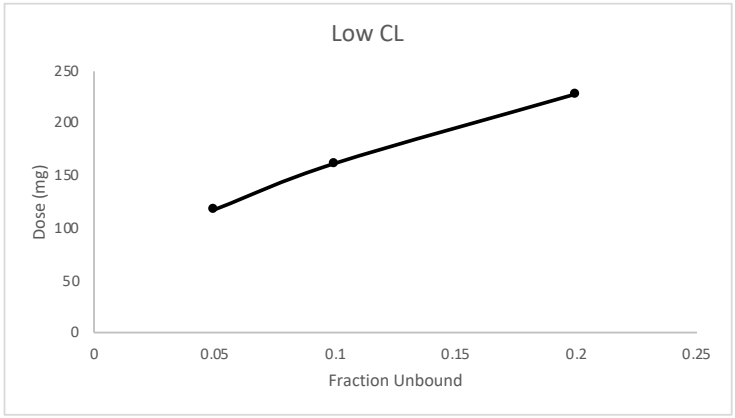

Figure 9: Characterization of the effect of changes in free-drug levels on predicted active dose with PD target being >50% inhibition of target for 24 hours with once daily dosing. Baseline hypothetical compound assumes 5% unbound drug and an IC_{50} of 12.5 nM free/unbound drug (calculated using 5% free and an IC_{50} of 250 nM total drug), and apparent Vd= 5 L/kg in a 70 kg patient. A). Effect of changes in free concentrations (f_u) on predicted drug dose for a high clearance drug. B) Effect of changes in free concentrations (f_u) on predicted drug dose for a moderate clearance drug C) Effect of changes in free concentrations (f_u) on predicted drug dose for a low clearance drug Total IC_{50} was adjusted for each change in f_u to maintain IC_{50} of 12.5 nM free/unbound drug.

As noted previously, the effect of an alteration in free concentrations is more complex in that it not only changes clearance but also alters total IC_{50}. Specifically, as f_u increases, the overall clearance also increases, and we would anticipate an increase in predicted dose. However, increased free concentrations would also result in more drug interacting with biological target receptors leading to lower total IC_{50} target concentrations. Lower IC_{50} target concentrations, would result in an anticipated decrease in predicted efficacious dose. The final predicted efficacious dose is dependent on whether the change in CL or the change in total IC_{50} dominates.

These results indicate that for high clearance compounds, increases in f_u do not affect clearance greatly, thus the effect of changes in total IC_{50} dominates resulting in a decrease in predicted dose. However, when the drug is a low or moderate clearance compound, an increase in f_u's effect on clearance is more marked, and thus the predicted dose shows modest increases.

Overall, these studies suggest that in most cases, the effect of clearance is the dominant factor in determining a predicted efficacious dose. While both potency and clearance play a role in a predicted active dose, the effect of improving clearance in general has a larger effect on reducing the predicted efficacious dose.

3.2 Results Where Pharmacodynamic Target is an Efficacious AUC

The following describes results where our PD target was maintenance of an efficacious area under the curve (AUC). The properties tested include only potency and total body clearance.

3.2.1 Effect of Improvements in Potency and Clearance on Dose

PK/PD simulations were performed using Model 1 in order to identify predicted efficacious doses associated with improvements in both compound potency and clearance. As described in section 1, our goal was to reduce the dose of our hypothetical compound (described in Table 1 of Methods section) from 2910 mg to 50 mg in a 70 kg human. The difference in this set of simulations is that rather than having a pharmacodynamic target of maintaining >50% inhibition of the biological target for 24 hours with once daily dosing, we now have a pharmacodynamic target of an efficacious AUC

Figure 10 summarizes the change in predicted active dose associated with improvement in both potency and clearance. In both cases, improvements in potency or clearance resulted in reasonably linear changes in the predicted active dose. Furthermore, similarly to data from our first study, changes in potency also caused proportional changes in dose (i.e. 2x improvement in potency resulted in a 2x decrease in dose) (Figure 10A). The exact values from each simulation are also summarized in Table 5 and Table 6.

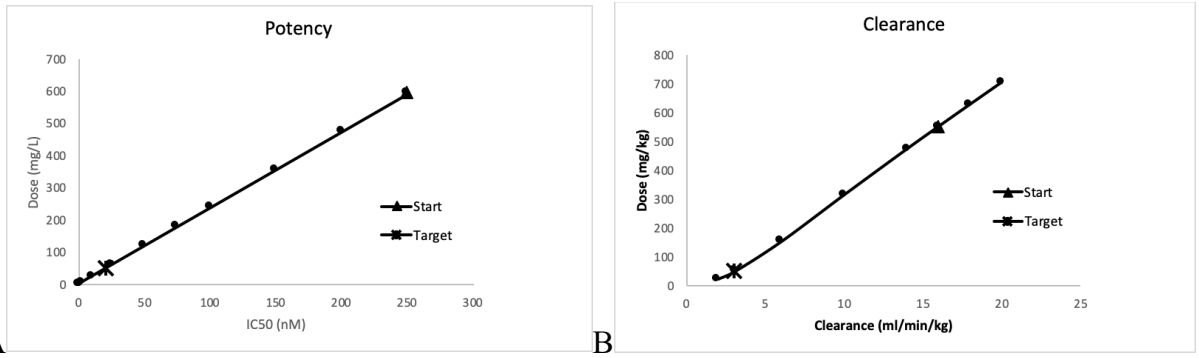

Figure 10: Effect of improvements in potency and clearance on predicted active dose with PD target being an efficacious AUC. The start represents the starting conditions for a hypothetical compound with the following properties: IC_{50}=250 nM, CL=16 ml/min/kg, and apparent Vd= 5 L/kg in a 70 kg patient. The target represents conditions where the dose has been improved to 50 mg in a 70 kg patient. A: graph shows relationship of potency versus dose. B: graph shows the relationship of clearance versus dose.

Table 5: Changes in dose in response to changes in potency with PD target being an AUC

IC_{50} (nM)	Dose (mg)
0.25	0.6
1	2.4
2.5	5.9
10	23.8
25	59.4
50	118.8
75	178.2
100	237.6
150	356.5
200	475.3
250	594.1

Table 6: Changes in dose in response to changes in clearance with PD target being an AUC

Clearance (ml/min/kg)	Dose (mg)
2	22.6
3	50.0
6	152.5
10	314.6
14	474.1
16	551.6
18	628.2
20	704.3

Our simulations suggest that when efficacious AUC is our PD target, there is no advantage to improving clearance over potency in the optimization process.

Chapter 4: Discussion and Conclusion

In this thesis, we have demonstrated the ability to use quantitative pharmacokinetic-pharmacodynamic models for the purpose of guiding drug optimization. While it has been long understood that various drug properties determine the active or efficacious dose of a drug, interrelationships between these drug properties and how they quantitatively determine the efficacious dose are rarely studied using a dynamic integrated system. Often times, optimization of chemical matter involves improvements of compound properties independently of one another. Considering the multitude of factors that can affect dose, it can be a daunting process to iteratively optimize each one, and wasteful to simply do large scale testing for all parameters [37]. Our work with PK/PD models illustrates how changes in compound properties and their impact on predicted efficacious dose can be evaluated through computer simulation. In this way, the relative importance of each property can be prioritized allowing compound optimization to be performed in a more rational manner.

It is important to note that our drug optimization goal was to reduce the dose needed to achieve a certain pharmacodynamic endpoint with daily dosing. One reason for this is that one cannot feasibly give too large of a dose to a patient, as it can lead to discomfort for the patient. Furthermore, it has been shown that simpler dosing regimens in which medication was given less frequently were associated with better adherence and compliance, which could lead to lower health care costs and better outcomes [50, 11]. Thus, our overarching goal in these studies was to use our PK/PD model to identify the most efficient way to modify drug properties in order to lower the active dose of our hypothetical compound to a reasonable once daily dose (i.e. 50 mg).

4.1 Potency and Clearance

Our first studies established the important role clearance plays in lowering the drug dose when the PD endpoint is maintenance of >50% inhibition of a biological target for 24 hours following daily dosing which involved maintenance of an IC_{50} concentration. Many common therapeutics, such as antibiotics that work using time-dependent killing (penicillins, cephalosporins), work in this fashion where minimum inhibitory concentrations have to be maintained for certain periods of time [13, 53]. It is important to note that our findings can be applied to any drug where specific target concentration or percent inhibition need to be maintained for activity. Maintenance of 50% inhibition was chosen for simplicity's sake, since we are also using the IC_{50} as a measure of potency.

The simulations demonstrated that improvements in potency reduces dose in a linear/proportional fashion, providing clearance does not change. Potency is one of the most commonly modified properties [37], and it is intuitive to think that improving potency is the most important drug property for reduction of dose. In fact, early chemical hits often have terrible potency, and early hit optimization is often focused on bringing the potency into an acceptable, if not yet ideal, range to create a lead for further testing [37]. However, even in the late optimization phase, the effect of clearance, while acknowledged as important, is often not given the weight that it deserves. For example, studies that point out the importance of addressing clearance in apparent dose have suggested that the unfavourable aspects of a high clearance compound (increase in active dose) can be offset by improving potency [9]. Our data have shown that this is not entirely the case, since small changes in clearance can lead to large changes in predicted dose, especially for high-clearance compounds. Furthermore, the effect of

changes in clearance on dose is much more dramatic than that of potency. This suggests that improved potency would likely be unable to compensate for an unacceptably high clearance when it comes to achieving an acceptable dose. This finding is of note since it suggests that one should consider prioritizing clearance as a parameter to optimize rather than potency as there are bigger gains in the reduction of the active dose.

4.2 Intrinsic Clearance

Hepatic intrinsic clearance (Cl_{int}) estimates for compounds are typically obtained from metabolic stability studies using microsomes or hepatocytes. Intrinsic clearance is defined as the ability of the liver to clear drug in the absence of blow flow limitations and binding to cells or proteins in the blood. In our Model 2, we related Cl_{int} to hepatic clearance using the well-stirred model [22, 45, 55]. Our assumption is that our hypothetical compounds are metabolized primarily by liver such that hepatic clearance approximates total clearance. The relationship between changes in intrinsic clearance and total clearance is not linear, but rather described by the equation (Equation 5 from methods):

$$\boldsymbol{CL} = Q\,\frac{f_u * CL_{int}}{f_u * CL_{int} + Q}$$

Thus, reductions in intrinsic clearance does not correspond to proportional changes in total clearance. For example, if we half the intrinsic clearance, as demonstrated by the reduction in CL_{int} from 1242 ml/min/kg to 621 m/min/kg, we would see total clearance drop from 15.5 ml/min/kg to 12.4 ml/min/kg (Table 4). However, since total clearance has changed, we would

expect to see dramatic decreases in dose, as seen in our first set of studies with Model 1 (Figure 9). As expected, decreases in intrinsic clearance resulted in reductions in dose (Figure 8A), and when we compare changes in dose to the total clearances that we back-calculated from the intrinsic clearances tested, we see that it follows the same non-linear relationship as seen in our PK/PD analysis using Model 1(Figure 7 and Figure 8B). We conclude then that reduction of a drug's intrinsic clearance is an effective way to reduce dose due to the resulting decrease in total clearance.

The importance of clearance in reducing active dose can be observed in drugs being used in clinical settings. One example is propranolol, a high clearance drug that is hepatically cleared. In patients with severe liver disease, their inherent ability to clear propranolol is lowered due to a lowering of propranolol's intrinsic clearance. When given propranolol, these patients with liver disease exhibit a lower than normal total clearance, as well as longer half-life [5]. Propranolol is associated with recommended dose reductions in patients with liver disease where the hepatic intrinsic clearance of these drugs is reduced.

4.3 Free/Unbound Concentrations

The effects of increase in free/unbound drug concentration on dose is more complex. As free fraction increases, there is more drug available to interact with both the biological targets, as well as metabolic enzymes. Thus, changes in available free/unbound drug can change both drug clearance and in vivo drug potency (total IC_{50}). Increases in free drug concentrations results in a decrease in total IC_{50}, which acts to decrease the efficacious dose. In contrast, increased free drug concentrations (i.e. $\uparrow f_u$) results in an increase in total clearance, which acts to increase the

efficacious dose. This effect of increases in free concentration (i.e. ↑ f_u) is summarized in Figure 11.

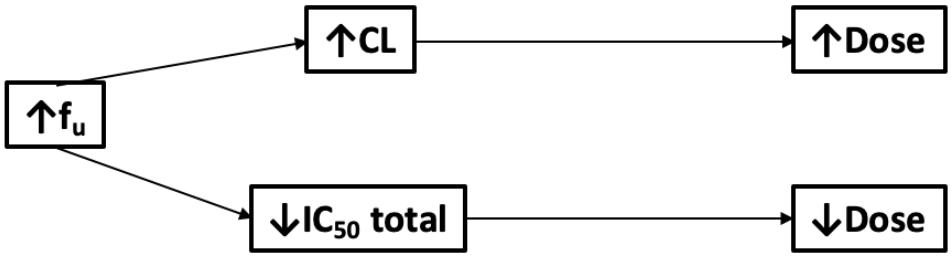

Figure 11: Schematic describing the opposing effects of increasing free drug levels on drug potency, clearance, and active dose.

Overall, the change in active dose due to increases in free drug concentrations is subject to the opposing effects of increasing potency and increasing clearance on the predicted efficacious dose.

As mentioned previously, the well-stirred model is a model of hepatic clearance model that is described by the following equation (Equation 5 from methods):

$$CL = QE = Q\frac{f_u * CL_{int}}{f_u * CL_{int} + Q}$$

According to the well-stirred model, hepatic drug clearance can be categories into three categories that are defined by the extraction ratio (E). Conventionally, high clearance drugs are defined as having E ≥ 0.75, moderate clearance drugs have an E between 0.25 and 0.75, and a

low clearance drug has an $E \leq 0.25$ [49, 55]. Changes in f_u and Cl_{int} have different effects on high, moderate and low clearance drugs.

For a high clearance compound, the CL_{int} is much larger than Q, and thus the denominator of the equation would approximate $f_u * CL_{int}$. In this case, the well-stirred model equation to simplify to the following:

$$CL \sim Q \qquad \textit{Equation 6}$$

For a low clearance compound, the Q is much larger than CL_{int}, and thus the denominator of the well-stirred equation would roughly equal to Q. In this case, the well-stirred model equation would simplify to the following:

$$CL \sim f_u * CL_{int} \qquad \textit{Equation 7}$$

For moderate clearance compounds, the full equation for the well-stirred model applies (Equation 5).

The described approximations of the well-stirred model for high, moderate and low clearance compounds aid in interpreting our results from our PK/PD simulations exploring the effect of increased free drug concentrations on predicted active does. As seen in Figure 9A, when free concentrations increase for a high clearance compound, the predicted dose decreases. As hepatic

clearance approximates liver blood flow for a high clearance compound, the decrease in dose is driven by improved potency (i.e. lower total IC_{50}) (Figure 11).

When we examine similar PK/PD simulations with moderate and low clearance, we see increases in predicted dose with increases in free drug concentrations (↑ f_u) (Figure 9B and 9C). For moderate and low clearance compounds, increase in f_u results in increase in clearance (Equation 5 and Equation 7). Thus, for moderate and low clearance compounds, the increase in clearance (causes ↑ in dose) have a greater effect on dose than the increase of potency (causes ↓in dose). The effects described by our model are consistent with real life observations of real drugs where alterations of f_u and free drug levels occur during certain disease state. Going back to our earlier example with propranolol (a high clearance drug), several studies have suggested that when levels of free drug are increased, there is a correlated increase in both drug effects and side effects [2, 58, 5]. These studies also indicate that the clearance of propranolol is not influenced by changes in free concentrations. [18, 58]. Dose reductions are recommended in this scenario, with one recommendation being 50% decrease in dose [2]. These results are consistent with what is predicted by our PK/PD analysis (see Figure 9A) which suggests that active doses decrease when free concentrations increase for a high clearance drug.

If we examine drugs with moderate to low clearance, we see different patterns. In a systematic review by Uldemolins *et al* in 2011, several antimicrobials were found to be impacted by changes in protein binding in patients with severely low albumin levels (hypoalbuminemia) on account of disease, which resulted in increased free drug concentrations [54]. Higher than normal clearance levels were reported for several antimicrobials including fusidic acid [40, 53],

teicoplanin [38, 6, 51], ceftriaxone [36, 28], and ertapenem [7, 8, 4]. The changes in clearance of these drugs resulted in changes to dosing to in order to maintain steady-state levels that are still efficacious. These changes included more frequent dosing regimen, altering the route of administration to an intravenous infusion such as in the case of ceftriaxone [28], or recommendations of a higher loading dose, such as for teicoplanin [6, 38]. In general, we can surmise that increases in free drug levels are associated with dosing strategies aimed at delivering a greater amount of drug to patients. These results are consistent with what our model predicts: changes in free drug levels increase clearance, and thus requires a higher dose or a greater amount of drug administered over time to maintain the same effect despite theoretically increasing potency (lowering total IC_{50}) (Figure 9B and 9C).

4.4 Maintaining Efficacious Exposure (AUC)

Our final set of PK/PD simulations were aimed at examining the effect of improving drug potency and clearance when the PD target is to maintain a specific efficacious AUC. Targeting a certain plasma concentration-time curve AUC is less common, but sometimes observed for drugs such as anti-cancer drugs. For example, axitinib is a vascular endothelial growth factor receptor tyrosine kinase inhibitor used in the treatment of renal cell carcinoma whose plasma exposure (AUC) has been shown to be correlated with clinical efficacy as well as adverse effects [27, 59]. Thus, we felt it important to examine the effects of clearance and potency changes on dose when the PD target is AUC.

Our simulations found that when the pharmacodynamic target is to maintain an efficacious AUC, there is no advantage of optimizing clearance over potency, since dose decreased linearly with

improvements in both properties. This also demonstrates that the choice to prioritize one property over the other depends on the pharmacodynamic endpoint in question.

4.5 Limitations and Future Directions

One of the main limitations of this study is the limited number of scenarios covered. For example, we explored only two PD targets, being: 1) maintaining >50% effect, and 2) maintaining an efficacious AUC. However, there are merely two common PD targets, and may not apply to mechanisms of action for other drugs.

Furthermore, we utilized only forms of the direct effect model as our mathematical framework. Other models exist models that can incorporate indirect or delayed effects [57]. It is possible the conclusions made here about how these drug properties affect dose may differ in drugs with differing mechanisms of action that were not mathematically characterized and explored. Exploring relationships between drug properties and their effect on efficacious doses for mechanisms of action, such as drugs that display delayed onset of effect, that we did not mathematically explore is a future direction to consider.

Our studies only considered efficacy as the main goal. However, another aspect of developing good drugs is safety. While we did not explore this factor in detail during our studies, since it would add another layer of complexity beyond the scope of this thesis, our model may be used to examine this aspect as well. For example, we have shown that decreases in potency shifts the concentration-time profile downwards, which could have important implications when it comes to side effects that may occur at higher concentrations. As well, decreases in clearance creates a

flatter concentration-time profile, which could be important for drugs with narrow therapeutic windows. Application of this sort of model when it comes to looking at candidate safety profiles is something to consider for further exploration and combining both safety and efficacy into our model will likely help further refine the results and guide optimization decisions.

Finally, it is important to also acknowledge that these are only mathematical models, and as such may not completely describe a drug's PK and PD in its entirety. The behavior of drugs in living systems are affected by many other factors, such as competition for binding, barriers to absorption, drug-drug or drug-food interactions, which were not covered in our simple model. Our main goal was to illustrate the value in utilizing PK/PD modeling and simulation as a tool to guide drug optimization decision making. Our use of simple case studies was conducive in demonstrating its utility and serves as a starting point to build refined models that could more accurately represent real biological conditions in the future.

4.6 Conclusion

We have demonstrated the ability to use PK/PD modeling to guide drug optimization. We have found that the prioritization of drug properties during the optimization stage can be dependent on the specific pharmacodynamic endpoint targeted. Overall, it seems that in most situations, prioritizing the optimization of clearance rather than potency can provide larger gains in reducing the predicted active dose.

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