DEVELOPMENT OF HYSTERESIS ANALYSIS AS A MODEL-INDEPENDENT APPROACH TO ASSESS TEMPORAL DISSOCIATION IN PHARMACOKINETICS AND PHARMACODYNAMICS

By

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Pharmacodynamic hysteresis is a loop, typically anti-clockwise, in the effect-concentration profile, resulting from temporal dissociation between elaboration of effect and changes in drug concentration. Hysteresis behavior impairs recovery of reliable numerical estimates of pharmacodynamic parameters. Existing methods for analyzing data with a hysteresis have focused on integrated pharmacokinetic-pharmacodynamic modeling, which is based on hypothetical constructs and unverifiable assumptions. The work presented herein was aimed at developing and evaluating model-independent metrics to quantify time delays in pharmacokinetic/pharmacodynamic systems. Relationships between various descriptors of hysteresis morphology and parameters associated with distributional (pharmacokinetic) or effect (pharmacodynamic) delays were explored in
silico. The ratio of x- versus y-coordinate of the hysteresis centroid was identified through simulation studies as the most useful descriptor in characterizing pharmacokinetic delays. The utility of this metric was demonstrated with mined data examining distribution of methotrexate into human brain, and indicated that hysteresis analysis can provide robust and sensitive results when traditional parametric approaches fail. The utility of hysteresis analysis in pharmacodynamic experiments was examined with simulated data generated from a multiplicity of commonly-encountered pharmacokinetic-pharmacodynamic systems, including systems with delayed distribution to the receptor target (effect-compartment systems) and delayed elaboration of effect after binding of the drug to the target (indirect response systems). In addition, the influence of the shape of the effect-versus concentration relationship (linear, hyperbolic, or sigmoidal) on hysteresis analysis was explored. Results of these experiments indicated that hysteresis analysis is most useful when the effect-concentration relationship is linear. Consistent with observations for nonlinear pharmacokinetic systems, nonlinearities in the effect-concentration profile resulted in nonlinear relationships between hysteresis descriptors and model parameters, with a consequent loss in analytical sensitivity and specificity. Results obtained with simulated data were confirmed with data mined from 12 published reports. Taken together, the results of this project indicate that the hysteresis centroid provides information useful in quantifying delays in pharmacokinetic/pharmacodynamic systems without the need for underlying assumptions. In particular, the centroid is useful for hypotheses-testing, rather than descriptive, purposes. As with non-compartmental approaches for pharmacokinetic
analysis based on statistical moment theory, hysteresis analysis appears to be useful only for linear systems.
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Chapter 1

TEMPORAL DELAYS IN
PHARMACOKINETICS AND PHARMACODYNAMICS:
HYSTERESIS BEHAVIOR AND DATA ANALYSIS APPROACHES

This chapter will be submitted to the *Journal of Pharmaceutical Sciences* as a mini-review,
and is presented in the format of that journal
**Introduction**

In both pharmacokinetics (PK) and pharmacodynamics (PD), it is important to understand the time course of changes in various aspects of the biological system, especially at the target site where the desired pharmacologic response or an undesired toxicity is elicited. Such aspects include the concentration (PK) of the agent in question and the immediate and down-stream responses to interaction of the agent in question with its biologic receptor (PD). It is recognized that the time course of drug concentrations in the systemic circulation is not be reflective of the time course of concentrations in the target tissues or at the receptor biophase prior to attainment of distribution equilibrium between those sites [1]. The differences in concentration-time profiles between the systemic circulation and the target tissue are often rectified through use of multi-compartment models with nonlinear least-squares regression [1]. In such cases, by definition there is a temporal delay between presentation of the drug to the systemic circulation and presentation of the drug to the target site.

In PD, in order to quantify the pharmacologic response that a therapeutic agent elicits, the relationship between effect and concentration usually is modeled to obtain an estimate of relevant PD parameters. Parameters of interest often include EC$_{50}$, the concentration at which 50% of the maximum response is achieved if the effect versus concentration relationship is modeled with an $E_{\text{max}}$ or sigmoidal equation [2]. In many cases, there is a temporal delay between presentation of the drug to the systemic circulation and elaboration of the effect elicited by the drug. Often, but certainly not always, such delays
are due to slow equilibration of the drug between the systemic circulation and the target tissue. Regardless of the underlying mechanism for delayed elaboration of effect, under non-steady-state conditions peak effect will occur at some time later than peak concentration in the systemic circulation.

A typical PD hysteresis loop is shown in Figure 1-1. When the time course of drug concentration is in phase with the time course of pharmacologic effect, plotting effect versus concentration results in a typical hyperbolic or sigmoidal relationship (Figure 1-1). Importantly, in this situation there is a singular relationship between effect and concentration: regardless of the time point examined during or after administration of the drug, a particular concentration is associated with a specific magnitude of effect. In contrast, when there is delayed onset of pharmacologic effect relative to generating concentrations in the systemic circulation, the effect versus concentration plot forms an anti-clockwise hysteresis loop. In this case, a particular concentration is associated with two different magnitudes of effect depending on whether the observation (measurement of effect and concentration) was made early in the profile (low effect) or later in the profile (high effect).

The absence of a singular relationship between effect and concentration under non-steady-state condition impedes the ability to generate fundamental PD parameters, resulting in the need to use relatively sophisticated analytical techniques to extract useful information from the data [3].

It has been suggested that the area bounded by the hysteresis loop (ABH) is positively correlated with how “deep” the effect compartment is relative to the central
compartment in a typical effect-compartment model [4]. As will be discussed later in this review, the size of the ABH is not necessarily reflective of the magnitude of delay. The overall morphology of the hysteresis loop, while indicative of the presence of a delay in response elaboration, has not been used routinely as a quantitative measure of the magnitude of that delay. It also should be noted that an anti-clockwise hysteresis loop indicates a delay between effect and concentration only when the direction of effect is positive; when the drug causes a decrease in the biologic metric (i.e., produces a negative effect), delayed elaboration of response would produce a clockwise hysteresis [5]. For the purposes of this review, it will be assumed that drug effect is positive, and that delays are always associated with anti-clockwise hysteresis behavior.

The presence of a hysteresis loop in the relationship between the drug response and drug concentration impairs the ability to obtain estimates of fundamental parameters (e.g., EC\(_{50}\)) that are the basis of understanding PD. It is crucial to identify and account for hysteresis behavior in order to obtain reliable estimates of PD parameters. For example, failure to account for hysteresis behavior for the antipsychotic aripiprazole led to overestimating the EC\(_{50}\); after accounting for the time delay using an integrated PK-PD model, a reliable prediction of the concentration-receptor occupancy relationship was obtained [6].

In this review, mechanisms of hysteresis behavior, drugs or drug classes that often exhibit hysteresis behavior, and data analysis methods that have been used to address
hysteresis behavior are discussed. While it is recognized, as described above, that PK hysteresis behavior can occur, the primary focus of this review is on PD hysteresis behavior.

**Mechanisms leading to hysteresis behavior in PD**

A temporal delay can be caused by a variety of mechanisms, including slow distribution of drug from plasma to the effect site [7-16], which actually is a PK phenomenon that impacts PD; slow receptor kinetics [17-19], multiple biological reactions after the binding of the drug to its receptor before the observed drug effect is produced [20-22], or slow accumulation of an active metabolite at the site of action [23-25] (also a PK process with a PD outcome). A variety of therapeutic agents display PD hysteresis behavior. For example, central nervous system agents, which must cross the blood-brain barrier (BBB) before eliciting a response, often evidence a temporal delay between drug effect and systemic concentration due to the time required to distribute from the plasma into brain. Delayed analgesic activity relative to plasma concentrations, for example, can be observed for opioids and non-opioids analgesics. In general, opioids differ with respect to the degree of hysteresis in effect versus concentration relationships. Some opioids such as alfentanil [7] have minimal delay between effect and concentration due to rapid equilibration (minimal BBB impedance) between the site of action and blood. Other opioids display a moderate delay in the production of response, including sufentanil and fentanyl [26], fentanyl and buprenorphine [9, 10], dihydrocodeine [11], remifentanil [12], methadone [13], and oxycodone [14]. It has been suggested that morphine and its active metabolite morphine-6-glucuronide (M6G) in general display the longest delay between the time
course of analgesic effect and that of systemic concentrations among all opioids [8, 16, 27, 28]. Other examples of pharmacologic classes that evidence delayed onset of pharmacologic response include, but are not limited to the anti-inflammatory effects of corticosteroids [29], calcium channel blockers[18], and beta-blockers [30].

**Approaches to establishing the true effect-concentration relationship for drugs with PD hysteresis behavior**

In order to reveal the true effect-concentration relationship (no hysteresis), several approaches can be utilized. First, experiments may be performed after distribution equilibrium has been established. This condition is most reliably attained by determining the effect versus concentration relationship under true steady-state conditions (during continuous or multiple-dose administration). At true steady-state, the systemic drug concentration is in equilibrium with the drug concentration at the receptor biophase; therefore, a singular (i.e., time-independent) relationship between concentration and effect can be established. Of course, this approach to eliminating the confounding factor of hysteresis is only effective if PD hysteresis has a PK basis (slow accumulation of either the parent drug or an active metabolite at the site of action). Disadvantages of this experimental approach include (1) it is labor-intensive and in some cases impractical to measure effects only after steady-state is reached; (2) in many cases onset of pharmacologic effect shortly after administration of drug is of interest (e.g for anesthetic agents); (3) for drugs with a long duration of action, in which case the effect may be present when drug concentrations in the systemic circulation are below the limit of detection, the effect-concentration
relationship cannot be established without modeling. In this latter case, hysteresis behavior is due to PD rather than PK factors, and so the steady-state approach simply would not be effective regardless of assay considerations.

An alternative experimental strategy, which also is relevant if PD hysteresis is due to a distributional delay, is to sample concentrations at the effect site instead of, or in addition to, sampling in the systemic circulation. This strategy often is used in preclinical studies of CNS-active agents, when terminal brain tissue samples [31] or continuous microdialysis sampling in brain [32] can circumvent slow equilibration (relative to the time scale of measuring drug response) between brain tissue and blood. When possible, measuring drug concentrations at site of action is preferable, as it allows unambiguous determination of important characteristics of the drug (e.g., potency). However, in most cases, drug concentration at the effect site is difficult or impossible to measure, especially in clinical studies.

A third experimental strategy, which is used most commonly, is to remove the influence of temporal delays between effect and concentration using PK-PD modeling [3]. Integrated PK-PD modeling provides a simple, if somewhat artificial, solution for analyzing data from non-steady state experiments. In the present review, several PD models that have been used to collapse the hysteresis loop, thereby generating a singular concentration-effect relationship, are presented. These include effect compartment modeling (the so-called linked model;[3], indirect response modeling (also referred to as
turnover models; [33]), transit compartment modeling [34], and more complicated modeling approaches that are combinations of several simple models.

**Effect compartment model**

The effect compartment modeling approach, which is based on a hypothetical effect compartment linked to another PK compartment by a first-order process (Figure 1-2), was proposed and elaborated by Dahlstrom et al.[16] and Sheiner et al. [35]. Briefly, the temporal dynamics of drug effect were modeled by linking a hypothetical effect compartment to the central (blood-containing) compartment of a PK model through a first-order distributional process (usually denoted as $K_{e0}$). In this construct, pharmacologic responses are assumed to be driven by concentrations in the hypothetical effect compartment. The rate constant governing effect offset ($K_{e0}$), which in actuality is the rate constant determining the time to equilibration of concentration between the central and hypothetical effect compartments, is estimated by collapsing the hysteresis loop in effects versus central-compartment concentration. This yields a singular relationship between effect and concentration at the effect site. In addition to the original effect compartment modeling approach (which is a parametric approach), semi-parametric variations have been proposed and elaborated by several groups [7, 36-39]. Although these approaches differ in mathematical detail, all semi-parametric approaches rely on the assumption of a hypothetical effect compartment, and most are directed at obtaining estimates of the rate constant $K_{e0}$ or its equivalent that then can be used to eliminate the hysteresis loop.
Effect compartment models are widely used in the PK-PD analysis of a variety of therapeutic agents [40] and the validity of this approach is evident when considering drugs with effect onset that is delayed due to slow distribution into effect site. Despite its popularity, the effect compartment model has its limitations. However, because estimation of some of the model parameters, particularly the EC50 value, is based on compressing the hysteresis loop, the method cannot distinguish among different factors that might contribute to temporal delay [35]. Viewed as a potentially mechanistic approach, the underlying assumption of the effect compartment model would restrict its usage to drugs that display PD hysteresis due to distributional delay. In practice, this strategy has been applied to drugs regardless of the mechanism of effect-onset delay. Applying the effect-compartment model to drugs with delays that cannot be explained by distributional factors could lead to misinterpretation of the results. For example, describing the inverse of Ke0 as an equilibrium half-life can be misleading in some cases, as the rate constant may reflect processes that are unrelated to establishing a concentration equilibrium between the effect site and the systemic circulation. In addition, the effect-compartment model assumes a simple first-order process for drug transfer from the systemic circulation to the hypothetical effect compartment; in some cases, this assumption may not be valid [7, 41]. In order to circumvent this problem, the effect-compartment model has been used in conjunction with other PD models, which will be discussed in the later in this review.

Conceptually the effect compartment model is appropriate for describing drug effects that lag behind drug concentrations in the systemic circulation due to distributional
delay into the receptor biophase. However, since this model has interpretational limitations when applied to other mechanisms of temporal delay, alternative approaches are attractive. One such alternative is the indirect response model (or turnover model) which was developed as a mechanism-based approach for pharmacologic responses that are indirect in nature [42].

**Indirect Response Model**

The indirect response (IDR) model has been proposed and elaborated by several research groups [42, 43]. Mechanism-based indirect response models incorporating different mechanisms of drug action were first systemically described by Dayneka and co-workers [42]. The theoretical basis for IDR models is the assumption that drugs can either stimulate or inhibit factors or processes that control production or loss of a response variable. The measured response change over time is expressed as the net result of production (controlled by zero-order rate constant $k_{in}$) and elimination (controlled by first-order rate constant $k_{out}$); a drug can stimulate or inhibit either one of these processes (Dayneka, Garg et al. 1993). Depending on the specific process altered by the drug, and whether the drug produces a “positive” or “negative” effect, IDR models can be divided into four subtypes (Figure 1-3): model I (inhibition of $k_{in}$, the zero-order rate constant that governs production of the response variable), model II (inhibition of $k_{out}$, the first-order rate constant that governs loss of the response variable), model III (stimulation of $k_{in}$), and model IV (stimulation of $k_{out}$) [42].
In addition to these four basic IDR models, other physiologically-relevant steps have been integrated into IDR models to describe drugs with complex pharmacologic responses including, but not limited to, (1) IDR models with a non-stationary baseline: precursor-dependent IDR model [44, 45], lifespan indirect response (LIDR) model for nature cells [46], and IDR models with physiologic limits [47]. Extended indirect response models have been reviewed in detail by Mager et al. [48].

The applicability of IDR models was shown by Jusko et al. using published data from various drugs such as warfarin, aldose reductase inhibitors, corticosteroids and others [49]. In addition, the effects of an interferon α-2a on MX protein synthesis were modeled by Nieforth et al. [50]; the antilipolytic effect of adenosine A1 agonist was adequately modeled using IDR model II [51], and the antihistaminic effect of bilastine was best described by IDR model I [52]. Lin et al. showed that an IDR model was a more appropriate approach for analyzing insulin response than the effect-compartment model [53]. Other examples of IDR model applications include prednisolone [54], clopidogrel [55], cibenzoline [56], and homocysteine [57].

As a mechanism-based approach, IDR models can be applied to situations in which PD hysteresis is due to PD, rather than PK, factors. Ideally, the appropriate model structure should be chosen based upon the mechanisms responsible for the effect delay. However, for new compounds, such information might not be known; in such cases, it can be challenging to choose among IDR models, or between a specific IDR model and an effect-compartment model, especially when parameters for model selection (e.g., Akaike
information criterion (AIC)) do not differ significantly among or between models. Multiple-dose or dose-ranging experiments may provide the data required to distinguish between models [58] as with IDR models the time to peak effect will change with dose (model II and IV) which is not the case for the effect compartment model.

**Other models**

In addition to linked and IDR models, other models have been used to analyze data that are characterized by hysteresis behavior, including, but not limited to, transit-compartment models (Figure 1-4) and receptor-based models that incorporate the association and dissociation rate constants for binding of the drug to the receptor. The transit-compartment model was proposed to account for the time delay in response when the signaling transduction process is believed to be the rate-limiting step in generating the pharmacologic response [59]. In addition, the transit-compartment model can serve as a “black box” to add delays resulting from unknown mechanisms into a PK-PD system. The rate at which drug associates with or dissociates from its receptor also can contribute to delayed effect onset, so this process has been incorporated into model structures [17].

The theoretical basis and applicability of the transit compartment model was discussed by Mager et al. [34]. The signal transduction/transit compartmental model has been subsequently utilized to model pharmacological effects of many therapeuticS such as corticosteroids [60], l-propranolol [61], scopolamine [62], metformin [63].

The transit-compartment model can be physiologically relevant when mechanisms of drug action are known. However, the signaling transduction pathway is a complex,
multiple-step process. When specific information regarding signal transduction for a particular drug is unavailable, the number of transit compartments included in the model is either arbitrarily decided or chosen based on goodness-of-fit criteria. Moreover, the transit-compartment model can be generalized to model drug effects that occur after a series of events. In this case, the best fit might be identified, but there is no guarantee that it represents the underlying physiologic processes that lead to the pharmacologic response.

Effect compartment models, IDR models, signaling transduction models, and other basic PD models can serve as building blocks for more sophisticated models required to analyze data for drugs that have complex mechanisms of action. Complex PD models are usually chosen after simpler models have failed to achieve an acceptable description of the biologic system. One scheme of a combined model has been suggested by Jusko et al. [64]. The influence of insulin on glucose clamp effects was modeled using an indirect response model along with the effect compartment to account for insulin concentration in the biophase [65]. The effect of ciloradine on heart rate also was described with a transit-compartment model coupled with an IDR model [66].

**Limitations associated with parametric compartmental modeling**

Integrated PK-PD modeling has been widely used as it provides a relatively simple approach for analyzing data associated with delayed pharmacologic responses. Although specific PK-PD models differ in structure, they all share similar limitations. For example, all parametric models rely on one or more assumptions that are hypothetical and difficult or impossible to test. Correct parameter estimation not only depends on meeting the
underlying assumptions of the model, but also on the nature of data set, including distribution of points along the time axis, variability in effect and concentration measurements, and the number of observations relative to the number of parameters in the model. Depending on experimental design and methods, model identifiability can be a limiting factor in the analysis [67].

Non-compartmental approaches

Despite the utility of integrated compartmental modeling, there are situations in which simpler approaches have value. For example, non-compartmental analysis based on statistical moment theory [68] is widely used in PK experiments. Unlike its compartmental counterpart, the non-compartmental approach has the advantage of not relying on assumptions of a specific model structure, and there is no need to use nonlinear least-squares regression techniques to obtain parameter estimates. Commonly-used non-compartmental parameters include, but are not limited to, the area under the concentration-time curve (AUC), the first moment of the concentration-time curve (AUMC), and the mean residence time (MRT). The only requirement for the application of statistical moment analysis to PK data is that the system from which the data were obtained is first-order. However, a truly non-compartmental approach to quantifying the time delay between elaboration of effect and production of concentration in the systemic circulation or in the target organ has not been extensively evaluated. A model-independent approach to obtaining information regarding time delays in PK and/or PD systems would represent a significant advantage for situations in which model-based approaches are inappropriate or
not feasible to implement. Because such delays result in an anti-clockwise hysteresis loop (between concentration in a target tissue and concentration in the systemic circulation for PK hysteresis, or between effect and concentration in either the systemic circulation or the target tissue for PD hysteresis), consideration of whether the morphology of the hysteresis loop itself may provide quantitative information on the magnitude of delay seems warranted.

**PK Hysteresis**

Hysteresis behavior has not been mentioned frequently in the PK (as opposed to PD) literature due to the fact that, in most situations, only systemic concentrations are measured and analyzed. When concentrations are determined at a peripheral site (tissue or extracellular fluid) in addition to the systemic circulation, data analysis strategies typically focus either on including both concentration-time functions in a compartmental model or on assessing the partition coefficient between the peripheral site and the systemic circulation. Plotting concentrations at the peripheral site against concentrations in the systemic circulation, which will result in a hysteresis loop if the rate of equilibration of drug between the two sites is slow relative to the time course of the experiment, rarely is done.

There are a few examples in which morphologic aspects of a hysteresis loop, including ABH, have been evaluated in a PK context [69]. For example, Ganzinger et al. evaluated the tissue distribution of antibiotics [69]. A hysteresis loop was produced by plotting drug concentrations at an extravascular site on y-axis against calculated
concentration in the peripheral compartment on the x-axis. The author showed that log-transformed ABH was proportional to log-transformed dose, and that a distribution coefficient could be obtained through linear regression of the data [69]. Upton et al. proposed an alternative method, an area fraction plot, to quantify transport delay during drug transit through an organ [70] as an analog of organ mean transit time (MTT). No studies, however, have focused on using graphical analysis of the hysteresis loop to characterize a PK distributational delay. The area under the systemic concentration-time curve (AUC) has proven extremely useful in PK data analysis because in all cases, at least for linear PK systems, it is simply the result of drug input (dose) and drug output (systemic clearance). In contrast, the area bounded by the hysteresis loop (ABH) formed by plotting a concentration at one site against a concentration at another site appears not to be useful in most situations because the size and shape of the hysteresis loop depends on multiple factors. Although ABH cannot be used to quantify the magnitude of distributational delay, other hysteresis descriptors such as hysteresis centroid [CH], and the unitless ratio of centroid x- vs. y-coordinate [CHR] may be more useful in characterizing distributational delay.

**PD hysteresis**

It was suggested in early studies on hysteresis behavior that the area enclosed by the hysteresis loop may contain information on how “deep” the effect compartment is relative to the reference compartment, which is usually the central compartment or the systemic circulation [71, 72]. However, similar to what was suggested previously for PK
hysteresis [70], a study of the pharmacologic effect on the QT\textsubscript{c} interval [73] showed that the size of the hysteresis loop does not always correlate with the magnitude of a time delay. In addition, there are other factors (e.g., the concentration scale) unrelated to the time delay that contribute to determination of the loop area. Thus, it is not surprising that the ABH by itself is uninformative in many situations. In addition, Rhodes et al. investigated the effect of inotropic drugs on the dynamic relationship between cyclic myoplasmic calcium levels and cardiac contractility [74]. Dynamic indices were derived from hysteresis loops formed by plotting left ventricular pressure versus intracellular calcium ([Ca\textsuperscript{2+}]). Their study showed that different inotropic drugs have a different impact on size, centroid, and orientation of hysteresis loops.

Only limited attempts have been made to investigate the utility of extracting information from a hysteresis loop to quantify a PD time delay. One study examined the area enclosed by the hysteresis loop, and the authors attempted to identify a metric that could be used as an indicator of a clinically-relevant effect delay without relying on parametric modeling [73]. A standardized convex area (H) that enclosed the original hysteresis loop was proposed as a metric instead of ABH. The authors showed that the standardized area was a better parameter than the T\textsubscript{max} difference (the difference between the time at which maximum concentration was observed and the time at which maximum effect was produced) for indicating an effect delay. To our knowledge, no previous studies have evaluated the use of the hysteresis loop for directly quantifying delays in effect elaboration.
Conclusion

The potential advantages of developing a non-compartmental or model-independent approach to identify and quantify distributional delays in PK or effect delays in PD are multifold. First, non-compartmental analyses are relatively simple to perform compared to their parametric modeling counterparts. Second, unlike model-based approaches, model-independent techniques rely on a limited set of assumptions or no assumptions whatsoever. Third, due to model identifiability issues some data sets simply are not amenable to parametric or semi-parametric analyses, and an alternative approach is required. Finally, results of model-dependent approaches may not be unambiguous, given either the hypothetical nature of the technique (e.g., effect-compartment models) or the inability to verify adherence to underlying assumptions. Given the scarcity of data addressing model-independent approaches to extracting information from PK or PD hysteresis loops, additional work in this area appears to be warranted.
Figure 1-1. Schematic illustration of concentration-effect profile with or without hysteresis behavior.

(A): Left Instantaneous equilibrium between drug concentration and effect; Right Resulting singular relationship between effect (E) and concentration (C). (B): Left Delayed production of effect relative to attainment of concentration; Right Resulting hysteresis loop in the relationship between effect and concentration.
Figure 1-2. Schematic illustration of effect compartment model.

Two compartment model with linked effect compartment model connected to the central compartment through a first order process. $K_{1e}$ and $K_{e0}$ represent the rates of flux into and out of the hypothetical effect compartment. CL is the systemic clearance, $K_0$ is input of drug by infusion. $CL_d$ is the distributional clearance between the central compartment and a peripheral compartment. $C_{blood}$ and $C_e$ represent the concentrations at central and effect compartment respectively.
Figure 1-3. Schematic illustration of the indirect response model (IDR) model.

The drug can act through downstream signaling pathways after its interaction with its receptor to either inhibit or stimulate $K_{in}$ (zero order rate constant for production of the response) and $K_{out}$ (first order rate constant for elimination of the response). Other symbols were defined in the legend of Figure 1-2.
Figure 1-4. Schematic illustration of transit compartment model.

The diagram describes a process in which delay between effect and concentration is modeled by adding number of transit compartments to include the signal transduction process that must take place before measurable effects are generated. $\tau$ is the mean transit time for each transit compartment.
Chapter 2

CHARACTERIZATION OF DISTRIBUTIONAL DELAY BY HYSTERESIS
ANALYSIS THROUGH SIMULATIONS

This chapter has been submitted for publication in *Pharmaceutical Research* and is presented in the style of that journal.

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Abstract

Purpose

Hysteresis often refers to an anti-clockwise loop in the effect-concentration profile when there is a temporal delay between the elaboration of effect and changes in drug concentration in the target tissue or the systemic circulation (pharmacodynamic [PD] hysteresis). However, hysteresis behavior also can be associated with the relationship between drug concentrations in tissue relative to drug concentrations in plasma (pharmacokinetic [PK] hysteresis). This study was conducted to explore the relationship between descriptors (area bounded by the hysteresis [ABH], the x- and y- coordinate of hysteresis centroid [CHX, CHY], and unitless ratio CHX/CHY [CHR]) of hysteresis formed by tissue vs. plasma concentrations under non-steady state conditions.

Methods

Various PK models were used to simulate drug concentration-time data with ADAPT (BSMR, Los Angeles, CA). ABH, CHX, CHY, and CHR for each data set were determined with Mathematica 8 (Wolfram Research, Champaign, IL) and OriginPro (OriginLab, Northampton, MA). The relationships between hysteresis descriptors and primary PK parameters (systemic clearance, distributional clearance, and apparent volumes of distribution) were examined.

Results

An increased delay between changes in drug concentrations in tissue relative to plasma resulted in non-linear and inconsistent relationships between ABH and the model
parameter(s) resulting in the time delay, which indicated that ABH cannot be used as a
surrogate parameter for the time delay. However, CHR was a linear function of the
magnitude of distributional delay, expressed as the reciprocal of the rate constant for egress
from the tissue compartment (e.g., $1/K_{21}$ for a linear two-compartment system).

Conclusions

This study demonstrated that CHR is a useful predictor of the time delay between changes
in concentrations in a target organ and changes in concentrations in plasma. Thus, a simple
non-compartmental analytical approach, one that is not dependent on the assumption of
linear disposition, can provide fundamental information regarding the distribution kinetics
of a substrate. Further studies are required to evaluate the utility of this approach with
experimentally-derived data, and to determine whether or not the mathematical technique
is applicable to PD hysteresis as well as PK hysteresis.
Introduction

For therapeutic agents with delayed onset of drug response, the plot of effect versus drug concentration will form a partial (after i.v. bolus administration) or complete (non-instantaneous administration) hysteresis loop. The presence of a hysteresis loop indicates that the time course of effect does not coincide with the time course of concentration, although it does not provide insight into the mechanism that might be responsible for delayed elaboration of response. Mechanisms that result in a temporal delay in the onset of drug response include slow distribution of drug from plasma to the effect site [8, 26], slow receptor kinetics [18], a cascade of biologic reactions after binding of the drug to its receptor before the observed drug effect is produced [75], or slow accumulation of an active metabolite at the site of action [23].

It has been suggested that the area bounded by the hysteresis loop (ABH) is determined by the magnitude of time delay, but that it cannot be used to directly quantify such a delay [71, 72]. Previous methods for assessing PD in the presence of hysteresis behavior therefore have focused on eliminating the hysteresis loop by parametric (or semi-parametric) PK-PD modeling. However, no method currently is available to utilize the hysteresis itself to quantify the magnitude of temporal dissociation between effect and concentration.

In order to identify a potential alternative method for analyzing concentration-effect data for drugs with a delayed response, the relationship among ABH (as well as other characteristics of the hysteresis loop), the magnitude of the time delay, and discrete PK-
PD parameters must be explored. For the specific example in which dissociation between the pharmacologic effect and concentration is due to an equilibration delay between the plasma-containing compartment (where drug concentration is usually measured) and the receptor biophase (where drug effect is elicited), the underlying reason for the hysteresis lies in the PK characteristics of the compound. In this case, plotting drug concentrations in the biophase or the target tissue (if measured, as in microdialysis studies for example) against concentrations in the plasma will result in a hysteresis. Similarly, plotting the concentrations measured in arterial blood entering an organ versus in venous blood leaving the organ also can result in hysteresis [70]. If fundamental information can be recovered from the hysteresis loop, such analyses may provide an analog of the commonly used non-compartmental approach, based on statistical moment theory, that allows recovery of important PK parameters without relying on mathematical modeling of the data [76]. Because hysteresis in PK represents a less complicated situation compared with that in PD, when hysteresis behavior may be due to the mechanism of drug action, and because many instances of PD hysteresis may in fact be due to disequilibrium between concentrations in the target organ and in plasma, examining hysteresis in PK is a good starting point for evaluating alternative data analysis approaches for hysteresis behavior. Previously, ABH has been examined in a PK context to a limited extent (e.g., to evaluate the distribution of antibiotic when the applicability of conventional PK modeling was limited [69]). However, comprehensive evaluation of this approach is lacking.
The present study was undertaken to explore the relationship between descriptors of hysteresis (ABH, the hysteresis centroid [CH], and the unitless ratio [CHR] of the centroid x-coordinate [CHX] to the centroid y-coordinate [CHY]) and PK parameters in a simple two-compartment system (with both linear and capacity-limited components) and in a transit-delay multi-compartment system. The specific goal of this study was to determine the utility of information contained in the hysteresis loop in characterizing the distributional delay between drug concentrations in plasma and in a target organ.

**Methods**

**PK models:**

Several series of simulations were performed to investigate the relationship between hysteresis and changes in PK parameters. Two simple PK models were used in these simulation experiments:

*Model one* was a simple two-compartment system (Figure 2-1). The distributional clearance (CL_d; unitless values ranging from 0.1 to 20, with a value of 0 collapsing the system to a single compartment) and volume of distribution in the peripheral compartment (V_t; unitless values ranging from 2 to 100) were varied individually while other model parameters were held constant. The differential equations describing this model are:

\[
\frac{dC_b}{dt} = \frac{k_0}{V_c} \left( \frac{CL + CL_d \times C_b}{V_c} + \frac{CL_d \times C_i}{V_c} \right) \quad (1)
\]

\[
\frac{dC_i}{dt} = \frac{CL_d \times C_b}{V_t} - \frac{CL_d \times C_i}{V_t} \quad (2)
\]
where $C_b$ is the concentration in the central compartment, $C_t$ is the concentration in the tissue compartment, $V_c$ is the apparent volume of the central compartment (unitless value of 10), and CL is the systemic clearance (unitless value of 1).

Because equilibration delay can be a function of nonlinear distributional processes, capacity-limited transfer between compartments was simulated by replacing the linear parameter $CL_d$ with the corresponding Michaelis–Menten parameters $K_m$ and $V_{\text{max}}$ ($V_{\text{max}} / (K_m + C)$). Values for $K_m$ and $V_{\text{max}}$ were selected so that the ratio of $V_{\text{max}} / K_m$, which represents the intrinsic clearance for the distributional process, corresponded to the values used for $CL_d$ in the linear-system simulations. Three conditions were used: (1) transfer from blood to tissue was controlled by a capacity-limited mechanism, but transfer from tissue to blood was a first-order process; (2) transfer from tissue to blood was saturable and transfer from blood to tissue was linear; (3) transfer in both directions was capacity-limited. The differential equations used in these three conditions were:

**Condition (1):**

\[
\frac{dC_b}{dt} = \frac{k_0}{V_c} - (CL + \frac{V_{\text{max}}}{K_m + C_b}) * \frac{C_b}{V_c} + \frac{CL_d * C_t}{V_c} \quad (3)
\]

\[
\frac{dC_t}{dt} = \frac{V_{\text{max}}}{K_m + C_b} * \frac{C_b}{V_t} - \frac{CL_d * C_t}{V_t} \quad (4)
\]

**Condition (2):**
Condition (3):

\[
\frac{dC_b}{dt} = \frac{k_0}{V_c} \left( CL + CL_d \right) C_b + \frac{V_{max}}{K_m + C_t} C_t
\]

\[
\frac{dC_t}{dt} = \frac{CL_d * C_b}{V_t} - \frac{V_{max}}{K_m + C_t} C_t
\]

Model two was constructed as shown in Figure 2-2. Transit compartments were added between the blood- and tissue-containing compartments to simulate a time delay that could cause hysteresis in the relationship between \( C_t \) and \( C_b \). One to three intervening compartments were added in separate simulations. For simplicity, the intervening compartments were assumed to have the same transit time (i.e., the same volume of distribution and distributional clearance) as the tissue compartment. The differential equations describing this system (for the example of two intervening compartments) were as follows:

\[
\frac{dC_b}{dt} = \frac{k_0}{V_c} \left( CL + CL_d \right) C_b + \frac{V_{max}}{K_m + C_t} C_t
\]

\[
\frac{dC_t}{dt} = \frac{V_{max}}{K_m + C_b} \frac{C_b}{V_t} - \frac{V_{max}}{K_m + C_t} C_t
\]

\[
\frac{dC_b}{dt} = \frac{k_0}{V_c} \left( CL + CL_d \right) C_b + \frac{CL_d * C_t}{V_c}
\]
Parameter selection:

The PK models used in this study were parameterized with starting values that were set to arbitrary units, and therefore were treated as unitless (CL = 1, CLd = 1, Vc = 10, and Vt = 15). A total of 80 simulations were performed with CL and Vc fixed at their starting values, while Vt and CLd varied between 2 and 100 and 0.1 and 20, respectively. The range of parameter values was designed to produce a sufficient range of the descriptors of hysteresis to facilitate interpretation of subsequent analyses. For model two, Vt was set to 20 and CLd varied between 0.1 and 50.

Descriptors and secondary parameters:

The non-compartmental descriptors of hysteresis examined in this study included the area bounded by the hysteresis loop [ABH], the hysteresis centroid [CH], and the unitless ratio [CHR] of the centroid x- [CHX] to centroid y- [CHY] coordinates. The mathematical equations that were used to determine their values are described below.

ABH was defined as the signed area of the loop formed by plotting concentration in the tissue compartment (Ct) against concentration in the blood compartment (Cb). The
hysteresis was simplified to a polygon defined by discrete data points, and ABH was calculated (Mathematica 8 and OriginPro 9.0) with an equation for signed partial area:

\[
Area = \frac{1}{2} \sum_{i=1}^{k} (x_i y_{i+1} - x_{i+1} y_i)
\] (13)

where \(x_i\) and \(y_i\) represent concentrations in the blood and tissue compartments, respectively, for each data point, and in summation \(x_{k+1} = x_1\) and \(y_{k+1} = y_1\) [10].

As a variation of ABH, ABHM, the area bounded by the hysteresis loop formed by plotting total mass of drug in the tissue compartment \((V_t*C_t)\) against total mass of drug in the blood compartment \((V_c*C_b)\), also was examined. Hysteresis based on mass was evaluated due to the likelihood that the numerical value of ABH would be affected by the value of the compartmental volumes of distribution, irrespective of the distributional time delay that may be present. Examining the behavior of the system in terms of mass, as opposed to concentration, would remove this confounding factor.

CH was determined by calculating the x- and y- coordinates of the centroid, respectively, using the following equations:

\[
C_x = \frac{1}{6A} \sum_{i=0}^{n-1} (x_i + x_{i+1})(x_i y_{i+1} - x_{i+1} y_i)
\] (14)

\[
C_y = \frac{1}{6A} \sum_{i=0}^{n-1} (y_i + y_{i+1})(x_i y_{i+1} - x_{i+1} y_i)
\] (15)
where A represents the ABH calculated for the corresponding loop, and the remaining variables are as described in equation (13).

Mean residence time in tissue (MRT$_t$) was calculated as:

$$MRT_t = \frac{AUMC}{AUC} - \frac{1}{K_{10}} - \frac{T_0}{2}$$  \hspace{1cm} (16)

Where AUMC and AUC were calculated using the concentration in plasma using trapezoidal methods [76]. $K_{10}$ is the rate of elimination calculated as $CL/V_c$ and $T_0$ is time of infusion which was 20 (arbitrary time units).

Pharmacokinetic simulations were conducted with ADAPT (BSR, Los Angeles, CA). Statistical analyses were performed using R (R Core Team, Vienna, Austria). The mathematical computation and data analysis were performed in both R and Mathematica 8 (Wolfram Research, Champaign, IL). Graphics were generated with R.

**Results**

Simulation experiments were performed to examine the influence of changes in PK parameters on descriptors of hysteresis and to compare hysteresis descriptors to the mean residence time in the tissue compartment (MRT$_t$). The first set of experiments utilized a linear two-compartment model (Figure 2-1). The second set of experiments was based on similar structural model (a two-compartment system) with capacity limited inter-compartmental transfer in one or both directions. The final set of simulations was based on the model described in Figure 2-2 in which one to three compartments were added between tissue and blood compartment to simulate prolonged transfer time.
**Experiment 1**

Concentration-time data were simulated in a linear two-compartment system with a variety of parameter value combinations selected to alter concentrations in the tissue compartment relative to concentrations in the central compartment, including the time to achieve distribution equilibrium. The data were subsequently analyzed as described in the Methods section to recover descriptors of the hysteresis loop.

**Influence of distributional clearance on hysteresis descriptors.** The influence of $C_{ld}$ on hysteresis descriptors, and how those relationships are modulated by $V_t$, is shown in Figure 2-3. The inter-compartmental distributional clearance, along with systemic clearance, determined the fraction of the administered dose that was presented to the peripheral compartment. The size of the hysteresis loop, whether expressed in terms of concentration (ABH) or mass (ABHM) revealed a nonlinear, polytonic relationship with $CL_d$. The ABHM vs. $CL_d$ relationship was clearly modulated by $V_t$. In contrast, the ABH-$CL_d$ relationship was relatively insensitive to changes in $V_t$. CHX decreased monotonically as $CL_d$ increased and asymptotically approached a minimum value; CHY increased monotonically as $CL_d$ increased, and reached a plateau when $CL_d$ increased beyond 5. For both centroid coordinates, $V_t$ modulated the relationship with $CL_d$. CHR evidenced a hyperbolic relationship with $CL_d$ because CHX and CHY changed in the opposite directions. $V_t$ had a minimum effect of the CHR-$CL_d$ relationship.

**Relationships between hysteresis descriptors and $K_{21}$.** The relationship between hysteresis descriptors and the secondary PK parameter $K_{21}$, which was calculated as the
CL_d / V_t ratio, also was explored. As with the primary PK parameters, most descriptors of the hysteresis loop (ABH, ABHM, CHX and CHY) evidenced a nonlinear relationship with K_{21} (Figure 2-4). The CHY - K_{21} relationship was approximately log-linear within a portion of the numerical range explored. The log-linear range was dependent on CL_d. CHR was proportional to K_{21} (R^2=0.99) across all simulation conditions (Figure 2-4).

**Relationships between hysteresis descriptors and MRT_t.** As shown in Figure 2-5, ABH did not display a linear relationship with MRT_t, as would have been expected if ABH was proportional to the magnitude of disequilibration between the tissue and blood compartments. The numerical value of ABH only corresponded to the magnitude of distributional delay when the delay was short and the change in delay (due to changes in PK parameters) was small. The numerical value of ABH is influenced by the numerical values of concentrations in the respective compartments (and in particular the range of lowest to highest concentration in tissue). As V_t increased, the resulting decrease in tissue concentrations led to a decrease in ABH, despite the fact that an increase in tissue volume would increase intercompartmental disequilibration. The simultaneous influence of V_t on concentrations and on MRT_t can be avoided by examining the hysteresis in terms of amount of drug, rather than drug concentration, in each compartment (ABHM). As expected, ABHM displayed a higher degree of correlation with distributional delay compared to ABH (Figure 2-5B). At a fixed value of CL_d, ABHM increased as MRT_t increased, although the relationship between ABHM and MRT_t was not proportionate. As the absolute amount of drug is rarely measured, ABHM has obvious practical limitations in analyzing
real data. Descriptors of the centroids of the hysteresis loop (CHX, CHY, and CHR) also were examined. CHX and CHY both evidenced nonlinear relationships with MRT. CHY was more sensitive to changes in MRT than CHX, because the scale of change in CHY is linked to concentrations in tissue. Interestingly, although both CHX and CHY evidenced nonlinear relationships with MRT, CHR (the ratio of CHX/CHY) increased linearly with MRT within each simulation group (same value for CLd but different values for Vt).

Experiment 2

The second set of simulation experiments was performed to investigate the impact of capacity-limited transfer on hysteresis behavior. Because methods of PK analysis based on statistical moment analysis are not applicable to nonlinear systems [68], a method for assessing distributional disequilibration that has no limiting assumptions may provide value. Therefore, it was of interest to determine how nonlinearity in the system affected the relationship between hysteresis descriptors and PK parameters. The relevant nonlinearity for modulating MRT would be capacity-limited transfer between compartments in either the uptake, efflux, or both directions. Such nonlinearities are relevant to protein-mediated translocation between blood and tissue [77]. The simulations were designed to explore the impact of each of these processes on hysteresis descriptors. The pharmacokinetic model used for these simulations is described in the Methods section. The ratio of Vmax to Km was constrained to the value of CLd (when applicable) to facilitate comparisons between linear and nonlinear systems.
Results of this series of simulations are shown in Figure 2-6 (although only data generated with conditions of CLd=2 are shown, due to space limitations). Three conditions were explored: capacity-limited influx (white circles), capacity-limited efflux (gray squares), and capacity-limited transfer in both directions (black triangles). Unlike in the linear system, none of the hysteresis descriptors were linearly correlated with any PK parameters (V_{max}, K_m, or MRT_t). As V_{max}/ K_m increased, hysteresis descriptors derived from different simulation conditions converged to the same value because with large V_{max}/ K_m, the system approached a simple linear system. With the exception of CHR, capacity-limited efflux had the largest impact on hysteresis descriptors (Figure 2-7). The differences in descriptors between capacity-limited influx and capacity-limited transfer in both directions were minimal under the conditions examined (Figure 2-7).

In order to account for the fact that the three different situations (capacity-limited influx, capacity-limited efflux, and capacity-limited bidirectional transfer) resulted in different dynamic ranges (difference between longest MRT_t and shortest MRT_t), the data were re-examined in terms of percentage change in MRT_t (Figure 2-8). When the data were examined in this manner, there was no longer an appreciable difference between nonlinear efflux and influx in total percentage change in MRT_t (25%-100%) while the nonlinear bidirectional process resulted in a smaller total percent change in MRT_t (data not shown). The rate of change (assessed via slope) for ABH, ABHM, and CHY with respect to the percent change in MRT_t was similar across the three situations. In contrast, the changes in CHX and CHR were different between saturable efflux and the other two conditions (influx
and bidirectional process). When efflux was changed, CHX was most sensitive to changes in MRTt, with minimal influence of changes in influx or bi-directional transport. In contrast, CHR did not change significantly when PK parameters controlling the efflux were manipulated, but was sensitive to changes in influx and bidirectional transport (Figure 2-8E).

Experiment 3

The final set of simulations was performed to explore the situation in which prolonged equilibrium delay is caused by the presence of intervening transit compartments between blood and tissue. The results of these simulations are displayed in Figure 2-9. For simplicity, the number of transfer compartments was used as a surrogate for the total distributional delay. In general, ABH and ABHM decreased with increasing degree of delay in distribution between the central compartment and the tissue compartment when CLd was small (CLd less than 3). When distributional clearance was large, additional transfer compartments did not affect either parameter. CHX and CHY decreased with increasing distributional delay across all values of CLd. CHR evidenced a positive relationship with distributional delay for all values of CLd exceeding 0.1.

Discussion

The presence of hysteresis in the concentration-effect profile has long been used as an indicator of temporal delay between the production of a specific concentration and the pharmacologic effect it elicits. Several PK-PD modeling approaches have been developed to deal with drugs with hysteresis behavior, with the predominant strategy being
compression of the hysteresis loop to obtain the relationship between effect and drug concentration in a “hypothetical effect compartment” [35, 78]. No particular emphasis has been placed on the hysteresis loop itself other than its use as an indicator of the existence of distributional or pharmacologic delay.

The area bounded by hysteresis loop (ABH) had rarely been examined closely in terms of how it relates to other PK-PD parameters, or its potential use as a stand-alone parameter to analyze PD data. Few studies have used ABH or evaluated other characteristics of the hysteresis loop to extract information [69]. For example, Ganzinger et al. explored the tissue distribution of antibiotics [69]. The hysteresis loop was formed by plotting drug concentration at an extravascular site on the y-axis together with concentration in the blood compartment on the x-axis. The authors showed that log-transformed ABH had a linear relationship with log-transformed dose, and that a distribution coefficient could be obtained through linear regression. Upton et al. proposed an alternative method (area fraction plot) instead of a traditional hysteresis plot to quantify transport delay when a drug transited an organ [70]. The area fraction plot proposed was an analogue of organ mean transit time (MTT). The authors suggested that the hysteresis loop itself cannot be used to quantify the time delay since it has concentration units on both x- and y-axes without a time unit [70], but no further analysis was performed. In this study, hysteresis descriptors (ABH, ABHM, CHX, CHY, CHR) were examined with respect to their potential in characterizing the distributional delay and in understanding the pharmacokinetic system.
The first experiment in this study (Figures 2-3 to 2-5) focused on the relationship between hysteresis descriptors and MRT\textsubscript{t} in a linear two-compartment system. The primary descriptor of the size of the hysteresis loop, ABH, was not proportional to MRT\textsubscript{t}. This was the expected result because MRT\textsubscript{t} was modulated by changes in V\textsubscript{t} as opposed to changes in CL\textsubscript{d}. The initial trajectory of the hysteresis plot is a reflection of how rapidly the drug distributed into the tissue compartment, while MRT\textsubscript{t} reflects how rapidly the drug exists the tissue compartment (and indeed is not affected by the initial rate of entry into the compartment). To further confound any potential relationships, numerical values of ABH decrease when V\textsubscript{t} is increased (due to the dilutional effect on concentration values of a larger volume) while simultaneously the equilibration delay is increased when V\textsubscript{t} increases. ABHM was evaluated simply to determine whether the dilutional effects on concentration truly do confound a potential relationship between hysteresis loop area and delays in distributional equilibration. While the present results suggested that ABHM may be more informative than ABH, the amount of drug in a tissue compartment is rarely measured, limiting the potential practical utility of this descriptor. Moreover, relationships between ABHM and MRT\textsubscript{t} segregated into two groups: those with CL\textsubscript{d} less than 1 and those with CL\textsubscript{d} larger than 1. For simulations with CL\textsubscript{d} less than 1 (0.1, 0.2 and 0.5), ABHM increased as CL\textsubscript{d} increased; in all other conditions, ABHM decreased as CL\textsubscript{d} increased. These specific results are due to the fact the systemic clearance was set to 1, and the directionality of change is dependent on whether distribution is the rate-limiting step (CL\textsubscript{d} <1) or systemic clearance is the rate-limiting step (CL\textsubscript{d} >1). Consistent with previous suggestions
analysis based on area bounded by the hysteresis loop was not useful because it does not result in a consistent relationship with the magnitude of distributional delay.

The centroid of a geometric figure has physical meaning as the center of mass for geometric shapes made with uniform materials. In the present study, the geometric figure was a hysteresis loop formed by plotting concentrations in one PK compartment against those in another PK compartment. The “uniform materials” requirement translates into an assumption that the confidence in the data obtained from each compartment is equivalent. In cases in which this assumption is not met, differential weighting of the data to reflect differential confidence (e.g., differential variability in measurement) could be used. Because the current simulations produced error-free data, differential weighting was not considered.

The parameters used to describe the centroid were the x-coordinate of the centroid (denoted as CHX), the y-coordinate of the centroid (denoted as CHY), and the ratio of x-coordinate to y-coordinate (CHR). Both CHX and CHY decreased as the MRTt increased. However, CHY decreased more rapidly than CHX because CHY is based on concentrations in the tissue compartment, which are sensitive to changes in Vt. The different rate of change in CHX and CHY with changes in MRTt was captured by CHR, which increased linearly with MRTt when CLd was kept constant within groups (i.e., when MRTt was modulated by changing Vt; Figure 2-5E). This result suggested that CHR may be a useful surrogate for the magnitude of distributional delay.
To examine the degree to which CHR may be a robust surrogate for a discrete pharmacokinetic parameter, the relationship between $\text{CL}_d/\text{V}_t$ (denoted as $K_{21}$) and the centroid of the hysteresis loop was explored (Figure 2-4). CHR evidenced a linear relationship with $1/K_{21}$ across all conditions, and data generated with different values of $\text{CL}_d$ collapsed to a single line with $R^2=0.99$. These results suggested that CHR was the most predictive parameter among all the hysteresis descriptors examined and can be useful in evaluating linear systems in which rates of equilibration between tissue and blood vary among experimental conditions (presuming concentrations in both the tissue and blood compartments are available).

Drugs can distribute from the blood to tissue by passive diffusion or via capacity-limited processes, which can behave like a linear system when the substrate concentration is low. It also was important to assess the extent to which the various descriptors of the hysteresis loop may be applicable to systems in which equilibration of substrate between tissue and blood is governed by at least one nonlinear process. Hysteresis descriptors in the presence of capacity-limited efflux were easily distinguished from those generated with capacity-limited influx or capacity-limited limited flux in both directions (Figure 2-7). With the exception of CHR, hysteresis descriptors in systems with capacity-limited efflux were most sensitive to changes in PK parameters. CHR was the only parameter found to be insensitive to changes in PK parameters in the model featuring capacity-limited efflux, but was relatively sensitive to changes in a model in which influx or bidirectional processes were saturable.
Not unexpectedly, and similar to other non-compartmental approaches for PK analysis, when nonlinear distributional processes were added to the system the relationship between hysteresis descriptors and PK parameters also became nonlinear. None of the hysteresis descriptors evaluated can be used as useful surrogates for distributional delay in a nonlinear system across a full range of conditions (from near-complete saturation of a nonlinear process to approximate linear behavior of that process). However, hysteresis analysis appears to have the capability of identifying a situation in which saturable transport is in the efflux-only direction. This capability may have limited utility in identifying the potential for transport-related drug interactions such as that which might occur with P-glycoprotein inhibition at the blood-brain barrier [79].

Non-mammillary systems also may have delays in substrate equilibration between a target tissue and blood. Transit compartments were added between the blood compartment and tissue compartment to model distributional delays that are not dependent on the volume of the tissue compartment or the rate of flux out of the blood compartment. In this system, with the exception of the lowest value of Cl_d (10-fold lower than CL), CHR tended to increase nearly proportionately with the number of transit compartments (and therefore with the delay in equilibration between the tissue and blood compartments). Taken together with the results of previous simulations, this observation suggests that CHR is a robust indicator of the delay in equilibration of concentrations between a tissue compartment and the systemic circulation.
It must be emphasized that, in order to accurately calculate the area bounded by hysteresis loop, an adequate number of measurements must be made in both blood and tissue. Equation (13) was used in the present study because of the ease of programming this equation to run analyses in batch mode. It also has the advantage of being a built-in function in a commercially available program (OriginPro). However, the equation is applicable for calculating ABH only when the hysteresis plot consists of a single loop [80]. This is the typical situation when variability in the data is low or when group-averaged data are used. When more than one loop is present in a hysteresis plot due to measurement errors, random variation, or the presence of multiple time-dependent functions, this equation would not be appropriate.

Conclusions

The presence of a hysteresis loop has long been used as an indicator of temporal disassociation between the production of pharmacologic response and the presentation of drug to the systemic circulation. Distributional delay between a target tissue and blood is one major contributor to the hysteresis behavior observed in effect-concentration profiles, and in and of itself results in a hysteresis when tissue concentrations are related to blood concentrations. This study evaluated the relationship between several hysteresis descriptors (ABH, ABHM, CHX, CHY, and CHR) and PK parameters in three basic PK systems, and the potential utility of these descriptors in characterizing distributional delay. CHR was a linear function of the secondary pharmacokinetic parameter $K_{21}$ ($CL_d/V_t$) under most conditions simulated, while other parameters displayed nonlinear (and therefore non-
predictive) relationships with PK parameters. These results suggest that CHR may be useful in characterizing the distributional delay between blood and target tissues in linear PK systems. Future efforts will be directed towards evaluating the applicability of CHR to observational, rather than simulated, PK data. In addition, this analytical strategy will be extended to the realm of PD, where the presence of hysteresis loops has attracted the most attention.
Figure legends

Figure 2-1. Schematic representation of the primary PK model.

CL is the systemic clearance; $V_c$ and $V_t$ are volumes of the central and tissue compartment respectively; $CL_d$ is the distributional clearance between blood and tissue compartment; $K_0$ represents the infusion rate. For simulations of nonlinear systems, $CL_d$ was replaced with $V_{\text{max}}$ and $K_m$ in one or both distributional directions.

Figure 2-2. Schematic representation of the transit-compartment model.

Multiple compartments (represented by 1 and 2 in the diagram) were added between blood and tissue compartment. The number of transit compartments varied between 0 and 3 in separate simulations. Symbols used are the same as explained in Figure 2-1.

Figure 2-3. Relationships between descriptors of the hysteresis loop and distributional clearance ($CL_d$).

(A) ABH, (B) ABHM, (C) CHX, (D) CHY, (E) CHR. Abbreviations and the method of calculation for each hysteresis descriptor are included in the Methods section. Symbols indicate different values of $V_t$: 0.5 (white circles), 2 (grey circles), 3 (black circles), 5 (white squares), 10 (grey squares), 15 (black squares), 30 (white triangles), 40 (grey triangles), 50 (black triangles), and 100 (white diamond).

Figure 2-4. Relationships between descriptors of the hysteresis loop and the secondary PK parameter $K_{21}$ ($CL_d/V_t$).

(A) ABH, (B) ABHM, (C) CHX, (D) CHY, (E) CHR. In Panel (E), the line represents the results of linear least-squares regression. Abbreviations and the method of calculation for
each hysteresis descriptor are included in the Methods section. Symbols indicate different values of $C_{ld}$: 0.1 (white circles), 0.2 (grey circles), 0.5 (black circles), 1 (empty squares), 2 (grey squares), 5 (black squares), 10 (white triangles), and 20 (grey triangles).

**Figure 2-5.** Relationships between descriptors of the hysteresis loop and mean residence time in tissue ($\text{MRT}_t$).

(A) ABH, (B) ABHM, (C) CHX, (D) CHY, (E) CHR. Abbreviations and the method of calculation for each hysteresis descriptor are included in the Methods section. Symbols indicate different values of $C_{ld}$ and are the same as Figure 2-4.

**Figure 2-6.** Relationships between descriptors of the hysteresis loop and $V_{\text{max}}$.

(A) ABH, (B) ABHM, (C) CHX, (D) CHY, (E) CHR, (F) MRT$_t$. Separate simulations were conducted for capacity-limited influx (white circles), capacity-limited efflux (gray squares), and capacity-limited processes in both directions (black triangles). Abbreviations and the method of calculation for each hysteresis descriptor are included in the Methods section.

**Figure 2-7.** Relationships between descriptors of the hysteresis loop and MRT$_t$ with capacity-limited transfer between the blood and tissue compartment.

(A) ABH, (B) ABHM, (C) CHX, (D) CHY, (E) CHR, (F) $V_{\text{max}}$. Separate simulations were conducted for capacity-limited influx (white circles), capacity-limited efflux (gray squares), and capacity-limited processes in both directions (black triangles). Abbreviations and the method of calculation for each hysteresis descriptor are included in the Methods section.

**Figure 2-8.** Relationships between descriptors of the hysteresis loop and standardized MRT$_t$ with capacity-limited transfer between the blood and tissue compartment.
(A) ABH, (B) ABHM, (C) CHX, (D) CHY, (E) CHR. Separate simulations were conducted for capacity-limited influx (white circles), capacity-limited efflux (gray squares), and capacity-limited processes in both directions (black triangles). Abbreviations and the method of calculation for each hysteresis descriptor are included in the Methods section.

**Figure 2-9.** Relationships between descriptors of the hysteresis loop and the number of compartments in a transit-compartment system.

(A) ABH, (B) ABHM, (C) CHX, (D) CHY, (E) CHR. Abbreviations and the method of calculation for each hysteresis descriptor are included in the Methods section. Symbols indicate different values of $C_{ld}$: 0.1 (white circles), 0.5 (grey circles), 1 (black circles), 2 (white squares), 3 (grey squares), 5 (black squares), 10 (white triangles), 20 (grey triangles), and 50 (black triangles),
Figures

Figure 2-1. Schematic representation of the primary PK model.
Figure 2-2. Schematic representation of the transit-compartment model.
Figure 2-3. Relationships between descriptors of the hysteresis loop and distributional clearance ($CL_d$).
Figure 2-4. Relationships between descriptors of the hysteresis loop and the secondary PK parameter $K_{21}$ ($CL_d/V_t$).
Figure 2-5. Relationships between descriptors of the hysteresis loop and mean residence time in tissue (MRT_t).
Figure 2-6. Relationships between descriptors of the hysteresis loop and $V_{\text{max}}$. 
Figure 2-7. Relationships between descriptors of the hysteresis loop and MRT\textsubscript{t} with capacity-limited transfer between the blood and tissue compartment.
Figure 2-8. Relationships between descriptors of the hysteresis loop and standardized MRT\text{t} with capacity-limited transfer between the blood and tissue compartment.
Figure 2-9. Relationships between descriptors of the hysteresis loop and the number of compartments in a transit-compartment system.
Chapter 3

CHARACTERIZATION OF DISTRIBUTIONAL DELAYS

BY HYSTERESIS ANALYSIS:

APPLICATION TO MICRODIALYSIS EXPERIMENTS

This chapter has been submitted for publication in Drug Metabolism and Disposition and is presented in the style of that journal
Abstract

Purpose

This study was conducted to examine the utility of descriptors of the hysteresis loop formed when plotting concentrations in a target organ vs. concentrations in plasma in quantifying delays in drug equilibration, and how this method compares to standard pharmacokinetic analysis approaches.

Methods

Methotrexate concentration-time data in plasma and brain tumor microdialysate were mined from a clinical study [81] of four subjects (two with normal blood-brain barrier function, two with disrupted barrier function) and analyzed by traditional compartmental and non-compartmental techniques, as well as by the hysteresis approach. Hysteresis descriptors (area bounded by hysteresis loop [ABH] and the unitless ratio of centroid x- vs. y-coordinate [CHR]) of each data set were determined with Mathematica.

Results

A singular pharmacokinetic model was not capable of describing the data in the two subjects with disrupted blood-brain barrier function. In addition, the concentration-time data in one subject were not sufficient to support valid extrapolations for area-under-the-curve analysis in tumor microdialysate. However, consistent with simulations, CHR was approximately 10-fold lower in the subjects with a disrupted blood-brain barrier, in whom equilibration of drug concentrations between brain tumor microdialysate and plasma should be relatively rapid, as compared to the subjects with normal barrier function.
Conclusions

The study demonstrated that hysteresis analysis, with CHR as the primary metric, is useful in characterizing distributional delays between concentrations in a target tissue and plasma. In agreement with previous simulations, application of the method to clinically-derived data produced consistent and sensitive results. This approach does not suffer from limitations associated with compartmental modeling (failure of a unique model to describe each data set) or non-compartmental analysis based on statistical moment theory (inability to extrapolate areas under the curve through infinite time with accuracy).
**Introduction**

For therapeutic agents that evidence demonstrate onset of response, the plot of effect versus drug concentration will form a partial (after i.v. bolus administration) or complete (after any form of non-instantaneous administration) counter-clockwise hysteresis loop. The presence of a hysteresis loop in the effect vs. concentration profile is used as evidence that the kinetics of pharmacologic response are dissociated from the kinetics of drug disposition [82]. It has been suggested that the area bounded by the hysteresis loop (ABH) is dependent upon the magnitude of kinetic-dynamic dissociation, but that it cannot be used to quantify the delay in response onset [70, 83]. Previous methods for assessing pharmacodynamics in the presence of hysteresis behavior therefore have focused on compressing the hysteresis loop by parametric or semi-parametric pharmacokinetic-pharmacodynamic (PK-PD) modeling [35, 38].

Hysteresis behavior also can be observed in drug disposition if concentrations are measured in blood and at an extravascular site. When there is a delayed equilibration of drug concentration between a target organ and plasma, a plot of target-organ concentration vs. plasma concentration will form a counter-clockwise hysteresis loop [82]. In comparison to pharmacodynamic hysteresis behavior, pharmacokinetic hysteresis is rarely examined. In most cases when drug equilibration between tissues and blood is slow, traditional compartmental modeling (i.e., recovering estimates of distributional clearance) or non-compartmental approaches (i.e., estimating mean residence time in tissue) provides satisfactory quantification of distributional delay [76]. However, there are situations (for
drugs that act in the central nervous system [CNS], for example) in which concentrations in the target organ, and the time required to achieve equilibration between the target organ and the systemic circulation, and are of great interest, but the data recovered in a given experiment may not be amenable to analysis by compartmental modeling or by traditional non-compartmental techniques.

Microdialysis has been widely used to measure concentrations of therapeutic agents in peripheral tissues when substrate equilibration between tissue and plasma may not be rapid. For drugs that act in the CNS, slow equilibration of concentrations between tissue and blood often is due to the impedence of drug distribution presented by the blood-brain barrier (BBB) [84]. The disposition of drugs that act on the CNS can be challenging to model due to the presence of capacity-limited efflux and/or capacity-limited influx in addition to passive diffusion [85]. Moreover, disease states that impact the integrity of BBB, variation in the expression of transporters, and the presence of other drugs or xenobiotics collectively can change the rate and extent of drug distribution into the brain [86, 87].

Microdialysis is an approach for obtaining serial estimates of drug concentration in extracellular fluid of tissues of interest. In humans, microdialysis has been used in several tissues, most commonly skeletal muscle [88] and the subcutaneous space [89]. Although microdialysis is used routinely for pharmacokinetic studies in brain tissue in preclinical species [90], its use for this purpose in humans is infrequent. Nevertheless, the impedance of drug distribution presented by the BBB, and the potential for that impedance to differ
between patients, makes brain tissue microdialysis an interesting case study for exploring relevant data analysis techniques.

A previous study (Chapter 2) utilized a series of mathematical simulations to examine the relationship between pharmacokinetic hysteresis descriptors (the area bounded by the hysteresis [ABH], the hysteresis centroid [CH], and the unitless ratio of centroid x- vs. y-coordinate (CHX/CHY) [CHR]) and the pharmacokinetic parameters (distributional clearance and the apparent volume of the peripheral compartment) that determine the rate of drug concentration equilibration between a peripheral compartment and the central compartment. Among the hysteresis descriptors examined, CHR was found to be the most useful in identifying equilibration delay. CHR increased proportionately with magnitude of delay (expressed as the inverse of the rate constant governing flux from the peripheral compartment to the central compartment) in a simple two compartmental model with linear pharmacokinetics. The present study was conducted to extend these simulation results by applying the analytical method to observational data. To this end, data were mined from a clinical study [81] in which drug concentrations were determined in brain (by microdialysis) and in plasma from four patients receiving chemotherapy for brain tumors. Two patients had disrupted BBB, and two had intact BBB, allowing assessment of the degree to which hysteresis analysis can identify differences in equilibration rate. For comparison, these data also were analyzed with traditional approaches (parametric modeling and non-compartmental methods) to characterize distributional delay under non-steady state conditions.
Methods

Data-mining

In order to compare hysteresis analysis and traditional approaches, data were mined from a representative study in which drug concentrations in the plasma and the target organ (brain extracellular fluid; B_{ECF}) were reported in four patients [81]. This study had been designed to characterize the distribution of methotrexate into brain tumors utilizing microdialysis to determine methotrexate concentrations in brain tissue. The microdialysis probe was placed in the contrast-enhancing region of the tumor (an indication of disrupted BBB) in two patients (patients A and B) or in the non-enhancing region in two other patients (patients C and D). Methotrexate sodium at dose of 12-g/m² was given to each patient by continuous infusion over 4 hr. Plasma samples were collected before administration and at 1, 2, 3, 3.9, 4.1, 4.25, 4.5, 4.75, 6, 8, 10, 12, 16, 20, 24, and 28 hr after starting the infusion. Microdialysate was collected at 30-min intervals from 1 hr before to 24 hr after the infusion.

Three approaches were used to analyze data: hysteresis analysis, compartmental modeling, and non-compartmental approaches.

Calculation of hysteresis descriptors

The hysteresis loop was constructed by plotting concentrations in brain microdialysate against concentrations in the plasma obtained at the same time points. The descriptors of the hysteresis loop examined in this study included the area bounded by the hysteresis loop [ABH], the hysteresis centroid [CH], and the unitless ratio of centroid x-
versus y-coordinate (CHX/CHY) [CHR]. The mathematical equations that were used to determine the values of these descriptors are described below.

**ABH** was defined as the signed area of the loop formed by plotting concentrations in the tissue compartment ($C_t$) against concentrations in the blood compartment ($C_b$). The hysteresis was simplified to a polygon defined by discrete data points, and ABH was calculated (Mathematica 8 and OriginPro 9.0) with an equation for signed partial area:

$$\text{Area} = \frac{1}{2} \sum_{i=1}^{k} (x_i y_{i+1} - x_{i+1} y_i)$$

(1)

where $x_i$ and $y_i$ represent concentrations in the blood and tissue compartments, respectively, for each data point, and in summation $x_{k+1} = x_1$ and $y_{k+1} = y_1$ [91].

Similarly, **CH** was determined by calculating its x- and y-coordinates respectively, using the following equations:

$$C_x = \frac{1}{6A} \sum_{i=0}^{n-1} (x_i + x_{i+1})(x_i y_{i+1} - x_{i+1} y_i)$$

(2)

$$C_y = \frac{1}{6A} \sum_{i=0}^{n-1} (y_i + y_{i+1})(x_i y_{i+1} - x_{i+1} y_i)$$

(3)

where $A$ represents the ABH calculated for the corresponding loop, and the identity of the other symbols is as described in equation (1). Finally, the ratio of the CH coordinates (CHR) was calculated by dividing $C_x$ by $C_y$. 
**Parametric modeling**

Compartmental modeling was performed in ADAPT (BMSR, Los Angeles, CA) by fitting the concentration-time profiles in both plasma and brain ECF simultaneously with appropriate model equations. Up to 6 structural models were evaluated for each data set (the concentration-time profiles for each patient). The models evaluated included one (no distributional delay), two, or three compartments and various combinations of linear and capacity-limited flux processes. The optimal model for each data set was identified among the alternative models based on lack of parameter covariance, and absence of bias in residual error in describing the concentration-time profiles. Parameter estimates from the optimal model for each patient were reported and used for comparison with the other analytical methods.

An attempt was made to fit the same model to data from all four patients, but no satisfactory solution was obtained. The data from patients A and B were best described by a two-compartment linear model with brain ECF (C_{Becf}) as part of central compartment (no equilibration delay). Brain ECF concentrations therefore were related to plasma concentrations with a partition coefficient (K_p). The data from patient C were best described with a three-compartment linear model (central compartment, peripheral compartment, and brain ECF compartment). The data from patient D were best described with a two-compartment model (central compartment and brain ECF compartment). Because the microdialysis approach is associated with a lag time (the time required for microdialysate to flow from the tip of the probe to the exit of the collection tubing), and
because tubing length and diameter were not reported (parameters that are required to calculate the dead volume in the tubing), inclusion of a lag time for appearance of methotrexate in microdialysate samples was evaluated. Based on the model selection criteria described above, inclusion of a lag time was necessary only for patient C.

**Non-compartmental analysis**

Mean residence time (MRT<sub>t</sub>) was estimated by statistical moment analysis [76]. Areas under the concentration versus time profile, and areas under the concentration*time product versus time profile (the first moment of the concentration-time curve) were calculated with the piecewise trapezoidal method through the final data point collected. Extrapolation of the area through infinite time was performed using standard approaches [76]. MRT<sub>t</sub> for patients A and B was calculated as:

\[
MRT_t = \frac{AUMC_t}{AUC_t} = \frac{\int_0^\infty t \times C_t dt}{\int_0^\infty C_t dt}
\]

where \( C_t \) represents the concentration in brain ECF. AUMC<sub>t</sub> and AUC<sub>t</sub> were calculated by numerical integration using the trapezoidal method and extrapolated to infinite time. MRT<sub>t</sub> for patients C and D were calculated from model-derived parameters according to equations (5) and (6) (for patients C and D respectively):

\[
MRT_t = \frac{V_s}{CL_{13}} - T_{lag-time}
\]

\[
MRT_t = \frac{V_s}{CL_{13}}
\]
MRT in the systemic circulation also was calculated using equation 4 and adjusted for half of the infusion time.

**Software**

Statistical analysis and graphics was performed in R (R Core Team, Vienna, Austria). The mathematical computation and data analyses were performed in both R and Mathematica 8 (Wolfram Research, Champaign, IL). Digitalization of published graphs was accomplished with the Digitizer tool included in OriginPro (OriginLab Corporation, Northampton, MA). Pharmacokinetic simulation and modeling were performed in ADAPT (BMSR, Los Angeles, CA).

**Results**

A previous simulation study (Chapter 2) demonstrated that CHR was proportional to the inverse of the egress rate constant controlling drug flux from a tissue compartment to the blood compartment in a two-compartment model with linear pharmacokinetics. CHR is a unitless parameter derived from concentrations in both blood and target tissue. The data obtained from a microdialysis study on methotrexate (MTX) in brain tumor tissue [81] were selected to demonstrate the applicability of the approach to actual data as opposed to simulated data. The time course of MTX concentrations in both plasma and brain ECF in each patient were mined from the study, and graphs were reconstructed as shown in Figure 3-1.

Although the authors of the original study reported results from compartmental modeling and non-compartmental analysis, the coefficients of variation for parameter
estimates were not reported, so the accuracy of parameter estimation could not be assessed. The first attempt in the present study was to remodel the data using the model and approaches described by the authors, but it was not possible to replicate the results reported in the paper. In order to provide an objective comparison among the three data analysis approaches, the data obtained from the paper were analyzed independently.

**Compartmental Modeling**

Results from compartmental modeling are summarized in Figure 3-1. For the two patients with disrupted blood-brain barrier (BBB) function (patients A and B), drug concentrations in brain ECF were in rapid equilibrium with those in plasma. The optimal models describing these two data sets (Figure 3-2) were consistent with expectations for distributional behavior when the microdialysis probe is located in a region with disrupted BBB. For both of these patients, concentrations in brain ECF were modeled as part of the central compartment, with a fixed (time-independent) partition coefficient ($K_p$). Relevant parameter estimates are provided in Table 3-1 for each patient. The performance of the model was judged to be satisfactory given the accuracy of the parameter estimates (Table 3-1) and the modest residual error associated with describing the data (Figure 3-1).

The concentration-time profiles for $C_{Beef}$ in patients C and D were distinctly different from those in patients A and B. Despite evaluating a wide range of model structures, the best-fit model evidenced identifiability issues (acceptable residual error but large coefficients of variation on parameter estimates, especially for parameters related to distribution into and within brain ECF) when concentrations in the brain were modeled
simultaneously with concentrations in plasma. The best-fit model for patient C also required addition of a lag time to account for the time required for dialysate to traverse the tubing.

**Non-compartmental approaches**

AUC and MRT with extrapolation to infinity were calculated for each subject and summarized in Table 3-1. In patients A and B, the mean residence time of MTX in brain ECF was identical to the mean residence time of MTX in plasma because the brain tissue compartment accessible by microdialysis merged with the central compartment secondary to BBB disruption. In the patients with intact BBB function, MRT\textsubscript{t} was larger than MRT, consistent with slow equilibration of MTX between the two compartments. As the time required for equilibration increased, the percentage of the total area contained in the tail of the curve (the time domain exceeding the final observed data point) increased (Table 3-1).

**Hysteresis analysis**

Hysteresis loops were constructed by plotting concentrations in the brain ECF against concentrations in the plasma for each patient (Figure 3-3). Because the sampling frequency was different for blood (samples obtained at discrete time points) and brain ECF (samples obtained over a time interval), and because microdialysis sampling provided more data points than blood sampling, the hysteresis plots represent a combination of observational data (for blood samples obtained at a time compatible with microdialysis samples) and interpolated values (when microdialysis samples were obtained at points in time between sequential blood samples). Hysteresis descriptors (ABH, CHX, CHY, and
CHR) were calculated as described in the Methods section, and results are summarized in Table 3-2. Patients with disrupted BBB function (A and B) had markedly lower values for both mean residence time in brain ECF and CHR as compared to patients with intact BBB function (C and D). Comparing the average values for intact BBB to disrupted BBB, the hysteresis approach had a larger dynamic range (15.9-fold) compared to the mean residence time approach (4.6-fold), suggesting that CHR may be a more sensitive identifier of differences in distributional delay. In addition, the hysteresis approach appeared to be more consistent (54% difference between patients C and D) than the mean residence time approach (287% difference between patients C and D) when equilibration between brain ECF and plasma was slow. The parameter estimation of $V_3$, $CL_{31}$ that was used to calculated $MRT_t$ had larger coefficient of variation. When equilibration was rapid (patients A and B), the two methods had approximately equivalent consistency (approximately 15% difference between patients A and B).

As suggested in previous simulations (Chapter 2), a larger ABH did not always correspond to a larger disequilibrium between tissue and blood compartments, and the relationship between ABH and $MRT_t$ was non-linear. In the present study, patients with a disrupted BBB (A and B) had a much larger ABH compared to patients with intact BBB function (C and D) which again suggested that ABH was not a reliable predictor of the magnitude of disequilibrium delay. CHX had minimal difference between patients, although patient B appeared to be an outlier. CHY appears to be inversely related to the magnitude of the time delay, with the largest CHY associated with no distributional delay.
This is consistent with simulation results (Chapter 2) in which CHY decreased monotonically as MRT\textsubscript{t} increased.

**Discussion**

The goal of this study was to test the utility of hysteresis descriptors such as CHR in characterizing distributional delay between the plasma and a target tissue based on published data mined from clinical studies. Previously, the relationship between morphological descriptors of the hysteresis loop, termed hysteresis descriptors (ABH, CHX, CHY and CHR), and pharmacokinetic parameters (CL\textsubscript{d}, V\textsubscript{t}, and the ratio of V\textsubscript{t}/CL\textsubscript{d}) were investigated in simulation experiments (Chapter 2). Of the descriptors examined, CHR was identified as the only parameter predictive of the magnitude of time delay in equilibration of concentrations between the target organ and blood. This analysis revealed that the relationship between CHR and V\textsubscript{t}/CL\textsubscript{d} was linear, which was consistent with the expectation that the slope of the hysteresis loop (equivalent to the inverse of CHR if the line bisecting the loop goes through the origin) is largely determined by the rate of transfer between compartments [70].

*Comparison of traditional pharmacokinetic analysis approaches with hysteresis analysis*

After CHR was identified as the best descriptor of distributional delay based on simulated data, the next step was to test this result by analyzing observational data. For purposes of expediency, it was decided to utilize clinical data from a previously-published study. The study performed by Blakeley et al. [81] provided suitable data for this purpose, which included (1) evaluating whether CHR changed significantly when the distribution
kinetics of a drug changed, and (2) comparing the hysteresis analysis approach to the well-accepted methods of compartmental-based modeling and non-compartmental analysis based on statistical moment theory. Of the four data sets obtained from this publication, two were representative of minimal distributional delay between plasma and brain ECF due to disrupted BBB function, and two represented a situation in which significant distributional delay existed due to the presence of a functional BBB.

In general, compartmental modeling of the data provided a satisfactory description of the concentration-time profiles for all four subjects. However, there was no singular unifying model that provided an acceptable description across all subjects, which impedes comparisons of parameters among data sets. Perhaps more importantly, the accuracy of parameter estimation in the presence of slow substrate equilibration between brain ECF and plasma (patients with intact BBB function) was poor (large coefficients of variation), which significantly reduces the confidence that the parameter estimates are representative of the actual disposition of the drug.

The difficulty encountered in modeling the data with traditional compartmental approaches might be due in part to the data structure of the brain ECF concentration-time profile. For patient C, fewer data points were available during the distributional phase; for patient D, in addition to the lack of data in the early distributional phase, the elimination phase was flat and therefore did not offer much information for parameter estimation. This type of data deficiency is not uncommon in pharmacokinetic studies, and results in the
identification of “optimal” models that are somewhat arbitrary in the sense that model structure is dictated by data structure.

Although the modeling performed by the authors of the original paper appeared to provide an acceptable description of the data, it was not possible to reproduce those results even when a similar model structure was used. There are several possible explanations for this outcome. For example, it might due to use of a different modeling approach: the authors fit plasma concentration-time data first, and used the optimized parameters for disposition in plasma as an input function for brain ECF. This stepwise modeling approach is not unique, but often is reserved for more complex modeling problems [32].

Regardless of the specific reason for the lack of correspondence between the original modeling results and the re-analysis reported herein, the disadvantages often associated with compartmental-based analysis of pharmacokinetic data are highlighted: (1) No singular unifying model could be identified to describe the data obtained from all patients. The need to use different model structures for different patients makes parameter comparisons less informative. (2) It was not possible to achieve accurate parameter estimation for some patients, an indication that more information was required to support the model. (3) Overall, compartmental modeling of these particular data was found to be inadequate for drawing conclusions regarding the magnitude of distributional delay in patients with intact BBB function compared to patients with a compromised BBB.

Traditional non-compartmental pharmacokinetic analysis based on statistical moment theory requires determination of the area under the concentration-time curve
(AUC), and the first moment of the concentration-time curve (AUMC), both extrapolated through infinite time. As a general rule, area extrapolations should not exceed 20%-30% of the total area reported in order to obtain estimates with acceptable precision [92]. The present data set, emphasized by the brain ECF concentration-time profile for patient D, illustrates an inherent difficulty with the statistical moment approach. When the rate of decline in concentration is slow relative to the overall time frame for the experiment, a larger degree of area extrapolation is required. In addition, the accuracy with which the terminal rate constant for the concentration-time profile can be estimated is diminished when the terminal phase is relatively “flat”, as was the case for brain ECF concentrations in patient D. Because an estimate of the terminal rate constant is required to extrapolate AUC through infinite time, and because the square of the terminal rate constant is used to extrapolate AUMC through infinite time, errors in estimating this key parameter result in magnified uncertainty in the calculated areas; this uncertainty carries through to the calculation of the mean residence time.

Compared with the two traditional data analysis methods, hysteresis analysis resulted in each data point contributing equally to determining the position of the centroid (CHX, CHY, and CHR), with no magnified importance of data in the distributional phase (as is the case in compartmental analysis) or in the terminal phase (as is the case in statistical moment analysis). In quantifying the difference in distributional behavior in patients with disrupted BBB function compared to patients with intact BBB function, the hysteresis analysis approach was clearly superior. Because different compartmental model
structures were required to fit the data obtained from different patients, it was not possible to use this approach to quantify distributional differences. The sensitivity of MRT₁ to differences in distributional equilibration was approximately three-fold less than the sensitivity of CHR, and MRT₁ appeared to be less consistent between subjects with intact BBB function (slow equilibration) than CHR.

Conclusions

The present study demonstrates that hysteresis analysis, with CHR as the primary metric, may be useful in identifying distributional delays between a target tissue and plasma in situations in which traditional data analysis methods fail or are inappropriate. While the present study is not definitive due to the limited number of concentration-time profiles evaluated, the fact that the results are consistent with those obtained in a previous simulation study (Chapter 2) suggests that the technique is generally applicable regardless of the specific nature of the distributional kinetics, and is dependent only upon the availability of concentration-time data in both the target tissue and in plasma. Further experimentation will be directed towards extending the principles for pharmacokinetic hysteresis analysis to hysteresis behavior in pharmacodynamic systems.
Figure legends

**Figure 3-1.** Compartmental modeling of data from Blakeley et al. [81]. Circles represent plasma concentrations; squares represent concentrations in brain tumor ECF for patient A (A), patient B (B), patient C (C), and patient D (D). Data were obtained by digitizing published figures; solid lines represent results of compartmental modeling (see Figure 3-2 for model structures).

**Figure 3-2.** Schematic representation of the model structure used. Schematic representation of best-fit model for patients A and B (panel A), patient C (panel B) and patient D (panel C).

**Figure 3-3.** Hysteresis analysis of data from Blakeley et al. [81]. Hysteresis loop from patient A (A), patient B (B), patient C (C), and patient D (D). Filled symbols indicate data that were obtained by digitizing published figures; the open circle in each panel or inset represents the calculated centroid of the hysteresis loop.
Figure 3-1. Compartmental modeling of data from Blakeley et al. [81]

Circles represent plasma concentrations; squares represent concentrations in brain tumor ECF for patient A (A), patient B (B), patient C (C), and patient D (D). Data were obtained by digitizing published figures; solid lines represent results of compartmental modeling (see Figure 3-2 for model structures).
Figure 3-2. Schematic representation of the model structure used.

Schematic representation of best-fit model for patients A and B (panel A), patient C (panel B) and patient D (panel C).
Figure 3-3. Hysteresis analysis of data from Blakeley et al. [81]

Hysteresis loop form patient A (A), patient B (B), patient C (C), and patient D (D). Filled symbols indicate data that were obtained by digitizing published figures; the open circle in each panel or inset represents the calculated centroid of the hysteresis loop.
### Tables

Table 3-1. Pharmacokinetic parameter estimates obtained with compartmental modeling or statistical moment analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>(CV%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (μM·h)</td>
<td>7480</td>
<td>7020</td>
<td>6060</td>
<td>7430</td>
<td></td>
</tr>
<tr>
<td>CL (L/h/m²)</td>
<td>3.55 (3%)</td>
<td>3.77 (3%)</td>
<td>4.10 (4%)</td>
<td>3.56 (3%)</td>
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</tr>
<tr>
<td>V₁ (L/m²)</td>
<td>7.01 (10%)</td>
<td>9.37 (5%)</td>
<td>8.12 (6%)</td>
<td>12.22 (4%)</td>
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</tr>
<tr>
<td>V₂ (L/m²)</td>
<td>3.87 (13%)</td>
<td>0.69 (11%)</td>
<td>0.69 (306%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CLd (L/h/m²)</td>
<td>1.49 (23%)</td>
<td>0.09 (28%)</td>
<td>0.13 (216%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MRT (h)</td>
<td>3.09</td>
<td>2.67</td>
<td>2.35</td>
<td>3.45</td>
<td></td>
</tr>
<tr>
<td>Brain ECF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kᵢp</td>
<td>0.31 (2%)</td>
<td>0.31 (2%)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lag time(h)</td>
<td>-</td>
<td>-</td>
<td>1.29 (2%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>AUC (μM·h)</td>
<td>2170</td>
<td>2070</td>
<td>166</td>
<td>634</td>
<td></td>
</tr>
<tr>
<td>V₃ (L/m²)</td>
<td>-</td>
<td>-</td>
<td>2.79 (3441%)</td>
<td>0.34 (287%)</td>
<td></td>
</tr>
<tr>
<td>CL₁₃ (L/h/m²)</td>
<td>-</td>
<td>-</td>
<td>0.01 (3440%)</td>
<td>0.002 (286%)</td>
<td></td>
</tr>
<tr>
<td>CL₃₁ (L/h/m²)</td>
<td>-</td>
<td>-</td>
<td>0.41 (286%)</td>
<td>0.016 (286%)</td>
<td></td>
</tr>
<tr>
<td>MRT₁ (h)</td>
<td>3.09</td>
<td>2.67</td>
<td>5.5</td>
<td>21.3</td>
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Table 3-2. Parameter estimates by hysteresis analysis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<td>82883</td>
<td>27445</td>
<td>16708</td>
<td>21079</td>
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<tr>
<td>CHX</td>
<td>610</td>
<td>789</td>
<td>619</td>
<td>554</td>
</tr>
<tr>
<td>CHY</td>
<td>161</td>
<td>246</td>
<td>9.21</td>
<td>13</td>
</tr>
<tr>
<td>CHR</td>
<td>3.78</td>
<td>3.19</td>
<td>67.25</td>
<td>43.56</td>
</tr>
<tr>
<td>MRT</td>
<td>3.09</td>
<td>2.67</td>
<td>2.35</td>
<td>3.45</td>
</tr>
<tr>
<td>MRT&lt;sub&gt;t&lt;/sub&gt;</td>
<td>3.09</td>
<td>2.67</td>
<td>5.5</td>
<td>21.3</td>
</tr>
</tbody>
</table>
Chapter 4

EVALUATION OF THE USE OF HYSTERESIS DESCRIPTORS TO QUANTIFY DISSOCIATION BETWEEN PHARMACODYNAMICS AND PHARMACOKINETICS

This chapter will be submitted to AAPS Journal and is presented in the format of that journal
Abstract

Purpose
This study was conducted to examine the utility of descriptors of the hysteresis loop, formed by plotting pharmacologic effect versus drug concentration after a single dose of an agent, in quantifying temporal dissociation between changes in drug response and changes in drug concentration.

Methods
Several different structural models (a simple two-compartment model with the effect site in the peripheral compartment, an effect-compartment model, and a series of indirect response models) were used to simulate effect-versus time and concentration-versus time profiles that would result in formation of a hysteresis loop when effect was plotted versus concentration. Hysteresis descriptors (area bounded by hysteresis loop [ABH]; centroid x- [CHX] and y- [CHY] coordinates, and the unitless ratio of centroid x- vs. y-coordinate [CHR]) of each data set were recovered. The relationships between hysteresis descriptors and discrete pharmacokinetic/pharmacodynamic parameters were evaluated.

Results
Analysis of ABH, CHX, and CHY revealed predominantly nonlinear relationships with various model parameters. Due to severe nonlinearity, including lack of a consistent directional change relative to model parameters, these descriptors were not found to be useful in describing or quantifying temporal delays in the production of pharmacologic effect. CHR had the most consistent relationships with model parameters, although also
evidenced nonlinearity in some cases or over some range of values. The nonlinearity in CHR versus parameter relationships was a function of the inherently nonlinear relationship between effect and concentration at the target site.

**Conclusions**

Consistent with previous observations for the use of hysteresis analysis in describing distributional delays, CHR was the most useful of the hysteresis descriptors for delayed elaboration of pharmacologic response. The utility of CHR as a fundamental metric for pharmacodynamic dissociation appears to be high for situations in which the relationship between effect and concentration is linear, or when the delay in effect onset is large relative to the time frame of the experiment.

Keywords: Hysteresis analysis, Hysteresis descriptors, temporal delay

**Abbreviations**

ABH: area bounded by the hysteresis loop based on drug concentrations; CH: centroid of the hysteresis loop; CHX: x-coordinate of the centroid; CHY: y-coordinate of the centroid; CHR: the unitless ratio of centroid x- vs. y-coordinate; AUC: area under the concentration-time profile; CL: systemic clearance; CLd: distributional clearance; Vc: volume of the central compartment; Vi: volume of the tissue compartment; k0: infusion rate; K1e \ K0: rate constant into and out of effect compartment; K_in: zero rate constant for synthesis of a measured response; K_out: first order rate constant for loss in response.
Introduction

An important goal of pharmacokinetic-pharmacodynamic (PK-PD) modeling under non-steady-state conditions is to reveal the fundamental relationships between pharmacologic effect and the drug concentration that elicits it, allowing prediction of future drug response. For some drugs, responses are in phase with plasma concentrations [93], and relating effect to concentration is no more challenging under non-steady-state conditions than at steady-state. Many drugs, however, display a poor relationship between the time course of drug response and the time course of systemic concentrations [10, 26]. As a result, strategies for analyzing data in the presence of a temporal delay between changes in pharmacologic effect and changes in systemic drug concentration have been of interest [40, 82, 94].

For therapeutic agents with a delayed onset of response, the plot of effect versus systemic drug concentration will form a partial (i.v. bolus administration) or complete (non-instantaneous administration) hysteresis loop. From an experimental standpoint, the presence of a hysteresis loop in the effect-concentration relationship is used as evidence for delayed elaboration of effect due to one of several mechanisms (slow accumulation of drug at the effect site and slow production of effect following binding of the drug to the receptor being the two most common) [40, 95]. It has been suggested, for example, that the area enclosed by the hysteresis loop is positively correlated with how “deep” the effect compartment (where pharmacologic effects are elicited) is relative to plasma compartment (where drug concentrations are usually measured) [4].
The presence of a hysteresis loop in the relationship between drug response and drug concentration impairs the ability to obtain estimates of fundamental parameters (e.g., EC$_{50}$) that are the basis of understanding pharmacodynamics [96]. For example, estimates of EC$_{50}$ for drugs with hysteresis behavior will be different depending on when relative to the administration of the dose effect and concentration are determined (during the time interval when concentrations are rising, during the time interval when concentrations are declining, or across the entire time domain). Failure to account for a kinetic-dynamic time delay leads to overestimates of EC$_{50}$ [6], while reliable evaluation can be achieved when the time delay is included in an integrated modeling approach.

Several parametric or semi-parametric PK-PD models have been proposed for analyzing the effect-concentration relationship in the presence of delayed response onset. The two most common approaches are the effect-compartment model and a series of indirect response/turnover models. The effect-compartment approach, which relies on the construction of a hypothetical effect compartment linked to the central compartment by a first-order process, was elaborated by Sheiner and colleagues [35]. The effect-compartment model has subsequently been used for analyzing PK-PD relationships for a variety of therapeutic agents [11, 97-100]. Despite its relatively widespread use, the effect compartment model has limitations. Estimates of PD parameters are based on compressing the hysteresis loop (in essence replacing the effect versus plasma concentration relationship with a relationship between effect and concentration in the hypothetical effect compartment). Because this approach is based on a model that treats all delays in response
onset as being a consequence of delayed distribution into the effect compartment, it cannot
distinguish different factors or mechanisms that contribute to the temporal delay [35]. For
cases in which delayed effect onset was not due to slow equilibration of concentration
between the effect site and plasma, parameter estimates cannot be extrapolated between
different doses even though the system is assumed to be linear [58].

Indirect response models (IDR) are a series of mechanism-based PD models
designed for drugs with slow elaboration of pharmacologic response (as opposed to slow
penetration of the drug to the effect site). A systematic description of IDR models has been
provided by Dayneka and co-workers [42]. The applicability of IDR models has been
demonstrated for a variety of drugs such as warfarin, aldose reductase inhibitors,
corticosteroids and others [49]. IDR models are preferable to effect-compartment models
when the mechanism for delayed response is known. IDR models can produce consistent
results in dose-escalation experiments, and can be expanded to incorporate other
physiologic conditions when response is constrained within a limited range. Despite
advantages associated with mechanism-based models, IDR models have practical
limitations. For example, knowledge of the mechanism of action and natural changes in
the biologic system in the absence of drug are required for parameter estimation [101];
such information may not be available in early drug development.

Hysteresis loops in effect-concentration plots usually only serve as an indicator of
temporal disequilibrium between pharmacologic effect and drug concentration. Few
studies have been performed to examine the utility of descriptors of hysteresis in
characterizing or quantifying the distributional delay. Recent studies have been directed
towards evaluating descriptors of hysteresis loops in PK, where the hysteresis is formed by
plotting concentrations in tissue versus concentrations in plasma when equilibration of
concentrations between the two sites is relatively slow (Chapter 2, Chapter 3). Among the
morphologic descriptors of the hysteresis (area bounded by the hysteresis [ABH], the
coordinates of hysteresis centroid [CHX and CHY], and the unitless ratio of centroid x- vs.
y-coordinate [CHR]), CHR was the best metric for distributional delay under a variety of
simulated conditions (Chapter 2). By applying hysteresis analysis to data mined from the
literature, hysteresis analysis with CHR as a metric was shown to be useful in
characterizing the distributional delay between a tissue (brain extracellular fluid as
determined by microdialysis sampling) and plasma (Chapter 3).

The goal of the present study was to examine the utility of hysteresis analysis in PD
experiments, as this is the situation in which hysteresis behavior is most often encountered.
Common structural models (mammillary models, effect-compartmental models, and
indirect response models) were used to generate simulated effect and concentration data.
Hysteresis analysis was performed to recover descriptors of the hysteresis loop, and the
relationships between hysteresis descriptors and PK-PD parameters were explored. The
potential utility and sensitivity of this data analysis approach, when transitioning from PK
to PD, was examined in detail.

**Methods:**

*Model structures and parameter selection*
Model structure 1 (Figure 4-1A): A two-compartment model was used to generate concentration-time profiles in plasma and tissue. In order to generate a time delay between changes in plasma concentrations and changes in response, the effect site was placed in the tissue compartment, and concentrations in the tissue compartment were used to drive pharmacologic response. Two PD functions were evaluated (simple $E_{\text{max}}$ and sigmoidal $E_{\text{max}}$). Values of the parameters associated with drug distribution between the two compartments ($\text{CL}_d$ and $V_t$) were varied, with the following ranges (arbitrary units): $\text{CL}_d$ (0.1-5) and $V_t$ (2-100). $V_c$ was fixed at 10; CL was fixed at 1; $E_{\text{max}}$ was fixed at 100; $EC_50$ was fixed at 20; the Hill coefficient was fixed at 1 (simple $E_{\text{max}}$) or 1.5 (sigmoidal $E_{\text{max}}$).

Model structure 2 (Figure 4-1B): To generate a further delay in response onset (compared to Model 1), an effect compartment was linked to the tissue compartment. Concentration-time profiles were simulated in plasma, tissue, and the effect compartment, and concentrations in effect compartment were used to drive pharmacologic response. Values of most of the pharmacokinetic parameters were varied in order to fully examine the behavior of this system; ranges of values (with arbitrary units) investigated were CL (0.1-10), $CL_d$ (0.2-20), $V_t$ (2-100), $K_{1e}$ (0.01-1), and $K_{e0}$ (0.01-1). $V_c$ was fixed at 10; $E_{\text{max}}$ was fixed at 100; $EC_50$ was fixed at 5. For simplicity, only the simple $E_{\text{max}}$ model was used with this model structure.

Model structure 3 (Figure 4-1C): Concentration-time profiles were simulated with a one-compartment model, and concentrations in this single pharmacokinetic compartment were used to drive pharmacologic response. Delays in response onset were achieved.
through use of an indirect response function. There are four fundamental IDR functions (stimulation of production of the response variable; inhibition of production of the response variable; stimulation of elimination of the response variable; inhibition of elimination of the response variable) [42]. All four IDRs were used in this simulation. Parameter ranges (arbitrary units) investigated were $K_{\text{in}}$ (0.5-10) and $R_0$ (0.5-50). CL was fixed at 1; $V_c$ was fixed at 10, $E_{\text{max}}$ was fixed at 4; EC$_{50}$ was fixed at 4; $I_{\text{max}}$ was fixed at 1; $K_{\text{out}}$ was calculated as $K_{\text{out}} = R_0/K_{\text{in}}$. For simplicity, only the simple $E_{\text{max}}$ model was used in this case.

The range of parameters examined for each of the models was determined in preliminary simulations so that a wide range of observable hysteresis behavior would be produced with each model. Combinations of parameter values that did not produce measurable hysteresis in the effect versus concentration plot were excluded from the analysis due to lack of relevancy.

**Hysteresis descriptors and secondary parameters**

The non-compartmental descriptors of hysteresis examined in this study included the area bounded by the hysteresis loop [ABH], the hysteresis centroid [CH] coordinates [CHX and CHY], the unitless ratio of centroid x- vs. y-coordinate [CHR], and the ratio of CHR [CHR ratio]. The mathematical equations used to calculate descriptor values are described as follows:

**ABH** was defined as the signed area of the loop formed by plotting pharmacologic response against concentration in the blood compartment ($C_b$) or concentration in the tissue compartment ($C_t$). The hysteresis was simplified to a polygon defined by discrete data
points, and ABH was calculated (Mathematica 8 and OriginPro 9.0) with an equation for signed partial area:

\[
Area = \frac{1}{2} \sum_{i=1}^{k} (x_i y_{i+1} - x_{i+1} y_i)
\]  

(1)

where \( x_i \) and \( y_i \) represent concentration and effect, respectively, for each data point, and in summation \( x_{k+1} = x_1 \) and \( y_{k+1} = y_1 \) [80].

Similarly, \( CH \) was determined by calculating the x- and y- coordinates of the hysteresis centroid, respectively, using following equations:

\[
C_x = \frac{1}{6A} \sum_{i=0}^{n-1} (x_i + x_{i+1})(x_i y_{i+1} - x_{i+1} y_i)
\]  

(2)

\[
C_y = \frac{1}{6A} \sum_{i=0}^{n-1} (y_i + y_{i+1})(x_i y_{i+1} - x_{i+1} y_i)
\]  

(3)

where \( A \) represents ABH calculated for the corresponding loop, and other symbols are as defined in equation (1).

\( CHR \) was calculated as ratio of \( C_x \) (CHX) divided by \( C_y \) (CHY) based on equations (2) and (3).

**Software used for data analysis**

Statistical analysis was performed using R (R Core Team, Vienna, Austria). The mathematical computations and data analyses were performed in both R and Mathematica 8 (Wolfram Research, Champaign, IL). Graphics were generated by R. Pharmacokinetic simulation and modeling were performed in ADAPT (BMSR, Los Angeles, CA).
Results

Relationship between hysteresis descriptors and PK parameters

Two-compartment model with the effect site in the tissue compartment

A simple two-compartment model was used to simulate concentration-time profiles in the blood and tissue compartments, and concentrations in tissue were modeled as driving the pharmacologic response with simple and sigmoidal $E_{\text{max}}$ functions. The hysteresis formed by plotting effect versus concentration was analyzed as described in the Methods section. The influence of distributional PK parameters ($V_t$ and $Cl_d$) on hysteresis descriptors is shown in Figure 4-2. ABH, CHX, and CHY showed a nonlinear relationship with $V_t$, as was the case with all values of $Cl_d$. For some combinations of $V_t$ and $Cl_d$, at least one of these hysteresis descriptors evidenced a biphasic relationship with $V_t$. The degree of nonlinearity in the relationship between hysteresis descriptors and PK parameters was more pronounced when the effects were generated by the sigmoidal $E_{\text{max}}$ model as compared to the simple $E_{\text{max}}$ model.

In contrast to the other hysteresis descriptors, the relationship between CHR and $V_t$ was linear over the predominant range of $V_t$ values. The region of nonlinearity was confined to small values of $V_t$, when the magnitude of dissociation between pharmacodynamics and pharmacokinetics would be predicted to be small. The predominant linear nature of the relationship was evident for both the simple and sigmoidal $E_{\text{max}}$ models.
Previous assessment of the applicability of hysteresis analysis in distributional PK (Chapter 2) demonstrated that CHR was a linear function of the inverse of the distributional rate constant $K_{21}$ (the rate constant that governs the movement of drug from the peripheral to the central compartment) for a simple two-compartment model. A similar, but not identical, result was obtained in analyzing the hysteresis formed by plotting effect versus blood concentration with data simulated in a simple two-compartment model (Figure 4-3). By relating CHR to the reciprocal of the distributional rate constant, which is the ratio of $Cl_d$ to $V_t$, a singular relationship was obtained regardless of the value of $Cl_d$ (or more importantly, the combination of $Cl_d$ and $V_t$ values). While the overall relationship was nonlinear (unlike the case for analysis of PK hysteresis, which was strictly linear), the region of nonlinearity was restricted to large values of $K_{21}$ (when temporal delay in effect onset would be short and hysteresis behavior would be minimal).

**Effect-compartment model**

An effect-compartment model with the effect site linked to the tissue compartment was used to increase the delay in effect onset. In this series of simulations, two separate sets of hysteresis descriptors were obtained: one based on the effect versus blood concentration plot, and one based on the effect versus tissue concentration plot. This strategy was used to mimic the experimental situation in which concentrations of a therapeutic agent are measured at different sampling sites in the same individual, with one sampling site representing the presumed target organ. Relationships between hysteresis descriptors and PK parameters are shown in Figures 4-4 through 4-8. The relationship
between each of the hysteresis descriptors and CL was nonlinear, with minimal differences between blood- and tissue-derived data (Figure 4-4). In all cases, hysteresis descriptors decreased as CL increased. The relationships between ABH, CHX, or CHY and $V_t$ also were nonlinear, and were further characterized by a decrease in the hysteresis descriptor with increasing $V_t$ (Figure 4-5). In contrast to CL, obvious differences were observed between descriptors derived from blood versus tissue data (all descriptors were consistently lower for tissue-derived as compared to blood-derived data). Unlike the other descriptors, CHR based on blood concentrations was a linear function of $V_t$ with a positive slope. CHR calculated from tissue concentrations changed nonlinearly with $V_t$ and decreased as $V_t$ increased.

The relationships between hysteresis descriptors and the rate constants that linked the effect and tissue compartments are shown in Figures 4-6 through 4-8. First, flux into and out of the effect compartment was assumed to be equivalent and were changed simultaneously (the common assumption for effect-compartment modeling; $K_{1e} = K_{e0}$). As $K_{1e}$ and $K_{e0}$ changed, all descriptors of hysteresis, including CHR, changed nonlinearly (Figure 4-6). When $K_{1e}$ and $K_{e0}$ were constrained to small values (that is, when kinetic-dynamic dissociation would be substantial) the relationship approached a linear function. Subsequent simulations were performed to evaluate the influence of the two rate constants independently. When $K_{1e}$ was changed alone (with $K_{e0}$ fixed at 0.1), all relationships were nonlinear (Figure 4-7). Again, when $K_{1e}$ was constrained to small values, a predominantly linear relationship was observed between CHR and the inverse of $K_{1e}$. When $K_{e0}$ was
varied (with $K_{1e}$ fixed at 0.1), all relationships between hysteresis descriptors and $K_{e0}$ were nonlinear with the exception of CHR, which evidenced a nearly linear relationship (Figure 4-8). To further probe the relationship between CHR and $K_{e0}$, $K_{e0}$ was varied at several different values of $K_{1e}$ (Figure 4-9). Regardless of the combination of values of the two rate constants, the approximately linear relationship persisted, with the value of $K_{1e}$ determining the slope of the line.

**Indirect response model**

Four types of basic indirect response models were investigated: inhibition of $K_{in}$ (model 1), inhibition of $K_{out}$ (model 2), stimulation of $K_{in}$ (model 3), and stimulation of $K_{out}$ (model 4). In order to make all hysteresis loops anti-clockwise for comparative purposes, in all models responses were expressed as absolute changes from baseline. By definition, the ratio of $K_{in}/K_{out}$ was fixed at $R_0$, and the relationship between parameters unique to the basic IDR model and hysteresis descriptors was explored by varying $K_{in}$ while $R_0$ was fixed at 20. Similar to the other simulated systems, hysteresis descriptors were related non-linearly with $K_{in}$ (Figure 4-10 A-D), and all descriptors except CHR showed a nonlinear relationship with the inverse of $K_{out}$ (data not shown). CHR, however, increased in an approximately linear fashion with increasing $1/K_{out}$ in model 1 and model 2 (Figure 4-10 E). $K_{out}$ had a limited influence on CHR in model 3 and model 4. Varying $R_0$ only served to scale up or down the baseline response, and had no impact on fundamental relationships (data not shown).

**Influence of dose-escalation and effect-concentration relationship on CHR**
The impact of changing dose on the hysteresis descriptor CHR was evaluated using an effect-compartment model. Results are shown in Figure 4-11. CHR remained unchanged when the relationship between effect and concentration was linear (data not show). CHR increased linearly with increasing dose when effects were simulated with a log-linear equation. The CHR-dose relationship became nonlinear when effects were modeled with a sigmoidal function.

Influence of the relationship between concentration and effect on the sensitivity of CHR to changes in PK-PD parameters

CHR has been shown to be the most useful parameter in PK hysteresis analysis (Chapter 2), so the sensitivity of CHR in response to changes in parameters with effect data (PD hysteresis) was compared to that of CHR based on concentration data alone (PK hysteresis). Since the underlying assumption of the effect compartment model is that hysteresis behavior is caused by the distributional delay between blood and biophase, the question of how the PD relationship between effect and concentration in the biophase would affect the sensitivity of CHR is relevant. To explore this issue, two common PD models were employed: a linear effect model (the relationship between concentration and effect can be described with a linear function) and a simple $E_{\text{max}}$ model (the relationship between concentration and effect can be described with a sigmoidal function). A log-linear model also was evaluated, but the results did not differ substantially from those of the sigmoidal model and so are not discussed herein. All three conditions were simulated, and in order to make fair comparisons the observed maximum effect was set to be less than or
equal to 100, and CHR based on hysteresis plots created with blood or tissue concentrations were recovered. The results of this simulation experiment are shown in Table 4-1 and Figure 4-12. When sigmoidal equations were introduced into the system, the sensitivity of CHR decreased compared to systems with a linear effect model. For example, when comparing the value range of CHR when $K_{1e}$ was varied, CHR ranged from 20.7 to 0.6 (value decreased as $K_{1e}$ increased) for the linear model, but only from 1.4 to 0.2 for the simple $E_{\text{max}}$ model.

Similar simulations facilitated comparison of model sensitivity with data generated by IDR models (Figure 4-13). In this situation, the linear relationship between effect and concentration also was constrained such that the maximum effect was comparable to $E_{\text{max}}$ or $I_{\text{max}}$. In this situation, CHR was also more sensitive to changes in $K_{\text{in}}$ when a linear effect model was used compared with sigmoidal model, and this result was consistent for all IDR sub-models. With model 1 and 2, CHR was relatively sensitive to changes in $K_{\text{out}}$ even when the effect-concentration relationship was not linear.

Discussion

The goal of this study was to examine the utility of hysteresis analysis in describing or quantifying PK/PD dissociation. To this end, several model systems were used to simulate effect-time data and concentration-time data in blood and in target tissue. These simulated data, produced with a PK/PD system of known structure and with known parameter values, were used to construct hysteresis loops, the morphology of which was determined according to previously-reported techniques (Chapter 2). The relationships
between each hysteresis descriptor and PK/PD parameters was then investigated to
determine the possibility that hysteresis morphology might serve as a mode-independent
alternative to parametric or semi-parametric PK/PD modeling.

**Two-compartment model**

In all situations, CHR was the only hysteresis descriptor that, at least under certain
conditions, displayed a linear relationship with egress-related PK parameters, and did so
across all models. In the simple two-compartment system, CHR increased as $V_t$ increased.
Because the mean residence time of a drug in the tissue compartment will be proportional
to the apparent volume of the tissue compartment [102], it may be inferred that CHR is a
predictor of mean residence time in tissue. This has been demonstrated previously in
simulation studies (Chapter 2) and with mined data (Chapter 3). When the site of action is
in a tissue compartment, an increase in the mean residence time in that compartment
relative to the mean residence time in blood will lead to an increase in PK/PD dissociation.
Given this reasoning, it can be concluded that, an increase in CHR would be predictive of
an increase in temporal delay of effect onset in this simple system.

Although nonlinearity in the relationship between CHR and $V_t$ was obvious for a
short time delay ($V_t$ was small), in general the CHR increased proportionally as the
magnitude of time delay increased. The reason for the transition from nonlinear to linear
behavior might be that, when a substantial delay was due to increased $V_t$, concentrations
in the tissue compartment (i.e., at the effect site) decreased to a value close to, or lower
than the EC$_{50}$, the region of the effect-concentration relationship more closely
approximates a linear function. Similar to previous observations in PK systems, when CHR was plotted against the inverse of the distributional rate constant in the egress direction (calculated as CLd/Vt), CLd no longer modulated the relationship (Figure 4-3). When the egress rate constant was high, consistent with small delays in achieving distributional equilibrium, the relationship was nonlinear.

The same dataset was used to characterize the distributional delay between blood and tissue by analyzing the hysteresis loop formed by plotting concentration in tissue versus concentration in blood. When the PK hysteresis behavior was analyzed, CHR was a linear function of Vt (or MRT), which suggests that the nonlinearity in the PD hysteresis case was introduced by the nonlinear equations that linked effect to concentration. Therefore, it is likely that if the relationship between effect and concentration was linear (as is the case for certain drugs over limited ranges of concentration), then the relationship between CHR and Vt (or MRTt) would be linear as well.

*Effect-compartment linked to a tissue compartment*

The relationships between hysteresis descriptors and changes in PK/PD parameters were examined with data simulated through an effect-compartment model with the effect site linked to, as opposed to contained within, the tissue compartment. In this model system, the influence of major PK/PD parameters on hysteresis behavior was investigated by changing one parameter value at a time while keeping the remaining parameter values constant. Hysteresis descriptors including CHR changed nonlinearly when CL or K1e
(where $K_{e0} = K_{1e}$) were varied (Figures 4-4 and 4-6). When $V_t$ was changed alone, only the CHRs based on the blood concentration was evidenced a proportional change.

Because these simulations were based on an effect compartment model, $K_{1e}$ and $K_{e0}$ were the parameters most relevant to the time delay between tissue concentration and effect. The temporal delay between changes in blood concentration and changes in effect was, of course, affected not only by $K_{1e}$ and $K_{e0}$, but also by $C_{ld}$ and $V_t$. As shown in Figure 4-6, when the rate constants for influx and efflux between tissue and effect compartment were simultaneously changed, all hysteresis descriptors were nonlinear functions of $K_{1e}$ or $K_{e0}$. CHR appeared to be almost linear with respect to the inverse of $K_{1e}$ and $K_{e0}$. When only efflux from the effect compartment ($K_{e0}$) was changed, CHR based on either blood or tissue concentration changed proportionally with $K_{e0}$ (Figure 4-8). Moreover, the linearity of relationship between CHR and $K_{e0}$ was not dependent on values of $K_{1e}$; the slope of the regression line was depended upon $K_{1e}$ (Figure 4-9).

Unlike the two-compartment model, CHRs formed a linear relationship with the efflux rate constant $K_{e0}$, but not its inverse. One possible explanation for this is that when $K_{e0}$ was increased by itself, it not only resulted in a shorter time to equilibrium between compartments but also decreased the maximum effect achieved because the system was simulated under non-steady-state conditions. When $K_{1e}$ and $K_{e0}$ changed simultaneously, more rapid equilibration resulted in higher effects. The inverse of $K_{e0}$ was used to calculate the equilibrium half-time for data modeled with an effect compartment [100, 103, 104]. It should be noted that traditional effect-compartment modeling utilizes the assumption that
Because this study simulated data to construct a hysteresis, as opposed to analyzing data when the objective is to collapse the hysteresis [105], there was no need to include this limiting assumption. As would be expected, CHR behaved differently when $K_{1e}$ and $K_{e0}$ were separate parameters that control a truly bidirectional process as compared to when they were consolidated into a single parameter.

**Indirect response model**

In addition to the effect-compartment model, indirect response (IDR) models are commonly used to model effect data with delayed response [22, 51, 52, 55, 106]. Unlike the effect-compartment approach, which relies on calculating concentrations in a hypothetical effect compartment [35], IDR models incorporate mechanism-based processes [48, 107-109]. A zero-order rate constant ($K_{in}$) is presumed to be responsible for production of the response variable, and a first-order rate constant ($K_{out}$) is presumed to control the loss of the response [42], and pharmacologic effect acts on (stimulates or inhibits) either $K_{out}$ or $K_{in}$. Similar to observations in other PK and PK/PD systems, CHR was a linear function of $1/K_{out}$ when $K_{out}$ or $K_{in}$ values were small, especially when the drug had an inhibitory effect on $K_{in}$ or $K_{out}$ (Figure 4-10). CHR seemed to be most sensitive to changes in parameter in model 1 (inhibition of $K_{in}$) and model 2 (inhibition of $K_{out}$); conversely CHR changed moderately in model 3 (stimulation of $K_{in}$) and was insensitive to changes in model 4 (stimulation of $K_{out}$) based on raw numerical values. However, when examining percentage change, CHR for model 1 and model 4 evidenced moderate sensitivity, CHR for model 3 displayed limited sensitivity, and the CHR for model 2
showed the highest degree of sensitivity. It was not unexpected that CHR for model 2 was most sensitive to changes in $K_{in}/K_{out}$ because in this model the drug would inhibit loss of response, thus prolonging the time delay. Similar to what has been observed in other systems, CHR was more sensitive to changes in parameters when the time delay was relatively large. With models 3 and 4, pharmacologic effect results in stimulation of processes, thereby shortening observable time delays between effect and concentration and rendering CHR less sensitive to changes in parameter values.

**Influence of dose escalation**

Previous observations suggested that PD hysteresis may be influenced by the administered dose [28, 110]. Dose-escalation simulations were performed using the traditional effect-compartment model. For both blood and tissue compartments, the linearity of the relationship between CHR and dose depended on the relationship between concentration and effect. For a linear PK-PD relationship, increases in dose produced proportional changes in blood concentrations, tissue concentrations, and effect. However, CHR did not change with dose (data not shown). For log-linear PK-PD relationships, the CHR versus dose relationship was approximated linear; when the concentration-effect relationship was sigmoidal, the CHR-dose relationship deviated from further linearity. Regardless of whether or not the relationship was linear, CHR increased monotonically with increased dose. While the influence of dose on CHR was expected, one goal of this experiment was to identify a parameter that can be used to normalize CHR across a dose range. The results of this set of simulations suggest that this is not a feasible goal. The rate
of change in CHR was different based on blood concentrations and tissue concentrations; moreover, the ratio between CHR also changed with dose when the effect versus concentration relationship was non-linear. However, based on the result, when the effect versus concentration relationship was sigmoidal, a 100-fold increase in dose only led to a less than 2-fold change in the ratio of CHR based on tissue and that based on blood concentration, which suggested that impact of changing dose was negligible.

Factors that influence the sensitivity of CHR

As shown previously, CHR can serve as a robust and sensitive parameter to quantify the time-delay in distributional PK (Chapter 2). An important question to be addressed in the current study was whether CHR is a similarly robust descriptor of temporal delays in PD. Sensitivity was evaluated with different PD sub-models embedded in each of the structural models evaluated in the current study. In all three models the sensitivity of CHR was lower when the effect-concentration was sigmoidal as compared to linear. When the effect-concentration relationship was linear, the results were comparable to those that were obtained in examining hysteresis loops produced by distributional PK factors. This was the anticipated result because the linear effect model simply multiplies the effect-site concentration by a constant. The shape of the relationship between effect and either tissue or blood concentration will therefore be identical to the shape of the relationship between effect-site concentration and either tissue or blood concentration, as long as the maximum effect-site concentration does not exceed the concentration required to produce 100% maximal effect. One possible explanation for the changes in sensitivity associated
with different effect models is illustrated in Figure 4-14. Representative hysteresis plots based on the effect-compartment model with different effect-concentration relationships were examined. As $K_{c0}$ increased from 0.01 to 1, the axes of the hysteresis loops based on a linear effect model changed significantly, while those based on a sigmoidal effect model changed much less. This suggests that, consistent with other non-compartmental analysis approaches, the utility and applicability of hysteresis analysis is also limited to linear processes.

**Conclusion**

Similar to what was observed with hysteresis analysis in PK, CHR was found to be the most useful hysteresis descriptor in terms of explaining or predicting temporal delays in pharmacologic effect. The relationship between CHR and PK/PD parameters of interest were typically linear or at least monotonic, while each of the other hysteresis descriptors evidenced nonlinear and often non-monotonic relationships with key model parameters. However, the loss of sensitivity for CHR as the effect-concentration relationship became increasingly nonlinear, which limits its applicability in data analysis because often the region of interest may fall in the nonlinear portion of the concentration-response profile. Because calculating CHR does not require a structural model or sophisticated quantitative tools, it may serve as an alternative non-compartmental approach for analysis of effect data with time delay when certain conditions are met.

Despite the fact that observing a non-zero ABH has long been used as an indicator of a delayed response [72], ABH has value only as a qualitative descriptor of temporal
delay. The magnitude of ABH is influenced by both the range of concentrations and the range of effects encountered. Accordingly, PK/PD changes that result in increased temporal delays but decreased concentration (e.g., due to an increase in the apparent volume of the tissue compartment) might actually decrease, as opposed to increase, the ABH. This anomaly, while clearly consistent with pharmacokinetic reasoning, results in non-monotonic relationships (which by definition are non-predictive) between ABH and some parameters related to distributional (and therefore effect) delay.

Future studies will focus on applying hysteresis analysis to data from PD studies mined from published literature in order to assess the potential utility of hysteresis analysis, with CHR as a metric, under actual experimental conditions.
Table 4-1. Sensitivity of CHR to changes in PK-PD parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value range</th>
<th>CHR value range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Linear (Blood)</td>
</tr>
<tr>
<td>K&lt;sub&gt;1e&lt;/sub&gt;</td>
<td>0.01-1</td>
<td>20.7-0.6</td>
</tr>
<tr>
<td>K&lt;sub&gt;ɛ0&lt;/sub&gt;</td>
<td>0.01-1</td>
<td>1.1-14.5</td>
</tr>
<tr>
<td>K&lt;sub&gt;1e/ɛ0&lt;/sub&gt;</td>
<td>0.01-1</td>
<td>6.3-1.7</td>
</tr>
<tr>
<td>CL&lt;sub&gt;d&lt;/sub&gt;</td>
<td>0.1-20</td>
<td>14.0-1.7</td>
</tr>
<tr>
<td>CL</td>
<td>0.1-100</td>
<td>2.3-2.5</td>
</tr>
<tr>
<td>V&lt;sub&gt;t&lt;/sub&gt;</td>
<td>1-100</td>
<td>1.6-11.8</td>
</tr>
</tbody>
</table>
**Figure legends**

**Figure 4-1.** Schematic representation of models used in simulations. A) Model structure 1, simple two-compartment model with concentration in tissue compartment driving the pharmacological response; B) Model structure 2, two-compartmental model with a linked effect compartment; C) Model structure 3, one-compartment model with indirect responses. Detailed explanations are provided in the Methods section.

**Figure 4-2.** Relationships between descriptors of hysteresis and $V_t$. (A) Area bounded by the hysteresis [ABH]; (B) x-coordinates of centroids [CHX]; (c) y-coordinates of centroids [CHY]; (D) the ratio of CHX to CHY. In all panels, data are grouped by values of $CL_d$ (see figure legend for corresponding values).

**Figure 4-3.** The relationship between CHR and egress rate constant ($CL_d/V_t$). (A) Effect simulated when Hill coefficient equals 1; (B) Effect simulated when Hill coefficient is 1.5.

**Figure 4-4.** Relationships between descriptors of hysteresis and egress rate constant. (A) ABH; (B) CHR; (C) CHX; (D) CHY. Hysteresis analysis was based on both blood concentration (open circles) and tissue concentration (filled squares).

**Figure 4-5.** Relationships between descriptors of hysteresis and volume of distribution in tissue compartment ($V_t$). (A) ABH; (B) CHR; (C) CHX; (D) CHY. Hysteresis analysis was based on both blood concentration (open circles) and tissue concentration (filled squares).

**Figure 4-6.** Relationships between descriptors of hysteresis and rate constants for flux into and out of the effect compartment ($K_{1e}$ and $K_{0e}$). (A) ABH; (B) CHR; (C) CHX; (D) CHY;
(E) inverse of $K_{1e}$ and $K_{e0}$ and CHR. Hysteresis analysis was based on both blood concentration (open circles) and tissue concentration (filled squares).

**Figure 4-7.** Relationship between descriptors of hysteresis and effect compartment influx rate constant $K_{1e}$ when $K_{e0}$ was fixed at 0.1. (A) ABH; (B) CHR; (C) CHX; (D) CHY; (E) inverse of $K_{1e}$ and CHR. Hysteresis analysis was based on both blood concentration (open circles) and tissue concentration (filled squares).

**Figure 4-8.** The relationship between descriptors of hysteresis and effect compartment efflux rate constant $K_{e0}$ when $K_{1e}$ was fixed at 0.1. (A) ABH; (B) CHR; (C) CHX; (D) CHY. Hysteresis analysis were based both on blood concentrations (open circles) and tissue concentrations (filled squares).

**Figure 4-9.** Relationships between descriptors of hysteresis CHR and effect compartment efflux rate constant $K_{e0}$ when $K_{1e}$ was fixed at different values. Hysteresis analysis was based on both blood concentration (left panel) and tissue concentration (right panel). In all panels, data are grouped by values of $K_{1e}$ (see figure legend for corresponding values).

**Figure 4-10.** Relationships between descriptors of hysteresis and rate constants $K_{in}$ and $K_{out}$ in indirect response models. (A) $K_{in}$ and ABH; (B) $K_{in}$ and CHR; (C) $K_{in}$ and CHX; (D) $K_{in}$ and CHY; (E) $1/K_{out}$ and CHR. $R_0=K_{in}/K_{out}$ was fixed at 20 for all models.

**Figure 4-11.** Influence of different doses on CHR. CHR was determined based on both blood concentration (upper panel) and tissue concentration (bottom panel). Effects were simulated with either a log-linear equation (grey squares), a simple $E_{\text{max}}$ equation (black triangles), or a sigmoidal $E_{\text{max}}$ equation (empty circles).
**Figure 4-12.** Sensitivity of CHR in pharmacodynamics with linear or sigmoidal effect. The relationship between CHR and (A) $K_{1e}$ and $K_{e0}$; (B) inverse of $K_{1e}$ and $K_{e0}$; (C) $K_{1e}$; (D) inverse of $K_{1e}$; (E) $K_{e0}$. In each graph, the linear effect was driven by concentrations in blood (filled triangles) or tissue (empty triangles), and sigmoidal effect was driven by concentrations in blood (filled circles) or in tissue (empty circles).

**Figure 4-13.** Influence of the concentration-effect relationship (linear versus sigmoidal effect) on CHR based on IDR model. The relationship between CHR and the inverse of $K_{out}$ is shown when the relationship between concentration and effect was linear (left) or sigmoidal (right). Simulated results were based on IDR model 1 (empty circles), model 2 (filled circles), model 3 (empty squares), or model 4 (filled squares).

**Figure 4-14.** Hysteresis loop when $K_{e0}$ was changed based on effect-compartment model. Concentrations in blood were plotted against effect when $k_{e0} = 0.01$ (A), 0.1 (B) and 1 (C) when the relationship between concentration and effect was linear (fill circles), sigmoidal (filled triangles) and log-linear (empty circles).
Figures

Figure 4-1. Schematic representation of models used in simulations.
Figure 4-2. Relationships between descriptors of hysteresis and $V_i$. 
Figure 4-3. The relationship between CHR and egress rate constant ($\text{Cl}_d/V_i$).
Figure 4-4. Relationships between descriptors of hysteresis and egress rate constant.
Figure 4-5. Relationships between descriptors of hysteresis and volume of distribution in tissue compartment ($V_t$).
Figure 4-6. Relationships between descriptors of hysteresis and rate constants for flux into and out of the effect compartment ($K_{1e}$ and $K_{e0}$).
Figure 4-7. Relationship between descriptors of hysteresis and effect compartment influx rate constant $K_{1e}$ when $K_{e0}$ is fixed at 0.1.
Figure 4-8. The relationship between descriptors of hysteresis and effect compartment efflux rate constant $K_{e0}$ when $K_{1e}$ is fixed at 0.1.
Figure 4-9. Relationships between descriptors of hysteresis CHR and effect compartment efflux rate constant $K_{e0}$ when $K_{1e}$ is fixed at different values.
Figure 4-10. Relationships between descriptors of hysteresis and rate constants $K_{in}$ and $K_{out}$ in indirect response models.
Figure 4-11. Influence of different doses on the hysteresis descriptor CHR.
Figure 4-12. Sensitivity of CHR in pharmacodynamics with linear effect or sigmoidal effect.
Figure 4-13. Influence of concentration-effect relationship (linear versus sigmoidal effect) on CHR based on IDR model.
Figure 4-14. Hysteresis loop when $K_{e0}$ was altered based on the effect-compartment model.
Chapter 5

ANALYSIS OF HYSTERESIS BEHAVIOR IN

PHARMACODYNAMIC EXPERIMENTS:

EVALUATION OF THE APPROACH WITH MINED DATA

This chapter will be submitted to Biopharmaceutics and Drug Disposition and is presented in the format of that journal
Abstract

A hysteresis loop present in the effect versus concentration plot is an indication of a delayed response. Previous studies have shown that among hysteresis descriptors (area bounded by the hysteresis [ABH], the x- and y- coordinate of hysteresis centroid [CHX, CHY], and unitless ratio CHX/CHY [CHR] of the hysteresis loop), CHR is the most useful in quantifying temporal delays. The goal of the present study was to recover CHR from published data across a variety of pharmacodynamic situations to evaluate its potential utility. To this end, CHR were compared to parameter(s) from compartmental modeling reported in the original literature. Analysis based on data mined from 12 studies confirmed previous simulation results. CHR was most useful when the effect model was linear and when temporal delays were relatively large. When delays are small, variability in the data can obscure hysteresis behavior, making CHR less reliable. When the effect model is nonlinear (log-linear or sigmoidal), the relationship between CHR and parameters responsible for temporal delays lose proportionality.

Abbreviations

ABH: area bounded by the hysteresis loop based on drug concentrations; CH: centroid of the hysteresis loop; CHX : x-coordinate of the centroid; CHY: y-coordinate of the centroid; CHR: the unitless ratio of centroid x- vs. y-coordinate; AUC: area under the concentration-time profile; CL: systemic clearance; CLd: distributional clearance; Vc: volume of the central compartment; Vt: volume of the tissue compartment; k0: infusion rate;
$K_{1e}$ and $K_{e0}$ : rate constant into and out of effect compartment; $K_{in}$ : zero rate constant for synthesis of a measured response; $K_{out}$: first order rate constant for loss in response.

**Introduction**

When there is a temporal delay in the production of pharmacologic response, an anticlockwise loop, usually referred to as a hysteresis, will be apparent in the effect versus concentration profile if effect and concentration are measured over time after a single dose [40, 82]. The presence of a hysteresis loop impairs the ability to obtain accurate estimates of important pharmacodynamic parameters, most notably $EC_{50}$, the concentration at which 50% of maximum effect is achieved. The analytical difficulty posed by hysteresis behavior centers on the loss of a unique relationship between effect and concentration. Instead, depending on the point in time after a dose that an observation is made, the same concentration can be associated with two markedly different magnitudes of drug response [111-117]. A wide range of therapeutic agents display hysteresis behavior that must be taken into consideration when analyzing pharmacodynamics data. For example, opioid analgesics [14, 118, 119], benzodiazepines [105, 120, 121], anti-inflammatory agents [122, 123], diuretics [124, 125], beta-blockers [126, 127], and calcium channel blockers [18, 128, 129] all have significant temporal dissociation between drug action and disposition.

The hysteresis loop is frequently used as an indicator of the presence of a time delay between changes in concentration and changes in effect, but rarely is used for quantitative purposes. Hysteresis analysis is based on morphological assessment of the hysteresis loop itself, and descriptors of the hysteresis loop include the area bounded by the hysteresis
[ABH], the hysteresis centroid [CH] x- [CHX] and y- [CHY] coordinates, the unitless ratio of centroid x- vs. y-coordinate [CHR], and the ratio of CHR [CHRratio] (when two or more CHR values are available in the same subject, such as when hysteresis loops can be constructed for effect versus concentration relationships in both tissue and plasma). As shown previously, CHR is a useful and sensitive descriptor for characterizing temporal delays in pharmacokinetics (PK; Chapters 2 and 3) and to a somewhat more limited extent in pharmacodynamics (PD; Chapter 4). The limitation in the utility of CHR in the realm of PD as opposed to PK is related to the nonlinearity that often is present in the relationship between effect and concentration. That nonlinearity carries through to relationships between CHR and key PK/PD parameters, most importantly those parameters that are associated with temporal dissociation between PD and PK (Chapter 4). The impact of this nonlinearity is that CHR loses some predictive power (it is more difficult to extrapolate or interpolate nonlinear as opposed to linear functions) and some sensitivity (changes in parameters associated with temporal delay result in a less-than-proportionate change in CHR in some regions of the profile). Despite these limitations, the facile nature of recovery of CHR from a data set makes it a potentially attractive descriptor of temporal delay.

Previous work has focused primarily on establishing the fundamental relational behavior of CHR to parameters in PK and PK/PD systems with simulated (controlled) data (Chapters 2 and 4). Additional work was devoted to testing the hysteresis analysis approach with PK data, primarily to determine whether CHR was capable of describing distributional delays in PK systems (Chapter 3), recognizing that such distributional delays often are
responsible for delayed onset of pharmacologic response. The test of an analytical approach with actual data is an important step in understanding the potential applicability of the approach. Because the interest in hysteresis behavior is mostly related to PK/PD systems, as opposed to simply distributional events, it is similarly important to test the approach with experimentally-derived PK/PD data. In this regard, assessment of the applicability of the approach is more complicated for PK/PD data as opposed to PK data. Specifically, the relationship between CHR and parameters associated with delayed onset of response (due either to delayed presentation of the drug to the receptor site or to delayed elaboration of response once the drug has interacted with the receptor) is dependent on several factors: the structural model (inclusion of a hypothetical effect compartment), the relationship between effect and concentration (linear, log-linear, or sigmoidal), and the presumed mechanism of the delay (distributional versus indirect responses). Therefore, a multiplicity of experimental situations must be evaluated in order to assess the potential applicability of the approach. The only practical approach to achieve the experimental diversity required is to mine appropriate data from the literature.

The present study was designed to explore the ability to recover CHR from PK/PD data, and to assess the degree to which this descriptor of the hysteresis may be predictive of temporal delay in drug response, across a wide range of conditions. The results obtained from this effort can be viewed in context of previous information generated from simulation studies, and can provide guidance as to how and when hysteresis analysis can be a useful tool in understanding PK/PD behavior.
Methods

Data-mining strategy

Literature reports included in the present study were identified by searching in PubMed for publications with PD data when hysteresis was shown using keywords including but not limited to “hysteresis”, “effect compartment model” and “indirect response model”. Only publications in which data were analyzed with parametric modeling, and in which data were presented in graphical or tabular form, were deemed acceptable for inclusion in this study. Modeled data were important in order to compare hysteresis descriptors with PK/PD parameters; recoverable data (numeric or graphical) were required to generate the data set for analysis.

Descriptors and secondary parameters

The non-compartmental descriptors of hysteresis examined in this study included the area bounded by the hysteresis loop [ABH], the hysteresis centroid [CH] and its x-[CHX] and y-[CHY] coordinates, the unitless ratio of centroid x- vs. y-coordinate [CHR], and the ratio of CHR [CHRratio] constructed with concentration data from different sites (where applicable). The mathematical equations that were used to determine the values of these descriptors are described as follows:

\[
\text{ABH} \text{ was defined as the signed area of the loop formed by plotting pharmacologic response against concentration in blood (C_b) or in tissue (C_t). The hysteresis was simplified to a polygon defined by discrete data points, and ABH was calculated (Mathematica 8 and OriginPro 9.0) with an equation for signed partial area:}
\]
where \( x_i \) and \( y_i \) represent concentrations and effects, respectively, for each data point, and in summation \( x_{k+1} = x_k \) and \( y_{k+1} = y_k \) [80].

Similarly, \( \text{CH} \) was determined by calculating its \( x \)- and \( y \)-coordinates, respectively, using following questions:

\[
C_x = \frac{1}{6A} \sum_{i=0}^{n-1} (x_i + x_{i+1})(x_iy_{i+1} - x_{i+1}y_i)
\]

(2)

\[
C_y = \frac{1}{6A} \sum_{i=0}^{n-1} (y_i + y_{i+1})(x_iy_{i+1} - x_{i+1}y_i)
\]

(3)

where \( A \) represents ABH calculated for the corresponding loop. The remaining symbols are as described in equation (1).

\( \text{CHR} \) was calculated simply as the ratio of the two centroid coordinates recovered from equations (2) and (3).

**Software used for data analysis**

Statistical analysis was performed using R (R Core Team, Vienna, Austria). The mathematical computation and data analyses were performed in both R and Mathematica 8 (Wolfram Research, Champaign, IL). Graphics were generated by R. Digitalization of published graphs was performed with the Digitizer tool in OriginPro (OriginLab Corporation, Northampton, MA).
Results

During the data mining process, publications were selected initially according to the method described in the data mining strategy section. Results then were grouped based on similarities in the experimental design, the specifics of the PK/PD system (e.g., the type of delay in response onset), and/or the type of study performed. These groupings were intended to add insight into specific experimental scenarios, and so are presented in that manner.

Differences in delay between different sample sites and effect

After being absorbed into the systemic circulation, drugs can distribute into various tissues and organs at potentially different rates. Therefore, temporal delays in effect versus concentration may be different at different anatomical locations. To explore this issue, studies reporting concentration-time data from more than one sampling site were mined, and CHR and the CHR$_{\text{ratio}}$ between sites were compared to parameters obtained through parametric modeling. The first study examined involved the antinociceptive effect of morphine [28], which is one of the opioids that is subject to efflux by P-glycoprotein (P-gp) at the blood-brain barrier [130]. The antinociceptive effect of morphine was delayed relative to establishing concentrations in plasma and in brain extracellular fluid (ECF). A two-compartment model with a hypothetical effect compartment linked to either the plasma compartment or the brain compartment was fitted to the data. The results of hysteresis analysis are summarized in Table 5-1 and a representative hysteresis loop (after administration of the lower dose in the study) is shown in Figure 5-1. CHR values based
on plasma concentrations were larger than those based on concentrations in brain ECF at each morphine dose level examined. The ratio of CHR (5.8 and 8) was similar to the ratio of the inverse of $K_{e0}$ (6.7) between the two concentration locations (plasma and brain ECF).

A similar study examined the antinociceptive effect of morphine-6-glucuronide (M6G) in rats, with concentration monitoring in blood and in brain ECF [100]. M6G displayed the longest delay (103 min) between concentrations in plasma and production of antinociceptive response among all agents examined. Identical experiments were performed on two consecutive days to identify potential time-dependent effects, and the hysteresis loop obtained on day 1 is shown in Figure 5-2. The authors were able to fit population PK-PD models to the data from all rats by incorporating different baseline responses for different days, so only one set of parameters was reported. CHR was calculated for each experimental day. Comparisons of CHR and parameters obtained from compartmental modeling are summarized in Table 5-2. In this study, the CHR ratios were comparable between the two study days (3.5 and 4.3). The CHR ratio was approximately 2-fold larger than the $1/K_{e0}$ ratio. There also was a two-fold difference in individual CHR (based on plasma or ECF) when comparing data from day 1 to day 2, which possibly was due to differences in baseline response level between the two days.

**Dose escalation experiments**

Dose escalation experiments are common in PD studies. If the PK system is linear, increasing the dose usually has no impact on distributional delay [58]; therefore, if PK/PD dissociation is due to slow equilibration of the drug between the effect site and the systemic
circulation, dose escalation should not have an effect on delays in response onset. However, if mechanisms other than distributional delay are responsible for hysteresis, the PK/PD dissociation might change with the dose administered. Based on simulations, when there is no change in PK-PD parameters, CHR remains constant as dose increases if the effect model is linear whereas the increase is proportional if the effect model is log-linear (Chapter 4). A two-fold increase in dose leads to a change in CHR that is less than or equal to two-fold, depending on the relationship between effect and concentration.

Several studies that presented data from dose-ranging experiments were analyzed to examine the behavior of CHR in dose-escalation experiments. In the study of morphine antinociceptive effects in rats described in the preceding section, a low dose (10 mg/kg) and a high dose (40 mg/kg) were tested. The four-fold increase in dose resulted in an approximate two-fold increase in CHR based on concentrations in plasma or brain ECF (Table 5-1). Because increasing dose led to similar changes in CHR in plasma and brain ECF, the CHR ratio did not change markedly (5.8 versus 8). Estimated values of $K_{c0}$ were similar for the two doses [28]. A second study focused on tacrolimus- and cyclosporine-associated inhibition of calcineurin phosphatase activity, with two doses tested for each drug [99]. Results are summarized in Table 5-3, and a representative hysteresis loop is shown in Figure 5-3. A 10-fold increase in cyclosporine dose resulted in a 4.3-fold increase in CHR and a similar (5.3-fold) increase in $K_{c0}$. In contrast, a 50-fold increase in tacrolimus dose resulted in a 3.5-fold increase in CHR and a much larger (12.8-fold) increase in $K_{c0}$. The reason for the difference in compatibility between changes in CHR and changes in $K_{c0}$
are not readily apparent, but likely are due to the fact that the relationship between CHR and $K_{e0}$ is nonlinear in most cases.

**Different therapeutic agents with the same pharmacologic effect**

Another common area of PD investigation is on a class of drugs that have similar effects or share a common target. The PK/PD of seven opioids were evaluated in mice [131]. These opioids were chosen to cover a wide range of agents that have distinct potencies and different physiochemical properties. Results for these compounds are summarized in Table 5-4. Representative data (after administration of morphine) mined from the study are shown in Figure 5-4. The rank-order of CHR among these compounds was significantly correlated with the rank order of $t_{1/2,Ke0}$ (Spearman rho = 0.900, p < 0.05).

Another study in this category was on the electroencephalographic (EEG) effect of midazolam and diazepam in human subjects [105]. Three doses of midazolam and diazepam were administered to three individuals. The data (Figure 5-5) obtained after midazolam (25 mg) and diazepam (50 mg) from a single subject were used, and results are summarized in Table 5-5. The data from only one subject was used because original data from the other two subjects were not included in the original publication. The ratio of CHR values between the two drugs (2.54) was similar to the ratio of $K_{e0}$ (2.72), suggesting compatibility of the two metrics.

**Single drug with multiple effects**

Studies on drugs that have multiple targets, or act on one target that induces changes in various physiologic processes, often include measurement of multiple biologic effects
after a single dose. Different effects may have similar or different time delays relative to production of systemic concentrations, depending on the target organ for each effect and the mechanism by which each effect is produced. Data were mined from two such studies to assess how CHR compares to model-dependent parameters across different biologic effects. The first of these studies was on the effects of verapamil in sheep [132]. The parameter estimates for the (-)–enantiomer of verapamil are summarized in Table 5-6, and representative data are shown in Figure 5-6. The three effect measurements differed greatly in scale and in magnitude of delay based on $K_{e0}$ values. CHR did not correlate well with $K_{e0}$ across this set of responses. The second study in this category was on central nervous system (CNS) effects of lorazepam measured with computerized continuous tracking (TRKN), body sway with eyes open (SWAY OPEN), and digit symbol substitution (DSS) tests [120]. Although parameter estimates were reported for all six subjects, data for calculating CHR could be recovered for only one subject (subject 6; Figure 5-7). Results for this single subject are summarized in Table 5-7. Out of three measured effects, TRKN and SWAY OPEN were on similar numerical scale (ranged from 0-500), while DSS were on a smaller scale (ranged from 0-90). Overall, there was not a consistent relationship between CHR and $K_{e0}$ across the three effect measurements.

**Linear effect model**

Previous simulation studies indicated that the sensitivity of CHR in response to changes in PK/PD parameters was highest when the relationship between effect and concentration are linear (Chapter 4). Two studies with a linear effect-concentration
relationship were identified and are included here. EEG effects of risperidone in healthy volunteers were investigated by Lee et al. [133]. Hysteresis was observed in the EEG effect versus plasma concentration of risperidone (Figure 5-8) or the sum of risperidone and its active metabolite (noted as risperidone(+)) relationships. Results are summarized in Table 5-8. Consistent with previous simulations that indicated CHR is more sensitive than Ke0 to system changes when the effect-concentration relationship is linear, the change in CHR between risperidone and risperidone(+) (3.36) was two-fold larger than the change in Ke0 (1.68). The next study in this category examined a drug interaction with itraconazole and domperidone [91]. PK/PD of domperidone was evaluated after 5 days of treatment with either itraconazole or placebo (Figure 5-9). The relationship between effect and concentration at the effect site was linear. The AUC of domperidone was increased 3-fold by itraconazole, but maximum response was not affected. As with the risperidone study [133], the change in CHR between itraconazole treatment and placebo (3.16) was larger than the relative change in Ke0 (1.94) (Table 5-9).

Drug-Drug interaction studies

Two studies were found on the interaction of irbesartan and hydrochlorothiazide (HCTZ), one in hypertensive dogs (Figure 5-10) [134] and one in normotensive human volunteers (Figure 5-11) [135]. In the first study, effects on systolic (SBP) and diastolic (DBP) blood pressure were linked to concentrations through a sigmoidal effect model. Results are summarized in Table 5-10. In this study, the larger CHR (in the absence of the drug interaction) corresponded to the longer time delay as identified by PK/PD modeling.
However, the ratio of CHR between the two different treatments (approximately two-fold) was lower than the ratio of $1/K_{e0}$ between the two treatments (approximately three-fold), suggesting somewhat lower sensitivity for CHR. Results of the study performed in healthy volunteers are summarized in Table 5-11. Neither CHR nor $K_{e0}$ was different between treatments when the effect was SBP. When changes in DBP were modeled, there was a two-fold increase in $K_{e0}$ in the interaction phase, without a corresponding increase in CHR. However, the authors of the original publication indicated that the difference in $K_{e0}$ was not statistically significant, and the apparent difference in the estimate might simply reflect a large variance (80%). Regardless, in this particular case, it is apparent that both CHR and $K_{e0}$ indicate that the drug interaction has little or no effect on temporal delay in response.

**Individual variability of CHR and $K_{e0}$**

Intersubject variability in CHR was compared to intersubject variability in model-dependent parameters by mining data from publications in which data and parameter estimates were reported for individual subjects. The QTc interval prolongation effects of dofetilide were measured in 10 subjects after intravenous infusion [136]. A hysteresis loop was observed in all subjects when dofetilide was given intravenously, and a representative plot is shown in Figure 5-12. CHR was calculated for each subject and compared with the reported value of $K_{e0}$ obtained through PK/PD modeling (Table 5-12). Because $K_{e0}$ values were not reported for subjects 1 and 3, these subjects were not included in the hysteresis analysis. The coefficient of variation (CV%) for CHR (0.0079 ± 0.0012) was 16%. In
comparison the CV% for $K_{e0}$ (7.51 ± 3.46) was 46% (Table 5-13). These data suggest that CHR is at least as reproducible a metric as the model-derived $K_{e0}$.

Discussion

The relationship between CHR and $K_{e0}$ in PD experiments

In PK, the hysteresis descriptor CHR was found to be a linear function of the inverse of the rate constant ($K_{21}$) governing drug flux from the peripheral compartment to the central compartment (Chapter 2). In other words, a larger CHR resulted from, and was therefore predictive of, a longer time delay in establishing equilibrium between two compartments. CHR therefore represents an alternative non-parametric approach to characterizing the distributional delay between compartments. However, CHR was found to lose sensitivity to changes in temporal delay when applied to simulated PD data, primarily because of the inherently nonlinear relationship between effect and concentration in many cases (Chapter 4). Because $K_{e0}$ is, in essence, the PD analog of $K_{21}$ ($K_{e0}$ governs loss of drug from the hypothetical effect compartment), this study was undertaken to assess the degree to which changes in CHR are compatible with changes in $K_{e0}$ across a wide range of experimental situations.

The previous simulation study indicated that the relationships between CHR and PK/PD parameters are model structure-dependent; CHR can either increase or decrease when the parameter value was increased depending on the specific structural model generating the data (Chapter 4). For example, in a simple two-compartment model with the effect site in the tissue compartment, CHR increased as the egress rate constant from the
tissue compartment decreased. When data were simulated with an effect-compartment model, CHR had a positive linear relationship with $K_{e0}$ when only $K_{e0}$ was changed, but it changed linearly with $1/K_{1e}$ when only $K_{1e}$ or both $K_{1e}$ and $K_{e0}$ (given $K_{1e} = K_{e0}$) were changed simultaneously. In indirect response models, CHR increased linearly with the inverse of $K_{out}$, a parameter equivalent to $K_{e0}$. As a result, it was difficult to pinpoint the direction of change in time delays based on CHR alone when the underlying mechanism of the delay was unknown. Although it is possible to distinguish longer delays from shorter delays based on common sense or visual inspection of the graph itself in some cases, CHR ceased to be a stand-alone alternative approach that quantifies changes in time delay between effect and concentration. Somewhat counter-intuitively, changes in CHR can be used as a quantitative measurement of the change in temporal delay, but the direction of change in temporal delay cannot be inferred from CHR. In addition, if the relationship between effect and concentrations is sigmoidal, changes in CHR might be less than changes in $K_{e0}$ because the sensitivity of CHR decreases when the effect-concentration relationship is nonlinear.

The relationship between relative changes in CHR and relative changes in $K_{e0}$

The ratio of CHR between two experimental treatments (two different drugs, for example) has not been tested previously as a predictive parameter. The potential utility of the ratio of CHR was explored because it has the potential to quantify changes as a relative difference in time delay between two experimental conditions. In the first study [28], the ratio of CHR between two sample sites, plasma versus brain ECF, were similar when dose
was changed. The ratio of CHR was compatible with the ratio of $K_{e0}$ (5.8 and 8 versus 6.5). Similar results were obtained from a study in which the antinociceptive effect of M6G was measured; CHR ratios were comparable between study days, even though CHR based on individual sample site showed two-fold differences (Table 5-2), suggesting that, as a relative measure, the ratio of CHR was consistent with results obtained through compartmental modeling. It can be concluded from these examples that, when CHR can be calculated under two or more experimental conditions, the relative change in CHR as a ratio is reasonably predictive of the relative change in the model-dependent parameter $K_{e0}$.

**Influence of dose on CHR**

The influence of an increase in dose on CHR was investigated in previous simulations (Chapter 4). When the effect model was linear, CHR did not change with dose. When a log-linear effect model was used, CHR increased almost linearly with dose; when a sigmoidal effect model was used, increases in dose resulted in a less-than-proportionate increase in CHR. In the first study, the CHR ratio ($CHR_{plasma}$ versus $CHR_{brain\ ECF}$) for the lower dose was similar to that for the higher dose. This result suggests that if concentrations were sampled in two places with distinct concentration-time profiles, the ratio of CHR would remain relatively unchanged if the temporal delay was unchanged with increasing dose. This observation is consistent with the underlying assumption of the effect-compartment model, in which the time delay between effect production and concentration establishment should remain unchanged with dose.
Despite the expectations associated with the effect-compartment model, there were some studies in which estimates of $K_{e0}$ changed with dose. For example, both $K_{e0}$ and CHR increased with increasing in dose in a study of tacrolimus and cyclosporine [99], but the ratio of CHR was smaller than the ratio of $K_{e0}$ for tacrolimus and similar to that of $K_{e0}$ for cyclosporine.

**Influence of PD properties on CHR**

In the previous simulation study (Chapter 4), comparisons between different therapeutic agents with different potencies were not considered because $EC_{50}$ and $E_{max}$ were fixed in all conditions. However, both parameters would be expected to have an impact on CHR. Mining data offered an opportunity to examine the behavior and sensitivity of CHR to differences in potency or affinity of drugs. One difficulty associated with addressing this issue with actual, as opposed to simulated, data is that experiments often account for potency by adjusting the dose to ensure a comparable range of responses for different drugs. Results (Table 5-4) suggested that despite the relationship between $K_{e0}$ and CHR being, in most cases, nonlinear, CHR had a strong rank-order correlation with $t_{1/2}$ $k_{e0}$ in experiments examining a wide range of agents. By utilizing a rank-order approach, as opposed to linear regression of the raw data, the influence of nonlinear behavior is minimized.

A study of verapamil provided an example in which difference in PD parameters ($E_{max}$ and $EC_{50}$) can impact the value of CHR (Table 5-6). In another study on lorazepam and its effects on CNS (Table 5-7), the scale of three effect measurements were closer to
each other than those in the verapamil study, but CHR still did not have a consistent relationship with $K_{e0}$. The differences in the concentration-effect relationship of three effect measurements ($EC_{50}$, $E_{max}$ and $\gamma$) had differing impact on CHR and $K_{e0}$. Standardizing effect measurements to percentage change from baseline eliminated the impact of range of changes in effects on CHR, but might lead to undesirable effects on CHR such as limiting the possible values of CHR and decreasing sensitivity. In summary, the results of this set of studies suggested that CHR was not suitable for making comparisons between drugs with different PD properties, although when a large number of drugs were included in the analysis, CHR could be indicative of $K_{e0}$ overall (Table 5-4).

**Linear effect versus sigmoidal effect model**

As found in simulations, CHR was sensitive to changes in PK/PD parameters when a linear effect relationship was used to link concentration to effect. In contrast, sensitivity diminished when the effect-concentration relationship was nonlinear. Although a linear effect model is rarely observed in clinical studies, a few examples were identified. As shown in Tables 5-8 and 5-9, when the relationship between effect and concentration was linear, the differences between CHRs were larger than difference in $K_{e0}$ based on ratios, consistent with a higher sensitivity for CHR. Moreover, CHR ratios were positively related to $K_{e0}$ which is consistent with simulations with the effect-compartment model when $K_{e0}$ was changed by itself. The results based on mined data were similar to those from simulations, and suggested that when the effect model is linear, CHR is sensitive to changes in the magnitude of delay between effect and concentration.
The sigmoidal effect model is undoubtedly the most commonly used function for describing the effect-concentration relationship. This commonality is reflected in the studies included here, most of which were modeled with a sigmoidal function. Out of 10 mined studies in which a sigmoidal effect model was used, two produced CHR values that did not have a consistent relationship with $K_{e0}$ (Table 5-6, 5-7). In the remaining studies, when there was a consistent relationship between CHR and $K_{e0}$, ratio of CHR was either similar to or smaller than the ratio of $K_{e0}$ or its inverse (Table 5-1, 5-3, 5-5, 5-10). Only in one study (Table 5-2), the ratio of CHR was larger than ratio of $K_{e0}$. In one study (Table 5-4) the rank order of CHR was significantly correlated with rank order of $t_{1/2K_{e0}}$. Therefore, consistent with previous simulations, the sensitivity of CHR in PD was dependent on having a linear relationship between effect and concentration. When the relationship between effect and concentration is nonlinear, the relationship between $K_{e0}$ and CHR was often nonlinear which produced inconsistent results among studies examined.

**Inter-individual variability of CHR and $K_{e0}$**

Because the value of CHR can be influenced by many factors, it did not correlate well with PK/PD parameters such as $K_{e0}$ when multiple factors were significantly changed between conditions. It might be suitable for analyzing data from systems in which parameters remained relatively unchanged, or varied within a limited range, such as in studies in which data are collected in multiple individuals. The inter-individual variability of CHR was compared to the variability in parameters using data obtained in multiple individuals (Table 5-12). The coefficient of variation (CV) of CHR was smaller than that
of $K_{e0}$. The likely reason for smaller CV of CHR might be that the CHR was not as sensitive to changes in system in PD unless the relationship between concentrations and effect was linear. In addition, the CV of $K_{e0}$ also reflects estimation error resulting from compartmental modeling due to uncertainties in parameter estimation.

**Conclusion**

The presence of hysteresis has been used as an indicator of temporal delay between effect and concentration. The goal of the present study was to investigate the utility of CHR in quantifying that delay based on published data. Data were mined from 12 publications to investigate the utility of CHR across a wide range of experimental goals and protocols. Similar to previous simulation studies, CHR was less sensitive than $K_{e0}$ when the effect-concentration relationship was sigmoidal. When the effect-concentration relationship was linear, CHR was more sensitive than $K_{e0}$. Because multiple PK and PD factors can impact CHR, this metric did not correlate well with $K_{e0}$ when comparisons were made among drugs with distinct PD properties, or in situations in which different effects were obtained for the same drug.
Figure Legends

Figure 5-1. Average antinociception versus concentration relationship of morphine [28]. The antinociceptive-concentration relationship based on averaged data in venous blood (empty circles) and brain ECF after 10-min infusion of morphine of 10 mg/kg (low dose). The hysteresis loop shown was recovered from figure 3 in the original paper and is representative of other hysteresis loops [28].

Figure 5-2. Relationship between effect and concentration in rats [100]. Relationship between averaged effect and concentration in venous blood (filled squares) and in brain ECF (empty circles) after an exponential infusion of M6G over 4 hours on day 1. Representative hysteresis loop recovered from figure 3 in the original paper [100].

Figure 5-3. The relationship between averaged calcineurin activity and tacrolimus concentrations in whole blood [99]. The relationship between averaged calcineurin activity and tacrolimus concentration in whole blood (measured effects) after administration of tacrolimus at 0.1 mg/kg (filled squares) and 5 mg/kg (empty circles). The hysteresis loop shown was recovered from the original paper [99].

Figure 5-4. Concentration-time profile of morphine in blood (filled squares) and in brain ECF (empty circles) (A) and time course of antinociception (B) after a 3.6 mg/kg s.c. dose of morphine [131]. The hysteresis loop (C) was constructed from the data shown in (A) and (B), which were recovered from original paper.

Figure 5-5. The effect versus plasma concentration plot after a dose of diazepam in subject S [105]. The hysteresis loop shown was directly recovered from the original paper.
Figure 5-6. The concentration-time profile of (-)verapamil in arterial blood (A) and the time course of myocardial blood flow (Qh) (B) after administration of verapamil hydrochloride as a 2-min infusion [132].

The hysteresis loop (C) was constructed using the data shown in (A) and (B), which were recovered from the original paper.

Figure 5-7. Subcritical tracking (TRKN) versus plasma lorazepam concentration in subject 6 after a single oral dose of lorazepam [120]. The hysteresis loop presented in the figure was recreated from the original publication.

Figure 5-8. The mean EEG effect versus mean concentration of risperidone after oral administration in healthy human volunteers [133]. The figure was created through digitalization of the corresponding figure in the original publication.

Figure 5-9. The relationship between serum prolactin levels and plasma concentration of domperidone in a representative subject after placebo (empty circles) or itraconazole (filled circles) once a day for five days [91]. The figure was created based on digitalization of the corresponding figure in the original publication.

Figure 5-10. The relationship between inhibitory effects on SBP and plasma concentration of irbesartan after a single dose of irbesartan (30 mg/kg) with or without HCTZ (10 mg/kg once daily) [89]. The hysteresis loop shown was created with digitalization of the corresponding figure in the original publication.
**Figure 5-11.** The relationship between the change in SBP and plasma concentration of irbesartan (IRB) after a single dose of IRB alone (filled circles) or with combination of IRB and hydrochlorothiazide (HCT) (empty circles) [135]. The figure was created by digitalization of the corresponding figure in the original publication.

**Figure 5-12.** The relationship between QTc intervals duration and plasma concentration of dofetilide after intravenous administration of dofetilide in subject # 9 [136]. The hysteresis loop is based on digitalization of the corresponding graph in the original publication.
Figure 5-1. Average antinociception versus concentration relationship of morphine [28].

The antinociceptive-concentration relationship based on averaged data in venous blood (empty circles) and brain ECF after 10-min infusion of morphine of 10 mg/kg (low dose). The hysteresis loop shown was recovered from figure 3 in the original paper and is representative of other hysteresis loops [28].
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Relationship between averaged effect and concentration in venous blood (filled squares) and in brain extracellular fluid ECF (empty circles) after an exponential infusion of morphine-6-glucuronide (M6G) over 4 hours on day 1. The representative hysteresis loop is recovered from figure 3 in the original paper [100].
Figure 5-3. The relationship between averaged calcineurin activity and tacrolimus concentrations in whole blood [99].

The relationship between averaged calcineurin activity and tacrolimus concentration in whole blood (measured effects) after administration of tacrolimus at 0.1 mg/kg (filled squares) and 5 mg/kg (empty circles). The hysteresis loop shown was recovered from the original paper [99].
Figure 5-4. Concentration-time profiles of morphine in blood (filled squares) and in brain ECF (empty circles) (A) and time course of antinociception (B) after a 3.6 mg/kg subcutaneous dose of morphine [131].

The hysteresis loop (C) was constructed from the data shown in (A) and (B), which were recovered from original paper.
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The hysteresis loop shown was directly recovered from the original paper.
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The hysteresis loop presented in the figure was recreated from the original publication.
Figure 5-8. The mean EEG effect versus mean concentration of risperidone after oral administration in healthy human volunteers [133].

The figure was created through digitalization of the corresponding figure in the original publication. [The effect of risperidone on the EEG was described as the difference in absolute power in delta frequency band between risperidone and placebo.]
Figure 5-9. The relationship between serum prolactin levels and plasma concentration of domperidone in a representative subject after administration of placebo (empty circles) or itraconazole (filled circles) once a day for five days [91].

The figure was created based on digitalization of the corresponding figure in the original publication.
Figure 5-10. The relationship between inhibitory effects of irbesartan on systolic blood pressure and plasma concentration after a single dose of irbesartan (30 mg/kg) with or without hydrochlorothiazide (10 mg/kg once daily) [134].

The hysteresis loop shown was created with digitalization of the corresponding figure in the original publication.
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The figure was created by digitalization of the corresponding figure in the original publication.
Figure 5-12. The relationship between QT<sub>c</sub> intervals duration and plasma concentration of dofetilide after intravenous administration of dofetillide in subject # 9 [136].

The hysteresis loop is based on digitalization of the corresponding graph in the original publication.
## Tables

Table 5-1. Parameter estimations and CHR based on data obtained after 10 min infusion of morphine of 10 or 40 mg/kg in Rats [28].

<table>
<thead>
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<th></th>
<th>CHR</th>
<th>CHR&lt;sub&gt;ratio&lt;/sub&gt;</th>
<th>K&lt;sub&gt;e0&lt;/sub&gt; (min)</th>
<th>t&lt;sub&gt;1/2ke0&lt;/sub&gt; (min&lt;sup&gt;-1&lt;/sup&gt;)</th>
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<td>0.148</td>
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<tr>
<td><strong>High dose</strong></td>
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</tr>
<tr>
<td>(40mg/kg)</td>
<td>Plasma</td>
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<td>8</td>
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<tr>
<td></td>
<td>Brain ECF</td>
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Table 5-2. Parameter estimations and CHR of morphine-6-glucuronide (M6G) in rats [100].

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<thead>
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<th>CHR</th>
<th>$K_{e0}$ (min⁻¹)</th>
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<tr>
<td>Brain ECF (day 1)</td>
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<tr>
<td>Venous (day 2)</td>
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<td>Brain ECF (day 2)</td>
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Table 5-3. Parameter estimations and CHR of tacrolimus and cyclosporine in rats [30].

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<tbody>
<tr>
<td>Tacrolimus (0.1 mg/kg)</td>
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</tr>
<tr>
<td>Tacrolimus (5 mg/kg)</td>
<td>189</td>
<td>0.0015</td>
</tr>
<tr>
<td>Cyclosporin (1 mg/kg)</td>
<td>336</td>
<td>0.124</td>
</tr>
<tr>
<td>Cyclosporin (10 mg/kg)</td>
<td>78</td>
<td>0.024</td>
</tr>
</tbody>
</table>
Table 5-4. CHR and parameter estimations [131].

<table>
<thead>
<tr>
<th></th>
<th>CHR</th>
<th>K_e0 (min⁻¹)</th>
<th>t_{1/2_k_e0} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufentanil</td>
<td>0.02</td>
<td>0.16</td>
<td>4.3</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.08</td>
<td>0.14</td>
<td>4.9</td>
</tr>
<tr>
<td>Methadone</td>
<td>7.13</td>
<td>0.073</td>
<td>9.6</td>
</tr>
<tr>
<td>Loperamide</td>
<td>26.3</td>
<td>0.025</td>
<td>27</td>
</tr>
<tr>
<td>Morphine</td>
<td>8.22</td>
<td>0.009</td>
<td>74</td>
</tr>
</tbody>
</table>
Table 5-5. Parameter estimations and CHR of electroencephalographic effect of diazepam and midazolam [105].

<table>
<thead>
<tr>
<th></th>
<th>CHR</th>
<th>$t_{1/2 \text{ ke}0}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam (50mg)</td>
<td>20.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Midazolam(25mg)</td>
<td>7.94</td>
<td>4.9</td>
</tr>
</tbody>
</table>
Table 5-6. Parameter estimations and CHR of verapamil in sheep [132].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CHR</th>
<th>$K_{o0}$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_h$</td>
<td>0.73</td>
<td>1.7</td>
</tr>
<tr>
<td>LV $dP/dt_{max}$</td>
<td>0.0003</td>
<td>0.33</td>
</tr>
<tr>
<td>PR interval</td>
<td>0.01</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Table 5-7. Parameter estimations and CHR of lorazepam in human subject ( # 6) [120].

<table>
<thead>
<tr>
<th></th>
<th>CHR</th>
<th>$t_{1/2 \text{k}e0}$ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRKN</td>
<td>0.14</td>
<td>0.31</td>
</tr>
<tr>
<td>SWAY OPEN</td>
<td>0.18</td>
<td>0.70</td>
</tr>
<tr>
<td>DSS</td>
<td>0.76</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Table 5-8. CHR and parameter estimations of risperidone in healthy volunteers [133].

<table>
<thead>
<tr>
<th></th>
<th>CHR</th>
<th>$t_{1/2 , ke0}$ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risperidone</td>
<td>1.67</td>
<td>0.74</td>
</tr>
<tr>
<td>Risperidone (+)</td>
<td>5.62</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Table 5-9. Influence of itraconazole on domperidone in healthy male volunteers [91].

<table>
<thead>
<tr>
<th></th>
<th>CHR</th>
<th>$K_{e0}(\text{hr}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domperidone</td>
<td>0.37</td>
<td>3.22</td>
</tr>
<tr>
<td>Domperidone + Itraconazole</td>
<td>1.17</td>
<td>6.25</td>
</tr>
</tbody>
</table>
Table 5-10. Parameter estimations and CHR of irbesartan in hypertensive dogs [134].

<table>
<thead>
<tr>
<th></th>
<th>CHR</th>
<th>$K_{e0}\text{(min}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irbesartan (SBP)</td>
<td>0.76</td>
<td>0.36</td>
</tr>
<tr>
<td>Irbesartan + HCTZ (SBP)</td>
<td>0.42</td>
<td>1.26</td>
</tr>
<tr>
<td>Irbesartan (DBP)</td>
<td>1.76</td>
<td>0.48</td>
</tr>
<tr>
<td>Irbesartan + HCTZ (DBP)</td>
<td>0.89</td>
<td>1.68</td>
</tr>
</tbody>
</table>
Table 5-11. Parameters estimation and CHR of irbesartan (IRB) in human subjects [135].

<table>
<thead>
<tr>
<th></th>
<th>CHR</th>
<th>$K_{e0}$ (hr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRB-SBP</td>
<td>0.47</td>
<td>0.44</td>
</tr>
<tr>
<td>FDC-SBP</td>
<td>0.44</td>
<td>0.50</td>
</tr>
<tr>
<td>IRB-DBP</td>
<td>0.74</td>
<td>0.32</td>
</tr>
<tr>
<td>FDC-DBP</td>
<td>0.69</td>
<td>0.62</td>
</tr>
</tbody>
</table>
Table 5-12. QTc interval prolongation after intravenous infusion of dofetilide [136].

<table>
<thead>
<tr>
<th>Subject #</th>
<th>CHR</th>
<th>$K_{e0}$ (hr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.006</td>
<td>10.2</td>
</tr>
<tr>
<td>4</td>
<td>0.007</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>0.0085</td>
<td>5.7</td>
</tr>
<tr>
<td>6</td>
<td>0.009</td>
<td>15.3</td>
</tr>
<tr>
<td>7</td>
<td>0.009</td>
<td>4.7</td>
</tr>
<tr>
<td>8</td>
<td>0.009</td>
<td>7.3</td>
</tr>
<tr>
<td>9</td>
<td>0.009</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>0.006</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Mean: 0.0079 | 7.5
SD: 0.0013 | 3.4
CV(%): 16 | 46
The presence of a hysteresis loop in the relationship between effect and concentration under non-steady-state conditions has long been used as an indicator of dissociation between response to a drug and production of drug concentrations in the systemic circulation or target tissue [58]. Hysteresis behavior impairs the ability to recover accurate estimates of parameters such as the EC$_{50}$ that determine the true relationship between pharmacologic response and concentration [82]. Moreover, while the hysteresis itself indicates the presence of a temporal delay, it does not provide information regarding the magnitude or nature of the delay.

As discussed in the introductory chapter of this dissertation, the most commonly-used approach to analyze data with hysteresis behavior is through integrated compartmental modeling, including but not limited to effect-compartment models developed by Sheiner et al. [35], indirect response models as described by Dayneka and co-workers [42], and other mechanism-based and hybrid models [34]. A common aspect of each of these approaches is that the mechanism underlying the delayed elaboration of response must be known, significant assumptions regarding model structure must be made, or hypothetical constructs must be included. The hypothetical or uncertain nature of these models diminishes their value and potential leads to misinterpretation of analytical results.

In contrast to pharmacokinetic (PK) analysis, for which non-compartmental approaches based on statistical moment theory [68] are widely used, non-compartmental
or model-independent approaches for analysis of pharmacodynamic (PD) data, in particular for data that is characterized by hysteresis behavior, was lacking at the time this project was initiated. The purpose of the work presented in this dissertation therefore was aimed at developing and evaluating potential model-independent approaches to quantify temporal delays in PK and PD phenomena. Specifically, this work focused on distributional delays between a target organ and the systemic circulation, characterized by dissociation of the concentration-time profile in tissue from the concentration-time profile in blood (Chapters 2 and 3), and delays in effect onset and the production of concentrations in the target tissue or the systemic circulation, characterized by dissociation of the effect-time profile from the concentration-time profile (Chapter 4 and 5). Comprehensive simulation experiments were utilized to identify the optimal metric(s) for expressing PK or PD delays based on hysteresis loop morphology, and the utility of those metrics were tested prospectively against data mined from the literature.

Similar to the concentration-time profile and the effect-concentration relationship, the hysteresis loop also is a geographic shape to which morphologic analysis can be applied, and it was hypothesized that loop morphology contained information relevant to the time delay between PK or PD events. The descriptors of the hysteresis loop that were considered as candidate metrics included the area bounded by the hysteresis [ABH], the x-coordinate of the centroid of hysteresis loop [CHX], the y-coordinate of the centroid of hysteresis loop [CHY], and the ratio of centroid x- vs. y-coordinate [CHR]. Previous work suggested that ABH was unlikely to be a useful metric [70]. However, the specific reasons
associated with this assessment were not entirely clear, and so it was included in the current effort.

In Chapter 2, the relationship between hysteresis descriptors and PK parameters associated with slow equilibration of drug concentration between a target tissue and blood was evaluated. Concentration-time data in tissue and blood were generated via simulation based on several commonly-used PK model structures, representing drugs with a variety of PK characteristics, including mammillary systems (all distributional compartments radiating from a central compartment that contained the systemic circulation), catenary systems (transit-compartment systems in sequence rather than radiating from the central compartment), linear systems (the rates of all flux processes being proportional to the driving-force concentration for the process), and non-linear systems (some flux processes being capacity-limited). Results of this initial simulation study corroborated previous work indicating that ABH was not a useful metric across a wide range of conditions as it evidenced a non-linear relationship with PK parameter(s) as the distributional delay increased. The unitless metric CHR was identified as the most useful predictor of distributional delay among all hysteresis descriptors examined in this study. Importantly, CHR evidenced a linear relationship with the inverse of egress rate constant from target tissue to plasma (K21). Because the inverse of K21 often is viewed as a measure of the mean residence time of the substrate in that compartment, and the magnitude of distributional delay is a function of the mean residence time in the distributional compartment, the correlation between CHR and 1/K21 was viewed as a positive result.
The work reported in Chapter 3 was directed towards evaluating the utility of CHR as a metric for distributional delay by applying hysteresis analysis to data from a microdialysis study in humans [81]. This approach was taken in part to test whether the conclusions based on the simulation experiments in Chapter 2 would apply to data mined from a published study. Similar to expectations based on simulations, CHR had a positive relationship with the magnitude of time delay between the concentration-time profile in brain extracellular fluid and the concentration-time profile in plasma for the chemotherapeutic agent methotrexate. Specifically, patients with a disrupted blood-brain barrier had low values of CHR compared to patients with intact blood-brain barrier function. Because the blood-brain barrier impedes the distribution of methotrexate from blood into brain tissue, disruption of the blood-brain barrier should decrease distributional delay decrease CHR. This was, in fact, what was observed in this data-mining experiment. Moreover, hysteresis analysis produced more consistent and robust results when compared to both traditional compartmental modeling and non-compartmental approaches. In particular, the compartmental approach suffered from the inability to identify a singular model that could be applied to all data sets in the blood-brain barrier condition, while the non-compartmental approach would need to rely on an unacceptable degree of extrapolation of the data to fully characterize the area under the concentration-time profile and the first moment of the concentration-time profile for brain extracellular fluid in patients with intact blood-brain barrier function. In other words, neither the model-based approach nor the non-compartmental approach was appropriate for this set of data, a
situation that is not uncommonly encountered in PK analysis, especially in the clinical arena.

Taken together, the results of experiments presented in Chapters 2 and 3 demonstrated that hysteresis analysis is generally applicable to PK data provided that concentrations were measured in both the target organ and the systemic circulation and that the PK system (or at least the processes governing distribution of the drug between the systemic circulation and the target tissue) is linear or predominantly linear. It must be noted that, because the traditional approaches to PK data analysis are well-established, well-accepted, and generally both robust and facile, that the opportunity for a significant contribution of hysteresis analysis to PK analytical techniques is relatively limited. This line of investigation was pursued, however, for two reasons. The first, and most important, reason for including PK analysis as a part of this dissertation project was that delayed onset of pharmacologic response often is the result of slow distribution of the drug or an active metabolite to the site of action. Therefore, if the approach to examining hysteresis analysis in PD systems is to be comprehensive, then application of that analysis to distributional (PK) phenomenon was a logical starting point. The second reason, however, was not without merit: as mentioned above, it is not uncommon to encounter data that simply are not amenable to analysis by compartmental or traditional non-compartmental approaches. Although its applicability may be somewhat limited, the hysteresis analysis approach now provides a third option for analyzing data that might be incompatible with the more well-established techniques.
After having demonstrated that CHR was the best metric from morphologic analysis of the hysteresis loop in terms of describing distributional delays, the next step in this project was to evaluate the utility of hysteresis analysis in PD systems, recognizing that the observation of hysteresis behavior is most commonly associated with PD, rather than PK, data. Because the simulation studies in Chapter 2 indicated that CHR was a reasonable metric for linear systems, but utility of the metric was eroded as the system became increasingly nonlinear, attention needed to be paid to the impact of nonlinear relationships between effect and concentration. Because most PD systems are characterized by a sigmoidal, hyperbolic, or log-linear relationship between effect and concentration [3], the impact of different types of concentration-effect functions on the relationship between CHR and parameters associated with delayed pharmacologic response was of particular interest.

In Chapter 4, the utility of PD hysteresis descriptors was explored in simulated data generated with several commonly-used PK/PD model structures, and with different functions governing the effect versus concentration relationship. As was anticipated based upon the results of PK simulations, the introduction of nonlinearity through the effect-concentration relationship had a substantial impact on the relationship between CHR and parameters associated with the time delay between concentration and effect onset. Most hysteresis descriptors (ABH, CHX, CHY) evidenced a profound nonlinear relationship with model parameters. Similar to observations with PK hysteresis, CHR had the most
consistent relationship with PD model parameter(s), but the relationship was nonlinear in many cases or over a subset of the parameter space.

The basis of the nonlinearity between CHR and PD parameters was evident when results produced by analyzing data simulated with a nonlinear (sigmoidal) effect model were compared to results produced by analyzing data simulated with a linear effect model: in the presence of a linear effect versus concentration relationship, CHR retained sensitivity to changes in PD parameter values and maintained a linear relationship with those parameters ($K_{21}$ or its equivalent depending on the specific model structure). Taken together with observations from PK hysteresis analysis, the results of the experiments presented in Chapter 4 suggest that CHR as a metric of effect delay would be useful when the effect-concentration relationship is linear or approximately linear, or when the effect-concentration relationship is sigmoidal and the delay in effect onset is relatively large. These results are consistent with those from the PK portion of this dissertation, and provide an important measure of internal consistency: Regardless of either model structure or hysteresis type (PK or PD), nonlinearities in the system that produce the data composing the hysteresis results in nonlinear relationships between CHR and the model parameter(s) responsible for the distributional or effect-onset delay.

Because data used in Chapter 4 were simulated in a controlled manner (only one parameter was changed at a time), it was possible that the results were not entirely representative of actual experimental situations in which multiple parameter values might differ between subjects in an experiment or, certainly, across different drugs or
pharmacologic classes. Therefore, an additional data-mining experiment was performed. Data were obtained from 12 published studies in which the effect versus concentration relationship was characterized by a hysteresis loop, and hysteresis analysis was performed on each data set. The results of that analysis were compared to relevant parameter estimates (e.g., $K_{e0}$) that were reported in the original publications (i.e., the model-dependent analyses were not repeated, but rather the published results were accepted). The results of this experiment, which are summarized in Chapter 5, were consistent with the observations presented in Chapter 4. CHR was useful metric for effect-onset delay when the effect versus concentration function was linear or when temporal dissociation between effect and concentration was relatively large. Due to the nonlinear relationship between CHR and $K_{e0}$ when the effect model is nonlinear, CHR became a less reliable predictor of the time delay when the effect versus concentration relationship was sigmoidal.

Considering both the PK and PD aspects examined in this dissertation project, the primary advantages of hysteresis analysis may be stated as follows: (1) CHR is easy to calculate and can be used to infer a dissociation between PD and PK processes without resorting to parametric modeling; (2) as a non-parametric approach, calculation of descriptors of hysteresis loop (most importantly CHR) was robust with respect to calculation errors that are associated with extrapolation beyond the final data point collected, as is the case in calculating AUC for example, because in the case of hysteresis analysis there is no need to extrapolate from the last data point to infinite time. As with
other non-compartmental approaches, however, the utility of CHR as a metric was limited to linear systems or when the time delay was long.

**Future experimental direction**

In general, the results of this dissertation project are viewed as definitive with respect to morphologic aspects of the hysteresis loop that may be used as quantitative metrics for expressing distributional or effect-onset delays. ABH simply is not a useful parameter because it is confounded by scaling issues; changes in system parameters that result in increased delay can affect the scale of one (or both) variables composing the hysteresis loop. Both the x- and y-coordinates of the hysteresis centroid evidence nonlinear relationships with system parameters associated with temporal delays in distribution or effect onset. Those nonlinearities limit the sensitivity and predictive power of those descriptors. CHR, however, appeared to be a sensitive and robust metric, at least in the presence of linear PK or PD processes. Thus, this work has clearly established that CHR is the optimal (and in actuality the only) aspect of hysteresis morphology that may be a useful metric of temporal delays.

A question that remains is related to the influence of experimental error on the predictive capabilities of CHR. The simulation studies performed in this dissertation project produced error-free data to facilitate exploration of the relationship between hysteresis morphology and system parameter values. It would be of interest to explore the relationship between calculated CHR and systems parameters associated with distributional or effect-onset delay when the variables composing the hysteresis
(concentrations at two different sites for PK hysteresis; effect and concentration for PD hysteresis) are subject to experimental error. Although the simulations themselves would be straightforward to perform, there are several complicating factors that would need to be considered. For example, the “size” of the hysteresis relative to the variability in both the x- and y-variables would be an issue to explore, because at some point the presence of a hysteresis loop would be obscured by variability in the data.

The issue of experimental error raises a second consideration for future evaluation: is it possible to develop a statistical framework to identify the presence or absence of hysteresis within a particular data set? As described in the preceding paragraph, sometimes hysteresis behavior may be obscured by variability in the data (that is, a system with a true delay may produce “hysteresis-free” data simply because the data themselves have sufficient variability to obscure the presence of a hysteresis loop). Conversely, a system without a delay may, by chance, produce data that appear to form a hysteresis loop simply because the data are variable. Having a statistical approach that can objectively identify the presence of a true hysteresis loop would be a helpful addition to the analytical toolbox.

A third simulation experiment that might have merit would focus on the potential reproducibility of CHR within a hypothetical clinical study. Due to both uncontrolled experimental error and inter-subject variability in both drug disposition and drug action, it is logical to presume that the data from different subjects would result in hysteresis loops with different values of CHR. It would be of interest to understand the relationships among uncontrolled error, inter-subject variability, the “average” magnitude of the temporal delay,
and the range of CHR values produced. This type of question is readily addressed with Monte Carlo simulations in which inter-subject variability in PK and PD parameters can be incorporated, and uncontrolled error in concentration and effect measurements can be superimposed.

An obvious area for additional experimentation lies with applying hysteresis analysis to observational, rather than simulated, data. In Chapter 2, for example, only one study was included in the analysis. With continuing refinement of microdialysis techniques, concentrations in various target tissues might become more routinely measured in humans, which would provide additional data for PK hysteresis analysis. Certainly, the continued use of microdialysis sampling in animals will provide ample opportunity to mine and analyze data from preclinical experiments. These efforts would allow some degree of verification of the general applicability of hysteresis analysis with CHR as a metric.

Finally, the present project has shown that the utility of CHR is largely dependent on PK or PD system linearity. Nonlinear effect models or nonlinear distributional elements in a PK model result in nonlinearity in the relationship between CHR and model parameters. This nonlinearity, in turn decreases the sensitivity of CHR as a metric and reduces the predictive power of the analysis. More studies are required to fully explore the impact of nonlinearity on the potential utility of CHR, and to assess whether other non-compartmental descriptors might be identified that could be applied in those situations in which CHR is not suitable.
REFERENCES


75. Earp, J.C., et al., *Modeling corticosteroid effects in a rat model of rheumatoid arthritis II: mechanistic pharmacodynamic model for dexamethasone effects in*


131. Kalvass, J.C., et al., *Pharmacokinetics and pharmacodynamics of seven opioids in P-glycoprotein-competent mice: assessment of unbound brain EC50,u and


