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Scaling Pharmacodynamics from *In Vitro* and Preclinical Animal Studies to Humans

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Summary

An important feature of mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) models is the identification of drug- and system-specific factors that determine the intensity and time-course of pharmacological effects. This provides an opportunity to integrate information obtained from *in vitro* bioassays and preclinical pharmacological studies in animals to anticipate the clinical and adverse responses to drugs in humans. The fact that contemporary PK/PD modeling continues to evolve and seeks to emulate systems level properties should provide enhanced capabilities to scale-up pharmacodynamic data. Critical steps in drug discovery and development, such as lead compound and first in human dose selection, may become more efficient with the implementation and further refinement of translational PK/PD modeling. In this review, we highlight fundamental principles in pharmacodynamics and the basic expectations for *in vitro* bioassays and traditional allometric scaling in PK/PD modeling. Discussion of PK/PD modeling efforts for recombinant human erythropoietin is also included as a case study showing the potential for advanced systems analysis to facilitate extrapolations and improve understanding of inter-species differences in drug responses.

Keywords

allometric scaling; cell life span models; mechanism-based modeling; pharmacodynamics, PD; pharmacokinetics, PK; receptor occupancy; recombinant human erythropoietin, rHuEpo; target-mediated drug disposition, TMDD

Introduction

The extrapolation of *in silico*, *in vitro*, and preclinical animal studies to predict the likely pharmacokinetic properties of drugs in humans now appears within reach, largely due to advancements in physiologically-based pharmacokinetic (PBPK) modeling.[1,2] Whereas traditional allometry continues to prove useful under certain conditions for inter-species scaling of PK properties, significant progress has been achieved by transitioning from models of data (e.g., classic compartmental models) to those of biological systems. The PBPK modeling approach provides a framework for integrating drug-specific calculated parameters (e.g., octanol:water and blood:tissue partition coefficients) and *in vitro* measurements (e.g., plasma protein binding and hepatocyte intrinsic clearance) with physiological system-specific parameters (e.g., tissue volumes and blood flows). Given the

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relative success of anticipating human exposures to drugs and toxicants, [3,4] there is considerable interest in the development of techniques for the scaling of pharmacodynamic systems. Although drug responses are considerably more complex than processes controlling pharmacokinetics, the shift from empirical to mechanism-based PK/PD modeling[5,6] should provide the best means for translating *in vitro* and animal data to human clinical pharmacology. [7]

In this review, we discuss the basic tenets of pharmacodynamics, namely 1) pharmacokinetics or drug exposure as the driving function, 2) capacity-limitation of drug-receptor interactions, and 3) physiological turnover processes and functional adaptation or homeostatic feedback mechanisms. As with PBPK models, these basic components identify drug and system specific properties that might be anticipated using *in vitro* assays, allometry, and/or preclinical animal experiments. A case study showing how human responses to recombinant human erythropoietin (rHuEpo) can be predicted from scaling a mathematical model developed in rats is provided as an example of utilizing mechanism-based PK/PD models to scale complex pharmacological systems.

Basic Principles of Pharmacodynamics

The basic tenets of pharmacokinetics (PK), pharmacology, and physiology continue to form the basis for contemporary pharmacodynamic systems analysis (Fig. 1). Pharmacokinetics, or the processes controlling the time-course of drug concentrations in relevant biological fluids, tissues, and sites of action (biophase), is the driving force for subsequent pharmacological and most toxicological effects. Although mammillary plasma clearance models (simple linear compartmental models) and area/moment analysis are the most commonly applied techniques for characterizing the absorption and disposition (distribution and elimination) properties of drugs, PBPK models provide a comprehensive platform for describing the major processes influencing the concentration time-course and net exposure of drugs in various fluids and tissues (Fig. 1, left panel). Each tissue of interest is anatomically arranged and described by a series of mass balance differential equations. Fick's law of perfusion/diffusion and drug partitioning are featured along with a capacity-limited function for various drug binding, transport, and elimination processes. This approach provides insights into expected drug concentrations in important tissues, and potentially sites of action, and the intrinsic scalability of predictions across species and molecular drug properties is unparalleled. Whereas traditional PBPK model development has relied on destructive sampling in preclinical studies, advances in noninvasive imaging (such as positron emission tomography and magnetic resonance imaging) and microdialysis may eventually provide even finer details of *in vivo* drug disposition.[8,9]

At the biophase, the law of mass action and the limited concentration of pharmacological targets often manifest as nonlinear, capacity-limited systems.[10] The rate of change of a drug-receptor complex (*RC*) can be defined as:

$$\frac{dRC}{dt} = k_{on} \cdot (R_{tot} - RC) \cdot C - k_{off} \cdot RC \quad (1)$$

where R_{tot} is the maximum receptor concentration, C is the drug concentration at the site of action, and k_{on} and k_{off} are the second-order association and first-order dissociation rate constants. Assuming equilibrium conditions, this equation can be rearranged to yield the general binding equation:

$$RC = \frac{R_{tot} \cdot C}{K_D + C} \quad (2)$$

where K_D is the equilibrium dissociation constant (k_{off}/k_{on}). Based on Clark's theory of receptor occupancy, the stimulus or drug effect (E) can be directly proportional to the fraction of occupied receptors, such that $E=\alpha \cdot RC$, thus deriving a classic form of the Hill equation or sigmoid E_{max} model:

$$E=\frac{E_{\max} \cdot C^{\gamma}}{EC_{50}^{\gamma}+C^{\gamma}} \quad (3)$$

where $E_{\max}$ is the maximum effect, γ (or Hill coefficient) is a slope term that reflects the steepness of the effect-concentration curve, and the EC_{50} is a sensitivity parameter representing the drug concentration producing 50% of $E_{\max}$. The typical stimulus/effect-log concentration relationship is thus curvilinear, and typical profiles for varying values of γ are shown in the center panel of Figure 1.

In contrast to the linear transduction of receptor occupancy (Eq. 3), Black and Leff introduced the operational model of agonism to provide a mechanistic interpretation of concentration-effect curves.[11] The stimulus or effect is assumed to be nonlinearly related to the drug-receptor complex:

$$E=\frac{E_{\max} \cdot RC}{K_E+RC} \quad (4)$$

where K_E is the RC value producing half-maximal effect. Combining Equations 2 and 4 yields:

$$E=\frac{E_{\max} \cdot \tau \cdot C}{K_D+(\tau+1) \cdot C} \quad (5)$$

where E_{max} is a *system* maximum and (represents a transducer or efficacy function (R_{tot}/K_E). This model can accommodate complex relationships, such as partial agonism, where observed capacity and sensitivity properties are actually hybrid terms composed of drug specific (K_D and τ) and system specific ($E_{\max}$) parameters. Regardless of whether linear or nonlinear transduction is operational, capacity-limitation is a hallmark property of pharmacology, and consequentially, a suitable range of dose-levels (or concentrations) are required to define the parameters of such systems. In addition, the implementation of Equation 5 requires pharmacodynamic data, or at least prior information, on the properties of a full agonist to identify the maximal system response.

Physiological turnover processes and homeostatic feedback mechanisms represent the third major component of pharmacodynamics (Fig. 1, right panel). An open system for a biological substance, R, with zero-order production (k_{in}) and first-order removal (k_{out}) can be defined by the following differential equation:

$$dR/dt=k_{in}-k_{out} \cdot R \quad (6)$$

Assuming a time-invariant baseline or steady-state, the initial or baseline value (R^0) can be defined as the ratio of the production and loss terms: $R^0=k_{in}/k_{out}$. A family of basic indirect response models apply to many drugs where interaction with the pharmacological target (Eq. 3) serves to inhibit or stimulate either k_{in} or k_{out}.[12] A series of transit compartments can also be factored into such models to emulate time-dependent transduction processes that often exhibit significant onset delays and exposure-response hysteresis.[13] Knowledge of the turnover rates for physiological system components is important for the identification of the rate-limiting steps for specific pharmacological responses and might impact study design. Such information might also facilitate the characterization of feedback mechanisms that

might result in tolerance and/or rebound phenomena.[6] As both drugs and diseases often interfere with normal physiological processes, the turnover aspect of both indirect response models[14] and transduction models[15] renders them well suited for the simultaneous consideration of these factors in the time-course of disease progression.

Mechanism-based models seek to integrate these basic components to identify critical pharmacological and (patho)-physiological system properties as well as the rate-limiting steps in responses to drugs.[6,16] Useful models with a potential for translational medicine also provide a structural framework for incorporating *in silico*, *in vitro*, and preclinical PK/PD measurements to predict the effects of new drugs in humans and across levels of biological organization (Fig. 2). A discussion of all these methods is beyond the scope of this review, which will focus on *in vitro* assays and allometric principles in the context of mechanistic PK/PD models. The derivation of quantitative structure-PK/PD relationships (*in silico* modeling) to predict the exposure-response profiles of new chemical entities has been recently reviewed.[2]

Extrapolation of *In Vitro* Bioassays

Pharmacodynamic modeling of several systems has revealed that properties of drug interactions with pharmacological targets measured *in vitro* may be correlated with specific model parameters often reflective of drug potency. Shimada and colleagues developed an ion-channel binding model based on *in vitro* binding data of calcium channel antagonists, which demonstrate relatively slow rates of association and dissociation.[17] The pharmacologic effect was assumed to be proportional to the concentration of the drug-receptor complex and, as an extension of Equation 1, the rate of change of the effect was defined as:

$$\frac{dE}{dt} = k_{on} \cdot (E_{\max} - E) \cdot C - k_{\text{off}} \cdot E \quad (7)$$

The inclusion of the binding parameters was sufficient to explain the hysteresis observed between the PK and antihypertensive effect of eight calcium channel antagonists in Japanese patients. The calculated K_D values based on estimates of k_{on} and k_{off} were shown to be significantly correlated with those obtained from *in vitro* experiments. These results suggest that PK and *in vitro* binding data alone could be used to predict the pharmacodynamic profile of future drugs in this class. Kalvass and colleagues[18] performed extremely insightful PK/PD studies with seven opioids in mice showing the importance of time-course of brain distribution and binding in determining their antinociceptive effects. The EC_{50} of unbound drugs in brain showed excellent correlation with *in vitro* receptor binding affinities (K_D). From a drug development perspective, these examples demonstrate how *in vitro* assays may be coupled with useful PK/PD models to anticipate the outcomes of similar compounds and may guide lead compound selection.

Relative receptor affinity has been shown to be correlated with *in vivo* estimates of drug potency for several drugs, and *in vitro* measurements could be used in scaling of EC_{50} values across species. In a 5-way randomized placebo-controlled crossover study aimed at evaluating the dosing equivalency of four systemically administered corticosteroids, mechanism-based PK/PD models were used to estimate EC_{50} values for several immunomodulatory effects, including cortisol suppression, lymphocyte and neutrophil trafficking, and *ex vivo* inhibition of lymphocyte proliferation.[19,20] The estimated potencies for all of these responses were highly correlated with relative receptor affinity (*in vitro* K_D values normalized to dexamethasone). Differences in protein homology and other genetic sources of variability may result in altered drug binding affinity among species. Chien and colleagues corrected an EC_{50} value for a competitor drug measured in humans, using several

factors including receptor binding, to predict the *in vivo* human EC_{50} for a new chemical entity (*NCE*):[21]

$$EC_{50,\mathrm{NCE,human}} = \mathrm{EC}_{50,\mathrm{Competitor,human}} \cdot \left(\frac{\mathrm{EC}_{50,\mathrm{NCE}}}{\mathrm{EC}_{50,\mathrm{competitor}}} \right)_{\mathrm{rat}} \cdot \delta f_u \cdot \delta K_D \quad (8)$$

where δf_u and δK_D are correction factors for differences in the free fraction in plasma (f_u) and binding affinity (K_D). For example,

$$\delta K_D = \left(\frac{K_{D,\mathrm{human}}}{K_{D,\mathrm{rat}}} \right)_{\mathrm{NCE}} \cdot \left(\frac{K_{D,\mathrm{rat}}}{K_{D,\mathrm{human}}} \right)_{\mathrm{competitor}} \quad (9)$$

The scaled EC_{50} from animal and *in vitro* data (Eq. 8) was coupled with other projected parameters to simulate a dose-response curve (Eq. 3 with an added baseline) for a new antihypertensive agent, relative to a competitor, in the preclinical phase of development. Monte Carlo simulations included a relatively large confidence interval about expected outcomes; however, data from clinical studies would eventually be used to confirm and update the model.

Traditional Allometric Scaling in PK/PD

Although the structural nature of physiologically-based models makes them uniquely suited for scaling and predicting human drug exposures, the extrapolation of PK-PD models from animals to humans is primarily based on classic allometric principles.[22] There is a general expectation that many physiological processes and organ sizes (θ) tend to obey a power law:[23]

$$\theta = a \cdot W^b \quad (10)$$

with W representing body weight and a and b as drug/process coefficients. The exponent, b, tends to be around 0.75 for clearance processes, 1.0 for organ sizes or physiological volumes, and 0.25 for physiological times or the duration of physiological events (e.g., heart-beat and breath duration, cell lifespans, and turnover times of endogenous substances or processes).[24] A theoretical basis for allometric scaling has been proposed by West and colleagues based on the fractal nature of biological systems and energy conservation principles.[25] Empirical models have also been coupled with allometric relationships and *in vitro* metabolism experiments using nonlinear mixed effects modeling to improve the scalability of such models.[26,27]

The basic expectations in pharmacodynamics are that physiological turnover rate constants of most general structures and functions should be predictable among species based on allometric principles, whereas capacity ($E_{\max}$) and sensitivity (EC_{50}) parameters tend to be similar across species. Brodie and colleagues were the first to examine some PK-PD properties across species, revealing inter-species differences in duration of action and biological half-life, but similarity in plasma concentrations on awakening (i.e., concentration producing a standard response analogous to an EC_{50}), following hexobarbital administration.[28] There has long been a general belief that the plasma drug concentration required to elicit a certain (intensity of) action is often similar in experimental animals and humans.[29] While interspecies differences in relative receptor affinity and plasma protein binding occur (Eqs. 8 and 9),[21] there are several examples that show reasonable agreement of such properties between rats and humans for chemically-related series of drugs. Ito and colleagues demonstrated a linear correlation between the logarithm of K_D values of benzodiazepines in rat and human cerebral cortex tissue over several orders of magnitude.[30]

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