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Mini-Review

Theme: Pharmacokinetic and Pharmacodynamic Concepts in Oncology Drug Development
Guest Editors: Robert M. Straubinger and Donald E. Mager

The Pharmacokinetic/Pharmacodynamic Pipeline: Translating Anticancer Drug Pharmacology to the Clinic

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Abstract. Progress in an understanding of the genetic basis of cancer coupled to molecular pharmacology of potential new anticancer drugs calls for new approaches that are able to address key issues in the drug development process, including pharmacokinetic (PK) and pharmacodynamic (PD) relationships. The incorporation of predictive preclinical PK/PD models into rationally designed early-stage clinical trials offers a promising way to relieve a significant bottleneck in the drug discovery pipeline. The aim of the current review is to discuss some considerations for how quantitative PK and PD analyses for anticancer drugs may be conducted and integrated into a global translational effort, and the importance of examining drug disposition and dynamics in target tissues to support the development of preclinical PK/PD models that can be subsequently extrapolated to predict pharmacologic characteristics in patients. In this article, we describe three different physiologically based (PB) PK modeling approaches, i.e., the whole-body PBPK model, the hybrid PBPK model, and the two-pore model for macromolecules, as well as their applications. General conclusions are that greater effort should be made to generate more clinical data that could validate scaled preclinical PB-PK/PD tumor-based models and, thus, stimulate a framework for preclinical to clinical translation. Finally, given the innovative techniques to measure tissue drug concentrations and associated biomarkers of drug responses, development of predictive PK/PD models will become a standard approach for drug discovery and development.

KEY WORDS: anticancer drugs; drug discovery and development; pharmacokinetic/pharmacodynamic model; physiologically based pharmacokinetic model.

INTRODUCTION

Since the first description of using nitrogen mustard for the treatment of Hodgkin's lymphoma by Cornelius P. Rhoads in 1949, the use of effective chemotherapeutic agents has been shown to relieve symptoms and improve survival in patients with various types of cancer. However, major drawbacks of conventional anticancer chemotherapy have been the lack of specificity along with narrow therapeutic indices and the occurrence of tumor resistance. Over the last three decades, advances in genetics and molecular/cell biology have greatly extended our knowledge of the molecular pathogenesis of cancer, and subsequently opened up new avenues for developing molecular targeted therapy through the identification of potential therapeutic targets involved in intracellular signaling processes responsible for transformation and tumor progression (1,2). Moreover, rapid progress in drug discovery technologies made over recent years has also provided modern approaches to hasten the drug discovery process and improve productivity (3,4). However, despite the high yield of potential new agents through integrative and innovative approaches in drug discovery, truly meaningful gains in the economics of drug discovery have not yet been achieved, and the success rate of new chemical entities still remains low, with only about 8% of cancer therapeutic or vaccine candidates approved in the USA. Given the concern of the rising percentage of late-stage clinical failures, generating high-quality detailed and predictive pharmacokinetic (PK) and pharmacodynamic (PD) information in the early phase of the drug development process could help reduce costly bottlenecks, inefficiencies, and unacceptably high attrition rates in the discovery and development pipeline.

The development of new drugs relies heavily on animal studies to provide a framework for human clinical trials. Often time, a drug that works well in animals may be ostensibly ineffective in humans. One explanation for the lack of effectiveness is the inappropriate translation of a drug dose from animal to human. For example, in the current oncology drug development paradigm, selection of starting doses for phase I clinical trials usually involves the use of various parameters in relation with prior data obtained from pharmacokinetic and toxicology studies in animals, such as the fraction of no adverse effect level (NOAEL) dose, fraction of lethal dose (LD_{10}), and allometric scaling using body surface area (BSA). Unfortunately, deriving a starting

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dose for phase I studies of anticancer drugs from preclinical animal toxicology and systemic pharmacokinetic data often proves to be the inappropriate conversion of drug doses from animal studies to human studies. The use of models to describe PK/PD data has received more and more attention from academia, industry, and regulatory authorities and is considered an advanced approach for exposure-response analysis (5) and clinical trial simulations (6). In many cases, plasma PK properties have been successfully related to pharmacological effects, thus being used as a surrogate for drug disposition at the site of action. However, the heterogeneous nature of tumor tissue often hampers drug delivery from plasma to tumor, leading to complex relationships between concentrations in plasma, interstitium, and tumor cells. In this article, some considerations for how preclinical PK and PD investigations on anticancer drugs should be conducted and integrated into a global translational effort will be discussed. The discussions and issues raised are to draw attention to different approaches to experimental design and data analysis. It is hoped that the issues discussed will provide enhanced insight into the importance of examining drug disposition and dynamics in target tissues that subsequently allows preclinical PK and PD models to be derived and extrapolated to predict pharmacologic characteristics in patients (7,8).

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING IN DRUG DEVELOPMENT

PK models are based on mass balance differential equations that characterize drug absorption and disposition within the body. The most common PK modeling approach is known as a mammilliary compartmental model, often referred to as a classic PK approach, which conceptualizes the body as a series of compartments reversibly linked to a central compartment (9). Despite its simplicity and widespread use, this concept has limited biological or physiological significance, and therefore, limited predictive power, and underpinnings to extrapolate from one animal species to another. In contrast, physiologically based PK (PBPK) models recognize anatomical and physiological realities, and attempt to account for the role of differential distribution within and between organs as well as their varying blood flows (10,11). A typical PBPK model is often comprised of multiple compartments that represent organ and tissues of interest that are linked by the blood circulation and sometimes in addition by the lymphatic system. Even though classic compartmental PK models and PBPK models both share a fundamental reliance on mass balance rate equations, the specific parameterization differs for PBPK models by incorporating physiological terms. However, a significant limitation to their application in drug research and development has been the requirement of vast quantities of data for model construction (12).

Nonetheless, there is a resurgence in PBPK models at least to the point in which the area has threaded into the dialog of drug discovery and development (13,14), if not in its actual application. A recent literature search using the single keyword phrase "physiologically based pharmacokinetic model" resulted in a total of 1,148 references, over one third of which were published after 2003, suggesting an increasing research activity in this area. The data not only support a growing interest in PBPK models but also coincide with a renewed focus on PK/PD-directed drug development (15,16). In any case, there are hurdles that can be overcome, particularly in the area of anticancer drugs, to further promote the use of PBPK models.

WHOLE-BODY PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

Drug distribution to tumors is an important component for understanding antitumor efficacy. Although drug concentrations in plasma may closely follow those in target sites for many therapeutic classes, this is not the case for most anticancer drugs. The heterogeneities of tumors involved in blood flow, vascular permeability, and interstitial fluid pressure have been adversely linked to complex relationships between concentrations in plasma, interstitium, and tumor cells, which often result in decreased and/or nonuniform drug uptake in tumors (17–19). PK models that depict a tumor compartment provide enhanced insight not only into the mechanisms that characterize drug disposition but also into pertinent PK–PD relationships, thus offering a quantitative basis to design and adjust therapies.

PBPK modeling of tumor drug disposition is concerned with the physiologically oriented description of the kinetic behavior of the drug of interest, thereby possessing the potential to address the distributional complexities of drug delivery into tumors. Whole-body PBPK models have been developed for several clinically used chemotherapeutic agents, such as adriamycin, 5-fluorouracil, doxorubicin, and methotrexate, as well as for several newer compounds with antitumor activities, including everolimus (20) and genistein (21). Attempts have been made to explore the predictive capability of those models and their ability to incorporate detailed biological information with the available data to yield more accurate parameters to describe distinct characteristics of drug disposition in various tissues of interest. A blood flow-limited PBPK model consisting of five tissue compartments was developed for docetaxel that incorporated specific binding to intracellular components, liver metabolism, biliary and intestinal elimination, and urinary excretion with active secretion (22). The resultant model was then modified with human model variables to predict the plasma distribution of docetaxel in human. A whole-body PBPK model developed for everolimus took into account the nonlinear binding of the drug to red blood cells and various organs, while that for genistein was able to characterize a dose-dependent reduction in biliary flow. These models are indicative of how PBPK models can represent mechanistic drug-dependent features that should lead to more accurate predictions, and provide insight on physiological variables can influence drug disposition and whether coadministered drugs might interact. In addition, PBPK modeling and simulation approaches can be used to evaluate and design novel drug formulations and delivery systems (23,24).

A comprehensive study by Xu *et al.* demonstrated a number of these features (25). The investigation derived a whole-body PBPK model of the ansamycin benzoquinone antibiotic 17-(allylamino)-17-demethoxygeldanamycin (17AAG) and its

active metabolite 17-(amino)-17-demethoxygeldanamycin (17AG) based on drug concentration measurements in both normal mice and mice bearing human breast cancer xenografts. Specifically and in sequential manner, a PBPK model for 17AAG and 17AG was constructed for normal tissues that were combined with a model for the uptake and distribution of 17AAG and 17AG in tumor including intracellular concentration profiles of 17AAG and 17AG. The predicted tumor concentrations of 17AAG and 17AG were linked to detailed PD indirect response models characterizing tumor cell oncoproteins ($p185^{erbB2}$ and Raf-1) and heat shock proteins (HSP70 and HSP90). Overall, the modeling effort of this study yielded a mechanistically driven PBPK/PD model of 17AAG and 17AG and their target molecules, and serves as a fine illustration of a whole-body PBPK approach (25).

PBPK models normally rely on two types of input data: independent physiological data, such as blood flows, organ volumes, tissue structure, and tissue composition; and drug-specific data, such as the unbound fraction in plasma, membrane permeability, and tissue-to-plasma partition coefficients. With regard to the independent physiological parameters associated with tumors, tumor blood flow is recognized to play a significant role in tumor drug uptake and exposure (26,27). Determination of blood flow rate *in vivo* usually involves the use of a diffusible indicator that is removed from tissue in proportion to blood flow based on the Kety–Schmidt principle (28) followed by measurements with external detectors, such as quantitative autoradiography, positron emission tomography, and magnetic resonance imaging. Unlike most normal tissues, tumor vasculature is highly heterogeneous and does not conform to the normal vascular morphology. Tumor blood flow in one tumor differs from another, even in tumors of equal histology and grade. PBPK models that depict tumors as single, well-stirred, and perfusion rate-limited compartments will not accommodate the heterogeneity of tumor blood flow that leads to heterogeneous tumor drug uptake. Nonetheless, PBPK modeling has the capability to predict variability in tumor PK beyond the typical assumption of tumor homogeneity with the use of Monte Carlo simulations that reflect parameter variability, and thus, an appreciation of the effect of tumor blood flow variability on predicted tumor drug concentrations can be ascertained.

Although the whole-body PBPK modeling approach is highly informative, one drawback that has deterred the wide application of whole-body PBPK models is the considerable resources needed to conduct the studies. These resources register as an array of animal PK/PD investigations to obtain drug concentration measurements in numerous tissues, and the associated computer modeling efforts needed to distill the data into validated models. Certainly, efforts that rely more on *in silico* and *in vitro* strategies to extract model parameters are attractive (29,30). For example, Poulin and Theil demonstrated the incorporation of *in vitro* data on drug lipophilicity, plasma protein binding, and intrinsic hepatic clearance into PBPK models to enable automated calculation of tissue: plasma partition coefficients ($P_{t:p}$s) under *in vivo* conditions (29). Rodgers and Rowland later derived a new mechanistic equation to predict tissue:plasma water partition coefficients for a broad range of compounds, including acid, neutrals, and weak-to-strong bases (30). The *in silico* and *in vitro* systems are particularly useful in the early phases of studies where the screening of large number of potential therapeutic candidates may be necessary. Nonetheless, the validation of those models still requires animal data albeit less than without the use of these interesting techniques.

In addition, as an increasing body of physiological and biological data has become available over the years, reasonably accurate predictions of the PKs of specific compounds are becoming feasible, even with limited drug-specific data. However, the lack of standardized parameter estimation methods and the inherent variability in physiological parameters often lead to a wide diversity of values in the literature. Moreover, determinations of physiological parameters under different experimental conditions will likely result in different values. For example, the brain blood flow rate in healthy rats (1.3 mL/min (31)) is different from that in the brain tumor-bearing rats (2.7 mL/min estimated based on the brain weight of 1.8 g (32)). Therefore, the selection of appropriate physiological data from the literature for PBPK modeling should consider experimental procedures, animal strains, and disease models, yet to reflect the ever present uncertainty in such variables may also require incorporation of statistical and Monte Carlo techniques.

It should also be appreciated that application of a PBPK approach to humans is most appealing for prediction of the systemic PK properties and those in the target tissue. In this regard, the dimensionally smaller but still physiological approach of hybrid PBPK models that focus on the target tissue(s) may be sufficient to translate the preclinical information to the clinic, and further, minimize the resources needed for whole-body PBPK investigations.

HYBRID PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

The origins of hybrid PBPK models can be traced to the 1970s with papers by early pioneers (33–35) in PBPK modeling; however, the approach has not received consistent attention although our lab has made frequent use of the strategy (7,8,36–39). Hybrid PBPK models, which incorporate components of whole-body PBPK and classic compartmental PK models, focus on tissues of interest, such as tumors, and thus avoid the complexities and considerable resources of developing a global or whole-body PBPK model. The drug concentration profile in blood or plasma may be cast as a forcing function [sequential model development] or derived along with the model for the target tissues [simultaneous model development]. Employment of a sequential modeling strategy proceeds by first fitting a forcing function, often cast as a polyexponential equation, to the available plasma drug concentration-time data, and then, the resultant function is set constant and serves as driving force for drug input into the tissue model. In the simultaneous modeling approach, differential mass balance equations would be fit to both the plasma and tissue drug concentration-time data until a suitable model is defined. Regardless of the strategy to obtain a hybrid PBPK model, the tissue compartments are represented as in whole-body PBPK models, and thus, possess the advantages of elucidating drug transport and elimination mechanisms within the context of physiological and pharmacological accuracy. In addition, hybrid PBPK models possess an

advantage in interspecies extrapolation relative to whole-body PBPK models that can be attributed to the explicit designation of a model for the plasma drug concentration profile. In whole-body PBPK approaches, the latter is defined by the net balance of arterial input and venous output from the tissues. The explicit designation of a model for the plasma drug concentration profile and the associated clearance processes provides a format that facilitates scale-up from animals to humans. Furthermore, there is an option to utilize readily available human plasma data, as highlighted below, to derive the desired function directly and use this as the input into the scaled models for target tissues. Thus, hybrid PK models can overcome some of the hurdles posed by whole-body PBPK models.

The advantages of the hybrid PBPK approach may also be seen as its disadvantages, namely its narrow focus, and being overly data-driven relying on *in vivo* experiments for model definition. Newer trends in PBPK modeling are making greater use of *in silico* and *in vitro* parameter estimation approaches with whole-body drug distribution studies serves as confirmatory. Certainly, hybrid PBPK models can also make use of analogous model building approaches and the use of animal data for model revision and validation. Hybrid PBPK model is attractive for quantitative pharmacological analyses of cancer chemotherapy of solid tumors, the site of drug action since an understanding of drug concentrations in tumor (rather than plasma) is essential to evaluate PK–PD relationships and antitumor efficacy. The aforementioned advantages of hybrid PBPK models relative to global PBPK models in terms of utilizing less resources and using human plasma drug concentration time data may be appreciated in our studies with temozolomide (TMZ) in which a comprehensive hybrid PBPK model was derived in rats bearing brain tumors (7,38). The hybrid PBPK model for TMZ consisted of two-compartment structures for normal brain and brain tumors that separated vascular and extravascular spaces by the blood–brain barrier (BBB), and a single compartment for the CSF. A systemic forcing function based on unbound plasma TMZ concentration measurements derived from serial blood samples was coupled to each CNS compartment in which measurements of unbound TMZ concentrations in CSF, normal brain, and brain tumor were obtained by microdialysis (Fig. 1a). TMZ's diffusional flux in and out of the CSF is represented by the permeability-area product (PA_{csf}), whereas unidirectional efflux and elimination of TMZ consisted of CSF bulk flow (Q_{csf}), and a CSF metabolic clearance (CL_{csf}), respectively. Normal brain and brain tumor compartments were characterized by unique blood flow rates and BBB transport constants. Once the preclinical model was finalized, means to scale the model to brain tumor patients were considered. At the time, Ostermann and coworkers (40) had obtained and modeled TMZ's CSF distribution in brain tumor patients as a surrogate for actual brain tumor measurements that are rare. Thus, our first exercise was to scale our preclinical model for TMZ CSF distribution to patients using the Ostermann data as validation. Using two scaling approaches referred to as the naïve and scaled predictors, accurate predictions of TMZ CSF distribution in patients were accomplished based on analogous observed and model-predicted ratios of the AUC_{csf}/AUC_p (Fig. 1b; 7). Predicated upon the successful extrapolation of the preclinical model for TMZ's disposition in CSF, the preclinical PBPK normal brain and brain tumor models for TMZ were extended to patients. In this case, human physiologic variables [i.e., blood flow rates and tissue volumes] assigned from the literature, and a TMZ plasma concentration forcing function adapted from available patient data were substituted into the preclinical model structure that also utilized the BBB influx and efflux parameters determined in rats (Fig. 1a). This PBPK TMZ model for patients was subsequently used to compare concentration-time profiles for clinically used multiple-dose regimens (Fig. 1c). A recent paper that employed brain tumor microdialysis of TMZ in patients indicated that our model predicted their observed results on TMZ tumor distribution and suggested it could be used to design therapeutic dosing regimens (41). The study illustrates an attractive feature of how PBPK models may distill various physiological and pharmacological variables into a quantitative tool that may be extrapolated between species to evaluate and design effective drug dosing regimens.

HYBRID PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC MODELS

The interest and importance of pharmacodynamics (PDs) to cancer chemotherapy are on the rise. This can be attributed to technological advances that permit the measurement of multiple protein targets in cells and tumors, and the explosion in translational and personalized medicine. Technological advances based on flow cytometry, imaging, and mass spectrometry have made high-content assays for protein quantitation possible, and thus, support drug discovery and systems biology efforts leading to an increased understanding of cell signaling networks. Individualized medicine based on genotypic variation will require a thorough understanding of target protein responses to drugs to define genotype–phenotype relationships that can provide a basis to improve drug dosing protocols and devise novel treatment strategies. Thus, PDs should be central to these endeavors, both conceptually and in sustaining key advances in cancer chemotherapy.

The PDs of anticancer drugs, and more specifically PD models, have lagged behind the available technological tools and the broad appeal of translational research and individualized therapy. This deficit can partly be attributed to the traditional nature of cancer drugs, being highly toxic and the use of maximum tolerated doses, making the use of a therapeutic PD endpoint of limited value. Although this latter point may be disputed, it is clear that the majority of PD modeling efforts for anticancer drugs have been models for drug-induced myelosuppression, a common dose-limiting toxicity (42–45). With the advent of imatinib at the turn of the century, the gateway for targeted anticancer therapy was opened that escalated the opportunities and altered the landscape for multidrug combination cancer chemotherapy as less toxic targeted drugs could be considered (46). These targeted drugs interfere with one or more cell signaling cascades and may offer numerous targets to monitor to assess drug activity. Many of the targeted drugs are mono- or pan-kinase inhibitors, whereby phosphorprotein measurements will indicate effectiveness; however, the development of drug

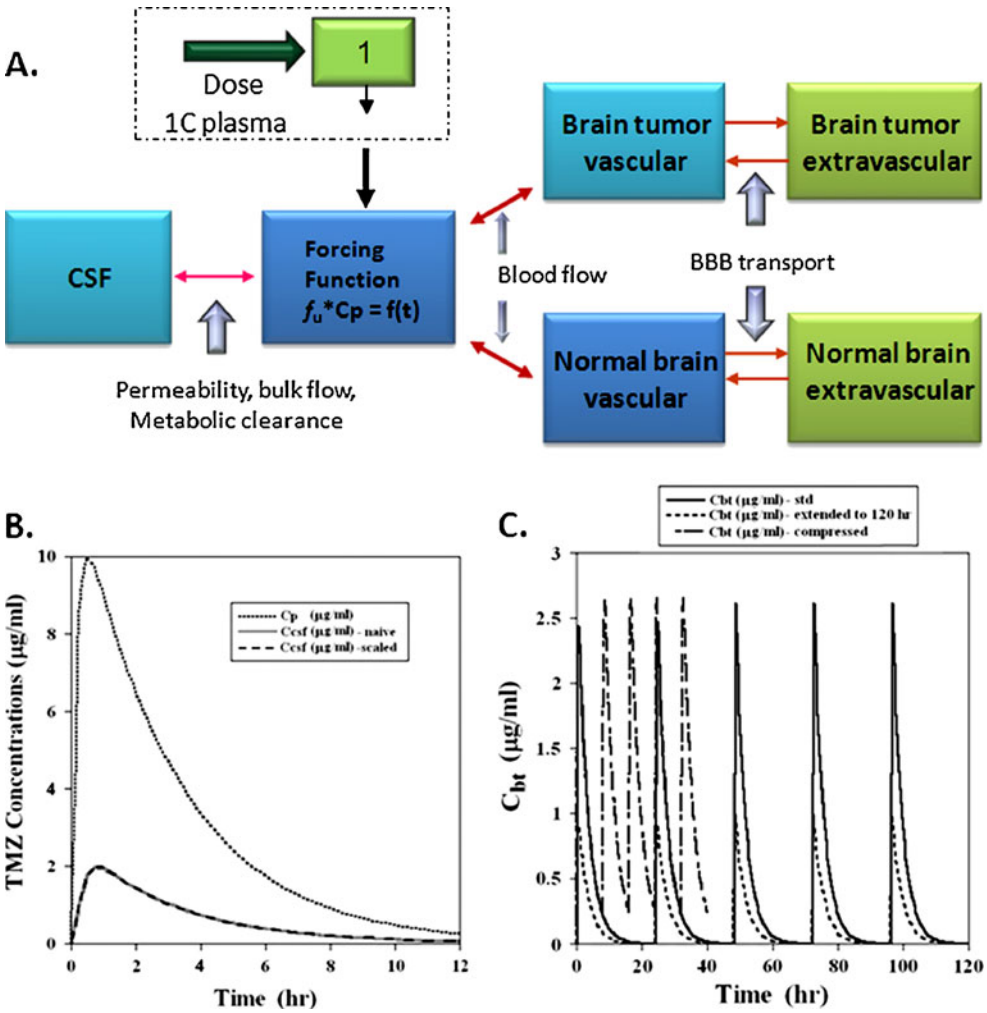

Fig. 1. **a** Schematic presentation of the hybrid PBPK model of TMZ in brain. The dose input for rats was IV, whereas for humans, it was oral. The structure of the brain model is the same in rats and human. **b** Human model-predicted plasma (*dotted line*), and CSF TMZ concentrations using naïve (*solid line*) and scaled (*dashed line*) model predictors. The CSF TMZ concentrations for these two predictors closely overlap. **c** Human model-predicted TMZ brain tumor concentrations comparing three different dosing regimens; standard (200 mg/m^2 daily×5 days every 28 days), compressed (200 mg/m^2 every 8 h for 5 doses every 28 days), and extended (75 mg/m^2 daily×21 days every 28 days). The simulations are shown to 120 h, the end of the standard dosing cycle (7)

resistance may require additional measurements to interpret and guide therapy. Therefore, it would seem that the primary direction of modern anticancer drug therapy should be to build a pharmacological foundation on PD characteristics that can be cast into quantitative models.

A PD model is a mathematic model that describes the effect of a drug as a function of drug concentration. A detailed discussion on types of PD models is beyond the scope of this article, but may be found in other excellent publications on the subject (47,48). Various PD models have been developed to quantify the time course of pharmacological effect of anticancer drugs in relation to drug concentrations in plasma or target tissues, providing a theoretical framework to understand experimental data and a quantitative basis to design and adjust dosing regimens. In general, therapeutic responses to most anticancer drugs may be considered indirect in nature, and thus, can be described by linking drug concentration to effect through an intermediate response variable (R), by means of a number of linear (L) or nonlinear (NL) functions, which may be either static (S) or dynamic (D; 49). The various modified forms of the general indirect PD model may be categorized into five groups based on the different types of PD measurements: (a) molecular surrogate marker model, which is based on therapeutic agent-targeted signaling transduction pathways (39,50); (b) cellular PD endpoint model, including cell cycle phase-specific model (51), transit compartment model (51,52); (c) tumor growth/shrinkage model, which is based on changes in tumor volume over time (53–55); (d) toxicity model, which describes the anticancer drug-induced dose-limiting toxicity (45); (e) combined PD model, which integrates biomarkers (e.g., signal transduction pathway) and PD endpoints (e.g., tumor volume) into one model (8,56).

A couple of studies by Gallo's group have applied physiologically based PK/PD modeling in a prospective manner to assist in the development of equivalent dosing

regimens for an epidermal growth factor receptor (EGFR) inhibitor, gefitinib, in a preclinical study in nude mice bearing LN229 human glioma xenografts that expressed either wild-type or vIII mutant EGFR (8,39). Before the PK/PD investigations in tumor-bearing mice, *in vitro* studies were performed to identify PD markers that could subsequently serve as putative PD endpoints *in vivo*. As a tyrosine kinase inhibitor, gefitinib downregulates tyrosine kinase-induced EGFR phosphorylation and subsequently inhibits downstream signal transduction proteins. Based on pertinent signal transduction pathways, the effect of gefitinib on several likely PD endpoints, including AKT, ERK, p38 mitogen-activated protein kinase, signal transducers and activators of transcription 3, and c-Fos, was examined in the pair of EGFR wild-type and vIII mutant EGFR cell lines as a function of gefitinib concentrations. Results from the western blot analysis demonstrated that pERK was inhibited in a dose-dependent manner in both cell lines, and was chosen as a PD marker to assess the effect the drug on tumor xenografts. To ensure that the most salient features of drug disposition and dynamics are characterized, a series of dose-dependent investigations of gefitinib in both LN229-wild-type EGFR and LN229-EGFRvIII mutant tumor-bearing mice were done, which consisted of a PK phase for the measurement of plasma and tumor drug concentrations and a PD phase for the quantitation of tumor pERK (Fig. 2a). The subsequent development of preclinical hybrid PK/PD models of gefitinib describing the accumulated PK/PD data from the three different dosing regimens was developed in a sequential manner with the definition of the PK model followed by the PD model. By fitting the PK models simultaneously to the combined gefitinib concentrations from all treatments, a single PK model for each tumor type was obtained. The best-fit plasma PK models were then cast as the forcing function to describe gefitinib plasma concentration input into a single tumor compartment model. Once the hybrid PK model for each EGFR wild-type and mutant xenograft was established, the PK model and associated variables were linked to a target-response PD model representing the inhibition of pERK in tumors. The results obtained from the model fitting showed that there was an approximate 1.6- to 1.8-fold decrease in IC_{50} values for the vIIIEGFR tumor group

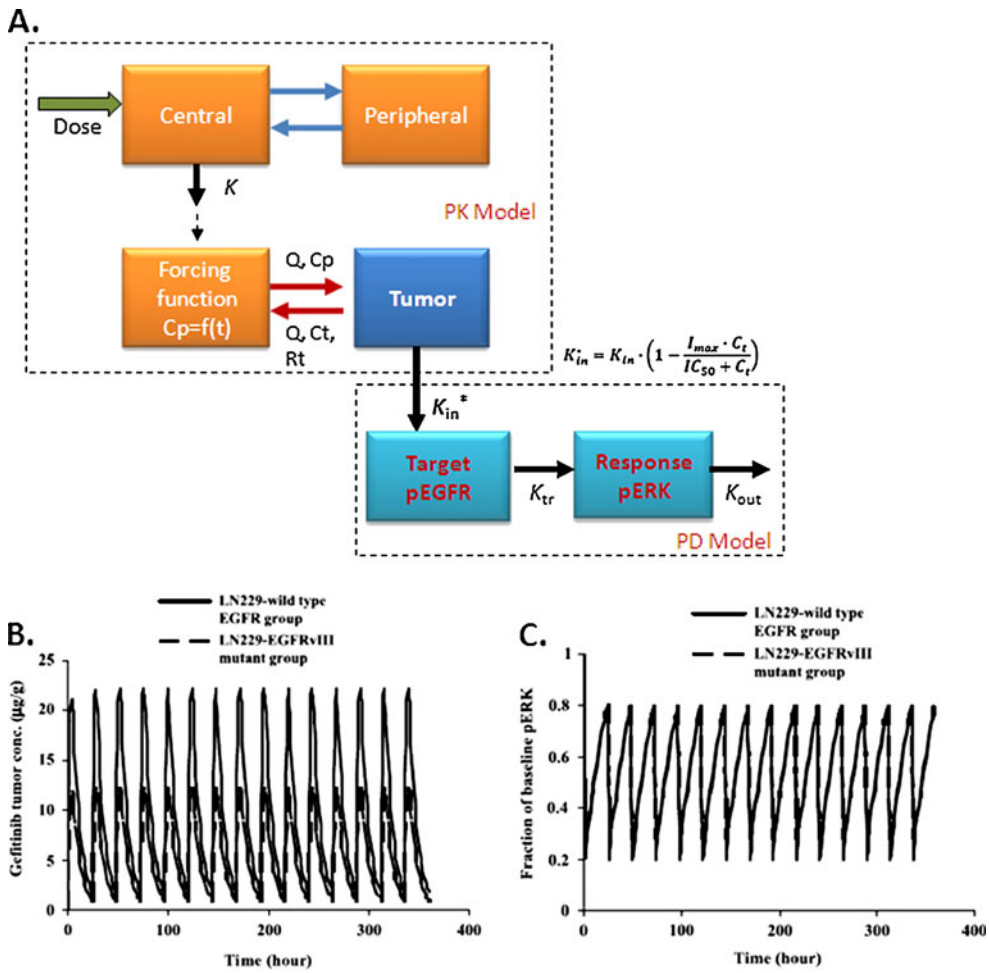

Fig. 2. Equivalent PK/PD dosing based on model predictions. **a** Schematic representation of a hybrid PK/PD model consisting of a two-compartment systemic disposition model, a one-compartment tumor model, and a two-compartment target-response model. Model simulations of **b** tumor gefitinib concentration-time profiles and **c** corresponding tumor pERK inhibition-time profiles following 150 mg/kg p.o. daily×15 day in LN229-wild-type EGFR tumor-bearing mice and 70 mg/kg p.o. daily×15 day in LN229-EGFRvIII mutant tumor-bearing mice (39)

compared with the wild-type EGFR group, consistent with the pharmacogenetic-based difference in gefitinib sensitivity and further agreed with *in vitro* cytotoxicity differences. Multiple-dose simulations of gefitinib in the wild-type EGFR and vIIIEGFR tumor groups predicted that by employing different daily doses, analogous pERK profiles could be achieved. The approach was referred to as the "equivalent dosing strategy," an exercise that acknowledged that different drug dosing schedules could be implemented to yield equivalent PD response profiles in genetically distinct tumors with varying sensitivities.

To confirm that the equivalent dosing strategy worked, multiple-dose studies of gefitinib in the two selected tumor groups, wild-type EGFR and vIIIEGFR, were completed. In this case, the gefitinib multiple-dose regimens for EGFR-wild-type and vIII mutant tumor-bearing mice were designed in order to identify gefitinib doses that produced equivalent pERK profiles in each tumor group based on maximum gefitinib tumor concentrations of 15 μg/g in wild-type EGFR tumors, which within the range of values reported in brain tumor patients (Fig. 2b, c). The two tumor types were implanted subcutaneously so the PK and PD measurements could be linked to a tumor size efficacy model. The PK, PD, and tumor size measurements obtained over a 15-day gefitinib treatment period allowed physiologically based hybrid PK/PD models to be developed for each tumor group, and finally linked to Gompertz-based tumor growth models. The net result of the preclinical multiple-dose studies supported the equivalent dosing strategy. Since analogous growth inhibition was achieved in both the wild-type and mutant EGFR groups, it is suggested that a relationship between a PD endpoint and efficacy, as embodied in tumor size, could be established (8).

The final exercise demonstrated the translation of the preclinical PD gefitinib models to brain tumor patients (8). First, a human hybrid physiologically based PK model was developed by linking the human forcing function derived from the available gefitinib plasma concentration data in glioma patients to a human brain tumor model derived from the reported patient data. The PK model was coupled directly to the preclinical target-response PD pERK models for either wild-type EGFR or mutant vIII EGFR tumors. Two simulation exercises were completed (a) to determine the gefitinib dose that predicted equivalent pERK profiles in the two tumor types and (b) to examine the effect of gefitinib brain tumor concentrations as set by the tumor/plasma partition coefficient on the percent inhibition of pERK at the nadir. Although the patient PK/PD models are speculative due to the paucity of data, the exercise demonstrates how model-based approaches can address pharmacologic questions mechanistically, and hopefully stimulate interest to gather clinical data to facilitate further development and validation of tumor-based models.

Since combination therapy with two or more anticancer drugs has become a common approach to treating most types of cancers, development of mechanistic PK/PD models that can help to select an optimal dosing regimen to achieve the maximal synergistic antitumor effect is highly desirable. A recent study by Soto *et al.* (57) demonstrated the feasibility of using PK/PD information derived from monotherapy data to predict neutropenia-related effects of a new combination therapy by assuming that the effects of the drugs were additive. Although a good agreement was achieved between observed and predicted data, the limitation of applying this approach to evaluate other combination therapies is that an assumption has to be made about the type of interaction. In a study by Koch *et al.* (52), an interaction term was introduced into a semimechanistic anticancer PK/PD model to describe the pharmacological effect of combination therapy. Since the interaction term is an empirical factor that reflects the nature and degree of interaction and depends on the administration schedule, this parameter allows optimizing the administration schedules of the combination therapy. Neither of the above examples employed a PBPK model; however, nothing would prevent adapting such an approach with additional considerations about specific representations of tissue compartments.

APPLICATION OF PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS TO THERAPEUTIC MACROMOLECULES

Biological macromolecules, including therapeutic proteins and peptides, have emerged to offer promise for the treatment of various types of cancer, yet significant biological barriers limit the ability of these molecules to reach the site of action from the systemic circulation. The aforementioned advantages of PBPK modeling for low molecular weight anticancer drugs also apply to macromolecules.

PBPK modeling has been employed to characterize the disposition of several monoclonal antibodies (MAbs) for cancer therapy (58–61). Although the basic principles of PBPK modeling are still valid for MAbs, several factors need to be taken into consideration: (a) MAbs usually have long biological half-lives compared with conventional small molecular drugs due to the low vascular-wall penetration, protection against catabolism through binding to FcRn receptors, and limited renal elimination; (b) as a result of their large size, the transport of MAbs across blood vessels as well as through the interstitial fluid is determined by passive diffusion, convection, and in some cases endocytosis, and filtration between cells; and (c) some MAbs demonstrate target-mediated disposition, where the antibody disposition is mediated by specific binding to targets such as epidermal growth factor receptor (EGFR), CD33, CD11, and Her2 and is generally characterized by either a higher antibody clearance at lower antibody doses or prolonged circulation time upon saturation of the target-mediated pathway (62).

The two-pore model, which was first established by Rippe and Haraldsson (63), has been used to characterize the pharmacokinetics of macromolecules including therapeutic antibodies (Fig. 3). The model describes transcapillary drug exchange according to the two-pore formalism that depicts small and large pores, lymph flow, and an additional subcompartment that represents the endosomal space, which contains FcRn receptors that are able to protect the macromolecules from catabolism by binding. In a study by Baxter and coworkers (58), a PBPK model based on the two-pore model was developed to describe the biodistribution of a radiolabeled monoclonal antibody IgG1 (ZCE025) and its fragments (F(ab')2 and Fab) and of a nonspecific IgG1 (MOPC21) in normal tissues and in T380 human colon carcinoma xenografts in nude mice. This model contained two novel features: (a) the antibody and its fragments have nonspecific, nonsaturable binding in both normal and tumor

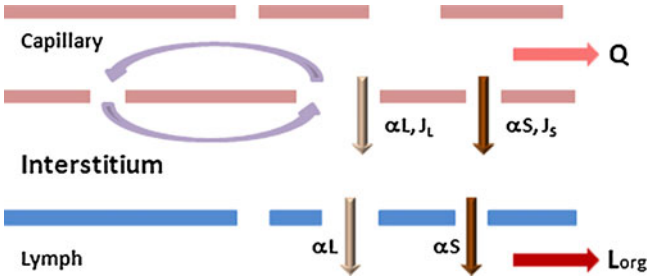

Fig. 3. Schematic representation of the two-pore model for describing the flux of MAb across the capillary wall. Q plasma flow rate, L_{org} lymph flow rate, α_S and α_L fractions of the hydraulic conductivity attributable to the small and large pore pathways respectively, J_S and J_L transcapillary fluid flow rate (from vascular to interstitial) via small and large pores respectively, J_{iso} fluid recirculation flow rate

tissues and specific, saturable binding in the tumor tissue; and (b) the net flux of antibodies and fragments across the capillary between plasma and interstitial fluid is described by a two-pore model. According to the two-pore model, there would be recirculation of fluid caused by filtration from large pores and absorption via small pores. Even when net fluid flow is zero, the filtration of large molecules through the large pores is counterbalanced by osmotic absorption of the macromolecule-free fluid through the small pores. This preclinical model was used to predict the biodistribution of ^{111}In-labeled ZCE025 IgG in a 70-kg human with a 20-g tumor by scaling the physical and physiological parameters from mice to human. Human physiological parameters that are drug-independent, such as blood flow rates and tissue volumes, were obtained from the literature, whereas other biochemical and physiological parameters that are drug-specific or unavailable in humans, including the osmotic reflection coefficient, binding rate constants and affinities, antigen concentration, and the fractional volume of the interstitial space, were assumed to be the same in the human and murine systems. Moreover, an exponent of 0.75 applied to body weight was arbitrarily set to scale the lymph and recirculation flow rates, permeability-surface area products, and urinary clearance. The model predictions compared favorably with clinical data for ^{111}In-labeled ZCE025 IgG in patients, although an alternate simulation with the transcapillary fluid recirculation flow rate scaled up by (body weight)$^{1.0}$ would yield a more precise fit to the data in the plasma and liver (59). As the PBPK modeling approach allows a wide range of dispositional characteristics to be considered and is amenable to all aspects of binding kinetics, the complex pharmacokinetics of therapeutic macromolecules with nonlinear distribution and elimination can be incorporated into the models. Future work directed to improve the predictability of the PBPK models could investigate whether obtaining parameter estimates from *in vitro* studies with human tissues, when the *in vivo* studies in human are unavailable, yield improved predictions compared to empirical scale-up of the animal data by human body weight.

SCALING AND INTEGRATION WITH SYSTEMS BIOLOGY

Drug discovery and development paradigms have been challenged, in part, due to the failures of typical "linear" path approaches and the greater emphasis on translational medicine. The pharmaceutical sciences may benefit from the dilemma of devising new strategies for drug discovery by advancing PK/PD-driven drug development. From the above discussion, it can be appreciated that PBPK/PD methods offer a means to anchor drug discovery and development from early preclinical investigations to the clinic. Nonetheless, there are areas that require further analyses if such methods are to become integrated and part of innovative drug discovery programs. One area is concerned with interspecies scaling of PK and PD models. Interspecies extrapolation of PK parameters and models has been considered since the inception of PBPK models (64), and at one time, a standard protocol was based on using PK data from four species to devise allometric relationships (65) for macro-PK parameters such as total clearance. However, experimentally, the latter protocol was shown unnecessary as single animal–man extrapolations were successful (7,38,66–68). Even with these limited examples of scaling rodent-to-man models, the underlying theory of scaling drug-dependent parameters contained in PBPK models has not been formally addressed; however, at the same time, it is appreciated that even empirical relationships, such as for drug clearance, may not be found, emphasizing the unique nature of the models.

Another component in translating preclinical PBPK/PD models to patients is, of course, how to scale PD models. This is a largely untapped area that Mager *et al.* (69) and others have appreciated (8,70). One might argue that when PD models are cast in terms of relative efficacy (i.e., fraction inhibition), as we did with the target-response model for gefitinib (8,39), the scaling problem is minimized as the absolute quantities of response proteins are not explicit in the model. Nonetheless, the presumption that drug concentration-response relationships, even as relative responses, in target tissues are analogous in animal models and patients should be challenged and examined with actual data from the relevant species.

The advent of new multiplex and proteomics technologies that are able to provide high PD content to support systems biology in its quest to define cell signaling networks offers a new level of integration with PK/PD modeling efforts. These techniques can provide individual cell measurements on numerous PD markers that can define signaling pathways in different genetic backgrounds and thus define genotype–phenotype relationships. Valuable data in its own right but to combine such relationships with PK measurements may be more beneficial not only to elevate individualized treatment but also to guide preclinical drug development.

SUMMARY AND CONCLUSIONS

The unacceptable high attrition rates in the discovery and development pipeline of anticancer drugs demand new paradigms. Rather than the use of traditional more linear drug discovery and development strategies, matrix approaches that integrate different disciplines [e.g., chemistry, toxicology, genetics, proteomics, pharmacology] in preclinical testing could enhance success rates and at least eliminate noncontenders earlier in the drug development process. Enhancements in the pharmacological arena can rely on the use of predictive physiologically based PK/PD models that have the potential to comprehensively characterize underlying drug disposition

mechanisms, define pertinent PK/PD relationship, extrapolate the preclinical results to the clinic, and set a quantitative basis for dosing optimization in the clinical setting. The successful translation of PK/PD tumor-based models to the clinic will require enhanced efforts to obtain tissue measurements coupled to innovative study designs in patients.

A range of physiologically based models have been documented, including global PBPK models, and hybrid PBPK and PBPK/PD models, which have the ability to incorporate the biological information and pharmacological data to describe distinct characteristics of drug disposition in various tissues and to predict PK/PD properties across species for either small molecular drugs or therapeutic macromolecules. Incorporation of a predictive preclinical PK/PD model into preclinical studies and early-stage clinical trials can have profound implications on the drug approval process, reducing time, cost, and failure rates in later-stage clinical trials. The challenges lie in the generation of clinical data to facilitate further development and validation of those predictive physiologically based PK/PD models, and in devising means to integrate target tissue-based PK/PD with systems biology. As PK/PD relationships remain a key consideration in the drug development process, early establishment of predictive PK/PD model provides a quantitative tool to enable rational decision making on a compound's suitability for further development including predictions of its clinical pharmacological profile. Looking to the future, when more and more innovative techniques for measuring tissue concentrations, identifying biomarkers of drug responses and quantifying those markers become available, predictive tissue-based PK/PD modeling will become integral to the drug discovery and development, with the potential of accelerating the progress of promising new agents through the drug development matrix and clinical hierarchy, increasing success rates, and ultimately facilitating the implementation of personalized medicine in cancer treatment.

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