REVIEW

Importance of Drug Pharmacokinetics at the Site of Action

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INTRODUCTION

Pharmacological activity depends on adequate drug levels at the effect site, but access to these sites for pharmacokinetic sampling is often limited. Thus, we often rely on plasma concentrations as surrogates in efforts to understand exposure–response relationships. Recent advances in analytical and quantitative methodologies allow for more robust and often noninvasive assessments of drug pharmacokinetics at the site of action. This review highlights the advances made in estimating drug exposures in several compartments of interest.

BACKGROUND

Due to difficulty in accessing relevant tissues or biological fluids to measure drug levels, plasma drug concentrations are often collected with the hope that they will correlate with or be informative about exposures in compartments of interest, which is often found to be the case. Even between structurally similar compounds, however, penetration into these matrices may vary widely, and so exclusive use of plasma exposure in pharmacokinetic–pharmacodynamic (PK/PD) models may lead to suboptimal assessment of exposure–response relationships and inaccurate dose selection in certain circumstances. Differences in penetration between even structurally similar compounds into a given site may be due to a host of factors ranging from permeability to local metabolism to physicochemical properties to the role of drug transporters in efflux or uptake.

Novel experimental and quantitative methods have been developed to assess drug levels at the site of action across therapies and disease areas. For central nervous system (CNS) diseases, the measurement of drug levels in the cerebrospinal fluid (CSF) using lumbar puncture and even directly in the brain using imaging or microdialysis has provided insight into drug penetration across the blood–brain barrier and helped characterize the compartmental pharmacology of such drugs. In oncology, linking information about drug penetration into solid tumors with radiologic outcomes provides a greater understanding of relevant exposure–response relationships. For HIV, characterization of drug distribution into the genital tract and the colorectum, sites of potential HIV exposure, has informed the development of preexposure prophylaxis. For antibacterials, lung penetration studies using microdialysis or invasive bronchiolar lavage have been instrumental in projecting drugs and doses with suffi-

cient local exposure to be efficacious against the bacterial pathogens that cause pneumonia, while the assessment of PK in urine provides insight into the likelihood of successful treatment of urinary tract infections.

As analytical and quantitative techniques have continued to evolve to allow for the assessment of drug levels at these various sites of action, so has our knowledge regarding the role of drug-metabolizing enzymes and transporters in the uptake and efflux of drugs in relevant compartments.[1,2] Our ability to assess the role a transporter may play in the distribution and elimination of a given drug has expanded with the development of increasingly complex *in vitro* systems and growing knowledge about how to translate such *in vitro* results to clinical impact. Together with quantitative approaches such as physiology-based pharmacokinetic (PBPK) modeling, we now have the tools at our disposal to predict drug disposition and how factors such as patient demographics, pharmacogenomics, or coadministration with other drugs affect it. There is a wealth of knowledge regarding the development and application of PBPK models that has been well described previously,[3–5] both for small molecules and monoclonal antibodies.[6,7]

With sufficient information about drug levels at the site of action, advances in population PK modeling can allow for the effect site to be treated as a separate compartment, thus bridging effect site PK to plasma PK. Once mathematical linkages between effect site and plasma PK are established, inferences about effect site PK can be made in subsequent experiments in which only plasma PK is collected, especially in cases where site-of-disease PK measurement is invasive or inconvenient. However, one must be careful to consider situations where nonlinearities may limit the ability to extrapolate plasma PK to tissue levels under different scenarios. Such limitations may arise due to saturation of drug transport pathways, nonlinear or concentration-dependent protein binding that differentially impacts fluids and tissues, or similarly nonlinear or concentration-dependent drug clearance. Additionally, often information regarding effect site PK is gathered in situations where limited sampling takes place. The dynamic changes in drug level in the plasma and effect site are often not synchronous, and thus sampling limitations may lead to an inadequate understanding of the relationship between plasma and effect site drug levels.

Additionally, systems-level models such as quantitative systems pharmacology (QSP) and physiology-based pharmacodynamic (PBPD) models have progressed to enable linking the effect of drug levels at the site of action to

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the underlying causal biology.[8,9] Together, these quantitative approaches coupled with advances in sampling techniques allow for great advances in our ability to predict and describe the distribution of drugs in the body and the corresponding impact on disease modulation and treatment. However, it should be noted that each of these experimental and quantitative methodologies may come with significant effort, and thus the benefit of such approaches needs to be weighed against the utility of simpler methods. In many cases, fit-for-purpose translational models or the utilization of plasma PK may be perfectly suitable for the purpose at hand.

Understanding PK at the site of action and demonstrating direct evidence of target engagement is not simply of scientific interest, but is also being increasingly recognized as key learning required to improve the yield of pharmaceutical research & development. Systematic reviews of drug discovery programs at large pharmaceutical companies have led to publication of translational risk frameworks, and obtaining adequate exposure at the intended site of action has been identified as a critical component of achieving downstream success in clinical trials.[10,11] Thus, the earlier one can demonstrate with confidence that a drug is present at the disease site, the higher the likelihood of observing the desired pharmacology for novel drug therapies. In this review, advances made in the estimation of drug exposures in several compartments of interest are discussed , as illustrated in **Figure 1**, including key biological considerations of each compartment and techniques utilized to gain insight into PK at the various sites of action, as described in **Tables 1 and 2**, respectively.

BRAIN AND CEREBROSPINAL FLUID

The brain and the CSF are unique and difficult-to-access compartments. The blood–brain barrier (BBB) does an exquisite job of shielding the CNS from substances in the systemic circulation that are either nonessential or toxic. Tight junctions between capillary endothelial cells restrict paracellular diffusion, and most compounds that manage to enter (however briefly) are efficiently removed by efflux pumps.[12] P-glycoprotein (P-gp, or MDR1) is the best-known transporter, but others, like breast cancer-resistant protein (BCRP), contribute to a highly redundant, efficient, and dynamic "waste" removal system.[13] Drugs to treat CNS diseases such as brain cancer, meningitis, pain, psychiatric or neurodegenerative disorders must cross the BBB or blood–CSF (BCSF) barriers to be effective, and thus drug developers must outwit this highly evolved system. The ability of a drug to enter and remain in the CNS depends on several physicochemical properties—lipid solubility, ionization, molecular weight, the number of hydrogen bond donors, protein binding—and as predictive knowledge to assess these characteristics increases, scoring systems are being developed to assess a compound's drug-like properties for CNS penetration.[14] Conversely, drugs intended for non-neurologic indications that readily enter the CNS may have off-target liabilities, such as efavirenz (HIV drug whose metabolite is directly neurotoxic and causes neurocognitive impairment) or cycloserine (anti-tuberculosis (TB) drug that is an NMDA agonist and provokes psychosis and symptoms of "irritability, hypocrisy and querulousness").[15–17]
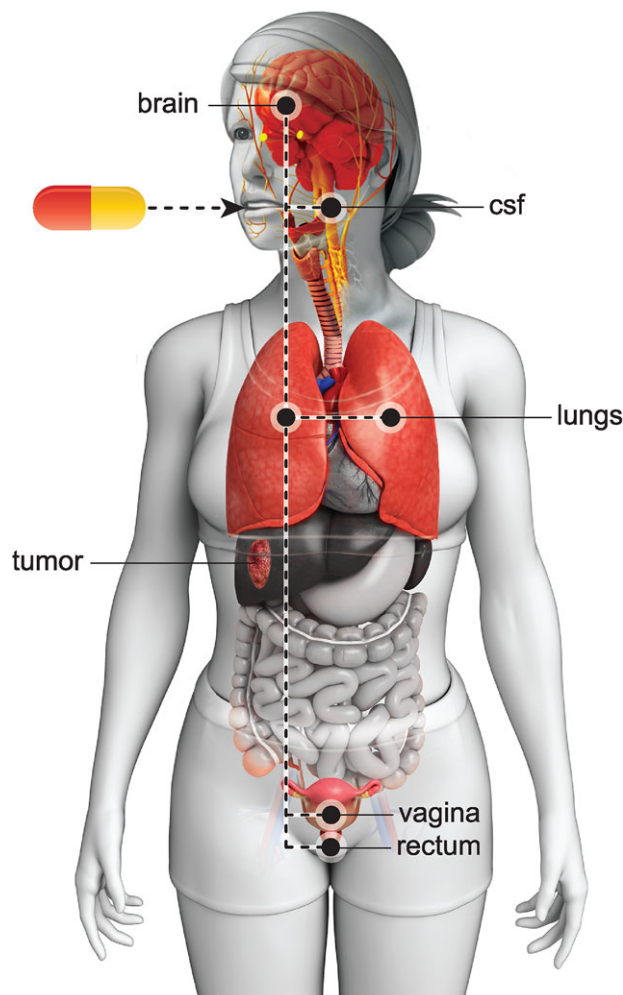
Figure 1 Schematic of relevant sites of action for assessing drug exposure, including the brain, cerebrospinal fluid (csf), lung, solid tumor, cervicovaginal fluid, and colorectal tissue.

For treatment of a disease of the brain or meninges, drugs must adequately penetrate into the brain and CSF when given at doses that are clinically safe. Knowledge of drug concentrations at the site of (on-target or off-target) action may be helpful in guiding PK/PD assessments that, in turn, direct drug dosing decisions. However, the brain and CSF are neither readily nor repeatedly accessible compartments, and given differences between drug clearance from plasma vs. CSF as well as variability in penetration of drugs to the brain and CSF related to inflammation and breakdown of vascular integrity, the dynamics of drug concentrations in CSF cannot generally be extrapolated from plasma concentration data or accurately estimated from a single CSF-to-plasma paired-sample ratio.[18,19] Moreover, CSF collected via lumbar puncture is often not a good proxy for drug concentrations in brain extracellular fluid (ECF) (drugs are pumped out of the brain into CSF, and CSF is produced cranially and then flows caudally).[20] There are also differences between BBB and BCSF with regard to the molecular architecture of membrane transporters. For example, while Pgp is the most abundantly expressed transporter on the apical side of the BBB, MRP1 is

Table 1 Organs/tissues of interest for disease site of action and drug PK

Site of action	Relevant diseases	Techniques to assess PK	Special considerations
Brain and cerebrospinal fluid	Psychiatric disease, brain cancer, neurodegenerative disease, encephalitis/meningitis	Microdialysis PET imaging with radiolabeled microdoses, functional MRI (fMRI)	Blood-brain barrier, blood:CSF barrier, transporters (P-gp)
Solid tumor	Cancer	Microdialysis, PET imaging	Blood flow, extravasation, interstitial diffusion
Genital tract and colorectum	HIV prevention, treatment of sexually transmitted infections	Cervicovaginal fluid sampling, tissue biopsies, imaging of radiolabeled drug	Gender differences, intracellular penetration
Lung	Bacterial pneumonia, tuberculosis, chronic obstructive pulmonary disease (COPD), asthma	Bronchoalveolar lavage (BAL) to measure PK in epithelial lining fluid (ELF), MALDI/mass spectrometry	Invasive and sparse sampling, high variability
Urine	Bacterial urinary tract infections	Urine interval collections	Collection over intervals as opposed to discrete time points may limit kinetic interpretation of data

Table 2 Comparison of tools used to measure PK at various sites of action

Technique	Relevance	Pros	Cons
Plasma PK	Standard and well-accepted method of assessing drug exposure	Serial sampling possible, can assess free and total drug levels	May not correlate with response when free drug hypothesis violated
Saliva PK	Useful for therapeutic dose monitoring (TDM)	Easy to sample	Risk of under- or over-estimation of lung PK for many drugs
Dried Blood Spots (DBS)	Animal studies, pediatrics, at home sampling	Avoids concerns around blood volume, can obtain PK at time of event at home (e.g., migraine), allow for PK and PD in same animals, some advantages in sample shipment and storage	Requires extensive work to enable accurate bioanalytical quantification and correlation with plasma PK, may need to correct for hemoglobin levels.
PET Scanning	Noninvasive strategy to measure drug in a tissue of interest	Noninvasive, can collect multiple images following a radiolabeled dose to determine concentrations in a tissue over time	Expensive, resolution not always sufficient, PET label may impact drug distribution, some PET labels have short half-life, signal may reflect parent drug or metabolite.
Microdialysis	Measuring drug concentrations in a hard-to-access site (brain, tumor) in a small number of patients	Allows assessment of free drug concentrations over the full dosing interval	Invasive, requires significant work to validate and calibrate
Microdosing	Initial assessment of drug distribution into a disease site of interest	Small dose size reduces risk of toxicity	May require advanced bioanalytical methods such as accelerated mass spectroscopy (AMS) due to low drug levels, microdose drug disposition may be different from therapeutic dose disposition

predominantly expressed at the choroid plexus (CP), facing the interstitial fluid. On the other hand, Pgp is expressed at the CP, facing the CSF. Based on the transporter localization, it is suggested that MRP1 is critical at the CP in eliminating drugs.[21,22] Therefore, using CSF PK studies to assess CNS penetration could potentially be inaccurate if drugs targeting brain parenchyma interact differently with Pgp and MRP1.

Up to now, CNS drug development has been fraught with peril, with CNS drugs taking an average of 18 years to progress from bench to bedside and only about an 8.2% success rate.[23,24] Late-stage failures abound, as CNS drugs tested in phase III trials are 45% less likely to be successfully registered than non-CNS drugs,[25] mostly due to failure of efficacy. Clearly, more effective strategies to assess both drug exposures in the brain and, more important, biomarkers of drug activity at the site of disease early in clinical development are needed. Cerebral microdialysis has emerged as a very informative, albeit invasive, means of continuously

measuring molecules in brain ECF (the fluid surrounding neurons and astrocytes).[20] Microdialysis can be used in animal models (e.g., rat) to get an early look at drug penetration into the brain and CSF. While one would hope to be able to characterize plasma-CSF-brain drug distribution relationships in an animal model and then simply collect CSF in humans and use relationships discovered in the rat to predict human brain ECF concentrations, it is, in fact (not surprisingly), not so easy.[26,27] In general, characteristics of the BBB and BCSF are preserved across species and plasma-CSF and plasma-brain ECF distribution for drugs may be qualitatively similar in rats and humans; however, human CSF concentrations are generally higher than rat CSF concentrations and display higher variability (the latter may be, in part, because CSF in studies is only collected in patients with a brain or meningeal disease, whereas rat CSF is collected in healthy animals), and brain drug distribution PBPK models developed using rat data underestimate human CSF

concentrations and often overestimate human brain ECF concentrations.[26] Dynamic information about free drug concentrations in brain ECF and CSF generated via microdialysis in rats, however, can be used to build semimechanistic models and characterize processes such as active transport, intracellular partitioning, and local tissue binding for drugs of different types in the CNS (e.g., lipophilic passively transported only (acetaminophen[28]); lipophilic and PGP substrate (quinidine[27]); hydrophilic and MRP substrate (methotrexate[29]), etc.[30]). Then human- and drug-specific information can be added to the models (drug physicochemical properties, fluid flows, transporter functionalities, disease states) to improve their predictive power for estimating human brain ECF concentrations. Microdialysis is increasingly being used to monitor cerebral metabolism clinically in patients with traumatic brain injury,[31] and techniques have been adapted to measure drug concentrations of putative neuroactive drugs in individuals requiring neurosurgery.[20] As more human microdialysis data are generated, intraspecies differences in drug distribution in CNS compartments as well as effects of disease on CNS PK will increasingly be characterized, which will lead to better predictive models of human brain ECF concentrations.[27] Positron emission tomography (PET) imaging has emerged as a noninvasive strategy for estimating CNS penetration of radiolabeled drugs. PET can also provide information about drug concentrations in specific regions of the brain known to have a high density of a receptor of interest (e.g., dopaminergic or serotonergic receptors for psychiatric drugs)[32] or in areas of known brain disease (e.g., amyloid-beta-rich areas in patients with Alzheimer's disease or tumors).[33] Functional neuroimaging, such as functional magnetic resonance imaging (fMRI), can be used throughout clinical development of CNS drugs, initially to demonstrate receptor occupancy in proof-of-concept studies,[34] then subsequently to assess exposure–response relationships by measuring biomarkers of target engagement or drug effect in response to an administered drug,[35] then finally to correlate drug exposure with brain structural features and function.[36] With fMRI, participants with a disease of interest can be asked to perform specific tasks (in the presence or absence of the drug of interest or following a therapeutic trial), and fMRI activity in the affected area of the brain can be quantified.

TUMOR

Small molecules and monoclonal antibodies are both employed in the treatment of cancer, either individually or, more often, in combination. Solid tumors are characterized by a complex and unique microenvironment that consists of infiltrating immune cells, low pH, a dense interstitial matrix, high interstitial pressure, and abnormal blood and lymphatic vascular structures.[37,38] Upregulation of vascular endothelial growth factor (VEGF), and chemokines such interleukin (IL)-8 and transforming growth factor (TGF)-beta1 by the tumors may trigger angiogenesis and increase vascular permeability and leakage.[39] *In vitro* studies suggest that drug uptake and local concentrations are dependent on the density of the tumor and the distance from the rim and the tumor stroma.[40]

Variations in perfusion, vascularization, interstitial transport, and nonlinear local binding and metabolism can all contribute to differences in tumor PK among individuals, and each of these processes has been captured in mathematical models that describe drug distribution in the tumor.[41]

P-gp, BCRP, and transporters of the multidrug resistance-associated protein (MRP/ABCC) family have established roles in conferring multidrug resistance by limiting intracellular drug accumulation in tumor cells.[42] Variability in drug penetration between patients has been demonstrated using molecular imaging with fluorescent or radiolabeled intact monoclonal antibodies or small molecules.[43–47] Labeled molecules have been developed against a variety of drug targets, such as HER2, EGFR, insulin-like growth factor 1, androgen receptor, estrogen receptor, estradiol, and platelet-derived growth factor receptor B.[48–50] Such molecular imaging techniques allow for increasingly precise and noninvasive methods of assessing targeted therapies.

Microdialysis can be a useful tool for assessing tumor-specific free drug concentrations over the dosing interval in animal models and in accessible solid tumors in patients with cancer. Given that the therapeutic margin for chemotherapeutic drugs is generally very narrow, knowledge of site of action PK for anticancer drugs is particularly valuable for dose selection. Drug disposition at tumor sites, however, is often hard to predict without direct sampling, as blood flow and volume, expression and activity of transporters, and, very importantly, local environmental factors often differ greatly between tumors and surrounding normal tissue.[51] Microdialysis, which is semiinvasive, allows sampling of free drug in the interstitial space of the tissue of interest at multiple postdose timepoints, which is more efficient and informative than the collection of tissue PK data at a single timepoint (e.g., at the time of sacrifice in animal models). Microdialysis has been best suited for small molecules, although recently probes have been introduced that also allow for the measurement of larger molecules.[52] As previously reviewed by Zhou and Gallo,[52] the utility of microdialysis as an approach relies on achieving adequate recovery of the analyte of interest, which may be impacted by a number of factors related to the probe, the tissue of interest, and the molecule of interest as well. Thus, each of these aspects should be taken into consideration when assessing the utility of microdialysis. Additionally, insertion of the microdialysis probe can disrupt the tumor microenvironment and impact drug distribution when care is not taken to allow for sufficient time for tissue to recover between samplings. Other macromolecules can be measured simultaneously in the tumor microenvironment, allowing for relevant site-of-disease treatment–response assessments.[53–55] As the treatment of cancer continues to become more personalized or individualized, the ability to quantify drug levels and tumor properties, not only in each patient, but at each individual tumor, could allow for a greater degree of understanding of the interplay between the tumor microenvironment, genetics, and drug distribution, and how all of these factors interact to result in a given response. Microdialysis has been used to assess solid tumor PK of chemotherapeutic agents for melanoma, brain tumors,[56,57] ovarian cancer,[58] and breast cancer,[59] among others, in murine models or in

patients with cancer and is useful in assessing targeted drug delivery strategies.[60,61]

The complicated tumor microenvironment, where drug distribution is controlled by pharmacokinetic processes such as expression of drug transporters together with physical boundaries subject to fluid flow limitations such as blood flow and interstitial pressure, has led to the development of multiscale modeling approaches that bring together well-understood mathematical representations of fluid dynamics with physiologically-based PK models to capture and couple processes occurring at the molecular and systemic levels.[62] As analytical, imaging, and computational approaches continue to evolve, the predictive power of such methods will continue to increase, allowing for more precise and accurate predictions of tumor drug penetration to enable personalized medicine approaches in oncology.

COLORECTUM AND GENITAL TRACT

Following decades of advances in the development of therapies for the treatment of HIV infection, recent clinical studies have demonstrated the utility of leveraging antiretroviral (ARV) agents for the prevention of HIV through preexposure prophylaxis (PrEP),[63] leading to the approval of the combination of tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) as PrEP to prevent HIV infection in adult men and women.

PrEP trial results are heavily influenced by adherence; specifically, PrEP treatments administered via a number of routes—ranging from oral to topical—demonstrate efficacy, but only when they are taken, and the proportion of study participants adherent to prophylactic treatment varies widely across studies.[64] For instance, both the FEM-PrEP and FACTS trials, where topical PrEP was administered, showed negative results but also very poor adherence.[65,66] In contrast, several studies in women where adherence rates were far greater, including the Partners PrEP, TDF2, and CAPRISA 004 trials, demonstrated definitive efficacy of PrEP regimens.[67–69] This has also led to the ongoing development of novel routes of administration, such as vaginal rings[70] and subdermal implants[71] that are designed to simplify and thus enhance adherence.

Therefore, in order to understand the potential for a regimen to be effective for PrEP, and, at the same time, to indirectly gain information on regimen adherence, PK measurement at the sites of potential exposure to HIV, namely, the colorectum and female genital tract (FGT), is highly informative. For instance, an analysis of drug levels of tenofovir in the cervicovaginal fluid (CVF) of participants in the CAPRISA 004 study showed a correlation between PK in the CVF and the level of protection against HIV transmission, with 65% protection achieved with tenofovir levels above 100 ng/mL and 76% protection with CVF levels above 1,000 ng/mL.[72] Protein binding in the CVF is thought to be limited, with the majority of measured drug being unbound.[73] While these results are encouraging regarding the utility of CVF measures, there is limited biological rationale or justification for these threshold targets and more work likely needs to be done to determine robust PK/PD targets in these peripheral fluids. Further, as previously noted, local drug concentrations

in an individual are a function of both drug disposition in that individual and adherence, complicating interpretation of site-of-action PK. An assessment of the exposure–response of tenofovir or other HIV nucleoside reverse transcriptase inhibitors (NRTIs) is further complicated by the fact that these agents are typically activated by intracellular enzymes, so intracellular concentrations of the active drug are of most relevance. In the case of tenofovir, intracellular kinases phosphorylate tenofovir to the active tenofovir diphosphate moiety, whose levels need to be considered when assessing linkages between drug exposure and response.

The PK of antiretroviral drugs measured in mucosal tissues is highly variable, due both to patient behavior (adherence), and to the influence of differential drug metabolism and transport at these sites relative to plasma, thus rendering plasma to be of limited value as a surrogate for target site exposure. However, the measurement of tissue PK in the genital tract can require invasive biopsies, which limits the richness of data that can be obtained, and so there is a greater reliance on statistical analysis or population PK modeling to pool and interpret the results. Collection of mucosal fluids in recent studies has provided valuable information about local PK without the need for invasive biopsies. More specifically, the collection of cervicovaginal fluid via direct aspiration provides insight into drug concentrations in the female genital tract, while the collection of rectal fluid via swab similarly provides insight into concentrations in the colorectum. The correlation between drug levels in these fluids and the surrounding tissues was investigated in one study, to validate use of local fluid measures as a surrogate for tissue levels.[74] Mucosal fluid levels positively correlated with corresponding tissue levels, suggesting they may potentially be used as a surrogate for the measurement of effect site PK. However, there is still limited information available regarding protein binding in these tissues, which may limit interpretation of such data in the context of related plasma concentration data.

Tissue-specific PK of drugs for HIV prevention may help us identify those groups that are more likely to benefit from their use. For instance, tenofovir exposures are approximately 100-fold higher in colorectum than plasma, while cervicovaginal levels are similar to plasma.[75] These findings might cause one to postulate that females at risk for HIV acquisition via vaginal sex may be more sensitive to differences in adherence than men who have sex with men (MSM) who are at risk to acquire HIV through anoreceptive intercourse, and indeed, results of the iPrEx study in men demonstrated appreciable protection even with sporadic dosing,[76] while the FemPrEP study in women, in which adherence was poor, failed.[66]

Advances in understanding the PK/PD relationship for HIV prevention using advances in animal and *ex vivo* clinical models have been extensively reviewed.[63] Our understanding of site-specific differences in drug metabolism and transport increasingly allows us to predict and understand differences in antiretroviral penetration into colorectal and vaginal tissues. The mRNA expression of CYP enzymes in the colorectum and cervical tissues has been demonstrated, and a recent study took this work a step further, examining the enzymatic activity of CYPs and UGT enzymes through dosing of dapivirine and maraviroc as probe substrates, given

the interest in developing these compounds for use as topical microbicides for HIV PrEP.[77] CYP activity was present in both the colon and vagina, and UGT activity was present in the colon. Additionally, the investigators found that the metabolite profiles for maraviroc in the colorectal and vaginal fluids were markedly different than in the plasma or urine, suggesting that relative expression levels of CYP and UGT enzymes in tissues may differ from those in the liver. A growing body of knowledge regarding the activity of drug transporters in the female genital tract and colorectum is also enabling a greater understanding of PK at the site of action. A recent review[78] of efflux and uptake transporters in these tissues of interest provides an in-depth overview of the body of work to date. High-level conclusions from this work indicate that drug transport can play a major role in the tissue distribution of antiretroviral drugs. A predictive model utilizing data from 58 drugs demonstrated that the cervicovaginal penetration (ratio relative to plasma) could be predicted, and that this ratio was largely dependent on whether or not the drug was a substrate of drug efflux transporters MRP1 and MRP4.[79] Additionally, P-gp, BCRP, and MRP4 have all been found to be consistently expressed along the entire female genital tract, while P-gp, BCRP, and MRP1 through MRP7 are all positively expressed in human colorectal tissue at levels higher than or comparable to expression in the liver.[78] The activity of these transporters is regulated by factors such as hormones, disease status, and concomitantly administered drugs, which may provide some basis for the large interindividual variability observed in studies that have measured tissue PK of antiretrovirals for HIV PrEP, and in turn, suggest that an understanding of drug transport is necessary to predict tissue penetration.

Observations from the above-described clinical trials in HIV prevention, together with continuing advances in our understanding of tissue-specific drug metabolism and transport, suggest that there may be the opportunity, through advances in animal models,[63] clinical measurements,[70–72,74–76] *ex vivo* models,[63] and quantitative analyses[76] to enhance our ability to predict the right regimen for the right population to prevent HIV infection.

LUNG

Antimicrobial resistance is an epidemic and now constitutes an urgent public health threat. The menace of untreatable tuberculosis (TB),[80] gonorrhea, Gram-negative bacterial infections, and malaria has occasioned increased government funding and support for the development of new therapies. In parallel, advances in epidemiology, microbiology, pharmacology, and pharmacometrics have enabled us to understand at a more fundamental level the interrelated contributors to the emergence of resistant pathogens. This knowledge, in turn, allows for a more refined understanding of dose optimization and prediction of which therapies are most likely to be not only effective at killing the pathogen but also robust against acquired resistance.

Bacterial pneumonia can be caused by both Gram-positive and -negative organisms. Community-acquired pneumonia is most commonly caused by the Gram-positive organism *Streptococcus pneumoniae*, while Gram-negative bacilli

and *Staphylococcus aureus* are the most common causes of hospital-acquired, or nosocomial, pneumonia. Antibacterial resistance can be a major concern when treating patients with pneumonia, and dosing must be high enough to prevent the emergence of resistant pathogens. Many methods have been used to estimate drug concentrations of anti-infective agents in the lung, including microdialysis,[81] whole-tissue homogenates, sputum, respiratory secretions, bronchial mucosa, pleural fluid, bronchoalveolar lavage (BAL), epithelial lining fluid (ELF), PET, and nuclear magnetic resonance (NMR) spectroscopy.[82] There are various advantages and disadvantages of each of the above methods that must be considered when choosing an approach. For instance, pathogens may primarily exist in the extracellular or intracellular space. For extracellular pathogens, the ELF is considered the site of action and is considered a matrix where protein binding plays a limited role and the drug exists in the free or unbound state. For intracellular pathogens, alveolar macrophages are viewed as the site of predominant interest. Thus, knowledge of the behavior of the pathogen(s) under investigation may impact the choice of measurement technique. Additionally, approaches such as whole-tissue homogenization may obscure differences in the penetration of drug extracellularly vs. intracellularly, or between different cell types. Other indirect techniques, such as the measurement of drug levels in saliva, may give biased results, either due to dilution (leading to falsely low measurements) or preferential partitioning into the saliva relative to the lung site of action (leading to inaccurately high measurements). In recent lung penetration studies to support the development of new antimicrobial drug candidates, investigators have opted to rely on bronchoalveolar lavage (BAL) techniques for the measurement of ELF,[83–85] which is thought to be the most relevant site for Gram-positive and -negative pathogens. The importance of the ELF has been further emphasized with the recent guidance issued by the European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) on the use of PK and PD in the development of antibacterials, where drug levels in the ELF are specifically cited as important data to supply in support of an indication of a drug for pneumonia.[86] The collection and use of ELF data for anti-infective agents is described well in a recent review in which roughly 20 years of work including 80 clinical studies in a number of antibacterial classes is summarized.[87] The review identifies a number of key issues to consider in the design of ELF studies, including the dosing regimen utilized, the PK sampling times and handling of PK samples, the personnel involved in conduct of the studies, the analytical techniques utilized to measure drug levels, and the subsequent data analysis and/or modeling,[87] all of which are important to facilitate appropriate decision-making for candidate drugs.

Drug levels in the ELF relative to those in plasma can vary widely, ranging from several-fold below levels achieved in plasma to ratios that exceed a value of 1. The relative ratio of ELF to plasma concentration can be influenced by many drug properties, including plasma protein binding, the role of transporters in drug uptake and efflux, and the physicochemical properties of the molecule. Thus, it is important to compare drug levels in the ELF to free drug levels in the plasma to gain a more robust understanding of lung

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penetration and translation. Recently, a novel methodology was introduced that aims to predict ELF-to-plasma penetration ratios based solely on physicochemical properties using a quantitative structure activity relationship (QSAR) model.[88] As such approaches continue to be refined and ultimately validated, drug developers may gain more confidence in using them to select those drug candidates that are most likely to be effective in treating pneumonia, and then perhaps invasive PK assessments can be minimized or avoided.

Pulmonary TB is characterized by heterogeneous lesions, including granulomas with caseous necrotic centers and larger necrotic lesions that undergo liquefaction and develop into the characteristic cavitary lesions that one sees on chest radiography. Most patients with *M. tuberculosis* infection harbor $\sim 10^8$–10^9 organisms, with a variable proportion of these bacilli exhibiting preexisting, chromosomally mediated resistance to at least one drug of a typical multidrug regimen. To be effective, drugs in TB treatment regimens must penetrate into the sites of infection (including macrophages and liquefied contents of cavitary lesions[89]), must be present at the site of disease in adequate concentrations to protect companion drugs from emergence of resistance,[90] and should have activity against semidormant "persister" bacteria. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging is now being used to characterize spatial distribution of candidate drugs into diseased lungs; early studies suggest that sterilizing (i.e., curative) activity of anti-TB drugs appears to correlate with drug distribution into TB lesions.[91] In clinical trials of TB treatment, cavitary lung disease is a predictor of poor treatment response for some drugs (rifapentine) but not others (rifampicin and moxifloxacin), which may be explained, in part, by differences in the ability of these drugs to penetrate into and accumulate in the proteinaceous caseum of these lesions.[92–94]

The lung is also a key site of interest in understanding and optimizing the dosing of inhaled drugs, such as those for the treatment of asthma or chronic obstructive pulmonary disease (COPD). In order to provide a predictive platform to describe both the pharmacokinetics in the lung, and the subsequent pharmacodynamic effects, Caniga *et al.* developed an experimental inhalation platform that consists of an animal model coupled with a mathematical model to describe the drug dissolution, transport, distribution, and efficacy following inhaled delivery of mometasone in both rodents and humans.[95] This work provides a novel platform that can be expanded to estimate drug penetration following inhaled delivery and subsequent PD effects for other inhaled corticosteroids.

URINE

The PK of anti-infective agents in the urine is of interest, primarily for the treatment of urinary tract infections (UTIs). Urine PK is relatively straightforward to measure and requires collection of urine during fixed intervals over the course of a dosing interval, and utilizes similar bioanalytical methods for measurement as those used for the assessment of plasma PK. Antibacterial agents may undergo various routes of metabolism and/or elimination, and thus it is important to ascertain the degree that a drug concentrates in the urine

when considering the treatment of UTIs. For instance, fosfomycin is approved for the treatment of uncomplicated UTIs and only requires single-dose administration. After a single 3-g dose, maximum plasma concentrations are achieved in 2 h, followed by relatively rapid elimination from the plasma. However, high urinary concentrations (1,000–4,000 μg/ml) are achieved and remain above 100 μg/ml for 30–40 h, allowing for single-dose administration.[96] In contrast, several fluoroquinolones such as moxifloxacin, sparfloxacin, and gatifloxacin cannot be used in the treatment of UTI because they are not renally cleared and achieve low levels in the urine.[97] For instance, only about 5.9% of the administered dose of moxifloxacin is excreted in the urine.[98]

ROLE OF TRANSPORTERS IN INFLUENCING SITE-OF-ACTION PK: AN ADDITIONAL LAYER OF COMPLEXITY

By mediating drug absorption, tissue distribution and elimination, transporters, in concert with drug-metabolizing enzymes, are commonly essential for therapeutic drug response.[1] From a PK point of view, transporters expressed in intestinal, hepatic, and renal epithelia govern plasma concentrations of many drugs, which may correlate with drug concentrations at the site of action.[99] However, transporters may function as a protective barrier for certain organs such as brain and testis, limiting the access of drugs to their pharmacological targets. Conversely, drugs may be more concentrated in certain cell types through transporter-mediated penetration. Therefore, to achieve better therapeutic outcome, it is important to understand the role of transporters as determinants of drug concentrations at the site of action.

Transporters as determinants of levels of drugs for intracellular receptors

As described above, the most extensively studied example of drug resistance acquired through increasing the expression level of the multidrug efflux pump P-gp is in tumor cells. Upregulation of this transporter consequently limits the access of anticancer drugs to their intracellular targets.[100] Besides efflux transporters, influx transporters may also be important determinants of intracellular level of drugs. *In vitro* and clinical studies suggest that organic cation transporter 1 (OCT1) is a key determinant of the intracellular levels of imatanib and its effects in patients with chronic myeloid leukemia.[101,102] The intracellular level of drugs may also be determined by combined effects of both influx and efflux transporters. For example, metformin is a substrate of both hepatocyte basolateral uptake transporter OCT1 and canalicular efflux transporter MATE1.[1] Therefore, the level of metformin in hepatocyte is dependent on activities of both transporters.

Transporters are also important for drugs that are activated by intracellular enzymes. For example, intracellular kinases mediate the phosphorylation of tenofovir to its active moiety, tenofovir diphosphate. MRP4 is a determinant of the intracellular concentration of tenofovir diphosphate. HIV-infected patients having a single-nucleotide polymorphism (SNP) (3463A>G) in the ABCC4 gene encoding MRP4 have a 35% higher concentration of tenofovir diphosphate in

peripheral blood mononuclear cells when compared to patients without this SNP.[103]

Transporters may determine the levels of drugs for receptors in subcellular compartments such as mitochondria. For example, vesicular monoamine transporters (VMATs) sequester the parkinsonian neurotoxin MPP$^+$ inside secretory vesicles, keeping it away from its primary site of action in mitochondria.[104] The role of mitochondrial transporters in the SLC25 family in pharmacologic response is poorly understood, but it is likely that mitochondrial transporters play critical roles in the toxicity or efficacy of drugs with mitochondrial targets.

Transporters as determinants of levels of drugs in the vicinity of plasma membrane receptors

The neurotransmitter reuptake transporters are responsible for removal of neurotransmitters from the synapse, limiting activation of receptors on postsynaptic cells.[105] A major class of antidepressants, the selective serotonin reuptake inhibitors (SSRIs) targeting the serotonin transporter (SERT, *SLC6A4*), increase the level of serotonin in the synaptic cleft available to bind to and activate postsynaptic receptors.[106]

Advances in PET imaging provide an opportunity to study transporters as determinants of drug levels at the site of action. For example, PET radioligands that inhibit the norepinephrine transporter (NET, *SLC6A2*), the dopamine transporter (DAT, *SLC6A3*), or SERT (*SLC6A4*) allow researchers to measure the occupancy of these neurotransmitter transporters by various drugs in patients with depression.[107] In addition, [^{11}C]-metformin, used as a PET probe, is taken up by multidrug and toxin extrusion protein 1 (MATE1, *SLC47A1*) in the liver and kidney, which may provide valuable information noninvasively about the biodistribution of the drug, drug–drug interactions, and identification of responders vs. nonresponders.[108]

SUMMARY

A key aspect of understanding the likelihood of a drug to achieve desired target modulation is the demonstration of free drug exposure at the site of action. However, gaining such an understanding can be complicated by practical (invasiveness of sampling), analytical, and biological considerations. Drug exposure at the site of action may not be in equilibrium with blood levels, limiting the utility of blood sampling as a surrogate, such as in the cases where active transport or site-directed administration is utilized. Further, differences in protein binding in various fluids or tissues may complicate translation and understanding of exposure–response, and should be carefully considered when pursuing such experimental and quantitative methods, ensuring that a framework centered on free drug levels is utilized. In this review, advances made in estimating drug exposures in several compartments of interest are summarized, including key biological considerations of each compartment, as detailed in **Table 1**, and techniques utilized to gain insight into PK at the various sites of action, as described in **Table 2**. Specifically, considerations regarding measurement of drug levels in the CNS, tumors, the cervicovaginal tract and rectum, lung, and in the urine were discussed, as illustrated in **Figure 1**.

Further, the additional complexity introduced by the role of drug transporters is highlighted. As analytical techniques and the underlying knowledge of the biology continue to evolve, quantitative systems models can leverage such knowledge and allow for further insight to be gained, increasing our ability to predict and describe the distribution of drugs in the body and the corresponding impact on disease modulation and treatment. In cases where the efforts associated with such methods are warranted, and reliance on fit-for-purpose approaches or the measurement of plasma PK is inadequate, such approaches represent a significant advance in framing our understanding of drug pharmacology.

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