A Paradigm Shift in Pharmacokinetic–Pharmacodynamic (PKPD) Modeling: Rule of Thumb for Estimating Free Drug Level in Tissue Compared with Plasma to Guide Drug Design

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ABSTRACT: A basic assumption in pharmacokinetics–pharmacodynamics research is that the free drug concentration is similar in plasma and tissue, and, hence, *in vitro* plasma data can be used to estimate the *in vivo* condition in tissue. However, in a companion manuscript, it has been demonstrated that this assumption is violated for the ionized drugs. Nonetheless, these observations focus on *in vitro* static environments and do not challenge data with an *in vivo* dynamic system. Therefore, an extension from an *in vitro* to an *in vivo* system becomes the necessary next step. The objective of this study was to perform theoretical simulations of the free drug concentration in tissue and plasma by using a physiologically based pharmacokinetics (PBPK) model reproducing the *in vivo* conditions in human. Therefore, the effects of drug ionization, lipophilicity, and clearance have been taken into account in a dynamic system. This modeling exercise was performed as a proof of concept to demonstrate that free drug concentration in tissue and plasma may also differ in a dynamic system for passively permeable drugs that are ionized at the physiological pH. The PBPK model simulations indicated that free drug concentrations in tissue cells and plasma significantly differ for the ionized drugs because of the pH gradient effect between cells and interstitial space. Hence, a rule of thumb for potentially performing more accurate PBPK/PD modeling is suggested, which states that the free drug concentration in tissue and plasma will differ for the ionizable drugs in contrast to the neutral drugs. In addition to the pH gradient effect for the ionizable drugs, lipophilicity and clearance effects will increase or decrease the free drug concentration in tissue and plasma for each class of drugs; thus, higher will be the drug lipophilicity and clearance, lower would be the free drug concentration in plasma, and, hence, in tissue, in a dynamic *in vivo* system. Therefore, only considering the value of free fraction in plasma derived from a static *in vitro* environment might be biased to guide drug design (the old paradigm), and, hence, it is recommended to use a PBPK model to reproduce more accurately the *in vivo* condition in tissue (the new paradigm). This newly developed approach can be used to predict free drug concentration in diverse tissue compartments for small molecules in toxicology and pharmacology studies, which can be leveraged to optimize the pharmacokinetics drivers of tissue distribution based upon physicochemical and physiological input parameters in an attempt to optimize free drug level in tissue. Overall, this present study provides guidance on the application of plasma and tissue concentration information in PBPK/PD research in preclinical and clinical studies, which is in accordance with the recent literature. J Pharm Sci 104:2359–2368, 2015

Keywords: ADME; unbound fraction; pharmacokinetics; pharmacodynamics; disposition; distribution; partition coefficients; PBPK; PKPD

INTRODUCTION

In preclinical and clinical studies, total drug concentrations in plasma or tissue are often correlated with pharmacodynamics (PD). However, the use of total tissue levels (e.g., tissue concentrations derived from homogenates) or biopsies to draw direct conclusions on drug activity is unwarranted and/or unreliable.[1–4] This is in contrast with the unbound (free) drug concentration at the target site, which should be more pharmacologically relevant.[1–4] Related to this, recent reviews highlighted various examples from the literature where the free drug concentration in tissue has demonstrated a superior correlation with the efficacy compared with the free drug concentration in plasma.[1,2] Therefore, the *in vivo* pharmacokinetics–pharmacodynamics (PK/PD) research should rely on the assumption that the free drug level at the site of action in tissue is the relevant measure of drug effect. However, tissue data are rarely available in humans, and, hence, a basic assumption in PK/PD research is that the free drug concentration is similar in plasma and tissue; thus, the free drug concentration in plasma can be used to estimate that concentration in tissues. In other words, the free drug concentration in the aqueous phase should be equal in the different organs under steady-state condition and when passive permeability is the main factor governing the drug transport. Hence, the traditional approach in drug design is to use the unbound free fraction in plasma (fu_p) determined *in vitro* to estimate the free drug concentration in tissue under *in vivo* condition (i.e., fu_p × total plasma concentration = free fraction in tissue (fu_t) × total tissue concentration).[1,2,5] These observations focus on *in vitro* static environments and do not challenge data with an *in vivo* dynamic system. Therefore, an extension from an *in vitro* to an *in vivo* system

Abbreviations used: C_{max}, maximal plasma concentration; fu_p, free fraction in plasma; fui_p, fraction unionized in plasma; fu_t, free fraction in tissue; fui_t, fraction unionized in tissue; Kp, tissue–plasma partition coefficient; Kpu, unbound tissue–water partition coefficient; log *P*, log *n*-octanol–buffer partition coefficient; i.v., intravenous; PBPK, physiologically based pharmacokinetics; PK, pharmacokinetics; PK/PD, pharmacokinetics–pharmacodynamics; p*K*a, ionization constant; RBP, blood–plasma ratio.

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becomes the necessary next step. This is because living systems are dynamic, with many simultaneous physiological actions on the free drug molecules, for example, pH gradient, binding to the target, binding to lipids and proteins, metabolism, movement between cellular and tissue compartment.[2] Accordingly, it is has already been suggested in the literature that the free drug concentration would depend, throughout the time course, on the intrinsic clearance, lipophilicity, and ionization potential of drugs.[2–12] For example, the free drug concentration in plasma and tissue should decrease when the intrinsic clearance value of a drug increases. Similarly, the free drug concentration in plasma and tissue should also decrease as the amount of drug bound to proteins and lipids increases because of the effect of drug lipophilicity. And the free drug concentration in plasma and tissue would also be influenced by drug ionization due to the pH gradient effect between the intracellular and interstitial spaces. Intracellular unbound (free) drug concentrations determine affinity to targets in the cell interior; however, there is a pH gradient effect compared to the interstitial space and plasma. Similalry, extracellular unbound drug concentrations determine affinity to targets in the interstitial space, but there is no pH gradient effect compared to plasma under normal conditions in healthy tissues. Therefore, in cultured cells under *in vitro* conditions, intracellular accumulation of unbound drug was consistent with pH-dependent subcellular sequestration and compound lipophilicity.[13] Hence, these observations should be further tested with a dynamic *in vivo* system.

In this context, for the ionizable drugs, the ionization state of both the drug and the binding site potentially change as a function of pH. It is indeed important to realize that for bases and acids there are ionized and nonionized fractions that are usually lumped into a single unbound concentration. However, considering that only the unbound unionized drug is able to permeate cell membranes *in vivo*, the free drug concentration in plasma and tissue, at equilibrium, would differ by the fraction of unionized drug (fui) on both sides of the membrane [i.e., $fu_p \times fui_p$ (fraction unionized in plasma) × total plasma concentration = $fu_t \times fui_t$ (fraction unionized in tissue) × total tissue concentration, and, hence, the free drug concentration in plasma and tissue would differ by the ratio of fui_p/fui_t].[5–11] Accordingly, in a companion manuscript, it has been demonstrated that the values of plasma fu_p and muscle fu_t determined *in vitro* in humans depended on the physiological pH.[5] In other words, the correlation between the fu_p and fu_t values was more robust when these two parameters are determined at the same pH value (i.e., 7.4) compared with when fu_p and fu_t are determined at a different pH value (i.e., pH 7.4 for fu_p vs. 7.0 for fu_t). These observations suggest a drug ionization effect in the aqueous phase; therefore, under *in vivo* condition, the free drug concentration in plasma at pH 7.4 would not equal the free drug concentration in tissue cells at pH 7.0 particularly for the ionizable drugs, which follows the pH partition hypothesis. The lower intracellular pH caused basic drugs to be trapped inside the cell, as they are not able to permeate the cell membrane in the ionized form, and, inversely, for the ionized acids.

For interstitial concentrations, the role of microdialysis should also be acknowledged. Microdialysis has become one of the major tools to sample endogenous and exogenous substances in interstitial spaces.[12] As a matter of fact, there are a number of papers using microdialysis, where the unbound drug concentrations in plasma and interstitial space were similar, which was expected as the pH of plasma and interstitial space is similar under normal conditions in healthy tissues, and, hence, the pH gradient effect would be minimal (i.e., $fui_p/fui_t \sim 1$). This is true particularly true when passive permeability is the predominant distribution process. For example, the unbound drug concentrations in plasma and the interstitial space of skeletal muscle determined by microdialysis were similar for acetaminophen and gemcitabine as well as for some ionizable drugs.[12] Conversely, the unbound drug concentrations in plasma and the interstitial space significantly differed for other drugs and tissues (e.g., brain); however, this is expected to be governed by efflux transport effects at the membrane level and/or permeation limitation effects at the capillary level.[12] Therefore, the free drug level in human tissue is the relevant metric to optimize on particularly under real dynamic *in vivo* condition.[1–5] Alternatively, the published tissue composition-based models, which were successfully validated in the past, can be used to replace the microdialysis as the free and bound drug concentrations can be predicted in both the cellular fraction and interstitial space in human tissues only on the basis of *in vitro* and physiological input data.[5–11] Hence, the tissue-to-plasma concentration ratios observed in humans for several drugs were accurately predicted by considering the dissimilarities in the binding and ionization on both sides of the membrane (i.e., by predicting $fu_p/fu_t \times fui_p/fui_t$).[5–11] To date, the pH gradient and transport effects as well as the binding to lipids and proteins have been incorporated in these models; therefore, they could also be used to explore the differences in the free and bound drug concentrations that can potentially be observed between tissue compartments and plasma under real dynamic *in vivo* condition. Moreover, the tissue composition-based models can be associated with a physiologically-based (PBPK) model to predict drug distribution in tissues by considering separately the cells and interstial space under dynamic *in vivo* conditions.

Overall, this implies that a drug with a high value of fu_p for plasma will indicate important free drug concentration in plasma, and, hence, in tissues, but this is true only in an *in vitro* static environment; conversely, in a dynamic *in vivo* system, the free drug concentration in tissue can be much lower than expected based on the *in vitro* fu_p value when the drug in tissue is rapidly cleared, highly bound, and/or ionized, for example.[2,5] At present, this theory needs to be further challenged to guide drug design. The objective of this study was to perform theoretical simulations of the free drug concentration in tissue and plasma by using a PBPK model reproducing the *in vivo* condition in humans. For the purpose of this study, the dissimilarities in the binding and ionization on both sides of the membrane were investigated for passively permeable compounds (i.e., the dissimilarities in the interstitial and intracellular free drug concentrations were investigated first). Therefore, the effect of drug lipophilicity, ionization, and clearance has been taken into account in a dynamic system. This modeling exercise was performed as a proof of concept to demonstrate that free drug concentration in tissue and plasma may also differ in a dynamic *in vivo* system, particularly for the ionizable drugs. Accordingly, a rule of thumb for potentially performing more accurate PK/PD modeling research and to guide drug design is suggested in this study.