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Incorporation of lysosomal sequestration in the mechanistic model for prediction of tissue distribution of basic drugs

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ABSTRACT

The prediction of tissue-to-plasma water partition coefficients (*Kpu*) from in vitro and in silico data using the tissue-composition based model (Rodgers & Rowland, J Pharm Sci. 2005, 94(6):1237–48.) is well established. However, distribution of basic drugs, in particular into lysosome-rich lung tissue, tends to be under-predicted by this approach. The aim of this study was to develop an extended mechanistic model for the prediction of *Kpu* which accounts for lysosomal sequestration and the contribution of different cell types in the tissue of interest. The extended model is based on compound-specific physicochemical properties and tissue composition data to describe drug ionization, distribution into tissue water and drug binding to neutral lipids, neutral phospholipids and acidic phospholipids in tissues, including lysosomes. Physiological data on the types of cells contributing to lung, kidney and liver, their lysosomal content and lysosomal pH were collated from the literature. The predictive power of the extended mechanistic model was evaluated using a dataset of 28 basic drugs ($pK_a \geq 7.8$, 17 β-blockers, 11 structurally diverse drugs) for which experimentally determined *Kpu* data in rat tissue have been reported. Accounting for the lysosomal sequestration in the extended mechanistic model improved the accuracy of *Kpu* predictions in lung compared to the original Rodgers model (56% drugs within 2-fold or 88% within 3-fold of observed values). Reduction in the extent of *Kpu* under-prediction was also evident in liver and kidney. However, consideration of lysosomal sequestration increased the occurrence of over-predictions, yielding overall comparable model performances for kidney and liver, with 68% and 54% of *Kpu* values within 2-fold error, respectively. High lysosomal concentration ratios relative to cytosol (> 1000-fold) were predicted for the drugs investigated; the extent differed depending on the lysosomal pH and concentration of acidic phospholipids among cell types. Despite this extensive lysosomal sequestration in the individual cells types, the maximal change in the overall predicted tissue *Kpu* was < 3-fold for lysosome-rich tissues investigated here. Accounting for the variability in cellular physiological model input parameters, in particular lysosomal *pH* and fraction of the cellular volume occupied by the lysosomes, only partially explained discrepancies between observed and predicted *Kpu* data in the lung. Improved understanding of the system properties, e.g., cell/organelle composition is required to support further development of mechanistic equations for the prediction of drug tissue distribution. Application of this revised mechanistic model is recommended for prediction of *Kpu* in lysosome-rich tissue to facilitate the advancement of physiologically-based prediction of volume of distribution and drug exposure in the tissues.

1. Introduction

Tissue-to-plasma water partition coefficients (*Kpu*) are important drug-related distribution parameters in physiologically-based pharmacokinetic (PBPK) models (Jones et al., 2015; Galetin, 2014; Zhao et al., 2011; Rostami-Hodjegan et al., 2012). The experimental determination of *Kpu* requires in vivo studies under steady-state conditions which are laborious and time-intensive. Therefore, several in silico models for the prediction of *Kpu* from drug-specific physicochemical properties and tissue composition data have been developed (Poulin and Theil, 2000; Rodgers et al., 2005; Rodgers and Rowland, 2006; Berezhkovskiy, 2004; Jansson et al., 2008). An initial model by Poulin and Theil (2000) described tissue distribution based on passive processes and accounted for drug dissolution in tissue water in addition to drug binding to neutral lipids, neutral phospholipids and macromolecules. Subsequently, Rodgers et al. incorporated the contribution of electrostatic interactions of moderate-to-strong bases ($pK_a > 7$) with acidic phospholipids (Rodgers et al., 2005; Yata et al., 1990); a separate

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mechanistic model was developed for the prediction of tissue distribution of acids, very weak bases, neutrals and zwitterions (Rodgers and Rowland, 2006). In the case of moderate-to-strong bases, only the nonionized drug was assumed to bind to neutral lipids and neutral phospholipids, whereas electrostatic interaction with acidic phospholipids was assumed to be the predominant factor for the lipid binding of cationic drug species (Rodgers et al., 2005). The Rodgers & Rowland model improved accuracy in *Kpu* predictions of 28 moderate-to-strong bases across a range of tissues (Rodgers et al., 2005). Despite improved model performances relative to Poulin et al., under-prediction in *Kpu* (lung) prevailed and both under- and over-predictions of *Kpu* (*brain*) were evident (Rodgers et al., 2005). The same trend in under-prediction of *Kpu* (lung) for lipophilic bases was confirmed in a comprehensive analysis of six tissue distribution prediction models applied to a set of 81 acidic, basic, neutral and zwitter-ionic compounds (Graham et al., 2012).

Disregarding lysosomal sequestration of basic drugs in the Rodgers & Rowland model (Rodgers et al., 2005) is one possible explanation for the prevailing under-prediction of *Kpu* (lung). Lysosomes are acidic organelles found in almost all animal and human cells where they act as the main digestive system (Appelqvist et al., 2013). They are abundant in lungs, liver, kidney and spleen, and in certain cell types such as macrophages where they may contribute even > 10% to the cellular volume (MacIntyre and Cutler, 1988a; Ufuk et al., 2017). Extensive accumulation of cationic amphiphilic drugs (CAD) in alveolar macrophages and other cell types has been reported and attributed to large extent to subcellular sequestration of basic drugs in lysosomes (MacIntyre and Cutler, 1988a; Vestal et al., 1980; Antonini and Reasor, 1991; Duvvuri et al., 2004; Daniel et al., 1995; Kazmi et al., 2013; Ufuk et al., 2015). This 'trapping' phenomenon is attributed to pH differences between the cytosol (pH ~ 7.2) and acidic lysosomes (pH ~ 4.5–5), as protonation in the lysosomal environment shifts the equilibrium of the basic drug towards the ionized species (MacIntyre and Cutler, 1988a; Hallifax and Houston, 2007). This extensive drug ionization in lysosomes and reduced permeability of ionized drug species across the lysosomal membrane may lead to potentially very high drug concentrations in this organelle relative to the cytosol. In addition to the pH gradient, binding of cationic drugs to phospholipids in lysosomal membrane contributes to this subcellular accumulation (MacIntyre and Cutler, 1988a; Hallifax and Houston, 2007). Prolonged accumulation of CAD in lysosomes has been associated with increased risk of drug-induced phospholipidosis, an important safety concern for potential drug candidates (Reasor et al., 2006; Choi et al., 2013). CAD-induced phospholipidosis is characterized by extensive accumulation of phospholipids in cells, formation of lamellar inclusion bodies, drug accumulation in association with increased phospholipids and reversibility after discontinuation of drug treatment (Reasor et al., 2006). Several mechanisms may be involved in the accumulation of phospholipids including direct or indirect inhibition of lysosomal phospholipases (Reasor et al., 2006). Potential additional implications of drug accumulation in lysosomes on either drug efficacy, drug resistance and/or drug-drug interactions at the level of lysosomes have all been highlighted (Ufuk et al., 2017; Logan et al., 2012).

Methodological difficulties, e.g., the fragility of lysosomes during homogenization procedures, potential contamination of lysosomes and diffusion of drug from this organelle during the isolation procedure, have hampered direct measurements of lysosomal drug concentrations and determination of the relative importance of lysosomal sequestration in vitro (MacIntyre and Cutler, 1988a). Studies often rely on indirect assessment of the extent of lysosomal sequestration by i) using agents to abolish existing pH gradient between cytosol and lysosomes (e.g., ammonium chloride, monensin) (Kazmi et al., 2013; Ufuk et al., 2015; Daniel and Wojcikowski, 1997) or ii) measuring cellular *Kpu* at low and high drug concentrations, assuming that the saturable component of the cellular uptake at a low drug concentration is attributed to the lysosomal sequestration (Hallifax and Houston, 2007). Indirect methods neglect the effect of ionic strength and changes in membrane surface potential at higher drug concentrations due to insertion of cationic drugs into lipid bilayers (such as lysosomes), which may all affect drug binding to the membrane (Seelig et al., 1996).

The aims of this study were to extend the existing Rodgers & Rowland mechanistic model (Rodgers et al., 2005) to account for lysosomal sequestration in the prediction of *Kpu* for moderate-to-strong bases, to investigate the impact of this process on *Kpu* predictions for lysosome-rich tissues and to assess whether consideration of lysosomal sequestration reduces the under-prediction trends previously observed for basic drugs and *Kpu* (lung) in particular. Physiological data on different cell types contributing to the overall tissue were collated for lung, kidney and liver, including also lysosomal volume fraction and lysosomal pH in individual cell types. Performances of the original and extended tissue-composition models were evaluated in a comparative manner using a dataset of 28 basic drugs employed for development of the original tissue distribution prediction tool (Rodgers et al., 2005). The impact of variability in physiological model input parameters collated from the literature on *Kpu* predictions was considered. The scope and limitations of the extended mechanistic model relative to the original Rodgers model and its application for the prediction of tissue distribution within physiologically-based framework are discussed.

2. Materials and methods

2.1. Derivation of a mechanistic equation for the prediction of drug tissue distribution accounting for lysosomal sequestration

The tissue-to-plasma water partition coefficient, *Kpu*, is defined as the ratio of the total drug concentration in tissue, $C_{Tissue,Total}$, and the unbound drug concentration in plasma, Cu_P, at steady-state:

$$Kpu = \frac{C_{Tissue,Total}}{Cu_P} \quad (1)$$

For each tissue, $C_{Tissue,Total}$ is obtained by diving the total amount of drug in tissue ($A_{Tissue,Total}$) by its volume (V_{Tissue}). $A_{Tissue,Total}$ can be expressed as the sum of amounts of drug in non-lysosomal compartments ($A_{Non-Lys}$) and in lysosomes (A_{Lys}), as shown in Eq. (2).

$$C_{Tissue,Total} = \frac{A_{Tissue,Total}}{V_{Tissue}} = \frac{A_{Non-Lys} + A_{Lys}}{V_{Tissue}} \quad (2)$$

Expressing A_{Lys} in terms of lysosomal volume ($V_{Lys} = f_{Lys} \cdot V_{Tissue}$) yields Eq. (3), where f_{Lys} represents volume fraction of tissue occupied by lysosomes:

$$C_{Tissue,Total} = \frac{A_{Non-Lys}}{V_{Tissue}} + \frac{A_{Lys}}{V_{Lys}} \cdot f_{Lys} \quad (3)$$

or expressed in terms of concentrations:

$$C_{Tissue,Total} = C_{Non-Lys} + C_{Lys} \cdot f_{Lys} \quad (4)$$

Rodgers et al. (2005) have proposed a mechanistic equation for the prediction of the drug concentration in tissue and *Kpu* for compounds with a basic $pK_a > 7$. Drug ionization and drug dissolution in intracellular and extracellular tissue water, in addition to drug binding to neutral lipids, neutral phospholipids and acidic phospholipids were taken into account. In the study by Rodgers et al., a distinction between the distribution of drug into cytosol and subcellular organelles, i.e., the contribution of lysosomal sequestration was not considered. Drug amounts and drug concentrations in tissue estimated by the original Rodgers model are referred to here as $A_{Tissue,R}$ and $C_{Tissue,R}$, respectively (Eqs. (5) and (6)):