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Title:

Predicting Regional Respiratory Tissue and Systemic Concentrations of Orally Inhaled Drugs through a Novel PBPK Model

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Abbreviations: OI, oral inhalation; PBPK, physiologically-based pharmacokinetic; OIDs, orally inhaled drugs; PD, pharmacodynamics; BE, bioequivalence; ELF, epithelial lining fluid; DME, drug-metabolizing enzymes; DMET, drug-metabolizing enzymes and transporters; PK, pharmacokinetic; CE, comparative clinical endpoint, ET, extrathoracic; ET1, extrathoracic (nasal passage); ET2, extrathoracic (oral passage); BB, bronchial; bb, bronchiolar; AL, alveolar or pulmonary; TH, thoracic airway; C, central region; P, peripheral region; ICRP, international commission on radiological protection; MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation of the aerodynamic diameter; IV, intravenous; C_{max} , maximum concentration; AUC, area under the curve; Q, volumetric or inhalation flow; V, tidal or inhalation volume; V_D , dead space volume; FRC, functional residual capacity, BDP, beclomethasone dipropionate; 17-BMP, beclomethasone 17-

monopropionate; logD, octanol-water distribution coefficient at pH 7.4; CL_{int}, intrinsic clearance; IVIVE, *in vitro* to *in vivo* extrapolation; k_{cat}, catalytic rate constant; V_{max}, maximum enzymatic reaction rate; J_{max}, maximum transport rate; K_m, substrate affinity or Michaelis-Menten constant; P-gp, P-glycoprotein; MRP, multidrug resistance-associated protein; BCRP, breast cancer resistance protein; OCT, organic cation transporter; OCTN, organic cation/carnitine transporter; PEPT1, peptide transporter 1; OATP, organic-anion-transporting polypeptides; CYP, cytochrome P450; UGT, UDP-glucuronosyltransferase; CES, carboxylesterases; SULT, sulfotransferase; f_{hyg}, hygroscopic growth factor; DF, deposition fraction; DF_{scalar}, deposition fraction scalar; P_{scalar}, permeability scalar; f_{gut}, fraction drug absorbed from the gut; RB, relative to the baseline.

Keywords: oral inhalation, drug deposition and absorption, regional respiratory and systemic drug concentrations, physiologically-based pharmacokinetic modeling and simulation

Abstract

Oral inhalation (OI) of drugs is the route of choice to treat respiratory diseases or for recreational drug use (e.g., cannabis). Following OI, the drug is deposited in and systemically absorbed from various regions of the respiratory tract. Measuring regional respiratory tissue drug concentrations at the site of action is important for evaluating the efficacy and safety of orally inhaled drugs (OID). Since such a measurement is routinely not possible in humans, the only alternative is to predict these concentrations, for example by physiologically based pharmacokinetic (PBPK) modeling. Therefore, we developed an OI-PBPK model to integrate the interplay between regional respiratory drug deposition and systemic absorption to predict regional respiratory tissue and systemic drug concentrations. We validated our OI-PBPK model by comparing the simulated and observed plasma concentration-time profiles of two OID, morphine and nicotine. Further, we performed sensitivity analyses to quantitatively demonstrate the impact of key parameters on the extent and pattern of regional respiratory drug deposition, absorption, and the resulting regional respiratory tissue and systemic plasma concentrations. Our OI-PBPK model can be applied to predict regional respiratory tissue and systemic drug concentrations to optimize OID formulations, delivery systems, and dosing regimens. Further, our model could be used to establish the bioequivalence of generic OID for which systemic plasma concentrations are not measurable or are not a good surrogate of the respiratory tissue drug concentrations.

Significance Statement

Our OI-PBPK model is the first comprehensive model to predict regional respiratory deposition, as well as systemic and regional tissue concentrations of OID, especially at the drug's site of action, which is difficult to measure in humans. This model will help optimize OID formulations, delivery systems, dosing regimens, and bioequivalence assessment of generic OID. Further, this model can be linked with organs-on-chips, pharmacodynamic and quantitative systems pharmacology models to predict and evaluate the safety and efficacy of OID.

Introduction

Oral inhalation (OI) of drugs is the route of choice to treat respiratory diseases (e.g., bronchodilators) and for recreational drug use (e.g., cannabis and nicotine). The advantages of administering drugs via this route are multifold. This route is non-invasive, consumer-friendly, results in rapid onset of action, local effect and, therefore, reduced systemic side effects (provided systemic absorption is low) (Gardenhire et al., 2017). The respiratory tract is not a homogenous tissue, and orally inhaled drugs (OID) can be differentially deposited and absorbed from various regions of the respiratory tract (Figs. 1 and 2) to produce local or systemic effect (i.e., after reaching the systemic circulation) or both (Gehr, 1994; ICRP, 1995; Derendorf et al., 2006). Often an OID is targeted to specific regions of the respiratory tract based on the condition being treated: e.g., the trachea for tracheomalacia, bronchi for bronchitis and asthma, bronchioles for bronchiolitis obliterans, and alveoli for infections such as COVID-19, emphysema, and pulmonary fibrosis. Thus, a mechanistic understanding of the regional respiratory drug deposition and absorption is crucial to determine local and systemic drug concentrations and, therefore, pharmacodynamics (PD) (i.e., both efficacy and toxicity) of the drug (Mobley and Hochhaus, 2001). Once understood, this mechanistic knowledge can be applied to optimize OID dosing regimens, drug formulation, delivery systems and evaluate bioequivalence (BE) of a generic OID (Zhao et al., 2019).

Conventionally, regional respiratory drug concentrations are assessed by imaging, and local lung pharmacokinetic (PK) studies (CHMP, 2009; Sadiq et al., 2021). Such studies are costly, and therefore not possible to implement routinely. In addition, local PK studies are invasive and the measured concentration-time data are usually sparse. Another approach is to scale local PK in animals to humans. However, when enzymes and transporters are involved, such allometric scaling may not be accurate. Unlike the above studies, systemic PK studies do not provide a measure of the regional respiratory drug concentrations (CHMP, 2009) and, therefore, cannot be used as a surrogate measure of the PD of the drug in the respiratory tissue. Even when the target site of the OID is systemic (i.e., not in the respiratory tract), plasma drug concentrations are not always measurable after OI of the drug. In that event, this classical PK approach cannot be used to assess bioavailability of the OID or BE

assessment of a generic version of the OID. Even if the systemic PK of the OID are measurable, they may not be a surrogate of the respiratory target site drug concentrations and, therefore, the PD of the drug (Hendrickx et al., 2018; Newman and Witzmann, 2020). In such cases, to demonstrate BE of a generic OID, pharmacodynamic (PD) effect or comparative clinical endpoint (CE) can be used as a surrogate of local respiratory drug concentrations. However, unless the PD effect or CE is readily quantifiable, this approach is not feasible and, even if feasible, potentially costly (Newman and Witzmann, 2020).

To overcome some of the above deficiencies of routine PK studies of OID, a promising alternative approach is to predict local and systemic drug concentrations and, therefore, the PD of an OID through **Physiologically-Based Pharmacokinetic (PBPK)** modeling and simulation (M&S) (Zhao et al., 2019). However, for such a model to have reliable predictive success, it must incorporate fundamental processes that affect the respiratory drug deposition, absorption, and local as well as systemic drug concentrations (Eriksson et al., 2020). Several commercial and open-source OI-PBPK models are available (Borghardt et al., 2015). However, these models have several limitations, namely: i) incomplete inclusion of important regions of the respiratory tract; ii) lack of hygroscopic particle growth to predict respiratory deposition of a highly water-soluble drugs (e.g., nicotine); iii) lack of monitoring of hydrolyzed or metabolized product(s) (active or toxic) in epithelial lining fluid (ELF) of the respiratory tract, a feature especially important when the OID is a prodrug and the active moiety is the hydrolyzed product (e.g., beclomethasone dipropionate [BDP] and its active moiety such as beclomethasone 17-monopropionate [17-BMP]); and iv) lack of incorporation of tissue retention to accurately predict local concentration and absorption of the drug (e.g., budesonide). Therefore, the goals of the present study were to 1) develop a regional OI-PBPK model, which incorporates all the above-listed features, to predict regional respiratory drug deposition and local and systemic concentrations of an OID; 2) validate these predictions using the two OID, morphine and nicotine; and 3) conduct a sensitivity analysis to determine which drug and physiological parameters are important for regional and systemic drug exposure and therefore PD of an OID.

Materials and Methods

OI Model Structure and Assumptions. Our OI model consists of four regions (Figs. 2 and 3) based on the human respiratory tract model of the International Commission on Radiological Protection publication 66 (ICRP 66) (ICRP, 1995). It includes the extrathoracic (ET) and thoracic (TH) airways. The ET airway includes the nasal passages (ET1, anterior nose) and oral passages (ET2, includes the posterior nasal passages, larynx, pharynx, and mouth). The TH airway (also commonly known as lung) includes the bronchial (BB, which consists of the trachea [airway generation 0] and the bronchi [airway generations 1-8]); the bronchiolar (bb, consisting of the bronchioles and terminal bronchioles [airway generations 9-15]); and the alveolar (AL, includes the respiratory bronchioles, alveolar ducts, and sacs with their alveoli [airway generations 16 and beyond]) regions (Fig. 2a). The term ‘airway generation’ refers to the point at which an airway separates into two or smaller airways. The BB and bb regions are collectively referred as the tracheobronchial or central (C) region, whereas the AL region is known as the pulmonary or peripheral (P) region. The ET1 region was ignored in the current OI model because it is important for drug administration through nasal inhalation but not for OI. However, if necessary, it can be integrated into the current PBPK model. This OI model was created using MATLAB (version R2021) and its SimBiology module (Mathworks, MA). The rate equations were solved using the ode15s solver. Detailed information on model equations and systems parameters of the ICRP reference adult male are provided in the Supplemental Data (Supplemental Method, Tables S1-S5). As described below, each region was further subdivided into five compartments, i.e., airway lumen, ELF, epithelial, subepithelial, and blood (Fig. 3).

Airway Lumen Compartment. The regional dose exposure for each respiratory tract region was calculated by multiplying the regional deposition fraction (DF, percentage of the total amount of inhaled drug that is deposited in various regions of the respiratory tract) with the overall OI dose. In the absence of *in vivo* regional DF data obtained through imaging studies, deposition of drug in the airway lumen of the human respiratory tract was calculated using the *in silico* ICRP 66 deposition model (ICRP, 1995). Briefly, the ICRP 66 deposition model calculates the DF for each respiratory tract region (ET2, BB, bb, and AL) using algebraic equations built from

experimental and theoretical data (Supplemental Method, Tables S1-S3). The ICRP 66 deposition model considers breathing maneuvers (mentioned below) based on various activities such as sleep, sitting or resting, light exercise, and heavy exercise for nasal and mouth breathers. Here, we used the ICRP 66 deposition model to predict regional deposition of OID for a mouth breather who is seated. The ICRP 66 deposition model is applicable to particles with diameters ranging from 0.001 to 100 μm (ICRP, 1995). To determine the extent and pattern of drug deposition of OID (solid or liquid) from an OI delivery device, the ICRP 66 deposition model requires the following information: a) aerodynamic particle size distribution parameters including mass median aerodynamic diameter (MMAD, the diameter at which 50% of the particles/droplets in an aerosol are larger and 50% are smaller) and geometric standard deviation of the aerodynamic diameter (GSD, measures the dispersion of particle/droplet diameter); b) drug particle/droplet density; c) drug particle/droplet shape; d) volumetric or inhalation flow (Q , the volume of air that travels per unit of time); e) physiological parameters such as tidal or inhalation volume (V , the volume of air that enters or exits the lungs during each respiratory cycle), functional residual capacity (FRC, volume of air remaining in the lungs after passive expiration), and dead space volume (V_D , the volume of air inhaled that does not participate in gas exchange); f) anatomy parameter such average diameter of the different regions of the respiratory tract; and g) hygroscopic growth factor (f_{hyg}) information, which is critical for highly water soluble OI drugs because it can be used to predict diameter changes caused by respiratory tract humidity. The above mentioned inputs parameters were collected from literature and ICRP publications 66 and 135 (ICRP, 1995; Paquet et al., 2015) (Supplemental Data).

ELF or Airway Liquid Compartment. After drug deposition, the solid drug first dissolves in the ELF (described by the Hintz-Johnson model (Hintz and Johnson, 1989)), where it may be chemically degraded or metabolized (e.g., via hydrolysis). Since the OID may be a prodrug and the metabolite/degradant may be active/toxic, our model included monitoring the concentration of the metabolite/degradant in the ELF. The mucus and aqueous layers were combined into a single ELF and considered to be in instantaneous equilibrium with each other but dynamic changes in the volume of ELF was not considered because of the lack of information on fluid

absorption and secretion. The undissolved or dissolved amount of drug from each compartment (j) travels to the preceding compartment (j-1) through first-order respiratory transit rate constant (direction: AL to bb (through macrophage clearance), bb to BB (through mucociliary clearance), BB to ET2 (through mucociliary clearance), ET2 to the gut region (through swallowing) (ICRP, 1995; Borghardt et al., 2015). The respiratory transit rate constants were obtained from the ICRP clearance model (Paquet et al., 2015). The model also included the amount of drug removed from the ET2 region via coughing and exhaled.

Epithelial or Intracellular Compartment. The OID can be actively transported (unbound) or passively transferred (unionized and unbound) in a bidirectional manner across any of the cell membranes (Fig. 2b). Passive apparent permeability was assumed to be bidirectionally the same and scaled based on regional surface area (Gaohua et al., 2015; Melillo et al., 2020). In the epithelial compartment the unbound drug can be metabolized by the enzymes present there (Fig. 2b). The model includes drug tissue retention (e.g., reversible fatty acid conjugation) in localized epithelial tissue of the respiratory system through second-order association and first-order dissociation rate constants (Fig. 2b).

Subepithelial or Interstitial Compartment. The OID can be actively transported (unbound) or bidirectionally passively transferred (unionized and unbound) to the subepithelial compartment through the basal cell membrane. The lymphatic circulation can carry the drug from the subepithelial to the lymph nodes and then to the venous blood.

Blood or Vascular Compartment. Rapid bidirectional transfer between subepithelial and the blood compartment of the drug can be by both para- and transcellular route due to the presence of fenestrated endothelial cells (Kuepfer et al., 2016). Then, the drug can be carried into the arterial and venous blood through the systemic (ET2 region), bronchial (BB and bb regions), and pulmonary (AL region) circulations. The pulmonary circulation carries drugs from the venous blood to the AL regions. In contrast, the bronchial circulation carries drugs from the arterial blood to the BB and bb regions (Baile, 1996). Likewise, systemic circulation carries drugs from the arterial blood to the ET2 region.

Integration of OI Model into a Whole-Body PBPK Model. The OI model was integrated into our previously published PBPK framework (Ke et al., 2012; Ke et al., 2013; Ke et al., 2014; Zhang et al., 2017; Zhang and Unadkat, 2017). The whole-body PBPK model included all the major tissues responsible for drug disposition (Fig. 3). Except for the liver and respiratory tract, drug distribution from the blood into tissues was assumed to be perfusion limited. In the liver, segregated into three sub-compartments (intracellular, interstitial, and vascular), permeability-limitation was allowed. Likewise, in the respiratory tract compartments, permeability limited entry and exit of the drug across the cellular and capillary/endothelial membranes was allowed and was parameterized with respect to Michaelis-Menten parameters (maximum rate of active transport, J_{max} ; and Michaelis constant, K_m) or intrinsic clearance (CL_{int}). To describe the absorption of the drug in the gut that was swallowed from the ET2 region, the first-order oral absorption model was implemented. The peripheral arm vein sampling model developed by Huang and Isoherranen was incorporated into the OI-PBPK model (Huang and Isoherranen, 2020).

Development of OI-PBPK Model for Morphine and Nicotine. To develop OI-PBPK model for these drugs, the reported morphine (Emoto et al., 2018) and nicotine (Kovar et al., 2020) PBPK models following their intravenous (IV) administration (see Tables S6 and S7 for drug-dependent parameters) were adopted. Then, validation of the OI-PBPK model for morphine and nicotine was conducted as follows.

Estimation of parameters for the Morphine and Nicotine OI-PBPK Models. 1) regional morphine and nicotine deposition in the respiratory tract following its OI administration via a nebulizer and cigarette, respectively, were predicted using the ICRP 66 deposition model (Tables S8 and S9); 2) the *in vitro* apparent permeability between ELF and epithelial regional for both drugs was predicted using logarithm of octanol-water distribution coefficient at pH 7.4 ($\log D$) value through *in silico* regression model developed using $\log D$ and *in vitro* Calu-3 permeability data (Brillault et al., 2010) (Supplemental Method); 3) regional apparent permeability was calculated by scaling the *in vitro* apparent permeability of the drug through the epithelial cell thickness of each region of the respiratory tract (Supplemental

Method); 4) the *in vivo* regional permeability of the drug was calculated by the product of the regional apparent permeability and surface area of each region of the respiratory tract (Supplemental Method); 5) the Henderson-Hasselbalch equation (Po and Senozan, 2001) was used to compute the fraction unionization of the drug in each sub-compartment of the OI model using the drug's pKa value (Table S10); 6) the fraction unbound of the drug in each sub-compartment of the OI model was calculated using the fraction unbound in plasma and the partition coefficient (Table S10); 7) the puff frequency and time interval between each puff of the administered OI drug were obtained from the literature (Table S13); 8) *In vitro* to *in vivo* extrapolation (IVIVE) of the metabolic and/or transporter kinetics of the drug in the respiratory tract was performed using the relative total molar abundance of drug metabolizing enzymes and transporters (DMET) protein in the various regions of the respiratory tract relative to that in the liver tissue. To do so, we assumed that the catalytic rate constant (k_{cat}) is invariant across all tissues (Supplemental Method). The total molar abundance of DMET protein per tissue (in $\mu\text{mol}/\text{tissue}$ unit) was determined by multiplying the abundance of DMET protein in the subcellular fraction (in pmol/mg unit) by the yield of the subcellular fraction per gram of the tissue (in mg/g unit) and the tissue weight (in g unit).

Assumptions for Morphine and Nicotine OI-PBPK Models. For both drugs, the following assumptions were made 1) due to lack of information, chemical degradation/hydrolysis in the ELF compartment and tissue retention of drugs in the epithelial compartment was assumed to be negligible; 2) regional passive permeability between epithelial and subepithelial, and subepithelial and blood compartments was assumed to be high (100 cm/s) and not rate-limiting; 3) the amount of drug eliminated through coughing was negligible; 4) due to lack of region-specific tissue composition of lipids, proteins and pH, the fraction unbound and the fraction unionized in each compartment were assumed to be identical for each region of the respiratory tract.

Validation of Morphine and Nicotine OI-PBPK Models. For validation of our OI-PBPK model, the PK endpoints (maximum concentration [C_{max}] and area under the concentration time curve up to the last measurable concentration [AUC_{last}]) of the drugs after their IV and OI administration were simulated and compared with their

corresponding observed *in vivo* values. Our model was considered validated if the ratio of the simulated and the observed PK endpoints fell within 0.8-1.25 (Ladumor et al., 2019a; Ladumor et al., 2019b).

Sensitivity Analysis to Assess the Impact of Drug and Physiological Parameters on Respiratory Drug Deposition and Systemic Absorption.

A sensitivity analysis was performed to demonstrate the impact of drug and physiological parameters on the regional deposition, regional tissue concentration, and systemic absorption after OI of hypothetical drugs X and Y (modeled based on morphine, i.e. input to the OI-PBPK model were assumed to be identical to morphine except as noted below). The following additional assumptions are made for the hypothetical drugs X and Y: 1) Drug X was administered as a solid form through an inhaler, and its regional absorption was assumed to be limited by its solubility in the ELF; 2) Drug Y was assumed to be permeability-limited, metabolized in the respiratory tissues, binds with fatty acid in the epithelial region of the respiratory tract, and administered as a solution through a nebulizer. The deterministic parameters of both these formulations were MMAD, inhalation flow, inhalation volume, f_{hyg} , drug dissolution, tissue retention, and DMET kinetics, based on literature (Brand et al., 2005; Derendorf et al., 2006; Borghardt et al., 2015; Ehrhardt et al., 2017; Zhang et al., 2006). Therefore, these parameters were included in our sensitivity analysis and their values varied between 2 or 5-times of the value used for morphine. Then, they were integrated into the OI-PBPK model to predict the drug's respiratory deposition, local tissue and systemic exposure.

Results

Comparison of the OI-PBPK Model-Predicted and Observed Plasma Concentration of Morphine or Nicotine after IV Administration

Using our OI-PBPK model, we reproduced the predicted mean plasma concentration-time data published by others following IV administration of morphine (Fig. 4a) or nicotine (Fig. 5a). The predicted:observed values ($C_{\max}$ and AUC_{last}) fell within our *a priori* acceptance criterion (0.8-1.25) (Tables S11 and S12).

Prediction of Systemic Exposure to Nebulized Morphine after OI

The OI-PBPK model predicted that 53% of the nebulized morphine was deposited in the respiratory tract while the remainder (47%) was exhaled (Table S8). This deposition followed the order ET2 (19.2%) > AL (10.4%) > BB (16.0%) > bb (7.1 %) (Table S8). When f_{hyg} was not included in the model, the simulated PK endpoints ($C_{\max}$ and AUC_{last}) of morphine after OI were underpredicted as compared to observed data (Dershwitz et al., 2000) (Table S11). After including f_{hyg} (= 3) (Table S8), the change in the predicted percent total and regional deposition increased (total: 72.9%; ET2 (33.0%) > AL (12.3%) > BB (19.5%) > bb (8.1%)). However, the predicted PK endpoints remained underestimated (Table S11). After integrating a permeability scalar (P_{scalar} , 5) to the *in vitro* apparent permeability of morphine between ELF and epithelial regional compartments, the predicted PK endpoints fell within 0.8-1.25-fold of the observed values (Table S11).

Prediction of Systemic Exposure to Nicotine after Cigarette Smoking

The OI-PBPK model predicted a total of 55.7% of nicotine was deposited in the respiratory tract and the remaining 44.3 exhaled (Table S9). The predicted regional deposition of nicotine in the respiratory tract followed the order bb (31.3%) > AL (21.4%) > BB (2.0%) > ET2 (1.0%). Using these data, the systemic exposure of nicotine following OI administration was underpredicted. After including the reported f_{hyg} (1.7) (Schroeter et al., 2001) of nicotine in the model (Table S9), the change in the predicted percent total and regional deposition improved (total: 64%; bb (41.1%) > AL (20.8%) > BB (1.3%) > ET2 (0.7%)) when compared with the measured *in vivo* values (Table S9) but the systemic exposure remained underestimated. However, after including an empirical DF scaling factor (DF_{scalar} , 1.5), the predicted total and

regional deposition fractions were in good agreement with those measured *in vivo* (Table S9). With this scaling, the final predicted total and regional depositions of smoked nicotine were 96% and followed the order bb (61.7%) > AL (31.2%) > BB (2.0%) > ET2 (1.1%) (Table S9). Then, the simulated nicotine plasma concentration-time profiles after OI administration (cigarette smoking) fell within 1.25-fold of the observed values (Fig. 5 and Table S13).

Sensitivity Analyses of the Impact of Drug and Physiological Parameters on Respiratory Deposition and Systemic Absorption of OI

With an increase in MMAD, hypothetical drug X deposition increased in the ET2 and BB region but decreased in the AL region. Also, the ratio of deposition in the central to peripheral region (C/P ratio) increased with an increase in MMAD (Fig. 6a). As a result, due to the rapid dissolution in the airway fluid, the $C_{\max}$ and AUC_{last} of drug X were decreased and increased, respectively (Fig. 6b1). In contrast, no change in the PK endpoints was observed when fraction of oral gut absorption (f_{gut}) was set to zero (Fig. 6b2). However, when poor dissolution was assumed, all the PK endpoints of drug X increased (Fig. 6c1). The PK endpoints returned to baseline when oral gut absorption was set to zero (Fig. 6c2). Also, with a decrease in MMAD, the overall and regional deposition decreased but the relative AL deposition increased compared to other respiratory regions such as ET2. Hence, the C/P ratio decreased (Fig. 6a). This led to a decline in PK endpoints, but no change in PK endpoints when dissolution rate and mucociliary clearance were altered.

A decrease in the inhalation flow, resulted in a higher fraction of drug X deposited in the ET2 and BB regions and higher C/P ratio (>1). Due to this, $C_{\max}$ decreased but AUC_{last} modestly increased (Fig. S1a1 and S1a2). When increase in the inhalation flow, resulted in a higher fraction of drug X deposited in the AL region (relative to the baseline (RB): ~2-fold) and lower C/P ratio (<1). Consequently, $C_{\max}$ increased (RB: ~1.5-fold) but AUC_{last} did not change (Fig. S1a1 and S1a2).

An inhalation volume was increased, deposition of drug X in the AL region increased (RB: ~2-fold) resulting in a lower C/P ratio and higher $C_{\max}$ (RB: ~1.5-fold) but no change in AUC_{last} (Fig. S1b1 and S1b2). When the inhalation volume was decreased, deposition of drug X in the AL region decreased but drug deposition in

remaining regions did not change or slightly increased resulting in a higher C/P ratio, and $C_{\max}$ decreased but AUC_{last} modestly increased (Fig. S1b1 and S1b2).

An increase in the hygroscopic growth factor resulted in a higher total and regional deposition in only the ET2 region and resulted in higher AUC_{last} but a minor change in $C_{\max}$ (Fig. S1c1 and S1c2).

Increased apical influx and efflux transporter activity at epithelial and subepithelial membranes resulted in increased and decreased systemic and regional exposure of drug Y, respectively, but no change in AL tissue (Fig. S2 and S3). Likewise, increased metabolism resulted in a decrease in both systemic and regional tissue exposure of drug Y (Fig. S4). The increased dissociation rate constant in the epithelial region resulted in an increase systemic and regional $C_{\max}$ but no change in exposure of drug Y (Fig. S5).

A decrease in the dissolution rate (z-factor) of drug X (solid formulation as opposed to the liquid nebulizer formulation of Drug X) resulted in a decrease in both $C_{\max}$ and AUC_{last} (Fig. S6).

Discussion

In the present study, we developed an OI-PBPK model to predict drug deposition, absorption and the resulting respiratory tissue and systemic drug concentrations of OID. Our model was developed using the ICRP 66 deposition and clearance model (ICRP, 1995; Paquet et al., 2015). The *in vitro* data required by our OI-PBPK model are aerodynamic particle size distribution parameters, inhalation flow, inhalation volume, dissolution (solubility), permeability, fraction unbound/ionized, metabolic and transport kinetics. All these values are available, either from the literature, estimated using *in silico* methods or determined from *in vitro* studies of the drug.

Our OI-PBPK model can be distinguished from the existing OI-PBPK models by several features. First, our model accounts for the hygroscopic growth of particles to predict regional deposition of water-soluble hygroscopic drugs (ICRP, 1995; Chalvatzaki and Lazaridis, 2018). Second, our model can monitor drug degradation kinetics in the ELF. For example, BDP is hydrolyzed to its metabolite, 17-BMP in the intestinal fluid, which also indicates possible degradation in ELF, which has a comparable pH of 6.5 (Würthwein and Rohdewald, 1990). Third, our model accounts for tissue retention relevant to inhaled corticosteroids (e.g., budesonide) that can reversibly conjugate with fatty acid esters present in the epithelial region. This conjugation will prolong the mean residence time of the drug in the lung tissue (Miller-Larsson et al., 1998) (Fig. S5). Fourth, our model includes solubility and dissolution processes important for orally inhaled suspension or dry powder formulation. For example, inhaled corticosteroids (e.g., BDP) are highly lipophilic (Kumar et al., 2017), where dissolution/solubility is the rate-limiting steps in their absorption (Fig. S6). Hence, dissolution (solubility) processes are important to include in OI-PBPK models to reliably predict tissue and systemic concentrations of these drugs. Fifth, the volume of each compartment was estimated based on compartment thickness, which is critical for prediction of regional respiratory tissue drug concentrations. Last, but not least, our model accounts for regional transport and metabolic processes, crucial to determine local tissue concentrations of OID (Figs. S2-S5). For example, drugs can be actively transported through apical P-glycoprotein (P-gp) efflux transporter (e.g., BDP, ciclesonide, and budesonide) and/or apical organic cation transporter (OCT) influx transporter (e.g., albuterol,

formoterol, ipratropium, and tiotropium bromide) present in the epithelia of the respiratory tract tissues (Bosquillon, 2010; Crowe and Tan, 2012; Ehrhardt et al., 2017). Also, our model allows incorporation of transporters in the subepithelial compartment (e.g., airway smooth muscle cells), a site of action for many OI (Fig. S3). For example, OCT(N) transporters, present in the subepithelial regions (e.g., airway smooth muscle cells), are a target for inhaled bronchodilator drugs (e.g., albuterol) (Horvath et al., 2007). Moreover, a few OIs are metabolized in the epithelia of the lung by Phase I and Phase II DME, such as esterase that hydrolyze prodrugs to their pharmacologically active metabolites (e.g., prodrug BDP and its metabolite 17-BMP) (Olsson et al., 2011; Oesch et al., 2019; Somers et al., 2007).

For validation of our OI-PBPK model, we used the PK bioequivalence criterion (i.e., predicted systemic exposure within 0.85-1.25-fold of the observed value) to assess the success of the simulation. Our OI-PBPK model was validated using systemic mean morphine and nicotine exposure rather than respiratory tissue exposure, because the latter is not available. Our prediction for systemic exposure to morphine following OI administration via nebulizer was improved after integrating f_{hyg} and P_{scalar} in our model. The need to incorporate f_{hyg} is not surprising because morphine is a water-soluble drug (64 mg/mL water solubility), and the nebulized solution is mixed with saline (sodium chloride, NaCl) (Schuster et al., 1997; Dershwitz et al., 2000). NaCl is hygroscopic and exhibits a 2-4 fold higher in particle diameter upon inhalation (ICRP, 1995). As a result, it may have an effect on the amount of drug deposited in the respiratory tract, as shown in the literature for commercial nebulizer formulations (Haddrell et al., 2014). Additionally, morphine is a substrate of OCT1 and UGT2B7 (Emoto et al., 2017), expressed in the respiratory tract (Sakamoto et al., 2013). The systemic PK parameters of morphine were underpredicted after integrating morphine DMET kinetics in our OI model. Therefore, to recover the observed systemic morphine PK profile, we needed to apply a P_{scalar} value to the *in vitro* apparent permeability at apical epithelial membrane perhaps because our IVIVE of morphine transport kinetics was not accurate. For nicotine, besides including f_{hyg} , the deposition fraction needed to be scaled by DF_{scalar} to recover the *in vivo* deposition of the drug. This may be due to the fact that ICRP 66 deposition model does not take into account cloud dynamics and particle coagulation of smoked

aerosol and the DF_{scalar} indirectly represents these mechanisms in the model. Interestingly, we found that the impact of lung metabolism and/or transport on systemic PK of nicotine and morphine was minimal (data not shown), likely due to the low expression of DMET proteins in lungs.

In our sensitivity analysis, we showed the impact of drug and physiological parameters on drug deposition as well as respiratory and systemic drug concentrations. Some studied parameters, e.g., MMAD, inhalation flow, and inhalation volume, affected drug deposition and therefore the PK endpoints. For example, increased MMAD, resulted in greater deposition of drug X in total, including the ET2 and BB regions but lower deposition in AL region (Fig. 6a), most likely due to increased particle impaction caused by the increased particle size (Brand et al., 2005). This resulted in an increased AUC_{last} of drug X driven by greater total deposition and a lower C_{max} driven by lower deposition in the AL region (Fig. 6b1). No change in the PK endpoints was observed when fa_{gut} was set to zero. This suggests that all the drug is dissolved in the airway fluid and there is little drug cleared by mucociliary clearance to the GI tract (Fig. 6b2). However, when we assumed poor drug dissolution rate in the airway fluid then the PK endpoints were increased with increase in MMAD, likely because mucociliary clearance transferred undissolved drug to the GI tract (Fig. 6c1). Indeed, the PK endpoints did not increase with increase in MMAD when oral gut absorption was set to zero (Fig. 6c2). This again highlights the role of mucociliary clearance in interpreting PK data of OI drugs. With reduced MMAD, the total deposition decreased, but AL deposition increased relative to other regions (e.g. ET2) likely due to increased particle sedimentation in the AL region driven by the smaller particle size. This resulted in decreased PK endpoints as driven by lower total deposition but little or no change in PK endpoints with different dissolution conditions and mucociliary clearance because these parameters mostly affect drug absorbed from the ET2 region which was minimal due to reduced deposition in this region (Fig 6).

Similar trends were obtained with inhalation flow rate. Reduced flow rate resulted in greater deposition in the AL region and therefore greater C_{max} due to rapid absorption of the drug from the AL region, which has a greater surface area. AUC_{last} did not change likely because the drug's total deposition was similar to that obtained

with the other flows (Fig. S1a1 and S1a2). Similarly, increased inhalation volume, resulted in higher deposition in AL region, likely due to particle sedimentation. As a result, $C_{\max}$ was increased (due to larger AL surface area and higher drug's AL deposition) leading to higher rate and somewhat higher extent (AUC) of drug absorption (Fig. S1b1 and S1b2). Increased hygroscopic growth resulted in increased particle size due to impaction, and therefore higher total drug deposition in the ET2 region as well as total. As a result, AUC_{last} increased as driven by total deposition (Fig. S1c1 and S1c2). A modest increase in $C_{\max}$ is likely due to slightly increased drug deposition in AL region and therefore increased rate of absorption from this region (higher surface area). The latter was not observed with an increase in MMAD because that was accompanied by a reduction in AL drug deposition (Fig. 6a).

We also explored the impact of metabolism and transport on respiratory tissue and systemic drug concentrations. Variability in DMET protein abundance in the respiratory tract can alter the drug concentration at target site and therefore drug response. We found that increased influx or efflux apical epithelial or subepithelial membrane transporter activity resulted in either increased (influx) or reduced (efflux) drug Y epithelial concentrations in all the regions of the respiratory tract except for the AL region (only in the case of influx apical epithelial membrane transporter). The latter is because of the lower fraction of drug transported (ft) in the AL region relative to the drug passive drug permeability which is high due to the large AL surface area (Fig. S2 and S3). We found that increased drug metabolic activity in the epithelial region, decreased systemic and regional exposure of drug Y (Fig. S4). In contrast, as expected, increased dissociation rate of the drug from its intracellular binding to fatty acids, resulted in an increase in systemic and regional $C_{\max}$ but no change in local or systemic exposure as measured by AUC_{last} (Fig. S5). Also, we found that decreased dissolution rate of drug X resulted in a decrease in both $C_{\max}$ and AUC_{last} due to slow drug release in the airway fluid (Fig. S6).

There are a few limitations to our OI-PBPK model. First, the model does not consider device-related parameters such as device geometry, spray angle, plume size and force, or orifice diameter. However, it does consider critical parameters for drug deposition prediction like aerodynamic particle size distribution, inhalation flow, and

inhalation volume. Second, for simplicity, we used a monodisperse rather than a polydisperse particle size distribution to predict regional respiratory deposition of both drugs; however, the latter can be accomplished using a normal or lognormal particle size distribution. Third, though our model considers regional deposition and absorption, it does not do so for each generation of the respiratory tract due to a lack of required physiological data for each generation. Fifth, the effect, if any, of the excipient on the diameter of the OID particles other than hygroscopicity, can be addressed using the DF_{scalar} . Further, inclusion of regional thickness to calculate regional apparent permeability can be further tested with regional *in vitro* permeability experiments for both the test drugs. In addition, once data are available (or curated from the literature), our OI-PBPK model can easily be populated with interindividual variability in physiological (including the effect of age and sex) or drug-dependent parameters as well as the regional abundances of DMET proteins in the respiratory tract. All these refinements will be important for IVIVE of local and systemic exposure to OID.

In summary, our developed OI-PBPK model was successfully validated using systemic plasma concentrations after OI administration of morphine or nicotine. In addition, we conducted sensitivity analyses to show how various drug or physiological parameters will impact regional lung tissue as well as systemic concentrations of OID. We believe, after further validation with additional OID, our OI-PBPK model could be used in the future to predict regional lung tissue and systemic concentration of drugs to optimize OID dosing regimen and evaluate BE of generic OID when systemic drug concentrations are not measurable or are not a good surrogate of the tissue drug concentrations relevant for the PD of the drug.

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Authorship Contributions

Participated in research design: Ladumor and Unadkat.

Conducted experiments: Ladumor.

Performed data analysis: Ladumor and Unadkat.

Wrote or contributed to the writing of the manuscript: Ladumor and Unadkat.

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Footnotes

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Figure Legends

Fig. 1. Drug movement pathway from the oral inhalation device to the respiratory tract and eventually to the systemic circulation.

Fig. 2. Regional classification of the human respiratory tract (a) and the processes that determine the movement of drugs through different layers of each region (b). Transporter and enzymes listed in Fig 2b are examples and not a comprehensive list. ET, extrathoracic; ET1, extrathoracic (nasal passage); ET2, extrathoracic (oral passage); BB, bronchial; bb, bronchiolar; AL, alveolar or pulmonary; TH, thoracic airway (which include BB, bb and AL regions); C, central region (BB+bb); P, peripheral region (AL); ELF, epithelial lining fluid; P-gp, P-glycoprotein; MRP, multidrug resistance-associated protein; BCRP, breast cancer resistance protein; OCT, organic cation transporter; OCTN, organic cation/carnitine transporter; PEPT1, peptide transporter 1; OATP, organic-anion-transporting polypeptides; CYP, cytochrome P450; UGT, UDP-glucuronosyltransferase; CES, carboxylesterases; SULT, sulfotransferase.

Fig. 3. a) Schematic workflow for the development of OI-PBPK model with input and output information for the ICRP 66 deposition and PBPK model, and b) the framework for our regional OI-PBPK model. Drug concentration in the peripheral vein (rather than the central vein) and the central artery were sampled.

Fig. 4. Representative OI-PBPK model simulated and observed arterial morphine plasma concentrations after a) intravenous (8.8 mg over 0.16 h) or b) oral inhalational (17.6 mg, 8 inhalation doses with 1 min interval) administration (nebulizer). Drug concentration in the central artery was sampled. The predicted:observed values (C_{max} and AUC) fell within our *a priori* acceptance criterion (0.8-1.25) (Table S11).

Fig. 5. Representative OI-PBPK model simulated and observed peripheral venous or arterial nicotine plasma concentrations after a) intravenous (4.38 mg over 0.5 h) or b) oral inhalational (2.2 mg, multiple dosing) administration (cigarette smoking). Drug concentration in the peripheral vein (rather than the central vein) was sampled. The predicted:observed values ($C_{\max}$ and AUC_{last}) fell within our *a priori* acceptance criterion (0.8-1.25). Although our model was validated using data from a number of IV and OI studies (Table S12 and S13), only the observed data from Gourlay et al., 1997 are shown.

Fig. 6. Sensitivity analyses to demonstrate the impact of change in mass median aerodynamic diameter (MMAD), on a) total and regional respiratory tract deposition, and pharmacokinetics (PK) endpoints of drug X with b1 and b2) rapid dissolution in the airway fluid (z-factor = 10 L/mg/h) without and with no oral gut absorption ($f_{\text{gut}} = 0$), and c1 and c2) poor dissolution in the airway fluid (z-factor = 0.0001 L/mg/h) without and with no oral gut absorption ($f_{\text{gut}} = 0$). ET2, extrathoracic (oral passage); BB, bronchial; bb, bronchiolar; AL, alveolar; C, central region (BB+bb); P, peripheral region (AL); f_{gut} , fraction drug absorbed from the gut; z-factor, dissolution rate constant.

Fig. 1.

Fig. 2.

Fig. 3.

a)

b)

Fig. 4.

Fig. 5.

Fig. 6.