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HIGHLIGHTS

- We show how data availability affects the prediction of PBPK models for ethambutol.
- Urinary excretion data is crucial for accurate prediction of the disposition of ethambutol.
- The accuracy of ethambutol exposure prediction depends on intestinal permeability estimates.
- Caution is recommended for the use of PBPK models if key in vivo data/parameters are unavailable.

Ethambutol disposition in humans: challenges and limitations of whole-body physiologically-based pharmacokinetic modelling in early drug development

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1. INTRODUCTION

Physiologically-based pharmacokinetic (PBPK) models are a class of mathematical models used to describe and predict the absorption, distribution, metabolism and excretion (ADME) of chemical substances, including small and large molecules (Ferl et al., 2016). In contrast to compartmental pharmacokinetic (PK) models, which provide a phenomenological description of drugs disposition in plasma by a relatively simple model structure, PBPK models are based on a more detailed and mechanistic representation of ADME processes (Tsamandouras et al., 2015). In whole-body PBPK (WB-PBPK) models, the body is divided into physiologically-relevant compartments, usually tissues and organs (Theil et al., 2003). For each organ, a mass balance equation is written; these equations form a differential equation system describing the fate of the substance in every compartment of the body (Zhuang and Lu, 2016).

One of the main features of PBPK models is the possibility to integrate data on organism anatomy and physiology with the physicochemical drug properties derived from *in vitro* experiments. This feature is of primary interest in early drug development, when models can be used prospectively to screen and select molecules, based on predefined developability criteria. In this context, a key advantage of the PBPK modelling approach is the possibility to predict drug levels in specific organs and in the biophase, which provides a stronger rationale for dose selection, enhancing the probability of success in subsequent stages of development (Jusko, 2013; Lau et al., 2017; Wagner et al., 2015; Sager and Yu, 2015). This is especially relevant when considering target organs for which sampling in clinical trials requires invasive procedures or is simply not possible. Thanks to mass balance principles, PBPK models offer the opportunity to simulate drug disposition across tissues and organs, and consequently support therapeutic decisions (Lesko and Schmidt, 2012; Shebley et al., 2018). In fact, supporters of the approach envisage the development of PBPK modelling as an essential step towards the implementation of systems pharmacology and reduction of attrition in drug discovery and development (Leil and Bertz, 2014; Knight-Schrijver et al., 2016).

Thus far, numerous publications have shown the use of (WB-)PBPK models in preclinical and clinical settings for different investigations, from early drug discovery to beyond phase III clinical studies (De Buck et al., 2007; Jones and Rowland-Yeo, 2013; Hobbs et al., 2017). Recent reviews indicate that the main application fields include the characterization of drug-drug interactions (DDI), *in vitro-in vivo* extrapolation (IVIVE), inter- and intra-species scaling, clinical pharmacokinetics including inter-individual variability and mechanistic understanding of drug disposition. It has also been used to predict pharmacokinetic differences in special populations such as children and pregnant women (Sager et al., 2015; Jamei, 2016).

To date, regulatory submissions including PBPK models mainly focus on DDI, pediatric populations and absorption studies (Sager et al., 2015) However, due to their versatility and mechanistic basis, PBPK modelling is gaining momentum for regulatory purposes as an alternative to allometric scaling in the dose selection and justification in humans, as recently highlighted by the European Medicines Agency (EMA, 2017; Espie et al., 2009; Miller et al., 2019). Whilst a comparison of the predictive performance of the two approaches is beyond the scope of this work, PBPK models offer promising advantages over allometry, such as the explicit description of drug absorption processes, the distribution dynamics among different tissues and the processes that may be specific to the elimination of each compound. In light of the advantages and regulatory acceptance a PBPK modelling strategy is required that complies with the recent EMA guidelines, i.e., preclinical data should be generated and used in a more mechanistic fashion to support the characterisation of both safety and clinical pharmacology profiles of novel candidate molecules (Shen et al., 2019). Hence, the number of studies and application range are expected to become larger when best-practice guidelines for model development and verification will be widely accepted (Sager et al., 2015).

In the studies mentioned above, models have been used both retrospectively (i.e., after considerable amount of data on the candidate molecule or medicinal product have been collected) and

prospectively to predict human PK profiles from *in vitro*/animal data. Prospective (bottom-up) evaluations include, for example, the prediction of PK profile in humans from *in vitro* data or preclinical species at early stages of drug development (Jamei, 2016), whilst retrospective (top-down) analyses can focus on the mechanistic understanding of drug disposition processes using clinical data at late stages (Zhuang and Lu, 2016). Prospective evaluations may benefit from a progressive inclusion of clinical data for model refinement in an integrative middle-out approach (Tsamandouras et al., 2015; Zhuang and Lu, 2016; Sager et al., 2015). However, previous studies aimed at assessing the operational characteristics of PBPK modelling for a large number of compounds have highlighted low accuracy in the prediction of human PK during early drug development (Poulin et al., 2011). So far, limited efforts have been made to assess data requirements to ensure acceptable predictive performance across the different stages of phases of drug development, during which data availability varies considerably. This situation contrasts with ongoing developments in pharmacometrics, where Bayesian statistics is being implemented along with other statistical methods to account for model and parameter uncertainty as well as ensure appropriate predictive performance of deterministic and stochastic models. A systematic elucidation of the predictive performance as a function of data availability/quality may have considerable impact for different applications, in which highly different modelling and data- or task-dependent approaches, like bottom-up, top-down or middle-out, can be adopted. In particular, we believe that an in-depth analysis of a clinically relevant case study is needed to illustrate the implications of critical steps and gaps, enabling the implementation of truly informative experimental protocols and rational use of PBPK modelling, irrespective of drug development phase. Moreover, such a case study may provide insight into the requirements for the evaluaton of model uncertainty, including understanding of the parameters and processes that are critical for drug disposition.

Here, we investigated the predictive performance of WB-PBPK modelling both for prospective and retrospective evaluation of the disposition properties of a compound, which is known to have multiple organs contributing to drug disposition and is used in combination with other drugs in a

rather long (chronic) regimen. The primary goal of this study was, therefore, to assess the impact of data availability and prior knowledge on model predictive performance. Six *what-if* scenarios, which mimic different degrees of prior information or data availability, were identified and evaluated as part of this exercise. We envisage that key points emerging from the proposed scenarios for this case study may provide relevant insight into data requirements for the implementation of PBPK modelling at different phases of drug development. It can also be anticipated that other challenges and limitations may arise for other compounds, as WB-PBPK modelling depends also on drug-specific features.

Ethambutol (EMB) has been approved as first line treatment in combination with rifampicin, isoniazid and pyrazinamide for the treatment of pulmonary tuberculosis (TB) treatment (Dartois, 2014; WHO, 2016). As *Mycobacterium tuberculosis* is primarily present both intra and extracellularly in different areas in the lung (Dartois, 2014), characterization of tissue pharmacokinetics is essential for predicting therapeutic doses in humans. In this work, we have collated a wide range of experimental data for EMB to explore different scenarios, which represent important milestones or deliverables in early drug development, namely: 1) prospective first-time-in-human (FTIH) studies in which *in vitro* data, compound-specific physicochemical data and, in some cases, knowledge of disposition process from preclinical experiments are used to predict pharmacokinetics (Jones et al., 2013), 2) model refinement by progressive inclusion of a limited to rich set of clinical data (Jamei, 2016), 3) prediction of inter-individual variability (IIV) (Sager et al., 2015) and 4) prediction of EMB concentration in the target tissue (Zhuang and Lu, 2016).

2. MATERIALS AND METHODS

2.1 Data

Observed and simulated EMB concentration profiles from different clinical studies were considered for WB-PBPK model development and subsequent assessment of its predictive performance (Table

1). The same models and dosing regimens reported in the original investigations (Lee et al., 1980; Jönsson et al., 2011) were used to simulate plasma concentration-time profiles (see Supplementary Material, section 1.1). Simulations were performed via the *simulx* R function of the *mlxR* package (SimulX v.3.0, R v.3.0.3) (R, 2015; Lavielle, 2016). We relied mainly on simulated data to build the model since they provide a richer and more balanced dataset of plasma concentration-time profiles than the sampled data. More specifically, pharmacokinetic profiles were simulated after a single dose and at steady-state for different subjects receiving EMB. Observed pharmacokinetic data from other studies, investigating steady-state plasma and lung concentration (Conte et al., 2001) or EMB bioequivalence (Strauch et al., 2011; Xu et al., 2013; Wang et al., 2013), were considered to assess bias and precision.

Additional *in vitro* data, describing the physicochemical properties of EMB, were used to parameterize the model and, depending on the scenario (see below), *in vivo* animal studies were also integrated to account for information on the elimination route (Table 2).

2.2 Scenarios

Six scenarios were considered, in which data were added progressively during the model development/refinement process, starting from *in vitro* and animal experiments up to human clinical trials. In total, two FTIH, two sparse data and two rich data scenarios were evaluated (Figure 1), representing typical data availability at different stages of drug development. The parameter values, which were fixed or estimated for each scenario are summarized in Table 2. Specifically, in FTIH scenarios drug disposition was predicted based on *in vitro* and preclinical experiments; in sparse data scenarios, i.e., when clinical data are limited to IV administration, the impact of the availability of human data on both PBPK model refinement and predictive performance were explored; finally, in rich data scenarios human data following IV and PO administration were included in the analysis.

2.3 WB-PBPK modelling

Analyses were performed using PK-Sim (v.7.0; Open System Pharmacology Suite) (Eissing et al., 2011), since this software ensures high flexibility and control of model structure. Scenarios 1 and 2 are simulation-only scenarios. In scenarios 3-5, the model was parameterized using mean data following a single EMB dose (Table 1). Simultaneous fitting of plasma and urine data following IV administration was performed for parameter estimation in scenario 4, whereas the standard 2-step procedure (Kuepfer et al., 2016) was adopted in scenario 5: first, drug distribution and elimination were estimated using IV data (as in scenario 4), then drug absorption was evaluated from PO administration profiles. Parameters were estimated via the Monte Carlo-based method implemented in the PK-Sim estimation toolbox. In scenarios 1-5, typical subjects with adequate biometrics and demographics were simulated. In scenario 6, a population with target biometrics was generated, as illustrated in the Supplementary Material, section 1.2.

To account for the amount of EMB actually administered, the molecular weight ratio between pure EMB (204.31 g/mol) and EMB hydrochloride (277.232 g/mol) was calculated, and the doses administered as salt form were always corrected by this ratio. In addition, based on the physicochemical properties of EMB, which is a strong base, the Rodgers and Rowland distribution model was used in all the scenarios to compute organ-plasma partition coefficients (Rodgers et al., 2005; Rodgers and Rowland, 2006).

When hepatic clearance was not estimated from human data or assumed to be negligible (scenario 1-2), it was derived from *in vitro* experiments via microsomal activity measurements, which are widely used to assess liver metabolism (Jones and Rowland-Yeo, 2013). Hence, *in vitro* microsomal activity data were used to determine hepatic clearance value (Iwatsubo et al., 1997; Naritomi et al., 2003), as detailed in Supplementary Material, section 1.3. Since microsomal activity for EMB was below the limit of quantification (LOQ) of the assay (Lakshminarayana et al., 2014), the 0-LOQ range was spanned, covering a large range of non-measurable activities to account for the uncertanty of this parameter.

Unless indicated otherwise, glomerular filtration rate (GFR) efficiency was fixed to 1. Since *in vivo* data showed that monkey renal clearance values are 2 to 3 times higher than renal blood flow (indicating active renal drug secretion) (Lee et al., 1977), information from *in vivo* animal clearance was used for human predictions in scenario 2 and 3.D by fixing GFR efficiency to 3, to account for both active and passive renal elimination (OSP, 2017) (Table 2).

A Weibull function empirically described the dissolution of EMB within the intestinal segments (Goldsmith et al., 1978; Costa and Lobo, 2001). When Weibull parameters were not estimated from the available human data (scenario 1-4), median dissolution time and shape were estimated by fitting the *in vitro* dissolution curve (Dekker, 2007). When the drug intestinal permeability transcellular (IPT) value was not estimated (scenario 1-4), its value was computed by PK-Sim, based on effective molecular weight (MW_{eff}) and membrane affinity (MA), or LogP if MA is not available (Eq. 1) (Thelen et al., 2011).

$$P_{int}(MW_{eff},MA)=265.796*MWeff^{-4.49968}\times MA[cm/s] \qquad \text{Eq.(1)}$$

2.4 Evaluation of model predictive performance

The fold change (predicted/observed in mean data) of the area under curve (AUC) and maximum value (Cmax) derived from steady-state plasma concentration following PO administation were used in scenarios 1-5, together with the alveolar cell (AC) concentration. Since the pharmacokinetics of EMB is linear, all scenarios were analyzed considering only the 800-mg dose. AUC and Cmax following IV administration, and the fraction excreted in urine were also used to study the impact of different elimination processes. In scenario 5, additional single dose/steady-state profiles were used to evaluate the model (Table 1). In scenario 6, empirical and theoretical percentiles of single dose/steady-state plasma concentration-time profiles were compared to assess variability in the population in addition to central tendency.

3. RESULTS

3.1 Implications of prior knowledge and experimental data on predictive performance

As shown in Figure 2A-J, the predicted steady-state plasma and AC concentration vs. time profiles following PO administration of EMB to humans varied, depending on the degree of information available. The poorly predicted drug levels in lung tissue in FTIH and sparse data settings (scenarios 1-2 and 3-4, respectively) (Figure 2B,D,F,H) is probably a consequence of incorrect predictions of plasma levels (Figure 2A,C,E,G) and wrong assumptions about the disposition characteristics. By contrast, EMB plasma concentration data were well described and accurate prediction of drug level in AC were obtained (Figure 2J) when all the necessary data and information are available (scenario 5).

The predicted profiles in Figure 2 confirmed that increasing data availability and knowledge regarding the disposition properties of EMB results into improved precision (i.e., lower variability ranges) and reduced bias (i.e., consistency between data and model prediction) for both plasma and AC concentrations. This trend is clearly summarized by the steady-state plasma AUC fold change (Figure 2K). AUC values changed up to 11-fold compared to human data for FTIH settings to less than 1.2-fold when rich data sets are available (scenario 5). Results did not substantially change when considering secondary parameters such as Cmax (Supplementary Material, section 2.5). Similar quantitative conclusions can be drawn when examining the predicted AC concentrations (Figure 2B,D,F,H,J).

Details, parameters and challenges faced in each scenario are presented below, highlighting their implication for human predictions.

In **scenario 1**, the steady-state AUC predicted following PO administration spans across a large range (Figure 2A,K) and varies from 0.1- to 4.3-fold compared with human data. Such ae wide range, also

observed in lung prediction (Figure 2B), was mainly due to the uncertainty in hepatic clearance in humans from *in vitro* data, whose impact will be analyzed in depth in the following section. Even though this limitation could not be fully addressed in **scenario 2**, animal renal clearance data improved the predictive performance of the model: AUC fold change was 0.1-2 compared to human data, resulting into a relevant reduction of the variation of AUC (Figure 2C,K) and AC concentration (Figure 2D) ranges. In fact, a wide range of predictions was observed for each lipophilicity value from *in vitro* data (Table 2), confirming that hepatic clearance represents the major uncertainty source in FTIH scenarios (Supplementary Material, sections 2.1-2.2).

Human plasma data after IV administration provided the opportunity to estimate lipophilicity and clearance parameters (**scenario 3**). The use of only plasma concentration data to simultaneously estimate both hepatic clearance and tubular secretion (**scenario 3.C**) led to model identifiability problems (not shown). For this reason, we estimated both clearance processes individually, by neglecting the other elimination process (**scenarios 3.A and 3.B**) or using animal data to fix active renal clearance (**scenario 3.D**). IV plasma data were well fitted by the model in sub-scenarios 3.A, 3.B and 3.D (Supplementary Material, section 2.3). Compared with FTIH scenarios, the precision of plasma AUC prediction improved (0.2-1-fold) (Figure 2K). However, predicted PK profiles still showed considerable variability due to the different assumptions about the elimination processes and lipophilicity parameter values, as well as a biased prediction of human AC concentration (Figure 2E-F).

In **scenario 4**, urine data were also used. Mean plasma concentration vs. time profiles and EMB fraction excreted in urine (Figure 4B) following IV administration were successfully described, with clearance processes correctly parameterized (Table 2). The estimated lipophilicity was similar to the one in scenario 3. As expected, the estimated clearances differed from the ones in scenario 3 for each of the different assumptions. However, biased predictions were observed in both AUC and AC concentration (Figure 2G-H,K).

In **scenario 5**, Weibull distribution parameters and IPT were estimated based on human data. Weibull parameter estimates were in accordance with *in vitro* data, whereas the estimated IPT was similar to the default values computed based on LogP (Eq. 1) used in the other scenarios (Table 2). The model fitted the mean plasma concentration profiles following a single PO administration (800, 1000 and 1200 mg). Excellent prediction accuracy was observed for both steady-state plasma AUC and AC concentration (Figure 2I-K). Estimates were not considerably different from the values used in scenario 4, suggesting that the model may be highly sensitive to absorption parameters. Finally, the model predicted satisfactorily the mean EMB plasma concentration profiles in humans observed in independent data sets (different drug formulations at different doses, after single dose or at steady-state, Figure 3A-D and Table 1).

In **scenario 6**, the IIV associated with the plasma concentration vs. time profiles wass predicted accurately: the comparison between empirical and theoretical percentiles showed good agreement after single dose (Figure 3E-F and Supplementary Material, section 2.4). At steady-state, it appears that the 95th percentile is slightly underpredicted.

An in-depth overview of the results regarding the uncertainty in elimination and absorption is provided in the following sections.

3.2 Uncertainty in the contribution of hepatic and renal elimination processes

Because of the uncertainty in the *in vitro* microsomal activity, a range of hepatic clearance values had to be considered, from zero to an upper limit, depending on the LOQ of the *in vitro* assay (Table 2). In the FTIH scenarios this uncertainty affected significantly the prediction of IV plasma concentration vs. time profiles after single dose. This deviation is further amplified after PO administration (Figure 4A-B and Figure 2K – scenarios 1-2). Similarly, uncertainty in the contribution of hepatic and renal processes affects the prediction of the fraction of EMB excreted in urine (Figure 4C). Some knowledge about the renal elimination in animals (scenario 2) slightly decreases the variability in the prediction of plasma AUC (Figure 4A-B) and fraction excreted in urine (Figure 4C)

Importantly, human plasma concentrations and urine excretion profiles could not be accurately predicted by a single parameter set, suggesting that further understanding of the elimination process is required (Supplementary Material, section 2.1-2.2). Even by using single dose human IV data (scenario 3), misspecification in the drug elimination processes is observed, with results showing highly variable EMB fraction excreted in urine (20-100%) (Figure 4D-E). As indicated previously, such uncertainty has comparable effect on the predictions after steady-state plasma profiles after PO administration (Figure 2K).

Passive renal excretion did not suffice to describe the fraction of EMB excreted via the kidneys alone, as demonstrated in scenarios 1-3A. In scenario 4, a first-order tubular secretion was included and tubular secretion rate TS_{spec} of 1.46 min^{-1} was estimated. We found that, 72 hours after IV infusion, 43% of the total amount excreted via the kidneys was excreted actively, indicating that tubular secretion is a critical process for drug elimination. In agreement with the fact that EMB is a renally cleared drug, a low liver elimination rate (0.07 min^{-1}) was estimated, with values below the LOQ of *in vitro* assay (Lakshminarayana et al., 2014). Following IV infusion, the total plasma clearance was 0.45 L/h/kg, consistent with the values reported previously (0.51 L/h/kg) (Lee et al., 1980). Similarly, following PO administration, the total plasma clearance estimate was 0.74 L/h/kg (mean), which is consistent with the value of 0.80 L/h/kg, reported in (Jönsson et al., 2011).

These results highlight the need of a more sensitive assay of microsomal activity to decrease the uncertainty in the estimation of hepatic clearance. They also show the need to collect urine samples in preclinical protocols and undoubtedly in FTIH studies to overcome model identification problems during the parameterization of elimination processes.

*3.3 Impact of bioavailability and absorption processes*Despite evidence of satisfactory goodness-of-fit for plasma and urine I data following IV doses, the model built in scenario 4 was not capable of predicting steady-state EMB plasma and AC profiles after PO administration without bias (Figure 2G-H). By contrast, the model used in scenario 5 yielded accurate predictions of both plasma and AC

concentrations (Figure 2I-J), suggesting the importance of bioavailability- and absorption-related parameters. Considering scenarios 4-5, a variation of Weibull parameters did not significantly alter the predicted profiles, whilst small variations of IPT resulted into significant changes in thef EMB concentration vs. time profiles following PO administration (results not shown), which was further confirmed by sensitivity analysis (Supplementary Material, section 2.6). By simulating steady-state plasma concentration profiles using a fixed IPT value (estimated in scenario 5, instead of using Eq. 1), variability and bias in the AUC fold change decreased significantly (Figure 5A vs. Figure 2K). This indicated that a relevant part of variability in the predicted plasma concentration following oral administration in scenarios 1-4 was due to lipophilicity: it did not significantly affect EMB absorption or elimination *per se*, but had a significant effect on the calculation of the IPT default value (the single dose plasma concentration profile following PO administration in scenario 4 is shown as example in Figure 5C-D). Even though plasma concentration vs.time profiles were not affected in Figure 5D, different lipophilicity values yielded to different lung concentration profiles (Figure 5E-F).

In the fully parameterized model (scenario 5, Figure 5B), a constant bioavailability (60-61%) was obtained for the three dose levels, in agreement with the known linearity in EMB pharmacokinetics. The drug fraction absorbed in the gastrointestinal tract (GIT) was 65-66%, slightly lower than previously reported values (75% to 80%) (DrugBank, 2019).

4. DISCUSSION

In this study, we have investigated the predictive performance of a WB-PBPK model in the presence of different levels of evidence regarding the pharmacokinetics and physicochemical properties of EMB. As efforts in early drug development increasingly focus on the need for translational data, we have explored how *in silico* approaches, such as PBPK, can facilitate such an endeavour, whilst recognising that no consensus exists about the experimental requirements to ensure accurate translation or prediction of the pharmacokinetic properties from *in vitro* to *in vivo*, and, subsequently, from *in vivo* to humans. It has to be acknowledged that the analysis of a single

compound may not be sufficient to draw general recommendations regarding data requirements for PBPK modelling, and similar studies on other drugs might show different WB-PBPK model performance levels or challenges. Nonetheless, we believe that the systematic, stepwise evaluation of a case study, including a compound like EMB offers insight into critical points to consider in prospective and retrospective data use for PBPK modelling purposes.

Our analysis has focused on three different settings, which reflect common practice in drug development, i.e., when WB-PBPK models i) are used prospectively for the prediction of human pharmacokinetics in the absence of any clinical data; ii) are refined by progressively incuding a limited amount of clinical data; iii) are used retrospectively in a rich data context, when modelling objective is primarily focused on the estimation of model parameters.

As one would expect, the current results reveal that the accuracy of predicted EMB concentrations is directly correlated with the amount and quality of the available data that, in turn, depends on the stage of drug development (Figure 2). As WB-PBPK models are used from preclinical settings up to beyond phase III clinical studies, it should be also noticed that the required level of accuracy in model predictions may vary in the different stages of the drug development process: more accurate predictions are required in advanced stages, when more data are available, whilst varying degrees of uncertainty may be accepted when performing prediction of FTIH studies. This case study aimed to evaluate a setting in which human dose predictions are derived based on limited data, in line with the potential added value of WB-PBPK as a tool for bottom-up predictions, over empirical methods, such as allometric scaling. Whilst a comparative analysis of the different methods is beyond the scope of our investigation, the current results show that the use of a physiologically-based parameterization does not ensure high predictive performance when limited data are available. Furthermore, this analysis highlighted the potential flaws and critical data requirements for parameter estimation for a drug with predominant renal clearance. This finding reveals the importance of knowledge and/or data on drug elimination mechanisms not only when sparse data

are available, but also in a rich data setting. Overall, *in vivo* understanding of ADME properties (in particular, bioavailability, absorption and elimination processes) is crucial for accurate prediction of exposure in humans.

Here, the ability of a PBPK model to adequately predict plasma concentration was emphasized not simply because of its relevance for the interpretation of safety and efficacy data. Accurate description of plasma concentration vs. time profiles forms the basis for the prediction and extrapolation of drug levels in tissues and organs of interest. As many drugs have multiple elimination pathways, one of the main problems we faced with EMB in the FTIH scenarios was the lack of knowledge regarding the different clearance processes. The uncertainty about hepatic clearance and the lack of active tubular secretion data from preclinical protocols prevented accurate prediction of drug exposure in plasma and lung tissue when *in vitro* experiments were the only data source. The biased prediction in AUC following IV administration is further amplified when data after PO administration are used. The integration of *in vivo* animal data improved predictions following IV administration (AUC within 2-fold, compared with >3-fold without using animal data). In addition, it should be noted that in the two sparse data scenarios, only partial model parameterization was possible. On the other hand, the PBPK model consistently described plasma IV data well, which represents a fact of the utmost importance at this stage of drug development. Only when human urine data were included (scenario 4), drug distribution and elimination could be adequately estimated. Drug exposure following PO administration was poorly predicted when the IPT value computed via an empirical formula was used (Eq. 1). More accurate predictions were obtained when IPT values were estimated, as displayed in Figure 5A. This finding confirmed that IPT is one of the most sensitive parameters in the WB-PBPK model for ethambutol, and the accuracy of predictions depends dramatically on its value.

In the rich data scenarios, the availability of both IV and oral data allowed full parameterization of

the PBPK model. Parameter estimates were in agreement with literature values and the final model showed good predictive performance. Interestingly, even when model parameters were estimated from mean single dose data, mean steady-state plasma concentration vs. time profiles following PO administration were well predicted. The variability in organ volumes, blood flow rates, etc., and demographics of the populations included in the PK-Sim internal database allowed accurate description of the IIV, in a similar manner to the estimates from nonlinear mixed effects models. However, PK-Sim currently shows limitations in simulating the variability in populations with different parameter distributions (in our case, severely underweight TB patients).

Lastly, it is worth mentioning that the predictive performance of the WB-PBPK model was considered satisfactory when assessing the predictions obtained for external studies, which had not been included in the current analysis. Mean EMB plasma concentration vs. time profiles following the administration of different formulations were adequately predicted. Mean EMB concentrations at steady-state in lung was also well predicted, without the need for model customization or inclusion of additional compartments (Gaohua et al., 2015). Taken together, these results suggest that the use of simulated plasma concentration data instead of observed concentrations may be preferred. Clearly, in this case, simulated data had no impact on the predictive performance of the model.

In summary, the use of EMB as a case study provided insight into data requirements for the implementation of PBPK modelling across a range of scenarios mimicking the progression of a candidate molecule from early development to FTIH. It also showed the implications of data availability for prospective model development based on physicochemical drug properties and *in vitro* data, including the importance of data integrationduring the middle-out model refinement process, prediction of IIV and drug concentration in tissues and biophase. Ultimately, our study has enabled the the characterization of EMB disposition in plasma and in target tissue and as such model parameter estimates may prove valuable for subsequent evaluation of the antibacterial activity of EMB as companion drug in novel combination regimens.

5. CONLUSIONS

In conclusion, these results show that the predictive performance of WB-PBPK models cannot be guaranteed by the mechanistic or physiological nature of their parameterization. Whilst their use as a translational tool seems promising, challenges and limitations exist for the prediction of therapeutic dose and systemic and tissue/organ exposure of novel molecules when applied prospectively in early drug development stages. The lack of knowledge about disposition properties or poor understanding of the differences in disposition mechanisms between species can have major impact on model performance. This analysis also highlights the operating characteristics of WB-PBPK models when used retrospectively after clinical data have been obtained, providing insight into tissue and organ drug disposition , which is usually not possible with traditional compartmental approaches. Consequently, an adequate model building workflow is essential for the use of WB-PBPK models as a translational tool. The implementation of a generic structure including only passive processes overlooks active processes, which can have a critical role in disposition properties and mass balance. It should be clear, however, that it is not the complexity of these models that seems to be a limitation; rather it is the availability and accuracy of few, but crucial parameters.

ETHICAL APPROVAL

Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Supplementary methods and results. Simulation of human plasma concentration data (1.1), Population-based simulations (1.2), Hepatic clearance calculation from microsomal activity (1.3), Simulations, fittings and analyses for all the scenarios: scenario 1 to 6 (2.1-2.4), Cmax fold change at steady-state in different scenarios (2.5), Sensitivity analysis (2.6)..

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FIGURE LEGENDS

Figure 1: Description of the *what-if* scenarios analyzed in the current study. The input parameters of the WB-PBPK model and observed variables are summarized in the block scheme on top of the figure. Scenarios are illustrated, describing scope, knowledge assumptions and input parameters. Specifically, in **scenario 1** only physicochemical drug properties were available. Since no information on EMB renal elimination could be derived from *in vitro* assays, it was assumed that the drug is not actively secreted nor reabsorbed. In **scenario 2**, the knowledge on animal clearance was added to the data used in scenario 1. In **scenario 3**, plasma data collected after intravenous (IV) infusion of EMB to healthy subjects were integrated into the analysis. Different hypotheses regarding EMB clearance processes were formulated, resulting into four different sub-scenarios: in **scenario 3.A**, EMB is actively metabolized by the liver and only passively eliminated via the kidneys (hepatic clearance was estimated); in **scenario 3.B**, EMB is eliminated only via the kidneys (active tubular secretion was estimated); in **scenario 3.C**, passive and active renal elimination, and drug liver metabolism were assumed (liver and active kidney clearances were estimated); in **scenario 3.D**, monkey renal clearance information was included into the model (hepatic clearance was estimated). Lipophilicity was also estimated where indicated (see Table 2 and Supplementary Material, section 2.3). In **scenario 4**, urine data were also available for the analysis: IV plasma and urine data were simultaneously fitted (lipophilicity and clearance parameters were estimated). In **scenario 5**, drug absorption properties (Weibull dissolution time and shape, and intestinal permeability transcellular - IPT) following oral administration were estimated by simultaneous fitting of the mean plasma concentration profiles after 800, 1000 and 1200 mg EMB doses. In **scenario 6**, the PBPK model built in scenario 5 was used to describe EMB plasma concentration profiles in a population.

Figure 2: WB-PBPK model prediction performance for steady-state plasma and alveolar cells (AC) concentration following oral administration. A-J) Predicted concentration-time profiles (solid line) and mean human data (circles with error bars representing standard deviation) are shown for plasma (red) and AC (blue) concentrations in scenario 1 (A-B), scenario 2 (C-D), scenario 3 (E-F), scenario 4 (G-H) and scenario 5 (I-J). When two predicted profiles are present, they represent minimum and maximum predicted profile, due to uncertainty sources in scenarios 1-3. K) AUC fold change for plasma concentration data at steady-state for each scenario. Boxes represent the minimum-maximum range of AUC fold change. Fold change values are presented in log scale. The log2 AUC fold change value of 0 (corresponding to AUC fold change of 1, i.e. no change) is reported (dashed blue line). The log2 AUC fold change values of -1 and 1 (corresponding to AUC fold change of 0.5 and 2, respectively) are also reported (dashed red lines).

Figure 3: Performance of the WB-PBPK model in scenario 5-6. A-D) Plasma concentration-time profiles predicted by the model developed in scenario 5 following oral administration of 800 mg – single dose (A), 1000 mg – single dose (B), 1100 mg – single dose (C) and 15 mg/kg – steady-state (D). Data in panels A-D were not used for model fitting and are from previous investigations reported in Strauch et al. (2011), Xu et al. (2013), Wang et al. (2013) and Conte et al. (2001), respectively. E-F) Population prediction for plasma concentration-time profiles after single oral dose (E) or steady-state (F) (800 mg) according to a once daily dosing regimen. The shaded area and solid blue line represent the 5^{th}–95^{th} percentiles of the PK-Sim population predicted plasma concentration-time profiles and observed human data, respectively. The red and blue dashed lines

represent the median, while the red and blue dotted lines represent the 25th and 75th percentiles of the PK-Sim population predicted plasma concentration-time profiles and observed human data, respectively.

Figure 4: WB-PBPK model predictions showing the impact of uncertainty in hepatic and renal elimination processes. A) AUC fold change for single dose plasma concentration data following IV administration (15 mg/kg) for each scenario. Boxes represent the minimum-maximum range of AUC fold change. Fold change values are presented in log scale. The log2 AUC fold change value of 0 (corresponding to AUC fold change of 1, i.e. no change) is reported (dashed blue line). The log2 AUC fold change values of -1 and 1 (corresponding to AUC fold change of 0.5 and 2, respectively) are also reported (dashed red lines). B-C) Plasma concentration (B) and fraction excreted unchanged in urine (C) in scenarios 1-2 (FTIH settings). Solid lines represent the predicted minimum-maximum profiles (scenario 1: red; scenario 2: blue), and circles represent mean values of experimental data, with error bars representing standard deviation. D-E) Plasma concentration (D) and fraction excreted unchanged in urine (E) in scenarios 3-4 (sparse data settings). In panel D, red solid lines represent the minimum-maximum fitted profiles for the sub-scenarios in scenario 3, the blue dashed line (overlapped with one of the red lines) represents the fitted profile in scenario 4, and circles depict mean estimates fromexperimental data, with error bars representing standard deviation. In panel E, red solid lines represent the minimum-maximum predicted profiles for the sub-scenarios in scenario 3 (in which urine data are not fitted), the blue dashed line represents the fitted profile in scenario 4 (in which urine data are available), and circles depict mean estimates from experimental data, with error bars representing standard deviation.

Figure 5: WB-PBPK model predictions showing the impact of uncertainty in absorption processes. A) AUC fold change for steady-state plasma concentration data following oral administration (800 mg) for each scenario, in which IPT was fixed to the value estimated in scenario 5. Boxes represent the minimum-maximum range of AUC fold change. Fold change values are presented in log scale. The AUC fold change values of 1 is reported (dashed blue line), together with the AUC fold change values of 0.5 and 2 (dashed red lines). B) Simultaneous fitting of single dose data after oral administration in scenario 5. Lines represent fitted curves and data points represent mean plasma concentration data. Three doses were considered, with colours described in legend. C-F) Predicted profile of first-dose plasma concentration (C-D) and AC concentration (E-F) following oral administration (800 mg) in scenario 4, in which different lipophilicity values were fixed, as reported in the legend. IPT was computed via Eq. 1 (panels C and E) or fixed to the value estimated in scenario 5 (panels D and F). Plasma concentration data are also reported for comparison: circles represent mean human data, with error bars representing standard deviation.

Abstract

Whole-body physiologically based pharmacokinetic (WB-PBPK) models have become an important tool in drug development, as they enable characterization of pharmacokinetic

profiles across different organs based on physiological (systems specific) and physicochemical (drug specific) properties. However, it remains unclear which data are needed for accurate predictions when applying the approach to novel candidate molecules progressing into the clinic. In this work, as case study, we investigated the predictive performance of WB-PBPK models both for prospective and retrospective evaluation of the pharmacokinetics of ethambutol, considering scenarios that reflect different stages of development, including settings in which the data are limited to *in vitro* experiments, in vivo preclinical data, and when some clinical data are available. Overall, the accuracy of PBPK model predicted systemic and tissue exposure was heavily dependent on prior knowledge about the eliminating organs. Whilst these findings may be specific to ethambutol, the challenges and potential limitations identified here may be relevant to a variety of drugs, raising questions about 1) the minimum requirements for prospective use of WB-PBPK models during the characterization of drug disposition and 2) implication of uncertainty for dose selection in humans.

Table 1. EMB concentration data considered in this investigation. For each study, the formulation, route of administration, dose and schedules are reported along with the sampling and data type (observed or simulated). The relevance and the purpose of the data for the current analysis is outlined in the last column.

Study	Ethambutol	Administration	Dosage and schedule	Data collected	Observed or simulated	Details	Used for
Lee et al. (1980)	Myambutol 250, Lederle, San Diego, CA, USA	IV	15 mg/kg, 1-h infusion, observations up to 12 h after dose	Plasma concentration	Simulated	6 healthy subjects	Model development and evaluation
Lee et al. (1980)	Myambutol 250, Lederle, San Diego, CA, USA	IV	15 mg/kg, 1-h infusion, observations	Amount excreted unchanged in the urine	Observed	6 healthy subjects	Model development and evaluation

			at 24, 48 and 72 h				
Jönsson et al. (2011)	Purderal (400 mg, Pharmacare Ltd. Port Elizabeth, South Africa), Rolab (400 mg, Rolab [Pty.] Ltd., Berhampur, India) and Rifafour e-275 (275 mg, Sanofi-Aventis; Paris, France)	PO	800, 1000 or 1200 mg, once daily 5 times a week, first dose and steady-state, observations up to 15 h after dose	Plasma concentration	Simulated	Two studies performed on 164 patients diagnosed with TB*	Model development and evaluation
Strauch et al. (2011)	ETB-91-400A	PO	800 mg, first dose	Plasma concentration	Observed	24 healthy subjects, bioequivalence study	Model evaluation
Xu et al. (2013)	EMB (Hong Qi Pharmaceutical CO, Ltd, China)	PO	1000 mg, first dose	Plasma concentration	Observed	18 healthy subjects, bioequivalence study	Model evaluation
Wang et al. (2013)	Myrin-P Forte (Pfizer, NY, USA)	PO	1100 mg, first dose	Plasma concentration	Observed	35 healthy subjects, bioequivalence study	Model evaluation
Conte et al. (2001)	Not reported	PO	15 mg/kg, once daily for 5 days, steady-state, observations at 2 and 4 h	Plasma concentration	Observed	20 healthy subjects**	Model evaluation
Conte et al. (2001)	Not reported	PO	15 mg/kg, steady-state, observations at 4 h	Concentration in alveolar cells (AC)	Observed	20 healthy subjects**	Model evaluation

*We did not include data on the 1500 mg dose (administered to only one patient) and HIV-positive patients (24) in the simulated data set.
**We did not include patients with HIV/AIDS (20).

Table 2: Parameter values of the WB-PBPK model used in the different scenarios. EMB physicochemical properties were assumed to be the same in all scenarios. WB-PBPK model parameter values can change across scenarios based on the assumptions, the available information and data. Values estimated from human data are shown in bold.

EMB physico-chemical properties							
PARAMETER	**Compound type**	**pKa**	**EMB hydrochloride MW**	**Pure EMB MW**	**Main plasma protein binding**	**Fraction unbound (fu)**	**F s (**
Value	Diprotic base (Gaohua et al., 2015)	9.55 and 6.5 (Gaohua et al., 2015)	277.32 (DrugBank, 2019)	204.31 (DrugBank, 2019)	Albumin (Gaohua et al., 2015)	0.75	7 2
UNIT	-	-	g/mol	g/mol	-	-	n
MEANING	The type of	Acid	Molecular weight of	Molecular weight of	Drug specific	Free fraction of	S

	compound: acid, neutral or base. The compound type always refers to the unchanged form of the molecules (OSP, 2017)	dissociation constant	EMB hydrochloride	EMB hydrochloride	binding to proteins (plasma, interstitial or intracellular space) (OSP, 2017)	drug in plasma (OSP, 2017)	s i (
WB-PBPK model parameters							
PARAMETER	**Lipophilicity (LogP)**	**Specific clearance**	**GFR specific**	**GFR fraction**	**TSspec**	**Weibull median dissolution time**	**V s**
UNIT	-	1/min	mL/min/100g organ	-	1/min	min	-
MEANING	Lipophilicity	Hepatic clearance	Glomerular filtration rate normalized to the volume of the kidney (OSP, 2017)	Glomerular filtraton fraction (i.e. efficiency)	Specific clearance for tubular secretion	Time at which 50% percent of the compound has dissolved (OSP, 2017)	S t d 2
SCENARIO 1	-0.4 (Rodgers and Rowland, 2007) and 0.12 (Lakshminarayana et al., 2014)	0, 0.01, 0.1, 0.2, 0.41, 0.82, 0.164‡ when healthy volunteers (Lee et al., 1980) were considered) 0, 0.01, 0.1, 0.21, 0.425, 0.85, 1.7‡ (when TB patients (Jönsson et al., 2011) were considered) 0, 0.06, 0.125, 0.25, 0.51, 1.03, 2.06‡ (when healthy volunteers (Conte et al, 2001) were considered)	26.60	1	0	10.3 (estimated from Dekker (2007))	1 I
SCENARIO 2	-0.4 [43] and 0.12 [34]	0, 0.01, 0.1, 0.2, 0.41, 0.82, 0.164‡ when healthy volunteers (Lee et al., 1980) were considered) 0, 0.01, 0.1, 0.21, 0.425, 0.85, 1.7‡ (when TB patients (Jönsson et al., 2011) were considered) 0, 0.06, 0.125, 0.25, 0.51, 1.03, 2.06‡ (when healthy volunteers (Conte et al, 2001) were considered)	26.60	3 (Lee et al., 1977)	0	10.3 (estimated from Dekker (2007))	1 I
SCENARIO 3.A	-0.4 [43], 0.12 [34] and **-0.62**	**0.38** (when LogP-0.4)	26.60	1	0	10.3 (estimated from Dekker	1 I

		0.35 (when LogP=0.12) and 0.39 **(when LogP= 0.62)**				(2007))	
SCENARIO 3.B	-0.4 [43], 0.12 [34] and **-0.65**	0	26.60	1	**2.91** (when LogP-0.4) **2.76** (when LogP=0.12) and **2.72** (when LogP= 0.65)	10.3 (estimated from Dekker (2007))	1 I
SCENARIO 3.C	-0.4 [43], 0.12 [34] and **not identifiable**	**Always not identifiable**	26.60	1	**Always not identifiable**	10.3 (estimated from Dekker (2007))	1 I
SCENARIO 3.D	-0.4 [43], 0.12 [34] and **-0.63**	**0.21** (when LogP-0.4) **0.20** (when LogP=0.12) and **0.20** (when LogP= 0.63)	26.60	3 (Lee et al., 1977)	**0** (when LogP-0.4) **0** (when LogP=0.12) and **0** (when LogP= 0.65)	10.3 (estimated from Dekker (2007))	1 I
SCENARIO 4	**-0.64**	**0.07**	26.60	1	**1.46**	10.3 (estimated from Dekker (2007))	1 I
SCENARIO 5[§]	**-0.64**	**0.07**	26.60	1	**1.46**	**16.41**	**0**
SCENARIO 6[§]	**-0.64**	**0.07**	26.60	1	**1.46**	**16.41**	**0**

[‡]Different values in the 0-LOQ ranges were used for the simulations.
[§]Scenario 5 and 6 use the same data and assumptions for model parameterization, thereby yielding the same estimates. However, the two scenarios differ in the model predictive performance, which was carried out on mean (scenario 5) and population data (scenario 6).