Applications of physiologically based pharmacokinetic modelling for the optimisation of anti-infective therapies

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Abstract

Introduction: The pharmacokinetic properties of anti-infective drugs are a determinant part of treatment success. Pathogen replication is inhibited if adequate drug levels are achieved in target sites, whereas excessive drug concentrations linked to toxicity are to be avoided. Anti-infective distribution can be predicted by integrating *in vitro* drug properties and mathematical descriptions of human anatomy in physiologically based pharmacokinetic models. This method reduces the need for animal and human studies and is used increasingly in drug development and simulation of clinical scenario such as, for instance, drug-drug interactions, dose optimisation, novel formulations and pharmacokinetics in special populations.

Areas covered: We have assessed the relevance of physiologically based pharmacokinetic modelling in the anti-infective research field, giving an overview of mechanisms involved in model design, and have suggested strategies for future applications of physiologically based pharmacokinetic models.

Expert opinion: Physiologically based pharmacokinetic modelling provides a powerful tool in anti-infective optimisation, and there is now no doubt that both industry and regulatory bodies have recognised the importance of this technology. It should be acknowledged, however, that major challenges remain to be addressed and that information detailing disease group physiology and anti-infective pharmacodynamics is required if a personalised medicine approach is to be achieved.

1. Introduction

1.1 The importance of anti-infective pharmacokinetics

Favourable pharmacokinetic properties of anti-infective drugs are essential for treatment success, as these drugs require access to the pathogen to elicit an effect. In recent years, numerous studies have clarified the relevance of anti-infective pharmacokinetics (PK) for successful treatment and identified predictors of exposure in different populations of patients. In addition, anti-infective PK optimisation is an essential component for reducing the risk of drug-related toxicity for the host. However, in many cases a clear relationship has not been established between host toxicity and exposure. A complication in determining optimal exposure of anti-infectives is the often diverse pharmacokinetic-pharmacodynamic (PK-PD) relationship observed among drug classes. For example, β-lactams need to occupy the majority of binding sites in the bacteria before any real antibacterial effect is achieved and, subsequently, there is not a direct relationship between exposure and effect in patients in this case[1].

Physiologically based pharmacokinetic modelling is a bottom up technique which simulates the pharmacokinetics using *in vitro* drug data (i.e. physicochemical characteristics, intrinsic clearance, permeability) through a mathematical description of drug distribution. PBPK exists as a powerful tool in the development of future treatments and pharmacokinetic optimisation. This review investigates the strategy behind PBPK modelling, with particular emphasis on anti-infective pharmacokinetics. Specific clinical scenarios are also discussed, where patient demographics, genetics, drug-drug interactions (DDIs) and anti-infective exposure are considered in model development and treatment outcomes.

1.2 Physiologically based pharmacokinetic models

The PK of anti-infective agents results from a complex interplay of molecular and physiological processes in tissues mediated by a large variety of proteins. The *in vivo* disposition of a drug can be divided in three main phases: absorption, distribution in tissues and organs, and metabolism/elimination (ADME). An increasing number of studies are focusing on the identification of proteins involved in these ADME processes and their quantitative description is currently available.[2, 3]. This broad base of knowledge is essential for a successful prediction of PK through mathematical modelling. Mathematical equations are being used to describe processes influencing PK, such as tablet dissolution rate following administration, renal clearance, actions of drug transporters, gastrointestinal pH, phase I and phase II metabolism, as well as numerous other biological processes. Interestingly, all these processes can be described in a dynamic way to reflect their evolution over time[4-6]. In PBPK models, the human body is divided into anatomically meaningful compartments which integrate specific properties of a given organ (i.e. blood flow, organ mass, permeation limits, percentage fat) with drug characteristics which creates a structural model reflecting the anatomical arrangement of the tissues connected by perfusing blood. An example of a typical PBPK model is represented in Figure 1. The *in vitro* intrinsic metabolism rate of a drug is usually quantified using tissue-derived microsomes or cell lines expressing relevant metabolic enzymes, and this information is subsequently scaled up to determine whole organ clearance by considering local enzyme expression and other tissue-specific factors. Additional variables, specifically the blood flow rate to the liver and the extent to which the drug binds to plasma protein, are also considered when deriving blood clearance. The prediction of renal elimination can present issues due to the presence of several distinct processes in renal drug elimination. Drug excretion in the kidney consists of glomerular

filtration (passive process), tubular reabsorption (both passive and transporter mediated process) and tubular secretion (transporter mediated process). Recently, *in silico* modelling approaches have been developed to generate a prediction of renal clearance based on physicochemical properties[7].

Importantly, all the aforementioned ADME processes are highly variable between individuals. PBPK modelling overcomes this, as virtual patients can be simulated considering specific anatomical and physiological factors in populations. Changes in organ size and other anatomical characteristics have indeed been correlated with demographic variables in anthropometric studies[8-10], and multifactorial equations have been defined to generate anatomical and physiological parameters and their inter-individual variability. This set of equations constitutes an essential component of PBPK approaches in order to correctly capture the variability that is present in the population of interest. Through this approach it is possible to generate a virtual (but realistic) description of anatomical characteristics of patients and therefore obtain a representative evaluation of the variability in populations.

This characteristic, as well as numerous others, makes PBPK modelling particularly suitable for the discovery and optimisation of anti-infective agents, which often require access to specific infected tissues or target cells to elicit an effect. As an example, antiretroviral drugs need access to immune cells, the primary target of the human immunodeficiency virus (HIV). Additionally, non-linear concentration-efficacy relationships have been reported for ciprofloxacin[11] and voriconazole[12], and the non-linear protein binding observed with molecules such as ceftriaxone[13], cefazolin[14], cefonicid[15], ertapenem[16] and tigecycline[17] could be modelled by providing the mathematical expression of the change in protein binding with varying concentrations of the drug in plasma.

2. Clinical scenarios and therapy optimisation

Patient demographics, genetics, anti-infective exposure, status of the immune system, pathogen characteristics, and adherence may each affect the efficacy of anti-infective therapy. PBPK modelling can simulate clinical scenarios to assess the impact of such factors on the PK of anti-infective drugs with the ultimate goal to optimize therapy. The use of PBPK modelling to simulate clinical scenarios in relation to special populations, genetics, drug interactions, formulations and penetration in tissues is discussed thereafter (Figure 2).

2.1 Special populations

Most pharmacokinetic clinical trials performed during the drug development processes are based on the inclusion of healthy volunteers and often exclude subjects with specific conditions and characteristics. Subpopulations of patients such as pregnant women, children and infants, cirrhotic and HIV/HCV co-infected patients, elderly, obese and malnourished individuals have been largely underrepresented in clinical trials. As a consequence, the optimization of therapies is particularly challenging in these special populations due to the paucity of relevant pharmacokinetic data. The pharmacokinetics of certain drugs is known to be substantially affected by anatomical and physiological characteristics of special populations, as summarised in Table 1. Alterations in pharmacokinetic characteristics of anti-infectives are have been identified in special populations, such as the increased plasma concentrations observed in paediatric patients administered with cyclosporine, and the increased renal clearance of amoxicillin observed in pregnant women[18-20]. Through the incorporation of anatomical characteristics of special populations, PBPK modelling can predict anti-infective distribution in these subpopulations of interest and thus enables a better understanding of the relationship between anatomical factors and pharmacokinetics.

The aging process is characterised by progressive changes in several anthropometric variables and changes in the expression of key ADME enzymes and transporters[21]. Several classes of drugs, including anti-infectives, are more frequently prescribed to elderly compared to younger individuals. Consequently, the management of therapies in older patients is further complicated by a complex polypharmacy which increases the risk of potential DDIs, toxicity and loss of efficacy[22]. A comprehensive database describing the effect of age on relevant factors for drug distribution has been recently published and represents a valuable tool to define a realistic set of parameters for PBPK simulations in older patients[23]. A first example of PBPK modelling for dose finding and clinical trials in elderly populations has been recently presented[24].

The optimisation of dosing strategies in paediatric patients is complicated by several ethical and pharmacological factors but also by the absence of optimal formulations. Dose finding studies are rarely performed in this population; often the selection of therapeutic doses for children and infants is based on empirical scaling from adults where the dose is adjustment for body weight. However, the ontogeny of metabolic enzyme and transporter expression is not linearly correlated with age and, consequently, a direct dose scaling for children does not represent an optimal strategy in most cases. Moreover, physiological changes are more prominent for infants, further complicating the selection of doses in this frail special population[25]. The description of metabolic enzyme ontogeny in the different stages of childhood is available and has been included in PBPK approaches for simulation of pharmacokinetics in paediatric patients[26]. The optimisation of anti-infective therapies in infants and children could greatly benefit from a broader application of PBPK models, considering the clinical relevance of effective pharmacological tools to treat infections in paediatric patients. Although treatment of paediatric HIV patients results in several short and long term clinical benefits, available clinical options are limited. PBPK modelling has been

effectively applied in the simulation of antiretroviral pharmacokinetics in children, hypothesising dose optimisation based on genetic factors and weight[27].

Obesity is characterised by numerous anatomical changes that alter anti-infectives disposition with potential downstream effects on drug efficacy and toxicity. The risk of nosocomial infections is higher in this subpopulation of patients and can be associated with the development of resistances due to suboptimal anti-infective dosing[28]. The changes in organ composition, tissue volume, cytochrome P450 expression and blood flow have been mathematically described and successfully included in PBPK simulations suggesting dose adjustments and identifying patients with an higher risk of sub-therapeutic concentrations[29, 30]. For instance, the antiretroviral efavirenz, when used at the standard 600 mg once daily dose, was shown not to achieve adequate plasma exposure in obese patients.

An additional special population that require dose adjustment and optimization of therapeutic strategies is pregnant women. During pregnancy, several physiological changes are occurring and the correct dosing of anti-infectives acquires extreme relevance considering the potential exposure of the foetus to life-threatening infections and/or drug related toxicities. The first PBPK model simulating drug distribution in pregnant women and foetus was published in 1994 and subsequent studies have followed[31-33]. This approach has the potential to define optimal therapeutic options for pregnant women infected by pathogens.

In several ways, disease groups can themselves be treated as special populations. An important factor in the optimisation of anti-infectives is the understanding of how the progression of disease affects the physiological characteristics of the patient important for drug disposition. Due to the heavy nvolvement of the liver in drug metabolism and elimination, diseases which alter the physiological state of the liver, such as viral hepatitis, have been investigated for their effect on liver-based drug metabolism enzymes and

transporters. The expression levels of numerous cytochrome P450 (CYP) and phase II enzymes (CYP1A2, CYP2E1, CYP2D6, UGT1A) and transporters (ABCB1, ABCC2, ABCC3, SLC10A1, SLC22A1) in hepatitis C patients were shown to decrease as the severity of liver fibrosis, or fibrosis stage (F), increased[34]. Other studies have also concluded that hepatitis, particularly in cases with more severe liver damage, can alter the expression level of metabolism enzymes, and drug transporters[35-39]. Not all cases show reduced expression, with a recent study showing the up-regulation of transporter ABCC4 and enzyme CYP1B1 in patients with end-stage liver disease[40]. Additionally, nuclear receptors which are involved in the regulation of liver enzyme and transporter expression, such as the aryl hydrocarbon receptor (Ahr), the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR), show reduced expression in hepatitis C patients with fibrosis development[34]. The pharmacokinetic parameters of ribavirin have been assessed in hepatitis C patients using population pharmacokinetic modelling. Although no PBPK models have been created which simulate the reduced abundance of enzymes and transporters in hepatitis C patients displaying various levels of liver damage, this would be achievable by adjusting the amount of enzyme/transporter expressed per mg of liver. The PBPK models created could then be used to predict the impact of the liver damage on drug clearance rate. An additional factor to consider adjusting would be hepatic blood flow, an important factor in the determination of drug clearance and which reduces in patients with chronic hepatitis C infection[41].

2.2 Genetics

The expression and activity of metabolic enzymes and transporters involved in ADME are influenced by genetic variants which can have a relevant downstream effect on PK. Phase I and Phase II metabolic enzymes have been investigated using *in vitro* and clinical pharmacogenetic studies and a broad knowledge describing the correlation between genetics and PK is currently available [3]. Numerous anti-infectives are metabolised or eliminated by polymorphic enzymes and therefore pharmacogenetics may influence efficacy and toxicity in patients.

The influence of genetic variables on anti-infective PK can be predicted through PBPK modelling. *In vitro* systems can be utilised to clarify how polymorphisms alter expression and activity of transporters and drug metabolizing enzymes, and these investigations can be included in PBPK models. This approach has been applied to clarify the impact of CYP2B6 genetics on efavirenz pharmacokinetics and to help hypothesise potential pharmacogenetics-driven dose adjustment strategies[42, 43]. For instance, it was demonstrated that the dose amount of efavirenz, which is normally administered at 600 mg once-daily, can be decreased to 400 mg once-daily in HIV-infected individuals carrying the CYP2B6 516GT genotype (heterozygous mutation associated with a lower metabolizing activity of CYP2B6) and to 200 mg once daily in carriers of 516TT genotype (homozygous mutation) without compromising HIV virological suppression. Another disease area that could benefit from an application of PBPK modelling is fungal infections. Voriconazole is an antifungal with a broad spectrum of activity and is the treatment of choice for invasive aspergillosis and oesophageal candidiasis. The pharmacokinetics of this drug is highly variable between patients and this can cause suboptimal exposure or concentration dependent toxicity[44]. Voriconazole is mainly metabolised by CYP2C19, which is characterised by different genetic variants that can influence the activity of the enzyme. The CYP2C19*2 and *3 alleles have been correlated

with a “poor metaboliser” phenotype and other genotypes, such as CYP2C19*17, have been associated with increased enzymatic activity[45, 46]. Dose adjustment strategies for voriconazole may constitute an effective intervention to limit sub-optimal pharmacokinetics and PBPK models could support the selection of potential pharmacogenetics-driven dose individualisation strategies.

2.3 Drug-drug interactions

Pharmacokinetic DDIs occur when the exposure of a drug (victim) is impaired by the co-administration of a drug inhibiting or inducing (perpetrator) the metabolic pathway responsible for the elimination of the victim drug. DDIs are of major concern in the clinical practice as they may impair drug efficacy or precipitate toxicity and, in case of life-threatening adverse events, may contribute to the withdrawal of a drug from the market[47]. Thus, the evaluation of a drug’s potential for DDIs has become essential during the process of preclinical drug development. Regulatory guidelines recommend that the initial risk assessment for metabolic DDIs is done using *in vitro* studies to investigate whether the investigational drug inhibits or induces the cytochrome P450 (CYP) enzymes[48, 49]. *In vitro* data are subsequently integrated in mathematical models to evaluate the *in vivo* risk of inhibition or induction and thereby the need for conducting clinical DDI studies[50].

In the recent years, approaches to DDI prediction have evolved from the use of single equations to the use of software tools which integrate physiological and drug parameters together with a dynamic model to describe pharmacokinetics in humans[51]. Such PBPK models incorporate the temporal changes in the concentration of the perpetrator and victim drugs and thus allow simulations of concentration-time profiles for the inhibition or induction[52-55]. Furthermore, the PBPK approach enables the user to assess the effect of

various parameters (i.e. dosing regimen, dose staggering, concurrent inhibition and induction of multiple CYPs) on the magnitude of DDIs. Thus, this approach provides a more comprehensive and precise report of DDIs[56, 57]. Finally, the inclusion of the inter-individual variability in CYP expression arising from genetic, demographic or pathophysiological differences assists in defining the extent of the interaction magnitude at the extremes of the population[58]. The regulatory guidelines were recently updated to include the use of PBPK modelling at different stages of drug development with the purpose of assessing the potential for DDIs (early stage), to update initial PBPK models once more when *in vivo* data are available (late stage) and to inform the design of *in vivo* DDI studies (at all stages)[49].

Given their well characterized effects on CYP3A, anti-infective agents have often been used in PBPK models. Such models aim to elucidate the mechanism and time course of DDIs observed in clinical studies[59-61], to build mechanistic models[54, 55] or to inform the design of clinical drug interaction studies (i.e. determination of the timing of administration of the perpetrator drug to achieve the maximal inhibitory or inductive effect)[57, 62-64]. For instance, ketoconazole, a reversible inhibitor of CYP3A and P-glycoprotein, has been used to assess the interaction with a tyrosine kinase inhibitor. The magnitude of the interaction was first determined in a clinical study in healthy volunteers who received the investigational drug alone and in presence of ketoconazole. The simulation of the DDIs using a PBPK model showed that the inhibition of P-glycoprotein by ketoconazole must be taken into consideration, in addition to CYP3A inhibition, to fully explain the magnitude of the observed DDI[61]. Time-dependent inhibitors such as clarithromycin and telithromycin have been used to build mechanistic models able to simulate their non-linear pharmacokinetics and the related effect on the clearance of the victim drug midazolam[54, 55]. The elaboration of such mechanistic models is of interest as they may provide a framework for the prediction of other time-dependent DDIs. Finally, ketoconazole and rifampicin, a potent inducer of CYP3A,

have been used in PBPK models to inform about the maximal inhibitory and inductive effect of CYP3A, respectively, and thereby inform the design of DDI studies[57, 62]. For instance, Zhao et al. showed that a single dose of ketoconazole resulted in maximal inhibition for CYP3A substrates with short half-life and low bioavailability. Conversely, multiple doses of ketoconazole were required to achieve maximal inhibition for CYP3A substrates with long half-life. Whereas, a more recent study seems to indicate that multiple doses of ketoconazole are needed to reach maximal inhibition independently of the victim drug half-life[63]. Baneyx et al. showed that the maximal inductive effect was achieved with rifampicin pretreatment for five days and the administration of the victim drug at least two hours after the last rifampicin dose. Collectively, such simulations are important as a suboptimal inhibition or induction can lead to the underestimation of the DDI magnitude with a given CYP3A substrate. Rifampicin has also been used to evaluate the interplay between CYP3A4 and the hepatic uptake transporter OATP1B1 and its impact on repaglinide exposure[65]. The PBPK modelling showed that the opposite effects of rifampicin on CYP3A4 (induction) and OATP1B1 (inhibition) impacted repaglinide exposure differently depending on the timing of administration of the two drugs. CYP3A4 induction and OATP1B1 inhibition were apparent when both drugs were administered in temporal proximity, whereas CYP3A4 induction was more pronounced when the drugs were administered >12 h apart. Thus, mechanistic models should also take into account transporters in order to accurately predict DDIs, especially for drugs such as repaglinide whose systemic clearance is impacted by both the hepatic uptake and the metabolism.

In the field of DDI prediction, PBPK models have been useful to simulate virtual clinical studies in order to characterize DDIs for drug combinations used in the clinical practice but for which limited clinical data are available. For instance, PBPK models simulating virtual clinical trials were applied to predict the magnitude of DDIs between efavirenz or boosted

protease inhibitors and commonly prescribed antidepressants[56]. These antiretroviral drugs are characterized by mixed inhibitory/inductive effects on CYPs. The approach consisted to initially build mechanistic models, and to subsequently validate their robustness by comparing the magnitude of simulated DDIs using classical probe drugs to that observed in clinical studies. These models were then applied to simulate the magnitude of DDI that would be observed if the investigated antiretroviral drugs were given in individuals during 14 days followed by 8 days of concomitant administration with a given antidepressant. By taking into account the concurrent inhibitory and inductive effect of antiretroviral drugs on CYPs, the PBPK simulation showed that the magnitude of DDIs with antidepressants was overall weak to moderate. The modest magnitude has been attributed to the fact that antidepressants are substrates of multiple isoforms and thus metabolism can still occur through CYP that are weakly impacted by efavirenz or boosted protease inhibitors.

Simulations of virtual clinical trials have not only been used to quantify a DDI but also to determine the dose adjustment to overcome a given interaction[66]. For instance, antimalarial drugs are often used concomitantly with antiretroviral drugs in African countries to treat co-infected patients. Such drug combinations are difficult to handle as antimalarial drugs are susceptible to DDIs whereas a suboptimal drug exposure can lead to treatment failure and drug resistance. A PBPK model was used to simulate the magnitude of the DDI between efavirenz (600 mg once daily) and artemether (80 mg twice daily) in virtual subjects. Efavirenz was shown to reduce artemether area under the curve (AUC) by 80%, tripling the dose of artemether enabled to compensate efavirenz inductive effect. Simulations in a virtual population were also performed to evaluate the magnitude of the DDI between rifampicin and efavirenz based on the body weight and CYP2B6 genotype. This study aimed to define the weight cut-off requiring an increase in efavirenz dose to counteract the interaction with rifampicin. The results showed that an increase in efavirenz dose to 800 mg was appropriate

only in individuals with a body weight over 50 kg[67]. Finally, simulations in virtual individuals were done to provide recommendations on how to switch antifungal drugs given the presence of residual CYP inhibition. The switch from fluconazole (CYP2C19 inhibitor) to voriconazole (substrate of CYP2C19) was simulated using various lag times during treatment. This study showed that fluconazole would continue to have an inhibitory effect on voriconazole for at least 24 hours after its discontinuation[68]. Collectively, these studies show that the use of PBPK modelling to simulate clinical scenarios has the potential to provide answers to specific clinical questions which may be difficult to study in patients or for which data are lacking. Some examples of clinically relevant scenarios involving anti-infective drugs for which PBPK modelling could potentially help optimizing therapy include:

- The simulation of the magnitude of DDIs between antiretroviral agents and direct acting antiviral agents for hepatitis C virus infection considering different stage of liver disease and determination of the related dosage requirement.
- The simulation of the magnitude of DDIs for first-line drugs used to treat simultaneously HIV and tuberculosis and/or malaria.
- The simulation of the magnitude of DDIs between antiretroviral agents and anticancer agents, as such data are difficult to obtain from clinical studies.

It is important to highlight that the accuracy of DDI prediction depends not only on the PBPK model but also on the data inserted in the model. Detailed investigations may be required to simulate physiological processes occurring both under normal and pathological conditions. For instance, it is well known that inflammatory conditions caused by infections can alter drug disposition processes and thereby impact the magnitude of DDIs[69]. While some processes are well characterized, others are poorly described, which may cause the model to erroneously predict the pharmacokinetics of some drugs. In addition, the inclusion of drug transporters may improve the prediction of the models to some extent, although

many challenges remain in this area. For instance, more data are needed to better define the interplay between CYPs and transporters. Other challenges include the species differences in the substrate specificity, tissue distribution and relative abundance of drug transporters. These differences complicate the extrapolation of animal data in humans to quantitatively predict the impact of transporters on DDIs[70]. Finally, another area that requires improvement is the integration of extra-hepatic or non-CYP-related metabolism in the PBPK model to predict DDIs.

2.4 Formulations

Many anti-infective drugs are administered orally and are therefore subject to several environmental factors dictating the extent and rate of oral absorption. These factors can include pH-dependent solubility, the formation of insoluble complexes with gastrointestinal contents, instability in the gastrointestinal environment and altered transit time[71, 72]. To limit the detrimental effects of these factors, drug formulations can be utilised to control the release rate of orally-administered drugs in the gastrointestinal lumen, allowing for optimal absorption. Specialised dosing strategies can include delayed-release formulations, such as the use of enteric-coated tablets which protect the drug from the acidic environment of the stomach by preventing dissolution at lower pH[73, 74]. Extended-release formulations also exist and are often used to reduce C_{max} (decrease host toxicity) and increase the overall exposure time (improve drug effectiveness)[75]. Drug absorption into the blood circulatory system depends upon the delivery of drug particles or solution to the site of absorption. This applies not only to oral absorption but also to other non-intravenous routes of administration, such as sub-cutaneous, intra-peritoneal, transdermal, trans-ocular, intra-muscular and pulmonary. Release of drug from the formulation at these sites will depend on multiple factors, such as

local pH, tissue fat content and protein constitution. The optimum route of administration for a drug is often difficult to determine, and PBPK modelling combined with in vitro experimentation can help predict exposure levels[76, 77].

When optimising PBPK models it may be necessary to determine how a formulation interacts with its environment. Specifically, the drug release rate, the existence of delayed release and the ability of excipients to alter the properties of the drug or environment are all potential factors to be integrated. The Advanced Dissolution, Absorption and Metabolism (ADAM) model was created to take into account the release characteristics of free drug from orally-administered formulations, as well as to include the intestinal metabolism and active transport of drug[78]. There are published PBPK models which specifically investigate formulation-dissolution-related issues for anti-infectives. For example, the pH-dependent dissolution rate of 400 mg film-coated tablets of raltegravir, an anti-HIV integrase inhibitor, was determined *in vitro* by our group and subsequently included in a PBPK model to predict the effects of altered gastrointestinal pH on the rate and extent of drug absorption[79]. The model predictions fell within the range of clinical PK profiles and supported previous data showing increased raltegravir plasma concentrations when co-administered with acid-reducing agents[80]. Furthermore, the model in combination with *in vitro* studies successfully simulated the reduced oral absorption of raltegravir due to the binding of drug to divalent metals present in certain antacids[81], which has also been observed clinically[82]. This has led to the design of a human trial investigating the use of raltegravir with an antacid containing only monovalent metals, which is unlikely to result in a significant interaction.

2.5 Penetration in tissues

In many cases a microbial disease can reside in tissues other than the circulatory system, where sufficient penetration of an anti-infective drug into the target tissue(s) can be essential for treatment success. The life cycle of the malaria parasite occurs in erythrocytes and hepatocytes, therefore few potential physiological barriers should exist in achieving high drug concentrations at these sites[83]. However, several infections reside in tissues where drug penetration is more difficult to achieve. Mycobacterium tuberculosis infects macrophages, which can then migrate to tissues across the body, primarily infecting the lungs but also to other major organs such as spleen, liver and kidneys[84]. HIV primarily infects CD4+ T cells and macrophages which can reside in viral sanctuary sites (locations in the body where antiretrovirals cannot sufficiently penetrate to prevent viral replication). These sanctuary sites can include but are not limited to the brain, genital tract, gut-associated lymphoid tissue and peripheral lymph nodes[85]. The penetration of drugs in these sites is essential for the complete inhibition of viral replication in the body. Antifungal drugs are used to treat fungal infections throughout the body, although tissue distribution of these drugs can vary greatly. For example, in humans fluconazole showed higher cerebrospinal fluid (CSF) concentrations (52-82% achieved in plasma) than itraconazole (<10% achieved in plasma) which can be understood by comparing the properties of fluconazole (10% plasma protein binding, log P of 2.17) and itraconazole (98% plasma protein binding, log P of 6.99)[86]. The brain is often a target for anti-infectives, but achieving effective drug concentrations at this site may be impeded by the blood brain barrier (BBB). The BBB is a selective permeation barrier that separates the extracellular fluid of the brain from the blood circulation[87].

In these and other cases, direct measurement of drug concentrations in tissue, rather than in plasma, may give more meaningful information when linking drug exposure to efficacy[88]. However, measuring drug concentrations in human tissue can be impractical, and surrogate

animal models are generally regarded as poor predictors of drug tissue distribution in humans[89]. A further factor to consider is that some anti-infectives, such as the majority of antibiotics, require access to the interstitial space fluid (ISF) to elicit an effect, whereas other anti-infectives, such as all but a couple of anti-HIV drugs, require intracellular access. Methods using tissue homogenate to determine drug concentrations are therefore unable to differentiate between drug in ISF and cellular compartments, which may lead to erroneous predictions[90]. PBPK modelling provides a useful and flexible strategy to overcome the above issues.

PBPK models, besides predicting drug plasma exposure, can also simulate the penetration of drug in individual tissues. Only the free drug in the systemic circulation is assumed to be able to move from blood into tissues. This process is influenced by the amount of unbound drug in the plasma (fu_p) and also by the blood-to-plasma ratio (B:P). Using this approach prior to *in vivo* studies, the steady state volume of distribution (V_{ss}) and the affinity of drug for penetrating each tissue compartment can be estimated using a PBPK model which incorporates drug-parameters (Log $P_{O:W\ (i.e.\ octanol:\ water)}$, pK_a, fu_p, B:P) with tissue volumes and composition (fraction of tissue consisting of water, neutral lipids and phospholipids)[91]. The general assumption is that a drug with moderately high log P, a lack of charge at physiological pH, a high fu_p and a low B:P has favourable characteristics for tissue penetration. The movement of drug into the tissue is calculated using either perfusion-limited mechanisms, which assumes an instant ratio is reached in drug concentrations between flowing blood and corresponding tissue, or permeability-limited mechanisms, where the cell membrane and interstitial fluid provide additional barriers to drug movement[92]. The majority of PBPK models utilise perfusion-limited mechanisms for determining drug distribution, whereas PBPK models of large hydrophilic drugs and protein often utilise permeation-limited mechanisms. Each tissue generally has its unique selection and expression levels of drug

455 metabolising enzymes (both perfusion-limited and permeation-limited) and drug transporting
456 proteins (permeation-limited), and this information can be included in PBPK models
457 provided that these proteins are believed to influence drug disposition. The expression level
458 and functionality of drug metabolising enzymes and drug transporters can be influenced by
459 drug-drug interactions, genetics, disease state and other factors discussed elsewhere in the
460 review, adding further complexities to the accurate prediction of drug tissue penetration.

461 When a tissue is of particular importance for the efficacy and/or toxicity of a drug,
462 specialised PBPK models with detailed anatomical sub-compartments can be created. A
463 PBPK model has been published to predict regional brain PK of acetaminophen in which the
464 transfer of drug was determined between sub-compartments of CSF, brain extracellular fluid
465 (ECF) and brain intracellular space (ICS)[93]. This allowed for the accurate matching of *in vivo*
466 data where acetaminophen concentrations were around 4-fold higher in CSF compared to
467 brain ECF. In specific cases, the interstitial space within tissues can be an important target
468 site for anti-infective drugs which operate outside the cell, such as for the hydrophilic β-
469 lactam antibiotics and moxifloxacin[94-96]. As an example, a PBPK model of β-lactam
470 antibiotics was produced which included both the interstitial and intracellular portions of
471 tissues. This was shown to have a small but significant effect of drug distribution
472 calculations[94].

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3. Integration of disease-treatment relationships into PBPK models: the phenomenon of PBPK/PD modelling

When developing anti-infective drugs it can be crucially important that the pharmacodynamic characteristics of candidates are accurately assessed and compared, in order to select for lead candidates. This is usually performed *in vitro*, with the minimum inhibitory concentration (MIC) generally being used as a standard measurement for activity of antibiotics and antifungals. Although the exact definition of MIC can differ depending on disease, for most antibiotics the MIC is defined as the minimum concentration of drug required to prevent visible growth of a target organism following 24 hours incubation at 35˚C using standard inoculums (around 0.5 to 5 million CFU/mL). This method gives no information on the time course of antibacterial activity or if the activity is bactericidal (kills pathogen) or bacteriostatic (inhibits pathogen reproduction) in nature, therefore time-kill experiments are often performed following the determination of MIC. The most common *in vitro* model used to assess antibacterial action is the Hollow Fibre model, which uses tubular fibers in a cartridge through which drug-containing medium is pumped[97]. Other approaches include biofilm models and animal infection models[98, 99]. Once MIC has been established *in vitro*, the following PK/PD indices have been found to be useful in estimating in vivo efficacy:

1) T>MIC: the time (T) of exposure of microbe to plasma concentrations exceeding the MIC.
2) C_{max}/MIC: the ratio of maximum plasma concentration (C_{max}) to MIC.
3) AUC/MIC: the ratio of area under the the plasma concentration curve (AUC) to MIC.

Antibiotics are generally classed as having an activity which is time-dependent (T>MIC), concentration-dependent (C_{max}/MIC) or dependent on both time of exposure and concentration (AUC/MIC)[100]. In contrast to this system, the assessment of antiviral PD is

often complicated by the fact that in many cases an *in vitro* screening method does not exist or is not fully representative of the *in vivo* situation. Viral replication relies on the sequestration of host cell machinery, and all viral infections have an intra-cellular component, which both make developing effective antiviral therapies more difficult. For assessment of anti-HIV drugs, the reduction in HIV RNA, DNA or protein can be measured in a system using HIV-infected immortalised or ex vivo CD4+ immunological cells and used to produce a concentration-efficacy relationship such as EC_{50} or EC_{95}.

As discussed above, standard PBPK modelling allows for an understanding of factors that affect the ADME properties of a drug. In addition, information can be included detailing the efficacy, toxicity and inhibitory/induction potential of a drug[101]. If a concentration-effect relationship can also be established, then this allows for the creation of an integrated PBPK/PD model. The lack of effective *in vitro* PD screening methods for many infectious diseases has impeded the widespread use of this system, although there are published examples which have integrated PD data into compartmental modelling. A simple 2-compartmental PK/PD model was created for the anti-HIV drug bevirimat, where a dose-dependent relationship between drug plasma concentrations and viral load could be used to predict necessary doses for viral suppression in humans[102]. A semi-mechanistic PK/PD model was published by Nielsen et al which assessed the activity of antibacterial agents on *Streptococcus pyogenes*[103]. Time-kill values were determined *in vitro* for benzylpenicillin, cefuroxime, erythromycin, moxifloxacin, and vancomycin, and used to create a maximum effect (E_{max}) PD model. The natural rate of bacterial growth and death was included, in addition to drug-induced death. The attached PK model only predicted the chemical degradation of otherwise static drug concentrations; therefore this model had limited use for optimisation of antibacterial treatments in patients. An improved PK/PD model was developed, where a multi-compartment (central and peripheral) system allowed for

distribution and elimination of the drug, and also allowed for delayed drug action and the development of drug resistance[104, 105].

Physiologically-based parameters were not included in the above mathematical PK models. As discussed previously, without this information it is not possible to simulate the distribution of anti-infectives into specific tissues, which are important targets for numerous diseases residing outside of the circulatory system. As can be seen, important steps have been made in PK/PD model design, but there are clear benefits to working towards "whole body" infection models, where the distribution and actions of both the infection and the treatment can be simulated for optimisation.

4. Conclusion

The PBPK approach gives the opportunity of integrating *in vitro* experimental data in a mathematical description of human anatomy and physiology for the simulation of drug pharmacokinetics. Overall, this predictive tool can have relevant use for the prediction of anti-infective pharmacology and efficacy, giving insight of their distribution during the development process as well as finding application in the simulation of relevant clinical scenarios (Figure 3). In this review we have described some of the most recent studies conducted using PBPK, and hypothesised future innovative applications for a more effective use of anti-infectives not only in the average patients but also for special subpopulations of infected individuals. There are useful PD models developed using *in vitro* and *in vivo* approaches which can inform PBPK models of "target" concentrations in specific tissues to achieve treatment success, although much work remains to be done on improving our knowledge of PK-PD relationships in most diseases.

5. Expert opinion

There is an urgent requirement for novel treatment strategies of infectious diseases. The treatments of many infections remain ineffective and can often result in concentration-dependent host toxicity. Anti-infective resistance development is not a minor concern and should be acknowledged as a global health crisis, as is emphasised by the current alarming spread of drug-resistant Mycobacterium tuberculosis and malaria[106]. Consequently, new drugs need to be developed which are effective against resistant strains of infection. Furthermore, in cases where special patient populations have sub-optimal drug exposure due to alterations in physiology, the pharmacokinetics of currently used anti-infectives should be improved to prevent drug resistance development. PBPK modelling provides a powerful tool in our attempts to tackle the above issues, and both industry and regulatory bodies have recognised the importance of this technology. Between 2008 and 2012, the FDA received submissions for 18 investigational new drugs (IND) and 16 new drug applications (NDA) which included PBPK modelling [107]. Of these 33 IND/NDA cases, the majority of models (61%) were used to investigate drug-drug interactions, with the remaining models investigating paediatrics (18%), absorption (9%), hepatic impairment (6%), pharmacogenetics (3%) and a combination of pharmacogenetics and drug-drug interactions (3%). Additionally, the FDA increasingly use PBPK modelling in reviews of clinical scenarios, with 16 cases recorded between 2009 and 2012. Any definitive measure of model adequacy for PBPK in clinical pharmacology is yet to be defined and will necessarily vary from case to case, depending on factors such as the therapeutic window of the investigational drug. However, the FDA has outlined the essential information of a PBPK analysis needed in a regulatory submission, and these fundamental questions need to be addressed by the

applicant (17). Does the model use system- and drug-dependent parameters which are based on accepted physiology? Have input parameters been produced from reliable and reproducible data, or, when assumptions are made, can they be justified? Is the model able to effectively predict existing in vivo data? Does the model contain all necessary parameters to address known PK-influencing factors for a specific drug? Regarding PBPK modelling of anti-infectives specifically, many more questions would require to be addressed in specific cases, and this regulatory assessment of PBPK models is a constantly evolving process.

It should be acknowledged that major challenges remain to be addressed if PBPK modelling is to be increasingly used in anti-infective research. Diseases can alter the physiological characteristics of patients, which can in turn change the disposition and effectiveness of anti-infective drugs. To give examples, HIV-infected patients, particularly those with advanced disease progression, have higher gastric pH than uninfected individuals and this may explain why acid-reducing agents show reduced impact on the absorption of antiretrovirals with pH-dependent solubility, such as raltegravir, in HIV-infected patients[108]. Numerous bacterial and viral infections have been found to alter expression levels of drug metabolising enzymes and transporters, although the majority of these studies have been performed in animals[109]. In reality, for the purposes of constructing PBPK models, an understanding of relevant systems parameters are lacking in many disease groups and it is essential that pharmacologists, physiologists and clinicians collaborate to address these knowledge gaps. An extra complication can arise in the development of fully comprehensive anti-infective PBPK/PD models in simulated disease groups: most infections would not act as a benign, static factor in the model. Infection replication and death rates may need to be accounted for, and an infection may display variability in genetics and phenotype within a “population” which is relevant for treatment success. Preferences of some infections for specific tissues and the ability of infectious agents to spread to new locations would complicate the production of

PK/PD relationships. Furthermore, infections can often exist in different forms during a lifecycle, some of which show varying sensitivity to treatment, for example in the case of anti-malarial drugs which are not effective against the life-cycle stage involving the liver. Investigations into these factors provide great challenges, due to the absence in most cases of models for determining infection dynamics. However, regarding the establishment of PK/PD relationships, it is important to aim for simplicity where it is available. In the case of certain antibiotics, a relatively simple correlation has been established linking PK and PD, as is detailed in section 3, and this information is easily included in current PBPK model design.

Regarding the future of *in silico* based "personalised medicine" strategies, the ultimate goal is to achieve a complete picture incorporating the system of both the specific patient and the disease, and to combine this with the pharmacodynamics and toxicological characteristics of the anti-infective. Considering that there is a paucity of information available to construct these complete PBPK-PD models, a factor to consider is education: in order to attract researchers to the area of PBPK-PD model development, it is important that users are able to understand the underlining principals and the science behind modelling. The authors believe that this learning process should be initiated early in the development of future pharmacologists, and have included PBPK modelling theory and technique in educational programs.

621 **Table 1**. Main physiological and anatomical changes in special populations and their

622 potential effect on PK

Special populations	Anatomical and physiological factors	Potential effect on PK
Elderly [110, 111]	Increased gastric pH	Decreased absorption
	Delayed gastric emptying time	Slower absorption
	Decreased splanchnic blood flow	
	Decreased gastro-intestinal mobility	
	Decreased absorption surface	Decreased absorption
	Reduced expression of intestinal enzymes and transporters	Increased absorption
	Increased adiposity	Higher distribution
	Lower lean body mass and total body water	
	Decreased albumin, increased α1-acid-glycoprotein	Higher/lower distribution
	Reduced liver weight	Lower clearance
	Decreased hepatic blood flow	
	Changes in the expression of CYP450 isoforms	Lower/higher clearance
	Changes in plasma protein binding	
	Decreased glomerular filtration rate	Lower clearance
	Decreased kidney mass	
	Decreased glomerular surface area	
	Decreased renal blood flow	
Obesity [112, 113]	Increase adipose tissue mass	Higher distribution
	Increased cardiac output and altered hepatic blood flow	Higher distribution and higher/lower clearance
	Increased glomerular filtration and tubular secretion	Higher clearance
	Altered expression of metabolic enzymes	Higher/lower clearance
Pregnancy [114]	Increased cardiac output	Higher distribution and higher clearance
	Increase adipose tissue mass	Higher distribution
	Increased total body water	
	Increased plasma volume	

	Decreased plasma protein levels	Altered protein binding
	Altered expression of metabolic enzymes	Higher/lower clearance

Article highlight box

- The pharmacokinetic and pharmacodynamic properties of anti-infective drugs are often not fully understood but are usually found to relate to treatment success, and the development of new and improved anti-infective agents is essential if improvements in current disease treatment, including the treatment of drug-resistant infectious strains, are to be achieved.
- The optimisation of anti-infective drugs in special populations, such as paediatrics, the elderly, pregnant patients and patients who have co-morbidities, is difficult due to small group sizes and the additional risks associated with experimental treatment.
- Physiologically based pharmacokinetic modelling is a useful tool which allows for the creation of “virtual” populations with defined physiological characteristics and variability, which can then be combined with the physicochemical and biological properties of a drug to simulate drug pharmacokinetic pharmacodynamic characteristics.
- Models can be designed which investigate how drug disposition in the human body is influenced by factors such as genetics, drug-drug interactions, formulation properties, and drug disposition when simulated in special population.
- Physiologically based pharmacokinetic modelling can be combined with the pharmacodynamic properties of an anti-infective agent in order to fully predict the

interaction between the drug and infectious agent in an *in vivo* situation, allowing for the simulation of treatment effectiveness.

- Many areas remain where further research is required to improve the predictive value of physiologically based pharmacokinetic modelling of anti-infectives, including establishing improved correlations between in vitro and in vivo anti-infective pharmacodynamic relationships, and increasing our understanding of physiological changes occurring in patients during disease progression.

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