Physiologically based pharmacokinetic model of docetaxel and interspecies scaling: comparison of simple injection with folate receptor-targeting amphiphilic copolymer-modified liposomes

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Abstract
1. To compare the disposition of docetaxel (DTX) in male/female rats after intravenous administration of simple injection and folate-poly(PEG-cyanoacrylate-co-cholesteryl cyanoacrylate)-modified liposomes utilising a physiologically based pharmacokinetic (PBPK) modelling method, and extrapolate this model to mice and humans by taking into account the interspecies differences in physiological- and chemical-specific parameters.
2. Four structural models for single organs were evaluated, and the whole-body PBPK model included artery, vein, lung, brain, heart, spleen, liver, gastrointestinal tract, kidney, muscle and remainder compartment.
3. Rats following modified liposomes administration were characterised by significant decrease in the partition coefficients for brain, spleen, liver and remainder compartment. The blood-to-plasma partition coefficient also decreased significantly, while a marked rise of partition coefficients for lung, kidney and muscle was revealed. Partition coefficient for heart was approximately 1.3-fold higher in females than males, while the decrease of intestinal clearance was revealed in females compared to males. The final model successfully characterised the time course of DTX in rats, mice and humans.
4. This PBPK model is beneficial to the prediction of the effects of DTX in different species. It also represented a platform to encompass both formulation- and sex-related effects on DTX disposition and elimination in the future.

Keywords
Biodistribution, docetaxel, folate-poly(PEG-cyanoacrylate-co-cholesteryl cyanoacrylate)-modified liposomes, interspecies scaling, physiologically based pharmacokinetic modelling

Introduction
Docetaxel (DTX), the first semisynthetic taxoid, is one of the most active cytotoxic agents prescribed for several kinds of malignancies, such as breast, gastric, prostate and non-small cell lung cancer (Belani, 2005; Engels & Verweij, 2005; Engels et al., 2005; Lyseng-Williamson & Fenton, 2005; Nicolini et al., 2006). In vitro studies showed that DTX enhance microtubule polymerisation and stabilise microtubules against depolymerisation (Gligorov & Lotz, 2004; Yvon et al., 1999). During cell division, DTX action results in the disruption of the functioning of the mitotic spindle, which leads to cell cycle arrest in the G2/M phase, which in turn triggers apoptotic cell death (Fabbri et al., 2006; Woods et al., 1995).

Docetaxel is a highly lipophilic agent, sharing common features with other taxanes (e.g. paclitaxel and larotaxel). Due to its poor solubility in water, DTX is currently dissolved for clinical use in Polysorbate 80 and ethanol (50:50, v/v) as Taxotere®. However, some adverse effects occurred in the majority of patients treated with Taxotere®, for example, myelosuppression, haemolysis, fluid retention and severe hypersensitivity reaction (Esmaeli et al., 2001; Ferraresi et al., 2000; Valero et al., 1995). These adverse effects caused by Taxotere® are a limitation to its clinical use. Consequently, some special formulation approaches need to be taken, such as lipid microspheres, liposomes, nanospheres and folic acid conjugated nanoparticles.

Nanotechnology has been extensively exploited to improve conventional cancer therapy recently (Cho et al., 2008; Farokhzad & Langer, 2009). The nanocarriers, such as liposomes (mean particle size ≤ 120 nm), are currently under intensive investigation. Liposomes are spherical vesicles composed of single or multiple lipid bilayers, and have a number of appealing features, including high biocompatibility, high delivery efficiency and ease of surface modification (Wang et al., 2011; Zhao et al., 2009). The performance of liposome drug delivery systems for tumour therapy could be further improved by using a ligand coupled to the surface of vesicles to achieve an active targeting effect. The selective
overexpression of folate receptors in tumour cells and the high affinity of tumours for folic acid provide a unique opportunity for folic acid to be used as a targeting ligand to deliver therapeutic agents to cancer cells via folate receptor-mediated endocytosis (Leamon & Low, 1991). Therefore, the development of drug-loaded intravenous folate-mediated liposomes should be a worthwhile and promising strategy (Lee & Low, 1995).

Physiologically based pharmacokinetic (PBPK) modelling, treating the body as anatomical compartments connected by blood flow, utilises physiological and chemical-specific parameters, as well as mathematical equations to quantitatively describe the in vivo disposition of xenobiotics (Barrett et al., 2012). Compared with traditional compartmental modelling and non-compartmental analysis, which usually only focus on analysing concentration–time data in plasma, PBPK modelling is a more mechanistic approach for studying xenobiotic disposition (Nestorov, 2007). PBPK modelling is also capable of extrapolating across dose levels, formulations, routes of administration and species (Barrett et al., 2012; Rostami-Hodjegan, 2012). Therefore, one application of PBPK models is predicting xenobiotic exposure in humans based on that in experimental animals. In addition, this type of model may allow for the evaluation of the effects of different factors including ages, diseases, genetics, drug–drug interactions, etc., on xenobiotic disposition (Edginton et al., 2008; Zhao et al., 2011). Combined with pharmacodynamic data, PBPK modelling aids the understanding of therapeutic benefits and adverse effects of drugs, leading to optimised dosage regimens (Khalil & Läer, 2011). Because of these advantageous features, the interest in applying PBPK models in pharmaceutical industries and research academies has been rapidly growing in recent years (Rostami-Hodjegan, 2012).

Pharmacokinetic studies of DTX have been conducted in mice, rats and humans (Figg et al., 2010; Wang et al., 2014; Zhao et al., 2013). However, it seemed valuable to develop a PBPK model for DTX, which could provide further insight into the in vivo disposition of this drug and have potential applications in the design of formulations and clinical studies. We hypothesise that the development of a PBPK model can improve understanding of DTX pharmacokinetics and pharmacodynamics, which may be utilised for evaluating therapy with existing DTX formulations, and guide development of novel formulations and routes of administration. Hence, in this study we developed a PBPK model for DTX in rats. Extrapolation of this rat PBPK model to mice and humans was also performed to examine the utility of the model for predicting the effects of DTX exposure across species.

Materials and methods

Chemicals, reagents and animals
Docetaxel and paclitaxel reference standards (purity >99.0 and 99.5%, respectively) were purchased from the National Institutes for Food and Drug Control (Beijing, China). Methanol of HPLC grade was purchased from Fisher Scientific (Fair Lawn, NJ). Distilled water was prepared from demineralised water throughout the study. Other chemicals were of analytical grade.

Sprague-Dawley (SD) rats (mean weight 250 g) were kindly provided by the Experimental Animal Centre of Shenyang Pharmaceutical University (Shenyang, China). The animal study was carried out in accordance with the Guideline for Animal Experimentation of Shenyang Pharmaceutical University, and the protocol was approved by the Animal Ethics Committee of the institution.

Preparation of DTX solution and DTX-loaded modified liposomes

The preparation of DTX solution was as follows: The drug was supplied as 91.7 mg of DTX in 2.36 mL of polysorbate 80. The solvent was supplied and composed of ethanol/water (13:87, w/w). Before administration, the drug was diluted in 5% glucose maintaining a concentration of drug of about 1.25 mg mL⁻¹.

The synthesis procedure of the copolymers of folate-poly(PEG-cyanoacrylate-co-cholesteryl cyanoacrylate) (FA-PEG-PCHL), the liposome preparation procedure and the characterisation of polymers and liposomes were all detailed in our previous paper (Li et al., 2011). The prepared FA-PEG-PCHL-modified liposomes had a similar particle size distribution, with mean diameter ranging from 111 to 127 nm (111.6 ± 9.6 nm). The entrapment efficiency (%) of modified liposomes was 97.8 ± 1.6. DTX concentration in modified liposome preparation was 1.25 mg mL⁻¹.

Biodistribution of DTX solution and DTX-loaded modified liposomes in rats

A plasma pharmacokinetic study was designed to evaluate the FA-PEG-PCHL-modified liposomes by comparing with DTX solution. Twelve rats were randomly divided into two groups (equal numbers of males and females in each group) with a single dose (5 mg kg⁻¹) of DTX solution and modified liposomes intravenous administration to the two groups of rats, respectively. Blood samples of about 0.3 mL were collected from the fossa orbitalis vein into heparinised centrifuge tubes, at 0.083, 0.17, 0.33, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h. Plasma samples were processed following centrifugation at 12 000 rpm for 5 min and then stored at −80°C until analysis.

The tissue distribution study was carried out on rats which were randomly divided into six groups with six rats in each group (equal numbers of males and females). DTX solution/modified liposomes were given intravenously at a dose of 5 mg kg⁻¹ via the caudal vein. After administration, the rats were killed at 0.17, 2 and 6 h, and organs (lungs, brain, heart, spleen, liver, intestine, kidneys and muscle) were collected at the same time. The tissue samples were rinsed in ice-cold normal saline, blotted dry with filter paper, and then stored at −80°C until analysis.

DTX assay

The quantitative analytical method of DTX in rat plasma/tissues is established based on high performance liquid chromatography equipped with mass spectrometry (HPLC-MS, Shimadzu Co., Nakagyo-ku, Kyoto, Japan) method. Briefly, chromatographic separation was performed using a
Thermo Hypurity C<sub>18</sub>, 5 µm particle size (150 x 4.6 mm, internal diameter, Thermo Fisher Scientific, Waltham, MA) column kept at 35°C with a constant flow rate of 0.8 mL min<sup>-1</sup>. The mobile phase consisted of methanol-0.01% formic acid (82:18, v/v). Mass spectrometry detection was performed in the positive ion mode following m/z 830.45 ([M + Na]<sup>+</sup>) for DTX, and 876.50 ([M + Na]<sup>+</sup>) for internal standard (IS, paclitaxel). The IS stock solution was prepared at a concentration of 0.1 mg mL<sup>-1</sup> and further diluted to achieve a final concentration of 5 µg mL<sup>-1</sup> with methanol. About 1.0 g of the tissue sample was accurately weighed and homogenised with 2.0 mL methanol, while about 0.5 g of the tissue sample was taken and homogenised with 1.0 mL methanol if the total weight of the tissue is in the range of 0.5 and 1.0 g. The entire tissue sample was taken and homogenised with 1.0 mL methanol if the total weight of the tissue is less than 0.5 g. A volume of 20 µL methanol and 20 µL IS solution were added into 100 µL of plasma/tissue samples. This mixture was extracted with a volume of 1 mL ether, vortex-mixed for 3 min and centrifuged at 3000 g for 5 min. The upper extract was then evaporated to dryness at 35°C under nitrogen stream. The residue was reconstituted with a 100 µL aliquot of the mobile phase, and a 10 µL aliquot was injected directly onto the HPLC-MS system.

The retention times for DTX and IS were approximately 4.4 and 4.1 min, respectively. No interference from any endogenous substances was observed. The method was linear over the concentration range of 12.5–5000 ng g<sup>-1</sup> for rat tissues (Table S1). The method showed good intra-assay precision and accuracy with relative standard deviation (% RSD) values from 2.71 to 9.44% and mean relative error (% MRE) from −9.62 to 7.60%, as well as good inter-assay precision and accuracy with % RSD from 2.90 to 11.9%. The recoveries for all the bio-samples were over 81.2% (81.2–87.8%). There was no significant matrix effect for all the ratios within the range 96.0–99.8% for all the bio-samples. The results of short-term stability (−8.40 to 4.50%), freeze-thaw stability (−5.50 to 5.80%), auto-sampler stability (−6.60 to 5.00%), and long-term stability (−11.2 to 6.30%) of DTX in all the bio-samples were found to be within the range ±15.0%.

Data sources

A Monte Carlo simulation (MCS) was conducted to generate the plasma and tissue pharmacokinetic data of DTX used for developing the PBPK model. Rat datasets for MCS were found to be within the range ±15.0%. Mass spectrometry detection was performed in the positive ion mode following m/z 830.45 ([M + Na]<sup>+</sup>) for DTX, and 876.50 ([M + Na]<sup>+</sup>) for internal standard (IS, paclitaxel). The IS stock solution was prepared at a concentration of 0.1 mg mL<sup>-1</sup> and further diluted to achieve a final concentration of 5 µg mL<sup>-1</sup> with methanol. About 1.0 g of the tissue sample was accurately weighed and homogenised with 2.0 mL methanol, while about 0.5 g of the tissue sample was taken and homogenised with 1.0 mL methanol if the total weight of the tissue is in the range of 0.5 and 1.0 g. The entire tissue sample was taken and homogenised with 1.0 mL methanol if the total weight of the tissue is less than 0.5 g. A volume of 20 µL methanol and 20 µL IS solution were added into 100 µL of plasma/tissue samples. This mixture was extracted with a volume of 1 mL ether, vortex-mixed for 3 min and centrifuged at 3000 g for 5 min. The upper extract was then evaporated to dryness at 35°C under nitrogen stream. The residue was reconstituted with a 100 µL aliquot of the mobile phase, and a 10 µL aliquot was injected directly onto the HPLC-MS system.

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Data sources

A Monte Carlo simulation (MCS) was conducted to generate the plasma and tissue pharmacokinetic data of DTX used for developing the PBPK model. Rat datasets for MCS were collected not only from the current experimental data, but from routine plasma pharmacokinetic files of rats following a single i.v. injection (5 mg kg<sup>-1</sup>) of DTX (Chen et al., 2013; Wang et al., 2014). DTX plasma levels and tissue pharmacokinetic data were simulated in four populations. Each population was characterised by a different dosage regimen, namely male rats following DTX solution administration, male rats following DTX-loaded FA-PEG-PCHL-modified liposomes administration, female rats following DTX solution administration and female rats following FA-PEG-PCHL-modified liposomes administration. A log-normal distribution (based on the result of Shapiro–Wilk test) was assumed for both plasma and tissue DTX concentrations at each sampling time point. In the MCS procedure, the number of replicates was increased from 10 to 1000 until stable results were achieved. The following process was then repeated from the first to the 1000th subject using Crystal Ball v.7.2.2 (Oracle, Redwood Shores, CA). Lung, brain, heart, spleen, liver, gut, kidney, muscle and plasma DTX concentrations were randomly generated according to each mean value and inter-individual variance of the observed concentrations at each time point. Simulated concentrations out of 1.5 interquartile ranges (IQRs) of the observations were excluded to avoid overestimated values.

Other DTX plasma pharmacokinetic profiles of rats, mice and humans were used to assess intra- or interspecies extrapolation of the PBPK models. A preclinical study by Wonganan and colleagues (2009) reported plasma pharmacokinetic data from 0.083 to 24 h after single i.v. doses (10 mg kg<sup>-1</sup>) of simple DTX injection administered to male SD rats. Another study by Li and co-workers (2011) reported plasma pharmacokinetic profiles following i.v. administration of simple DTX injection and modified liposomes (10 mg kg<sup>-1</sup>) to male SD rats. Moreover, DTX concentration–time data in mouse plasma (Bradshaw-Pierce et al., 2007; Rudek et al., 2014; Wang et al., 2014), as well as plasma pharmacokinetic profile in humans (Bradshaw-Pierce et al., 2007; Eskens et al., 2014; Figg et al., 2010), were all used to assess interspecies extrapolation of the PBPK model. All data were directly extracted from tables or captured by digitisation from figures.

Physiological parameters

Physiological parameters, such as tissue volumes, blood flow rates to different organs, and fractions of vascular space in tissues were fixed to literature values reported in Table 1 (Brown et al., 1997; Davies & Morris, 1993). All tissues that were not sampled were lumped into a remainder compartment. A density of 1 was assumed for all tissues. The average body surface area of humans (mean body weight 70 kg) was set at 1.79 m<sup>2</sup> (Sharma & McNeill, 2009).

Development of the basic PBPK model

The population physiologically based pharmacokinetic (PPBPK) model was built using non-linear mixed-effect modelling in the computer programme NONMEM (version 7.3, Icon Development Solutions, Ellicott City, MD). The first-order conditional estimation with interaction (FOCEI) method within NONMEM was employed throughout the model-building procedure. Development of the PPBPK model started with construction of the base model, including the structural PBPK model and models for the inter-individual and residual variabilities. Once the base model was developed, covariate (COV) models were developed to explain the inter-individual variabilities. The likelihood ratio test (LRT) was used for statistical hypothesis testing to discriminate among alternative base and COV models.

Based on the tissue distribution data available for DTX in rats, the whole-body PBPK model consisted of lung, brain, heart, spleen, liver, gut, kidney, muscle, the remainder compartment, artery and venous blood pool (Figure 1). All the compartments were connected by blood flow. As DTX is extensively eliminated by metabolism (70–80% of the
administered dose) (Baker et al., 2006; Marre et al., 1996), both hepatic (CL_{li}) and intestinal (CL_{gu}) elimination pathways, which were assumed to be involved in the clearance of DTX, were included in the PBPK model.

Development of the PBPK model started with construction of the single-organ models (step 1). The DTX plasma concentration–time profile was described by an explicit bi-exponential equation and four different structural models for organs (model a to d) were evaluated independently (Figure 2). In model a, the organ was represented as a single “well-stirred” compartment (where the plasma DTX concentration is considered to be in instant equilibrium with the tissue concentration) and characterised by a tissue-to-blood (blood-to-plasma for artery and venous blood pool) partition coefficient (K_{t, tissue}), assuming that the binding to both tissue and blood occurs in a linear and non-saturable manner. In model b, the organ was divided into two subcompartments (vascular and extravascular) with a permeability-limited distribution (K_{p, tissue}) and K_{t, tissue}. Model c and d, in addition to vascular and extravascular spaces, included an additional “deep tissue” subcompartment that was characterised by first-order association (K_{in, tissue}) and dissociation (K_{out, tissue}) rate constants. Model selection was also performed using a visual inspection of the model-fitted profiles.

Once the single-organ models were all developed, a whole-body PBPK model was constructed subsequently (step 2). Eight organs (lung, brain, heart, spleen, liver, gut, kidney and muscle), which were assumed to have a “well-stirred” structure, were added to the model. The remainder compartment encompassed all non-sampled tissues, and also added to the model as a “well-stirred” structure. The following equations were used to describe the model structure for DTX disposition following single i.v. administration:

Artery (ar):
\[ V_{ar} \times \frac{dC_{ar}}{dt} = K_{t,pl} \times \frac{C_{lu}}{K_{t,lu}} - C_{ar} \times K_{t,pl} \]  

Brain (br), heart (he), spleen (sp), kidney (ki) and muscle (mu):
\[ V_{tissue} \times \frac{dC_{tissue}}{dt} = \frac{Q_{tissue}}{Q_{he}} \times \left( C_{ar} \times K_{t,pl} - C_{tissue} \right) \]  

Liver (li):
\[ V_{li} \times \frac{dC_{li}}{dt} = \left( Q_{li} - Q_{sp} - Q_{gu} \right) \times C_{ar} \times K_{t,pl} + Q_{sp} \times \frac{C_{sp}}{K_{t,sp}} + Q_{gu} \times \frac{C_{gu}}{K_{t,gu}} - Q_{li} \times \frac{C_{lu}}{K_{t,lu}} - CL_{li} \times C_{li} \]  

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**Table 1. Physiological parameters for mouse, rat and human.**

| Tissue   | Mouse | Rat | Human | Mouse | Rat | Human | Mouse | Rat | Human |
|----------|-------|-----|-------|-------|-----|-------|-------|-----|-------|
| Lungs    | 0.73  | 0.50| 0.76  | 100   | 100| 100   | 0.50  | 0.36| 0.33^a|
| Brain    | 1.65  | 0.57| 2.00  | 3.30  | 2.00| 12.0  | 0.03  | 0.03| 0.04  |
| Heart    | 0.50  | 0.33| 0.47  | 6.60  | 4.90| 4.00  | 0.17^b| 0.26| 0.17^b|
| Spleen   | 0.35  | 0.20| 0.26  | 1.12^c| 0.85^c| 1.38^c| 0.17   | 0.22| 0.30^d|
| Liver    | 5.49  | 3.66| 2.57  | 16.1^e| 18.3^f| 22.7^f| 0.31   | 0.21| 0.11  |
| Gut      | 4.22  | 2.70| 1.71  | 12.9  | 14.3| 16.7  | 0.04   | 0.04| 0.04  |
| Kidneys  | 1.67  | 0.73| 0.44  | 9.10  | 14.1| 18.0  | 0.24   | 0.16| 0.36  |
| Muscle   | 50.0  | 49.0| 50.0  | 11.4  | 10.1| 13.4  | 0.04   | 0.04| 0.01  |
| Rest of the body | 30.5^i| 34.9^i| 33.9^i| 53.5^i| 50.6^i| 29.9^i| 0.04   | 0.04| 0.04  |
| Blood    | 4.90  | 7.40| 7.90  | 100   | 100| 100   | –     | –   | –     |

*Mean value from mouse, rat and dog.
^Mean value from rat and dog.
^Mean value from mouse, rat and dog.
Calculated as body weight subtract sum of weight of lungs, brain, heart, spleen, liver, gut, kidneys and muscle.
Calculated as 100 subtract sum of blood flow of brain, heart, liver, kidneys and muscle.

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**Figure 1. Schematic representation of the whole-body PBPK model used to characterise the biodistribution of DTX in rats, mice and humans.**

Q_{co}, blood flow rate to tissues; CL_{tissue}, tissue clearance.
Figure 2. Model structures evaluated in this study. Model a, two subcompartments (vascular and extravascular) with a perfusion-limited distribution ($K_t$, tissue); Model b, two subcompartments (vascular and extravascular) with a permeability-limited distribution ($K_p$, tissue); Model c, three subcompartments (vascular, extravascular, and deep tissue) characterised by perfusion-limited distribution and rates of association with ($K_{in}$) and dissociation from ($K_{out}$) deep tissue subcompartment; Model d, three subcompartments (vascular, extravascular and deep tissue) characterised by permeability-limited distribution and rates of association with ($K_{in}$) and dissociation from ($K_{out}$) deep tissue subcompartment.

Development of the covariate model

The influence of dosage form and sex on DTX PBPK parameters was assessed by visual inspection of the COV and IIV plots (step 3). As the linear models were enough to describe the correlation between the PBPK parameters and the two categorical COVs, only the linear models were tested in this step. Potential COVs were incorporated sequentially into the model in order of significance, whereby the inclusion of the COV was based on a significant drop in the objective function value (OFV), biological plausibility and a drop in the IIV values. COVs selected to enter the intermediate model had to decrease the OFV significantly ($\Delta$OFV > 3.84 or $p$ value < 0.05) from the COV-free model. Addition of COVs to the base model was conducted in a stepwise manner. Finally, in the backward-deletion step, each COV was independently removed from the full-covariate model to confirm its relevance. If the increase in the OFV was not statistically significant ($\Delta$OFV < 6.63 or $p$-value > 0.01), the relationship between this COV and the corresponding PBPK parameter was not taken into account in the final model.

Model qualification and interspecies scaling

All diagnostic graphs and statistical analyses were conducted utilising Matlab software (version 8.2.0.701, MathWorks Corporation, Natick, MA). The robustness of the final PBPK model estimates was assessed using bootstrap analysis (step 4), in which subjects were randomly sampled with replacement from the dataset that was used in model development to obtain 1000 datasets that have the same number of subjects as the original dataset. The mean and variability (as measured in coefficient of variation, % CV) of the parameter estimates from the 1000 runs were then calculated and compared with the point estimates (mean values) obtained with the original dataset.
The adequacy of the final model was appraised by a visual predictive check (step 5). Plots of the median values and 90% confidence interval (CI, 5–95%) of the simulated concentration–time profiles were generated to check that the observed profiles were reasonably contained within this CI. In this step, all parameters estimated during step 1–3 were kept constant, and the predictive power of the PBPK model was assessed using the 10 mg kg\(^{-1}\) dosing datasets. Plasma DTX concentrations after a single i.v. dose of simple injection and modified liposomes in male rats had been measured and were compared against concentrations predicted by the established PBPK model.

The rat PBPK model was then extrapolated to mice and humans, by taking into account the interspecies differences in physiological and chemical-specific parameters (step 6). Rat physiological parameters (organ volume and blood flow) were replaced with corresponding values for mice or humans (Table 1). Chemical-specific \(K_{tissue}\) and \(K_{tp}\) values were assumed to be identical between rats and mice/humans. CL\(_i\) and CL\(_{gu}\) were predicted from the values estimated for rats using an allometric equation (Hu & Hayton, 2001; Hu et al., 2014; Kagan et al., 2011; Sharma & McNeill, 2009):

\[
CL_{mouse/human} = CL_{rat} \times \left(\frac{BW_{mouse/human}}{BW_{rat}}\right)^{0.67}
\]

where CL\(_{rat}\) and CL\(_{mouse/human}\) denote tissue clearance for rats and mice/humans, respectively. BW is species body weight, and 0.67 is the allometric exponent.

**Results**

**Basic PBPK model**

Four competing model structures (Figure 2) were evaluated for plasma and all tissues using bi-exponential driving functions for DTX concentrations. The simplest model structure that was able to describe the shape of the concentration–time profile in plasma or tissue was selected. The lung, heart, liver and kidney profiles could be modelled using a single parameter (tissue-to-blood partition coefficient). These organs are rich in blood flow and were assumed to have a “well-stirred” structure (model a). In order to satisfactorily capture the shape of the DTX pharmacokinetic profiles in muscle and remainder compartment, incorporation of a “deep tissue” subcompartment (model c) was further tested. However, it led to unstable parameter estimates although it significantly improved the fit (\(p\) value < 0.05). Incorporation of permeability-limited distribution (model b) into the structure of brain did not significantly reduce the residual variability (\(p\) value > 0.05). The most complex concentration–time profile was observed in spleen, which was characterised by a prolonged DTX terminal phase. Using the permeability-limited distribution structure did not further improve the fit (\(p\) value > 0.05). Using a “deep tissue” subcompartment (model c and d) led to unstable parameter estimates, although it decreased the residual variability significantly (\(p\) value < 0.05). Therefore, it was decided to use only the “well-stirred” structure and not include any other subcompartment in the spleen model. After developing the single-organ models, the whole-body model was then constructed according to the scheme presented in Figure 1.

Since a significant increase (\(p\) value < 0.05) in the OFV was found when fixed each of the 12 PBPK parameters, all the parameters included in the base PBPK model were necessary to be estimated. The data also supported including IIV terms for all 12 PBPK parameters, which were estimated with high precision. The residual unexplained variability was best characterised using a logarithmic error model, while additive, proportional and combined error models provided inferior fits. The residual error model was defined as:

\[
C_{obs}(t_i) = C_{pred}(\theta, t_i) + e_{i, obs}^{\epsilon_{i, obs}}
\]

where \(C_{obs}(t_i)\) is the observed concentration of the \(i\)th data point, \(C_{pred}(\theta, t_i)\) is the \(i\)th predicted value from the pharmacokinetic model and \(e_{i, obs}^{\epsilon_{i, obs}}\) represents a normally distributed random variable with a mean of 0.

**Covariate model**

A summary of covariate model building steps is shown in Table S1 (Supplementary), which represents the steps that resulted in statistical significance in the OFV during the development of the pharmaco-statistical model. Screening the effects of COVs on PBPK parameters during the univariate stepwise forward selection procedure suggested that the inclusion of dosage form had significant (\(p\) values < 0.05) effects on \(K_{t,pl}\), \(K_{t,he}\), \(K_{t,sp}\), \(K_{t,li}\), \(K_{t,ki}\) and \(K_{t,lu}\). Inclusion of sex had a significant (\(p\) values < 0.05) effect on \(K_{t,lu}, CL_{si}\) and CL\(_{gu}\). A combination of dosage form and sex was found to be associated with a statistically significant difference (\(p\) value < 0.05) in \(K_{t,lu}, K_{t,gu}\) and \(K_{t,sp}\). In addition, removal of dosage form effect on \(K_{t,gu}\), and sex effect on \(K_{t,lu}, CL_{si}\), \(K_{t,gu}\) and \(K_{t,sp}\) in the stepwise backward elimination was associated with a non-significant increase in OFV (\(p\) value > 0.01). Hence, these COV effects were removed from corresponding PBPK parameters.

**Final PBPK model**

The estimation result from the final model showed all PBPK parameters and corresponding IIVs were precisely estimated, with a relative standard error (% RSE) of 43.3% or less (Table 2). When comparing the basic and the final model, the IIVs in the PBPK parameters were reduced by more than 28.2% after adding dosage form and sex as model COVs. The effects of dosage form and sex on DTX PBPK parameters are shown in Figure S1 (Supplementary). The final equations for the structural model parameters are presented in Equations (9)–(18):

\[
K_{t,pl} = 51.7 \times (1 + D \times \theta_1)
\]

\[
K_{t,lu} = 0.307 \times (1 + D \times \theta_2)
\]

\[
K_{t,he} = 0.0410 \times (1 + D \times \theta_3)
\]

\[
K_{t,sp} = 0.699 \times (1 + D \times \theta_4)
\]
Table 2. Final estimates of the pharmacokinetic parameters obtained using NONMEM and bootstrap analysis of the final PBPK.

| Parameter (Units) | Mean (% RSE) | IIV (% CV) | Bootstrap result | CI (5%, 95%) |
|-------------------|--------------|------------|-------------------|--------------|
| $K_{t,gu}$       | 51.7 (4.33)  | 22.0       | 43.8              | 33.5, 56.7   |
| $K_{t,li}$       | 0.307 (3.90) | 26.3       | 0.351             | 0.254, 0.529 |
| $K_{t,sp}$       | 0.0410 (3.99)| 22.3       | 0.0337            | 0.0260, 0.0494 |
| $K_{t,he}$       | 0.165 (4.16) | 22.3       | 0.181             | 0.144, 0.237 |
| $K_{t,br}$       | 0.699 (3.70) | 27.8       | 0.549             | 0.381, 0.845 |
| $K_{t,lu}$       | 1.68 (5.98)  | 18.2       | 1.39              | 1.24, 1.89   |
| $\text{CL}_{gu}$ (L h$^{-1}$) | 0.0108 (15.4) | 70.3       | 0.00859 | 0.00260, 0.151 |
| $K_{t,lu}$       | 0.577 (16.7) | 15.3       | 0.580             | 0.386, 0.706 |
| $\text{CL}_{li}$ (L h$^{-1}$) | 0.567 (43.3) | 19.2       | 0.501             | 0.0221, 0.611 |
| $K_{t,sp}$       | 0.0989 (4.02)| 43.2       | 0.143             | 0.0863, 0.256 |
| $K_{t,he}$       | 0.0691 (2.24)| 17.8       | 0.0741            | 0.0609, 0.0986 |
| $K_{t,br}$       | 7.50 (3.62)  | 19.7       | 6.53              | 4.85, 8.14   |

Effect of dosage form
- $K_{t,gu}$ ($\theta_d$) $-0.266$ $-$ $-0.267$ $-0.299$, $-0.243$
- $K_{t,li}$ ($\theta_d$) $0.371$ $-$ $0.369$ $0.344$, $0.394$
- $K_{t,sp}$ ($\theta_d$) $-0.228$ $-$ $-0.228$ $-0.250$, $-0.202$
- $K_{t,he}$ ($\theta_d$) $-0.352$ $-$ $-0.350$ $-0.384$, $-0.319$
- $K_{t,br}$ ($\theta_d$) $-0.226$ $-$ $-0.227$ $-0.243$, $-0.212$
- $K_{t,lu}$ ($\theta_d$) $1.20$ $-$ $1.20$ $1.10$, $1.32$
- $K_{t,sp}$ ($\theta_d$) $0.190$ $-$ $0.191$ $0.175$, $0.206$
- $K_{t,he}$ ($\theta_d$) $-0.272$ $-$ $-0.270$ $-0.288$, $-0.256$

Effect of sex
- $K_{t,li}$ ($\theta_h$) $0.271$ $-$ $0.268$ $0.250$, $0.286$
- $K_{t,sp}$ ($\theta_h$) $-0.262$ $-$ $-0.263$ $-0.283$, $-0.248$

Logarithmic error $0.766$ $-$ $0.766$ $0.765$, $0.767$

CI: confidence interval; $\text{CL}_{gu}$: intestinal clearance; $\text{CL}_{li}$: hepatic clearance; CV: coefficient of variation; IIV: inter-individual variability; $K_{t,br}$: partition coefficient for brain; $K_{t,gu}$: partition coefficient for gut; $K_{t,he}$: partition coefficient for heart; $K_{t,ki}$: partition coefficient for kidney; $K_{t,li}$: partition coefficient for liver; $K_{t,sp}$: partition coefficient for lung; $K_{t,sp}$: partition coefficient for spleen; $K_{t,he}$: blood-to-plasma partition coefficient; RSE: relative standard error. $\theta$ represents the fractional change in the PBPK parameters.

$^a$Based on 976/1000 successful runs.

$^b$Based on 976/1000 successful runs.

\[
K_{t,li} = 1.68 \times (1 + D \times \theta_5)
\]  \hspace{1cm} (13)
\[
K_{t,ki} = 0.0989 \times (1 + D \times \theta_6)
\]  \hspace{1cm} (14)
\[
K_{t,mu} = 0.0691 \times (1 + D \times \theta_7)
\]  \hspace{1cm} (15)
\[
K_{t,he} = 7.50 \times (1 + D \times \theta_8)
\]  \hspace{1cm} (16)
\[
K_{t,br} = 0.165 \times (1 + S \times \theta_9)
\]  \hspace{1cm} (17)
\[
\text{CL}_{gu}(L \cdot h^{-1}) = 0.567 \times (1 + S \times \theta_{10})
\]  \hspace{1cm} (18)

where, $D$ is the dosage form (0: simple DTX injection; 1: modified liposomes). $S$ represents sex of the rats (0: male; 1: female). The estimated $\theta_5$ are presented in Table 2.

Model qualification and interspecies scaling

In order to evaluate the precision of estimated PBPK parameters, the bootstrap analysis was performed and it showed narrow confidence intervals for all parameters. The mean, median, 5th and 95th percentiles of the parameter estimates from the fit of the final model to the bootstrap samples are summarised in Table 2. The asymptotic estimates obtained from the original dataset showed close agreement with the medians and were all included in the 5th and 95th percentiles of the bootstrap values, indicating model stability.

The predictive performance (goodness-of-fit) of the final population model was assessed graphically (Figure 3). Population-predicted concentrations were calculated using population parameter estimates (mean values) and COVs information. The median and non-parametric 90% CI (5–95%) of the simulated concentrations were overlaid on the observed concentrations and visual inspection was performed to determine whether the observed data were reasonably contained within this CI. Using the 5 mg kg$^{-1}$ dose dataset, the proposed PBPK model allowed for a good simultaneous description of the experimental data for DTX in plasma and tissues. The final PBPK model simulations accurately approximated the observed concentration–time profiles in lung, brain, heart, spleen, liver, gut, kidney and muscle. The median of the population-predicted concentrations adequately described the central tendency in the observed data, and the population-predicted concentrations versus time profiles were distributed randomly across the observations, indicating the final model adequately described the observed concentrations.
over the entire DTX concentration range. Overall, although the drug concentrations in plasma were slightly overestimated, the final model provided an adequate and unbiased fit to the DTX pharmacokinetic data included in this analysis.

We also evaluated the performance of the proposed PBPK model by simulating i.v. injections of DTX solution and modified liposomes (10 mg kg\(^{-1}\)) in male rats utilising the model structure and parameters determined at steps 1–3 (Table 2). Model predictions were compared with the observations. As a result, the final model resulted in a good simultaneous description using both datasets (Figure 4).

To further evaluate the predictive performance of the rat model, the ability of the model to predict DTX pharmacokinetics in mice was evaluated. Model predicted pharmacokinetic profiles were compared graphically with observed data obtained following single i.v. administration of simple DTX injections (5 and 20 mg kg\(^{-1}\)) to male/female mice (Figure 5). Although there were considerable inter-individual variations in the simulated plasma concentrations (5 mg kg\(^{-1}\)), a good agreement can be observed between experimental and median simulated data. Similar conclusions can be made for observed and simulated concentrations for 20 mg kg\(^{-1}\) dosing datasets.

We also simulated DTX plasma pharmacokinetic profiles in humans, and compared the predictions with the reported values in the literature. DTX plasma concentration–time profiles under different doses (i.v. injection of 16, 30, 75 and 100 mg m\(^{-2}\)) were simulated by scaling parameters estimated for rats. The model predicted profiles are in good agreement with experimental data, except that the experimental concentration–time profiles had a little more curvature than the simulated ones (Figure 6).

Discussion

Microtubule protein has been considered as an attractive target in cancer therapy. Docetaxel, as a microtubule active agent, has shown desirable pharmacokinetic and pharmacodynamic properties. However, DTX has also been found to have several toxic effects, such as fatigue, asthenia, neutropenia, skin and nail toxicities, dose-limiting toxicity and other acute toxicities in clinical studies (Bradshaw-Pierce et al., 2007). Hence, estimating the concentration–time profiles of DTX in human tissues, which is not readily achieved by experimental approaches, may aid the understanding of concentration-efficacy and
concentration-toxicity relationship, leading to rational design of novel formulations and clinical dosing schedules. Our PBPK model would be of value in estimating DTX exposure in other species’ tissues because it acceptably predicted DTX concentration–time profiles in mouse and human plasma. The fact that our model reasonably reflected the time course of DTX disposition in rat tissues under different dose levels, improves the model’s credibility in estimating DTX exposure in mouse and human tissues.

Figure 4. Observed and model-simulated DTX concentration–time profiles in rat plasma following i.v. dose (10 mg kg\(^{-1}\)) of DTX injections (simple DTX injection/FA-PEG-PCHL-modified liposomes). Circles in (a) represent data from Li et al. (2011); squares in (a) represent data from Wonganan et al. (2009); circles in (b) represent data from Li et al. (2011). Lines represent the whole-body PBPK model predicted concentration–time profiles. Shaded areas denote the 90% confidence intervals.

Figure 5. Observed and model-simulated DTX concentration–time profiles in mouse plasma after i.v. doses (5 and 20 mg kg\(^{-1}\)) of simple DTX injections. Circles in (a) represent data from Wang et al. (2014); squares in (a) represent data from Wonganan et al. (2009); circles in (b) represent data from Li et al. (2011). Lines represent the whole-body PBPK model predicted concentration–time profiles. Shaded areas denote the 90% confidence intervals.

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Some previous studies reported that about 75% of DTX is rapidly eliminated by hepatobiliary extraction in humans, with similar metabolic pathways in all the species (Li et al., 2014). Accordingly, hepatic metabolism was considered as the main elimination pathway in this study, and then the hepatic clearance item \( \text{CL}_{\text{li}} \) was enrolled in the current PBPK model. However, the nature of the gastrointestinal tract elimination pathway \( \text{CL}_{\text{gu}} \) also needs to be elucidated. Intestinal metabolism is usually not a major route of drug elimination, but may play an important role in the total clearance of those xenobiotics that have extensive metabolism. In our current PBPK model, the pattern of elimination for DTX in humans was assumed to be similar to that in mice and rats. The hypothesis that a similar pattern of elimination for DTX exists across species is also one of the rationales for interspecies extrapolation of this PBPK model.

Changes in DTX tissue distribution in male/female rats after different dosage form administrations are reflected in altered PBPK parameters based on the population analysis [Equations (9)–(18)]. As in the case of dosage form, significant decrease (22.8–27.2%) was revealed in the partition coefficients for plasma \( K_{\text{t,pl}} \), brain \( K_{\text{t,br}} \) and remainder compartment \( K_{\text{t,re}} \) in rats following modified liposomes administration, while a marked rise of \( K_{\text{t,lu}} \) by 37.1% and \( K_{\text{t,mu}} \) by 19.0% was revealed. One possibility of this redistribution is likely due to the prevention of DTX precipitation by drug carriers. DTX is highly lipophilic and almost insoluble in water, which may cause its precipitation in vessels after intravenous administration of simple injections. When DTX is loaded in a liposome vehicle, it is embedded in the lipophilic group of the phospholipids molecules to form a tight combination with phospholipids. This structure may delay the release of free DTX into blood, and then delay the precipitation of DTX crystals and the penetration of free DTX into some tissues.

In rats following modified liposomes administration significant decrease in \( K_{\text{t,sp}} \) by 35.2% and \( K_{\text{t,li}} \) by 22.6% was revealed. Simple lipid emulsions are rapidly taken up by the reticuloendothelial system (RES) in the spleen and liver after intravenous administration (Mizushima et al., 1982; Waxman et al., 1985; Yamaguchi et al., 1984). However, the presence of a long hydrophilic chain in PEG around the shell of FA-PEG-PCHL would delay the trapping by RES and lead to a longer retention time in the circulation. In addition, a marked rise of \( K_{\text{t,ki}} \) by 120% was noted in rats following modified liposomes administration. The phenomenon could be explained by the folate receptor effect-folate receptor levels are naturally rich in kidney (Yamaguchi et al., 1984).

Figure 6. Observed and model-simulated DTX concentration–time profiles in human plasma after i.v. doses (16, 30, 75 and 100 mg m\(^{-2}\)) of simple DTX injections. Markers in (a) represent data from Figg et al. (2010); markers in (b) represent data from Bradshaw-Pierce et al. (2007); markers in (c) represent data from Eskens et al. (2014); markers in (d) represent data from Bradshaw-Pierce et al. (2007) and Eskens et al. (2014). Lines are whole-body PBPK model predicted profiles after simulation using interspecies scaling of parameters estimated using rat data. Shaded areas denote the 90% confidence intervals.
Notably, the overall disposition of DTX is governed not only by the formulations, but also by sex. Female rats were characterised by significantly higher (27.1%) $K_{\text{t,he}}$ and slower $CL_{\text{tissue}}$ (26.2%) than males. However, our study showed that no significant difference of $CL_{\text{tissue}}$ was found between male and female rats, which indicated that the inconsistency of the pharmacokinetics may arise from not only the CYP3A enzyme levels, but also the differences of blood flow rate and proportion of lipid in organs.

In the extrapolation of the rat PBPK model to mice and humans, chemical-specific parameter $K_{\text{t,issue}}$ for the same type of tissue remained unchanged, while species-specific parameters ($CL_{\text{tissue}}$) were scaled with body weight using empirical allometric exponents. This manner of extrapolation for species-specific parameters is routinely used in PBPK modelling (Anderton et al., 2004; Bradshaw-Pierce et al., 2007; Meno-Tetang et al., 2006), but may not always be accurate or valid because there are considerable differences in abundance and function of drug-metabolising enzymes, drug transporters and other molecules across species (Hu & Hayton, 2001; Sharma & McNeill, 2009). All these confounding factors can contribute to the discrepancies between the simulated and observed data. In our study, most of the observed data were reasonably simulated by our PBPK model. However, in some other studies, tissue drug concentrations and/or plasma pharmacokinetic profiles were not accurately predicted (Hu et al., 2014). These over- or underestimations indicated that species-dependent affinity in the non-linear binding of drugs to tissue components may exist. Not taking into account these species-dependent differences could lead to imperfect inter-species extrapolation. Collectively, the discrepancies between simulations and the experimental data imply that once available, more species-specific and/or chemical-specific parameters should be incorporated to achieve better model prediction. Therefore, our PBPK model could be refined when more species-specific data and mechanisms regarding DTX disposition become available.

Conclusion

In summary, a PBPK model was successfully developed to describe the time course of DTX concentrations in plasma and eight tissues of rats. The perfusion-limited model structures were employed to characterise the prolonged terminal phase of concentration-time profiles in tissues. After extrapolating the rat model to mice and humans, model simulations reasonably predicted DTX concentration-time profiles in mouse plasma, as well as in human plasma under different doses. Our PBPK model provided a good basis for further improvement, which necessitates additional studies, especially those to illuminate the entire mechanism of DTX elimination. It also represented a platform that can be further modified to encompass both formulation- and sex-related effects on DTX disposition and elimination in the future. A refined PBPK model would have extensive potential applications, and ultimately benefit the optimisation of dosing regimens to maximise therapeutic benefits while minimising adverse effects of DTX.

Declaration of interest

The authors declare no conflicts of interest.

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Supplementary material available online
Supplementary Figure S1 and Supplementary Table S1