DEVELOPING A PBPK MODEL FOR THE HIGH LEVEL INTEGRATED SENSOR FOR NANOTOXICITY SCREENING

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Abstract

Toxicity of any substance is determined by its physico-chemical properties and tissue exposure. In the case of nanoparticles (NPs), the relevant properties include the size, aspect ratio, surface charge, chemical composition etc.; the tissue exposure represents the NPs' concentration and exposure duration. The HISENTS project aims to develop a platform of integrated modules for nanotoxicity screening. The PBPK model is an integral part of the platform as it provides information on the tissue exposure to nanomaterials via their ADME (absorption, distribution, metabolism, and excretion) behaviour. For this reason, a simple perfusion rate-limited PBPK model requiring only one NP-specific parameter per tissue was developed. The model treats all tissues as well-stirred compartments and the rate of NP exchange is only limited by the tissue perfusion. The model was calibrated against in vivo data for nano-TiO_2 and, despite its simplicity, gave a reasonable agreement between predicted and measured NPs concentrations in the tissues. The main advantage of the model is that the biodistribution of NPs is described using only thermodynamic parameters (partition coefficients) which can be estimated from equilibrium responses of the individual modules.

Keywords: Nanotoxicity, pharmacokinetics, modelling

1. INTRODUCTION

Toxicity of any substance is determined by its physico-chemical properties and tissue exposure. In the case of nanoparticles (NPs), the relevant properties include the size (aspect ratio), surface charge, chemical composition etc.; the tissue exposure represents the NPs' concentration and exposure duration. Thus, any adverse response observed in a living organism is related to delivered dose (that is, NPs amount in the target tissue) rather than to the total dose administered [1]. In other words, the delivered dose is the amount of toxicant available for interaction with tissues.

The phenomenon of NPs transportation and interaction within a living system is complicated and still poorly understood. The tissue exposure, distribution, and the time course of NPs amount can be predicted using a physiologically based pharmacokinetic (PBPK) model which determines the delivered dose based on the rate of absorption, distribution, metabolism, and excretion (ADME). In contrast to classical compartmental pharmacokinetic analysis, the PBPK model is built from realistic physiological elements (tissues, organs) which are interconnected with blood so that the model structure resembles anatomy of mammals [2].

The HISENTS project aims to deliver an integrated platform for nanotoxicity screening. The PBPK model is an integral part of the platform as it provides information on tissue exposure to nanomaterials via their ADME behaviour. The individual sensor modules (RNA, DNA, biomembrane, lung, gut, kidney, liver, and placenta) are designed so they provide response related to a particular damage. However, toxicity (or safety) of any substance cannot be judged solely on basis of this response. For example, certain NPs may trigger a response (damage) when directly applied to a specific cell culture *in vitro* while an *in vivo* test carried out with the same nanomaterial can lead to a negative result since the NPs would not reach the cells at all. Also, the opposite may be the case: the tissue *in vitro* does not give any short-term response upon NPs exposure, only extensive

NPs accumulation is observed *in vivo* which could be harmful on a larger time scale. The tissue exposure can be predicted using the PBPK model which is an essential tool for the toxicity assessment. In this paper, a minimal PBPK model for nanoparticles biodistribution is presented and calibrated against *in vivo* data.

2. THEORY

In the PBPK models, the transportation of NPs is assumed to follow simple first-order kinetics. Applying the mass balance principle to compartments (i.e., tissue or organ), the change of NPs amount (A, usually expressed as NPs mass) can be written as

$$\frac{\mathrm{d}A}{\mathrm{d}t} = F(c_{\mathrm{in}} - c_{\mathrm{out}}), \tag{1}$$

where F is the blood flow rate through the compartment and c_{in} and c_{out} is the NPs concentration in inflowing and outflowing blood, respectively. For organs with elimination (liver and kidneys), an additional term representing excretion should be included. PBPK models used in drug discovery and toxicology assume either perfusion rate-limited or membrane-limited kinetics. The blood flow-limited model assumes that NPs transportation into (and from) tissues is very fast, and blood and tissue NPs concentrations equilibrate almost instantly. Such an assumption applied to equation (1) leads to a differential equation [1]

$$\frac{\mathrm{d}A}{\mathrm{d}t} = F\left(c_{\mathrm{bl}} - \frac{A}{V \cdot R_{\mathrm{t:bl}}}\right), \tag{2}$$

where "bl" subscript relates to blood, V is the compartment volume and $R_{\mathrm{t:bl}}$ is the tissue-blood partition coefficient which corresponds to ratio of NPs concentration in blood to that in tissue at equilibrium. Equation (2) implies that in the perfusion rate-limited model, the transportation of nanoparticles into one tissue depends on its blood supply (F) and on NP-tissue affinity ($R_{\mathrm{t:bl}}$). Thus, a great advantage of the rate-limited models over the membrane-limited ones resides in a fact that a kinetic phenomenon is modelled using a physiologically determined parameter (F) and a thermodynamic parameter ($R_{\mathrm{t:bl}}$). On the other hand, membrane-limited models assume that there is a membrane whose permeability is the rate limiting factor for NPs transportation. In this approach, each organ is represented by two interacting parts (vascular and extravascular compartment) which are separated by capillary wall membrane. The membrane-limited model consists from a system of two differential equations per organ/tissue [3]:

$$\begin{aligned}\frac{\mathrm{d}A_1}{\mathrm{d}t} &= F\left(c_{\mathrm{bl}} - \frac{A_1}{V_1}\right) - PS\left(\frac{A_1}{V_1} - \frac{A_2}{V_2 R_{\mathrm{t:bl}}}\right),\\ \frac{\mathrm{d}A_2}{\mathrm{d}t} &= PS\left(\frac{A_1}{V_1} - \frac{A_2}{V_2 R_{\mathrm{t:bl}}}\right).\end{aligned} \tag{3}$$

Compared to the perfusion rate-limited model, an additional parameter describing the membrane permeability (permeability-surface product, PS) is needed. Thus, for each organ there is a physiologically determined parameter (F) and two NPs-specific parameters (partition coefficient $R_{\mathrm{t:bl}}$ and membrane permeability-surface product PS).

For some NPs, it might be the case that the blood-tissue barrier or transport within the tissue is the rate-limiting step since we are dealing with relatively large nanoparticles compared to dissolved small molecules [4]. Moreover, the parameter $R_{\mathrm{t:bl}}$ appearing in both flow-limited and membrane-limited models can hardly be considered the "classical" blood:tissue partition coefficient as NPs are insoluble in both blood and tissues [5]. Within the HISENTS platform, application of the membrane-limited model could bring about some complications. First, the modules of the HISENTS platform are not expected to reflect the tissues to the similarity level that would allow modelling of different types of capillary wall membrane. Moreover, such an

approach would require determining two independent parameters ($R_{t:bl}$ and PS) per module and NP type, which may not be experimentally feasible. Our literature overview suggests that there is a lack of *in vivo* experimental data of sufficient quality supporting the inadequacy of perfusion rate-limited models to NPs biodistribution and such conclusions are frequently based only on theoretical grounds. In fact, most *in vivo* studies show that after intravenous administration the NPs are almost immediately cleared from blood and distributed to the tissues. Another possible interpretation of capillary wall membrane effect on NPs pharmacokinetics is that it can act as a "cut-off" filter whose permeability strongly depends on NPs dimension.

2.1. PBPK model design

For a whole-body PBPK model, all the major tissues and organs should be included in the model structure to ensure its versatility. Further, mass conservation principle should be incorporated in the PBPK model even though not all tissues are included (for example, muscles, skin, and adipose tissue are only rarely incorporated). A generally adapted solution resides in adding a compartment (usually called "remainder") that consists of all the tissues and organs not modelled as individual compartments.

A perfusion rate-limited PBPK model with 8 compartments was build; its schematic representation is depicted in **Figure 1**. All the important tissues/organs are connected in parallel to the central compartment (blood). The model assumes that liver and kidneys are the only possible sites for NPs excretion. The mass-balance differential equation for the blood compartment is

$$\frac{dA_{bl}}{dt} = V_{bl}\frac{dc_{bl}}{dt} = -\sum_i F_i \frac{A_{bl}}{V_{bl}} + \sum_j F_j \frac{A_j}{V_j R_j}, \tag{4}$$

where, referring to **Figure 1**, the summation indices are i = (bones, intestines, spleen, liver, kidneys, lungs, remainder) and j = (bones, intestines, liver, kidneys, lungs, remainder).

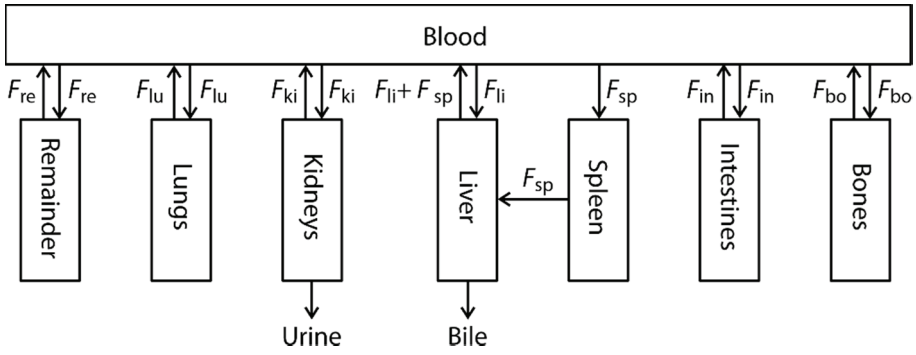

Figure 1 A perfusion rate-limited PBPK model used for calibration with *in vivo* NPs distribution data

The mass-balance differential equations for other compartments and excretory pathways can be derived in a similar manner:

$$\frac{dA_{bo}}{dt} = F_{bo}\left(\frac{A_{bl}}{V_{bl}} - \frac{A_{bo}}{V_{bo}R_{bo}}\right) \tag{5a}$$

$$\frac{dA_{in}}{dt} = F_{in}\left(\frac{A_{bl}}{V_{bl}} - \frac{A_{in}}{V_{in}R_{in}}\right) \tag{5b}$$

$$\frac{dA_{sp}}{dt} = F_{sp}\left(\frac{A_{bl}}{V_{bl}} - \frac{A_{sp}}{V_{sp}R_{sp}}\right) \tag{5c}$$

$$\frac{dA_{li}}{dt} = F_{li}\frac{A_{bl}}{V_{bl}} - (F_{sp} + F_{li})\frac{A_{li}}{V_{li}R_{li}} + F_{sp}\frac{A_{sp}}{V_{sp}R_{sp}} - CL_{bile}\frac{A_{li}}{V_{li}} \quad (5d)$$

$$\frac{dA_{ki}}{dt} = F_{ki}\left(\frac{A_{bl}}{V_{bl}} - \frac{A_{ki}}{V_{ki}R_{ki}}\right) - CL_{urine}\frac{A_{ki}}{V_{ki}} \quad (5e)$$

$$\frac{dA_{lu}}{dt} = F_{lu}\left(\frac{A_{bl}}{V_{bl}} - \frac{A_{lu}}{V_{lu}R_{lu}}\right) \quad (5f)$$

$$\frac{dA_{re}}{dt} = F_{re}\left(\frac{A_{bl}}{V_{bl}} - \frac{A_{re}}{V_{re}R_{re}}\right) \quad (5g)$$

$$\frac{dA_{urine}}{dt} = CL_{urine}\frac{A_{ki}}{V_{ki}} \quad (5h)$$

$$\frac{dA_{feces}}{dt} = CL_{bile}\frac{A_{li}}{V_{li}} \quad (5i)$$

The mammillary PBPK model consists of central blood compartment with all peripheral compartments connected in parallel; the only exception is spleen whose output is connected to liver in a similar manner as the portal vein in mammals. As already mentioned, *in vivo* data on NPs biodistribution suitable for PBPK modelling are relatively scarce; notable examples for TiO_2 NPs are [6, 7], for Ag NPs [8], for PEG-coated Au NPs [9], for graphene oxide NPs [10], and for PEG-coated polyacrylamide NPs [11].

2.2. Model calibration and testing

Suitability of the PBPK model defined by equations 4-5i was tested against TiO_2 biodistribution data in mice [6]. The physiological parameters (blood flow and organ masses) were taken from [12]. The computational model was implemented in the R language; the system of differential equations 4-5i was integrated numerically; the model parameters were optimized to data using the Nelder-Mead method. The objective function defined as

$$S = \sum_n \sum_m \frac{[A_{m,n}^{exp} - A_{m,n}^{PBPK}]^2}{s_{m,n}^2} \quad (6)$$

corresponds to the weighed total sum of squares; the *m* and *n* indices specify the NPs amount *A* in *m*-th tissue at the *n*-th time point. The results are graphically summarized in **Figure 2** and the corresponding PBPK parameters are listed in **Table 1**.

Table 1 Best fitting parameters of the perfusion rate-limited PBPK model (TiO_2 data from ref. [6])

R_{bo}	R_{in}	R_{sp}	R_{li}	R_{ki}	R_{lu}	R_{re}	CL_{bile} (kg h^{-1})	CL_{urine} (kg h^{-1})
35.1	1.76	885	1260	7.61	53.7	22.2	$1.04 \cdot 10^{-6}$	$9.01 \cdot 10^{-5}$

From **Figure 2** we can see that the model, despite its simplicity, describes the experimental data satisfactorily and captures all the important trends. The main sites of TiO_2 NPs accumulation (spleen and liver) correspondingly have by far the highest values of the partition coefficients (**Table 1**) which are one or two orders of magnitude higher than those of other organs. The data also demonstrate how quickly the NPs are cleared from the bloodstream and distributed among the tissues. After the liver, the second highest tissue burden can be found in the remainder which has a relatively low partition coefficient but its mass corresponds to more than 80 % of the total body mass.

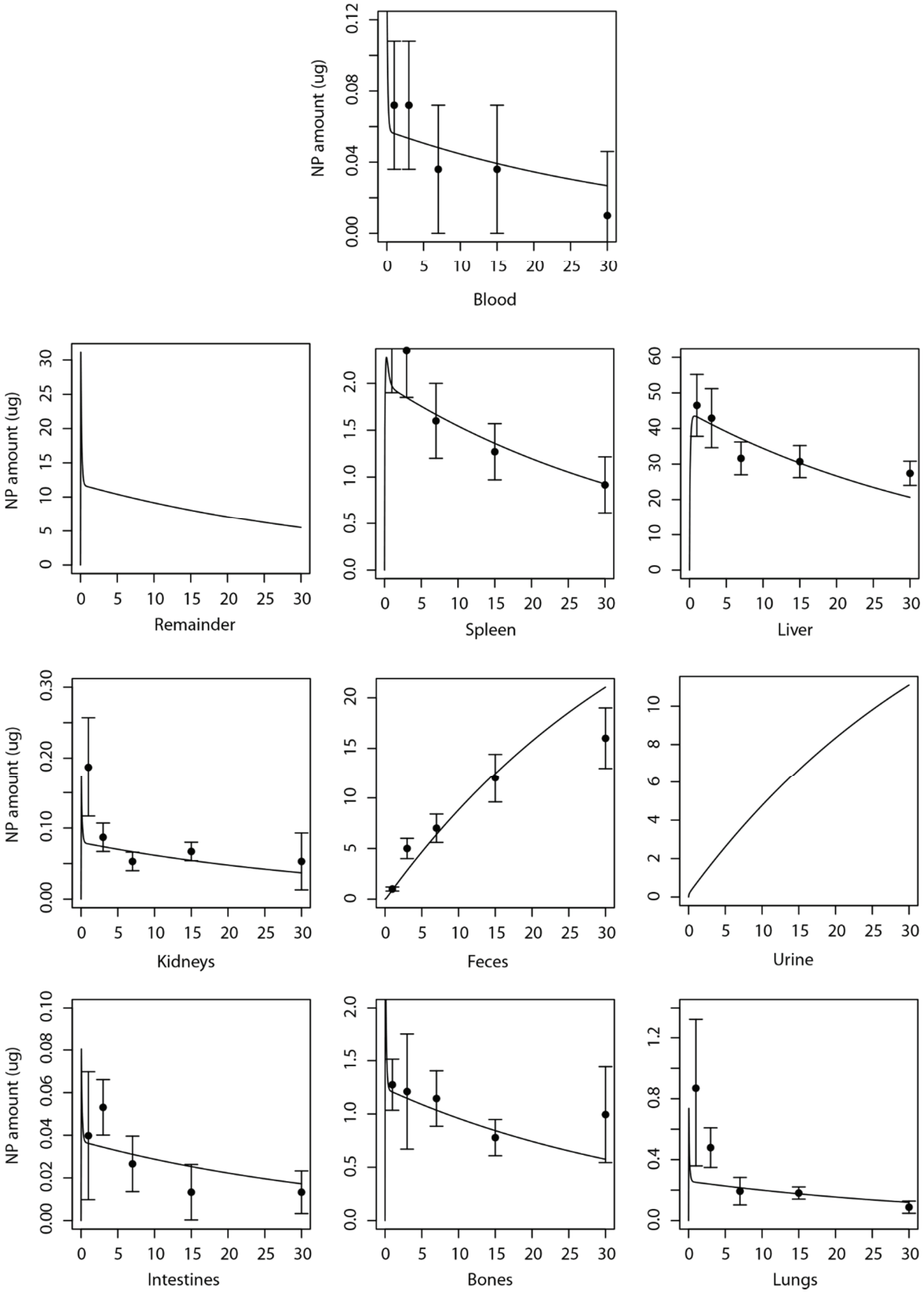

Figure 2 PBPK model applied to TiO_2 biodistribution data [6]. In all plots, the horizontal axis corresponds to the time after TiO_2 administration (in days). Data for urine were excluded due to a possible detachment of a radioactive label from NPs (see ref. [12])

3. CONCLUSION

A simple perfusion rate-limited PBPK model requiring only one NP-specific parameter per tissue was built. The model treats all tissues as well-stirred compartments and the rate of NP exchange is only limited by the tissue perfusion. The model was calibrated against in vivo data for nano-TiO_2. Despite its simplicity and minimum number of adjustable parameters the model gave a reasonable agreement between predicted and measured NPs concentrations in all the tissues under study. The main advantage of the model is that the biodistribution of NPs is described using only thermodynamic parameters (partition coefficients) whose values can be estimated from equilibrium responses of the individual "organ-on-a-chip" modules.

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