Using a PBPK model to study the influence of different characteristics of nanoparticles on their biodistribution

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Abstract. The studies on potential health risks possessed by engineered nanoparticles (NPs) have been growing rapidly. However, detailed and systemic knowledge on the uptake and biodistribution of NPs in body is still limited. Moreover, there is a need to characterize the relation between the characteristics of NPs (size, surface modifications, etc.) and their behaviours in the body. The aim of this study is to explore how these characteristics will influence the NPs uptake and biodistribution. We have successfully developed a Physiologically Based Pharmacokinetic (PBPK) model for the biodistribution of polyethylene glycol-coated polyacrylamide NPs in rats, modelling the capture and removal of NPs by phagocytizing cells. Based on this PBPK model, the behaviours of other nanoparticles (polymeric, quantum dot, silver, titanium oxide and cerium oxide NPs) are investigated, based on data from several experiments published in the literature. Size is one of the important properties to consider. Our model parameterization suggests that the uptake rate by phagocytizing cells will decrease as the size of nanoparticles increases when the removal rates for these nanoparticles are similar. This could indicate that the phagocytizing cells are saturated by the number of NPs rather than absolute mass. Nevertheless, surface modification, such as polyethylene glycol coating, may reduce the uptake rate by phagocytizing cells. With phagocytizing cells serving as a deposit of NPs, these influences of different characteristics of NPs to the behavior of phagocytizing cells could affect the fate of NPs in the body not only during the initial uptake within the first hour but also in long-term at the kinetic and dynamic levels.

1. Introduction
Wide production and use of nanoparticles (NPs) increase the likelihood of unintentional exposure at workplaces as well as in the general environment [1-4]. In vivo and in vitro studies have shown that NPs have the potential to induce oxidative stress, leading to inflammatory reactions [5, 6]. The degree of inflammatory response to NPs strongly depends on the biodistribution of NPs in the body [6, 7]. A few detailed investigations have revealed that at least some types of inhaled or intravenously injected NPs rapidly migrate to, and deposit in different organs via the systemic circulation [8-12]. However, the determinants of NP behavior in the body still need to be explored in a systematic way. It is therefore necessary to determine what extent and by which mechanisms nanomaterials translocate and bioaccumulate.
Recently a few studies have employed physiologically based pharmacokinetic (PBPK) models to describe the biodistribution of NPs. However, these models have not specifically addressed the role of phagocytizing cells [13, 14], although it has been observed that phagocytosis is a major element of NP biodistribution [7, 15]. The interaction between phagocytizing cells and NPs has been modeled based on in vitro experiments [16, 17], but such in vitro knowledge has hitherto not been incorporated in the attempts to describe the overall biodistribution in the body.

To further develop our knowledge of the biodistribution of NPs, several issues need to be addressed in the PBPK models, such as: a) how to account for nano-specific phenomena, such as phagocytosis; b) what are the most influential determinants affecting the biodistribution; and c) how would different characteristics (size, surface modification, etc.) impact these determinants.

In this study we build a PBPK model with the emphasis on the importance of phagocytosis using experimental data from rats given single doses of polyethylene glycol-coated polyacrylamide (PAA-peg) by intravenous injection. Then we adapted the model to other types of NPs without major structural changes in order to explore the influences of different characteristics of NPs on their biodistribution.

2. Method

2.1. Experimental data

The model was developed based on anatomical and physiological considerations and on an experimental biodistribution study published by Wenger et al [12]. In brief, five groups of three rats per group received a single intravenous dose of 7,000 μg 14C-labeled PAA-peg NPs via the tail vein. Urine, feces, cage residues, and blood samples were collected at designated times and tissue samples of the liver, spleen, kidney, heart, lungs, brain, lymph nodes (mesenteric, inguinal, and popliteal), and bone marrow (both femurs) were collected following euthanasia by CO2. Radioactivity levels were determined by liquid scintillation counting with correction for background chemiluminescence.

To adapt the model to other types of NPs, experimental data were taken from various studies: Wenger et al [12] for polyacrylamide (PAA) NP, Panagi et al [11] for poly(Lactide-co-glycolide)-monomethoxypoly(ethyleneglycol) (PLGA-mPEG) NP, Liu et al [18] for coated breviscapine-loaded poly(D, L-lactic acid) (BVP-PLA) NP, and Lankveld et al [14] for three different sizes of silver (Ag) NP.

2.2. PBPK model

The PBPK model consists of eight compartments: blood, lungs, heart, brain, kidneys, liver, immune organs (spleen, lymph nodes, and bone marrow), and the rest of the body. Within each compartment, there are three sub-compartments representing capillary blood, tissue and phagocytizing cells (PCs) and remaining tissue. The conceptual framework of the model is illustrated in Fig. 1.
The exchange of nanoparticle between blood and tissue in each organ is described as a flow- and diffusion-limited process and the latter is controlled by permeability parameters, which limits the effective blood flow [19]. The permeability coefficient for the brain compartment is set to zero, under the assumption of a highly efficient blood-brain barrier. The possibility of uneven distribution between blood and tissue at steady-state is taken care of by a blood-tissue partition coefficient.

Some of the nanoparticles that enter the tissue are captured by the tissue PCs. The uptake rate is a function of the efficacy and saturation level of the PCs and decreases as the PAA-peg nanoparticles in the PCs become saturated. For now, we assume that all PCs have the same efficacy and saturation level, independent of their location. However, the numbers of PCs differ between organs, causing different saturation levels. The saturated PCs become inactive and migrate to the immune organs. A removal rate of NPs, which includes excretion from the body and possibly biodegradation for some NPs, is adopted in the model.

The PBPK model was implemented in Berkely-Madonna™ (version 8.3.18). The model was optimised by fitting the unknown model parameters against the PAA-peg experimental data first. The following parameters were fitted: phagocytosis saturation level (one for each compartment), blood: tissue partition coefficient, permeability coefficients, uptake rate and desorption rate by PCs,
migration rate of inactive PCs, and clearance rate from kidneys and liver. All other parameter values were taken from the scientific literature. Then the model was adapted for other types of NPs. For this adaption, the phagocytosis saturation levels among all organs remained a constant ratio but were scaled either up or down to the same degree, expressed in the uptake capacity (UC), when fitting different datasets.

2.3. Model evaluation and sensitivity analysis

For model evaluation we determine the deviation from the 1:1 line between the log_{10} of measured and predicted values, calculating the standard deviation and the squared geometric standard deviation as well as the corresponding R^2.

Model sensitivity for each model parameter can be evaluated with the area under the curve (AUC) of blood and liver for the entire duration of each study. To test the sensitivity, each parameter’s value is increased by 10% and the AUC recalculated. A sensitivity coefficient can then be calculated using the following equation

\[
\text{Sensitivity coefficient} = \frac{dAUC_i}{dp_j} \frac{AUC_i}{p_j}
\]

Where \( AUC \) represents area under the curve (AUC) for compartment \( i \), and \( p_j \) represents the parameter \( j \). In this study, we chose the PCs uptake capacity (UC), uptake rate by PCs (\( k_{abs} \)), and the removal rate of NPs (\( k_{removal} \)) for the sensitivity analysis.

3. Results

3.1. Time course of the PAA-peg NP model

The time course for the PAA-peg NP is shown in Fig. 2 below. According to the experimental data, the fast perfused organs (including the lungs, liver, kidneys, heart, immune organs, and brain) exhibits a pattern of saturation of NPs and the model captures this phenomenon with the sub-compartment of PCs. For other types of NPs in this study, this saturation phenomenon is also observed, especially in the liver which has a high level of PCs.
3.2. Evaluation of the model
The predictions from the model are plotted against the measured data from the studies as shown in Fig. 3. The results for PAA NP and Ag NPs in sizes of 80 nm and 110 nm in diameters are not shown but they are very similar to the results for PAA-peg NP and Ag NP 20 nm. The $R^2$ range from 0.707 to 0.994, this shows the model’s predictions fit the experiment measurements relatively well. The two outliers for Ag NP 20 nm are in the blood. According to the measured data, towards the end of the study [14], no NPs were detected in the blood. In order to make these two data points available on the log scale, their values were set to 1 ng (the smallest value found in blood was 359 ng).

![Fig. 3 Model predictions versus experimental data for different types of NP](image)

3.1. Parameterization of the model
For the different types of NPs in this study, Table 1 summarizes their characteristics (sizes in diameter, whether have surface modification), injected doses, and the values of three fitted model parameters – UC, $k_{ab0}$, and $k_{removal}$. These NPs fall into two groups, one with low $k_{removal}$ (PAA-peg, PAA, and PLGA-mPEG) and one with high $k_{removal}$ (BVP-PLA and Ag). When comparing PAA-peg and PLGA-mPEG NPs (both have surface modification and low $k_{removal}$), the UC increased by 1.4 times and the $k_{ab0}$ decreased by 30 times when the diameter increased by 2.7 times; while the UC decreased slightly while $k_{ab0}$ increased for PAA NP when compared to PAA-peg NP (only difference is PAA-peg has surface modification). For BVP-PLA NPs and the Ag NPs, there seems to be a trend of increasing UC with increasing diameter but there is no clear pattern for $k_{ab0}$.
Table 1. Different nanoparticles and fitted parameters

| Nanoparticle   | Diameter (nm) | Surface modification | Dose (ug) | UC (−) | \( k_{ab0} \) (per h) | \( k_{removal} \) (per h) |
|----------------|---------------|----------------------|-----------|--------|----------------------|---------------------------|
| PAA-peg        | 31            | yes                  | 7000      | 1      | 17                   | 0.0028                    |
| PAA            | 31            | no                   | 11300     | 0.72   | 27                   | 0.0051                    |
| PLGA-mPEG      | 114           | yes                  | 1050      | 2.4    | 0.54                 | 0.025                     |
| BVP-PLA        | 319           | yes                  | 2200      | 5.8    | 1.7                  | 40.7                      |
| Ag(20)         | 20            | no                   | 119       | 0.019  | 15.2                 | 25.1                      |
| Ag(80)         | 80            | no                   | 132       | 0.055  | 30.7                 | 26.3                      |
| Ag(110)        | 110           | no                   | 138       | 0.031  | 57.6                 | 17.0                      |

1: All phagocytizing cells are assumed to behave the same; the number of phagocytizing cells per organs is independent of nanoparticles types, but parameters governing its dynamic may change.

2: UC="uptake capacity". Representing the relative uptake capacities of PCs to different NPs.

3: \( k_{ab0} \) is the maximum uptake rate by PCs

4: \( k_{removal} \) is removal rate from the body which may be caused by degradation or excretion.

3.2. Sensitivity of the model

The sensitivity coefficients calculated according to equation (1) for all types of NPs in this study are listed in Table 2. For all types of NPs except for BVP-PLA, the model is most sensitive to the parameter UC. \( k_{ab0} \) only becomes a sensitive parameter to the model for BVP-PLA. For the NPs with high \( k_{removal} \) (BVP-PLA and Ag), the model is also sensitive to this parameter. The reason for the model for BVP-PLA is not sensitive to UC but to \( k_{ab0} \) can be explained by the short duration of the BVP-PLA NP study, which lasted only for 3 hours while other experiments for the other NPs were at least 24 hours. It is possible that the PCs cannot be saturated within 3 hours and therefore making the UC an insensitive parameter but the \( k_{ab0} \) a sensitive one.

Table 2. Sensitivity coefficients for some parameters

| Nanoparticle   | UC    | \( k_{ab0} \) | \( k_{removal} \) |
|----------------|-------|--------------|-------------------|
|                | Blood | Liver        | Blood | Liver | Blood | Liver |
| PAA-peg        | -0.20 | 0.59         | -0.03 | -0.07 | -0.03 | -0.01 |
| PAA            | -0.07 | 0.62         | -0.0004| 0.0004| -0.04 | -0.02 |
| PLGA-mPEG      | -0.15 | 0.13         | -0.03 | 0.07  | -0.05 | -0.04 |
| BVP-PLA        | 0.22  | 0.004        | -0.06 | 0.48  | -0.90 | -1.78 |
| Ag(20)         | 0.97  | 0.45         | 0.0001| 0.33  | -0.03 | -0.48 |
| Ag(80)         | 0.98  | 0.30         | 0.01  | 0.18  | -0.02 | -0.52 |
| Ag(110)        | 0.97  | 0.73         | -0.0003| 0.07  | -0.02 | -0.18 |

1: The sensitivity coefficients were calculated by the percentage change divided by the percentage change in the parameter (set at 10%).

2: Also includes spleen in the columns for "liver" due to the nature of the experiment for this NP [11].
4. Conclusions and outlook
Introducing the phagocytizing cells enables the model to explain saturation phenomena in several compartments and model the dynamic well. With the increase in nanoparticle sizes, the phagocytizing cells uptake capacity (expressed as mass) increases in general when removal rates are similar. This could indicate that the phagocytizing cells are saturated by the number of nanoparticles rather than absolute mass. Compared to the other nanoparticles in this study, the BVP-PLA and silver nanoparticles are removed from the body 3 to 4 orders of magnitude faster than the other nanoparticles in this study.

For further study, it is worth designing more detailed experiments to test the hypothesis of increase in nanoparticle size would increase the uptake capacity of phagocytizing cells. It would also be of interest to pay more attention to the excretion within 1 hour of dosing since for the silver nanoparticles, a very fast removal of the nanoparticles from the body was observed but little is known about their fate.

Acknowledgement
This study was supported by U.S. EPA grant EPA-G2010-STAR-N1, the Swedish Council for Working Life and Social Research, and BioSimulation Consulting Inc., DE, USA.

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