A PBPK model to elucidate processes governing distribution and excretion of polyacrylamide nanoparticles

Y. Wenger*, O. Jolliet*, R. Schneider* and M. Philbert*

*University of Michigan, School of Public Health
Ann Arbor, MI, 48109, USA, yvan@umich.edu, philbert@umich.edu

ABSTRACT

This paper presents a physiologically based pharmacokinetics (PBPK) model that has been calibrated using experimental measurements of biodistribution and excretion of polyacrylamide nanoparticles in rats. Distribution to the organs of the reticular endothelium system is rapid and reach saturation depending on the dose level. The liver contains is dominating the accumulation of nanoparticles in organs. Excretion through urine occurs shortly after injection and is not dependent of the total concentration of nanoparticles in blood. In an opposite manner, excretion in faeces is slow and proportional to the blood concentration. Overall, excretion through faeces and urine are not major routes of eliminations since they only account for ~5% of the injected dose after 120hr. Finally, transfer from the blood to the carcass is responsible for the greatest amount of nanoparticles sequestered after 120hr, although the kinetic is slow (apparent half-life=40hr). The model predicts that accumulation in peripheral tissues would be significantly lower if the dose was not sufficient to saturate the organs.

Keywords: nanoparticles, pbpk, pharmacokinetic, polyacrylamide, nanomedicine

1 INTRODUCTION

Nano-scaled carriers are gaining considerable attention for the development of alternatives to conventional pharmaceutical administration. Compared to traditional drugs, nanoparticles can be effectively directed, and their content delivered in a more controlled manner. The pharmacologic agents are dissolved, entrapped, encapsulated, or attached to the nanoparticle and delivered to specific biological targets via targeting strategies that have been engineered on the surface of the NPs. Maintaining the dose delivered to targeted tissues at therapeutic levels, while reducing the dose delivered to healthy tissues, would be a significant improvement in drug treatments because side effects would be minimized or avoided and doses needed to achieve efficacious results reduced.

The most important clearance pathway of nanoparticles from the blood is generally thought to be confiscation by macrophages that comprise the reticular endothelial system (RES) or monocytic phagocytosis system (MPS) [1]. The rate of removal from the systemic circulation is influenced by opsonisation. Subsequently, opsonised nanoparticles are recognized by the RES and are removed by macrophage uptake mechanisms, such as phagocytosis [2]. Phagocytosis is not the only mechanism of uptake, as potocytosis (involving caveolae) and non-receptor meditated uptake have been reported [3]. The ultimate fate of NPs varies, depending on the ability of the NPs to degrade (hydrolysis, oxidation) or on other post-processing (metabolism) mechanism(s) that usually take place in the macrophage [4; 5; 6].

Polyacrylamide (PAA) nanoparticles (NPs) have been developed for implementation in biomedical applications such as targeted treatment of cancers through photodynamic therapy [7], quantification of key cellular ions at the single cell level [8; 9], and magnetic resonance imaging (MRI) enhancement [10]. Unlike the well described neurotoxicity of the acrylamide monomer used in the synthesis of PAA NPs [11; 12], the polymerized polymer is not neurotoxic and does not degrade back into its monomeric chemical form [13].

Before the utility of PAA NPs can be implemented in human applications, the toxicology of the PAA matrices needs to be defined. Previous experiments with PAA NPs show no visible alterations in histopathology, and chemical chemistry values indicate no inflammation up to a dose of 500 mg/kg over 4 weeks in rats. This paper builds on the characterization of this technology by investigating the pharmacokinetics, tissue distribution, and excretion of uncoated and PEG coated NPs in rats after a single i.v. administration.

2 METHODS

2.1 Nanoparticle Synthesis

Synthesis of polyacrylamide nanoparticles has been previously described [8] and has been adapted for incorporation of [^{14}C]-Acrylamide. Briefly, a water-in-oil (w/o) microemulsion was generated by stirring phosphate buffer (pH 7.4) and hexanes under argon. The amine-functionalized polyacrylamide nanoparticles were then polymerized by the addition of TEMED and ammonium persulfate (APS) to catalyze the free radical mediated cross-linking of acrylamide (17% w/v) with N-(3-amino-propyl) methacrylamide (4% w/v). Labelling was achieved by incorporation of a fraction of linear [^{14}C]-polyacrylamide

(American Radiolabeled Chemicals, St. Louis, MO) in the reaction mixture and it was taken advantage of amine-functionalized acrylamide to covalently link the monoethyl ester PEG to the amine groups on the newly synthesized NPs. Washing with ethanol and water was repeated to remove residual surfactants, and the suspensions were filtered at 0.22 μm and stored at 4°C.

2.2 Animals

Male Crl: 15 CD® (SD) IGS BR rats (weighing 218-241 g) were purchased from Charles River Laboratories, Portage, Michigan and individually housed seven days for acclimation. During acclimation, the rats were observed daily, and clinical signs of disease were monitored. Prior to the study all animals were weighed and subjected to a detailed clinical examination. A total of 15 rats received [^{14}C]-PAA NPs and 15 rats received surface coated PEG [^{14}C]-PAA NPs. During the study, rats were housed individually in metabolic cages and monitored for morbidity, mortality, and injury. Diet was available ad libitum to all rats, as was water through an automatic watering system. NPs were parenterally administered via a single i.v. injection in the tail vein. Due to differences in radioactivity contents of both formulations of nanoparticles, animals received an average dose of 11.6mg and 7.2mg of [^{14}C]-PAA NPs and PEG [^{14}C]-PAA NPs respectively, in order to achieve a delivery of approximately 10μCi per dose. The dose volume for all groups was approximately 1mL/kg. In general, triplicates of animals were used. It is not the case, however, for groups 1, 3, and 6 where a total of three animals have been discarded.

2.3 Tissue and Sample Collection

Blood, carcass, tissues [liver, spleen, kidney, heart, lungs, brain, lymph nodes (mesenteric, inguinal, and popliteal), bone marrow (femur)], and excreta (urine and feces) were collected at predetermined times (see table 1) and measured for radioactivity by liquid scintillation counting (LSC). Plasma, urine, whole blood, and cage residues were analyzed directly, while tissues, faeces and carcasses were analyzed following digestion with Solvable (Perkin-Elmer, Wellesly, MA. Scintillation cocktail; Ultima Gold). Residual radioactivity in cages revealed a negligible amount of material, and was thus neglected.

Group Number	n	Dose(SD) [mg-eq./kg]	Collection times Blood	Excreta	Tissues
1	2	45.1(1.1)	Pre, 5min, 1hr	0-1hr	1hr-organs
2	3	45.7(3.2)	Pre, 10min, 2hr	0-2hr	2hr-organs
3	2	45.4(0.5)	Pre, 20min, 4hr	0-4hr	4hr-organs
4	3	44.9(2.3)	Pre, 40min, 8hr	0-8hr	8hr-organs
5	3	42.5(2.4)	Pre, 1, 24, 48, 72, 96, 120hr	0-24/24-48/48-72/72-96/96-120hr	120hr-organs&carcass
*6	2	28.5(0.3)	Pre, 5min, 4hr	0-4hr	4hr-organs
*7	3	29.0(2.9)	Pre, 10min, 8h	0-8hr	8hr-organs
*8	3	26.9(1.7)	Pre, 30min, 8hr, 24hr	0-24hr	24hr-organs
*9	3	27.9(0.5)	Pre, 1, 24, 48hr	0-24/24-48hr	48hr-organs
*10	3	28.8(1.4)	Pre, 1, 24, 48, 72, 96, 120hr	0-24/24-48/48-72/72-96/96-120hr	120hr-organs&carcass

Table 1: Number of animals per group - n, doses, specimen collection times. Pre – predose. Min and hr are minutes and hours postdose. * indicates PEG formulation.

Blood samples (~2.0ml) were collected via the jugular vein at designated time points, with the exception of the final interval (120hrs), which was collected via cardiac puncture after euthanasia by CO_2. Blood was collected into EDTA containing tubes and stored on wet ice until separated into plasma and RBCs by centrifugation (4°C) at 3000 rpm, for 15min. In preparation for counting, tissues were removed after the final blood collection, blotted dry, weighed, digested, mixed with scintillation cocktail, and counted for at least 5 minutes or 100,000 counts. Whole carcasses were homogenized and prepared exactly like the tissues. Faeces were collected from animals and placed over wet ice at designated times, digested in an organic solubilizer, mixed with scintillation cocktail, and counted twice. Radioanalysis was performed using QuantSmart, version 3.01, (Perkin-Elmer), on a Perkin-Elmer Tricarb 2900 scintillation counter.

2.4 Pharmacokinetic analysis

A mass balance has been established, quantifying transfers between a central compartment (the blood), two peripheral compartments (organs, peripheral tissues), and the excreted flows (faeces, urine). Measured blood concentrations were normalized using individual animal's weights and doses. For the blood, the metric is expressed as the ratio of the measured blood concentration to an extrapolated initial concentration, assuming an instantaneous, complete, and homogenous transfer to the blood by i.v. administration. Additionally, blood masses were derived from animal weights at the beginning of the experiment, assuming a 0.065ml of blood per g body weight relationship [14] and a blood density of 1g/ml. In a comparable way, recovered fraction of doses for organs, tissues, faeces, and urine were calculated as the ratio of the equivalent mass of NPs recovered to the initial dose administered to each animal.

These calculated fractions of initial doses and transfer rates were estimated assuming the simplest mechanisms that satisfactorily described observed behaviors. Individual fractions of NPs remaining in blood over time were fitted using a bi-exponential function (eq. 1).

$$\mathrm{Blood}_{\mathrm{fraction}}(t) = \underbrace{A_1 \times e^{(-\lambda_1 \cdot hr)}}_{\text{First term}} + \underbrace{A_2 \times e^{(-\lambda_2 \cdot hr)}}_{\text{Second term}} \qquad (1)$$

Where $\mathrm{Blood}_{\mathrm{fraction}}$ is the measured actual concentration of radioactive material in blood divided by the calculated initial concentration in blood, t is the time postdose, A_1 and A_2 are unitless parameters, and λ_1, λ_2 are parameters with constant rate units (1/hr). To calculate pseudo half-lives

directly from these parameters $\left(t_{1/2} \cong \frac{\ln(2)}{\lambda_{1,2}} \right)$ assumes that one process drives the behavior of radioactive material in blood until its complete saturation. Relying on this assumption, λ_1 (1/hr) and λ_2 (1/hr) are equals to the rate constants associated with at least two independent processes. The variability of these four parameters has been assessed by fitting the data with SPSS (Rel. 13.0, 2004. Chicago: SPSS Inc) using a non-linear model, and imposing a biexponential function. The sequential quadratic programming procedure was selected, and the 95% C.I. on each parameters was used to estimate whether the parameters where significantly different for both NPs formulations.

The cumulative amount of radioactive material recovered in faeces was described by a first order elimination from the blood. The measured cumulative recovery (fraction of initial dose) was fitted using:

$$\begin{cases} \frac{\mathrm{dFaeces}_{\mathrm{cumul.}}(t)}{dt} = 0, \mathrm{t} < \mathrm{t}^* \\ \frac{\mathrm{dFaeces}_{\mathrm{cumul.}}(t)}{dt} = k_{blood\_faeces} \times \mathrm{Blood}_{\mathrm{fraction}}(t - t^*), \mathrm{t} \geq \mathrm{t}^* \end{cases} \quad (2)$$

Where $\mathrm{Faeces}_{\mathrm{cumul}}$ is the cumulative fraction of the dose recovered in faeces over time, k_{blood_faeces} (1/hr) is the first order rate constant corresponding to the transfer of radioactive material from the blood to the faeces. t* represents the delay observed from the removal from blood until the actual sampling, and could be seen as an average of the time spent in physiological processes such as the transfer from the blood to the liver, the latency in the liver, the transfer to the small intestine, and the latency in the intestine. Both t* and k_{blood_faeces} were fitted without any constraints.

A small portion of the injected radioactivity was recovered in urine with a kinetic that was consistent with the depletion of a pool independent from the total blood concentration. It was described by the equation:

$$\begin{cases} \frac{\mathrm{dUrine}_{\mathrm{cumul.}}(t)}{dt} = +k_{blood\_urine} \times M_{pool2}(t) \\ \frac{\mathrm{dM}_{\mathrm{pool2}}(t)}{dt} = +k_{blood\_urine} \times M_{pool2}(t) \end{cases} \quad (3)$$

Where, $\mathrm{Urine}_{\mathrm{cumul}}(\mathrm{t})$ is the cumulative fraction of dose recovered in urine over time, k_{blood_urine} (1/h) is the rate constant for the blood to urine transfer, and M_{pool2}, a pool of radioactivity in blood that is depleted by blood to urine transfer. The 2 subscript indicates that it this pool is distinct than the main pool of radioactivity in blood.

3 RESULTS

3.1 Distribution in organs and in tissues

Past studies demonstrate preferential uptake of nanoparticles into tissues affiliated with the reticular endothelial system (RES) [15]. The NPs content of RES organs quickly reached a plateau and did not show any additional accumulation after the initial loading except for the spleen, which was still increasing 4 hours post injection. The liver was the major site of deposition, accounting for about 10% of the initial dose. Taking into account other organs of the RES yield to approximately 20% of the dose. Sequestration by tissues contributed modestly at short times, but the amounts deposited in the carcass increased monotonically during the period (0-120hr).

3.2 Excretion

The amount of radioactivity excreted was measured after collection and radioanalysis of urine and faeces. Cumulatively, there was only a small amount of radioactivity recovered from either urine or feces for PAA or PAApeg nanoparticles. The small recovered fraction and the kinetic involved support a slow degradation of polyacrylamide nanoparticles in vivo during the first 120hr. R^2=0.998 and R^2=0.992 on the modeled cumulative percentage of dose recovered, for urine and feces respectively.

3.3 Kinetic of blood clearance

Measured pharmacokinetic parameters such as distribution to the organs and excretion rates were ued to reconstruct the mass balance and to simulate the apparent decrease of radioactivity in blood. An initial process account for less than 10% of the removal from the blood and is quickly saturated. The saturation time was different for PEGylated and non PEGylated NPs. This first phase is followed by an independent slower kinetic of clearance that take place up to the end of the experiment (120hr). Numerous past studies have suggested an association between the addition of surface coating agents such as polyethylene glycol and increased plasma residence times [16; 17]. However, the statistical analysis failed to reveal any statistical differences at the α=0.05 level between corresponding half-lives from both formulations.

REFERENCES

[1] S.M. Moghimi, A.C. Hunter, and J.C. Murray, Long-circulating and target-specific nanoparticles: Theory to practice. Pharmacological Reviews 53 (2001) 283-318.

[2] S.M. Moghimi, and A.C. Hunter, Recognition by macrophages and liver cells of opsonized phospholipid vesicles and phospholipid

headgroups. Pharmaceutical Research 18 (2001) 1-8.
[3] M.C. Garnett, and P. Kallinteri, Nanomedicines and nanotoxicology: Some physiological principles. Occupational Medicine 56 (2006) 307-311.
[4] K. Briley-Saebo, A. Bjørnerud, D. Grant, H. Ahlstrom, T. Berg, and G.M. Kindberg, Hepatic cellular distribution and degradation of iron oxide nanoparticles following single intravenous injection in rats: Implications for magnetic resonance imaging. Cell and Tissue Research 316 (2004) 315-323.
[5] R. Fernandez-Urrusuno, E. Fattal, J.M. Rodrigues Jr, J. Feger, P. Bedossa, and P. Couvreur, Effect of polymeric nanoparticle administration on the clearance activity of the mononuclear phagocyte system in mice. Journal of Biomedical Materials Research 31 (1996) 401-408.
[6] S.M. Moghimi, and J. Szebeni, Stealth liposomes and long circulating nanoparticles: Critical issues in pharmacokinetics, opsonization and protein-binding properties. Progress in Lipid Research 42 (2003) 463-478.
[7] R. Kopelman, Y.-E. Lee Koo, M. Philbert, B.A. Moffat, G. Ramachandra Reddy, P. McConville, D.E. Hall, T.L. Chenevert, M.S. Bhojani, and S.M. Buck, Multifunctional nanoparticle platforms for in vivo MRI enhancement and photodynamic therapy of a rat brain cancer. Journal of Magnetism and Magnetic Materials 293 (2005) 404-410.
[8] H.A. Clark, R. Kopelman, R. Tjalkens, and M.A. Philbert, Optical nanosensors for chemical analysis inside single living cells. 2. Sensors for pH and calcium and the intracellular application of PEBBLE sensors. Analytical Chemistry 71 (1999) 4837-4843.
[9] H.A. Clark, M. Hoyer, M.A. Philbert, and R. Kopelman, Optical nanosensors for chemical analysis inside single living cells. 1. Fabrication, characterization, and methods for intracellular delivery of PEBBLE sensors. Analytical Chemistry 71 (1999) 4831-4836.
[10] B.A. Moffat, G. Ramachandra Ready, P. McConville, D.E. Hall, T.L. Chenevert, R.R. Kopelman, M. Philbert, R. Weissleder, A. Rehemtulla, and B.D. Ross, A Novel Polyacrylamide Magnetic Nanoparticle Contrast Agent for Molecular Imaging using MRI. Molecular Imaging 2 (2003) 324-332.
[11] K.L. Dearfield, C.O. Abernathy, M.S. Ottley, J.H. Brantner, and P.F. Hayes, Acrylamide: Its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity. Mutation Research 195 (1988) 45-77.
[12] M. Friedman, Chemistry, biochemistry, and safety of acrylamide. A review. Journal of Agricultural and Food Chemistry 51 (2003) 4504-4526.
[13] M.J. Caulfield, G.G. Qiao, and D.H. Solomon, Some aspects of the properties and degradation of polyacrylamides. Chemical Reviews 102 (2002) 3067-3083.
[14] B.M. Mitruka, and H.M. Rawnsley, Clinical, biochemical and hematological reference values in normal experimental animals and normal humans., Masson Publishing, New York, 1981.
[15] S.M. Moghimi, and A.C. Hunter, Capture of stealth nanoparticles by the body's defences. Critical Reviews in Therapeutic Drug Carrier Systems 18 (2001) 527-550.
[16] D.E. Owens Iii, and N.A. Peppas, Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. International Journal of Pharmaceutics 307 (2006) 93-102.
[17] Z. Panagi, A. Beletsi, G. Evangelatos, E. Livaniou, D.S. Ithakissios, and K. Avgoustakis, Effect of dose on the biodistribution and pharmacokinetics of PLGA and PLGA-mPEG nanoparticles. International Journal of Pharmaceutics 221 (2001) 143-152.