Pharmacokinetics and clearance properties of nano-sized particles and molecules as multi-modality imaging agents: Considerations and caveats

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ABSTRACT

Nano-sized particles and molecules possess enormous potential as diagnostic imaging agents and hold promise for the development of multimodality agents with both imaging and therapeutic capabilities. Yet, some of the most promising nano-materials currently under investigation demonstrate prolonged tissue retention and contain heavy metals. This presents serious concerns for toxicity and additionally, prolonged particle retention may interfere with diagnostic imaging and testing modalities. The creation of nano-sized particles and molecules with optimal pharmacokinetics and clearance characteristics will minimize toxicity risks by reducing the duration of exposure to these agents. Given that many types of nano-materials possess easily modifiable surface chemistry, if nanoparticle characteristics associated with optimal pharmacokinetics and clearance from the body were well established, it would be feasible to design and create agents with more favorable clearance properties. In this talk, based on our experiences of dendrimer-based nano-sized MRI/Optical/Nuclear multimodality imaging agents, I will discuss the physiologic clearance of nano-sized particle and molecule, especially focusing on renal mechanisms, as well as provides an extended view of current research investigating clearance of other types of nano-sized particles and molecules, including dendrimers, quantum dots, and carbon, gold, and silica-based nanoparticles.

Keywords: nanomaterials, imaging, clearance, nano-toxicology, dendrimer

1 INTRODUCTION

Nanoparticles and nano-sized molecules possess enormous potential as diagnostic imaging agents and hold promise for the development of multimodality agents with both imaging and therapeutic capabilities. Generally defined as molecules with lengths that range from 1 to 100 nm in sizes, nanoparticles and nano-sized molecules show remarkable structural diversity and include nano-tubes, dots, wires, fibers, and capsules [1]. Although nanotechnology has exciting implications for medicine, this technology presents challenges regarding particle biocompatibility and much is to be learned regarding the understanding of small particle behavior *in vivo*. Unlike conventional imaging agents and therapeutics, many nanoparticles are highly stable *in vivo*—exemplified by a recent study suggested that quantum dots may be retained in the body (and remain fluorescent) for more than 100 days [2]. Additionally, some of the most promising nanoparticles currently under investigation contain heavy metals, which not only pose a risk for toxicity, but prolonged particle retention may interfere with diagnostic testing and imaging [3].

Creating nano-sized particles or molecules with optimal clearance characteristics will minimize toxicity risks and decrease concerns of nanoparticle and nano-sized molecules interference by reducing the duration of exposure to these agents. Given that many types of nanoparticles possess easily modifiable surface chemistry, if nanoparticle characteristics associated with optimal clearance were well established, it would be feasible to create agents with more favorable clearance properties. Properties currently known to affect clearance include particle material, size, shape, surface chemistry and charge—all of which vary depending on the individual particle type and modifications made for specific applications. This paper presents a thorough discussion of the physiologic clearance of nanoparticle and nano-sized molecules including dendrimers, quantum dots, liposomes and carbon, gold, and silica-based nanoparticles, focusing on renal mechanisms.

2 RENAL CLEARANCE OF NANO-SIZED AGENTS

The kidney is capable of rapidly removing molecules from the vascular compartment mostly as the injected forms and therefore, renal excretion represents a desirable pathway for nano-sizd agent removal with minimal catabolism or breakdown from the human body to avoid the possible side effects. Therefore, in this presentation, I am focusing mainly on the renal clearance. Renal clearance of intravascular agents is a multifaceted process involving glomerular filtration, tubular secretion, and finally elimination of the molecule through urinary excretion. As intravascular agents enter the glomerular capillary bed, molecules are either filtered through the glomerular capillary wall and into the proximal tubule, or remain within the vascular compartment. The glomerular capillary wall is composed of three layers: fenestrated endothelium; glomerular basement membrane (GBM); and the foot processes of glomerular epithelial cells, which are separated by filtration slits bridged by slit diaphragms [10]. It is generally accepted that glomerular filtrate flows through the

fenestrate, across the GBM, and through the filtration slits [10] (Fig. 1 right lower). Among the key nanostructural dimensions is the slit diaphragm, which is approximately 43 nm in diameter [10]. However, after taking into considering the combined effects of each layer of the glomerular capillary wall, the functional or physiologic pore size is significantly smaller—measuring only 4.5-5 nm in diameter [11].

Filtration of particles through the glomerular capillary wall—glomerular filtration—is highly dependent on molecule size and is referred to as the filtration-size threshold [10]. Molecules with an HD < 6 nm are typically filtered, while those > 8 nm are not typically capable of glomerular filtration. Filtration of molecules within the intermediate range, 6-8 nm in HD, depends upon both size and charge of the particle. The filtration-size threshold for globular proteins has been well studied and is generally accepted to be < 5 nm in HD. For example, inulin (HD: 3 nm) achieves 100% renal filtration with a blood half life of only 9 minutes [12]. The relationship between protein size and clearance is further demonstrated by antibody clearance data. The stabilized V region fragment of an antibody protein (HD: 4 nm) achieves 100% renal filtration with a serum half life of 5 minutes [13] as compared to the antibody Fab' fragment (HD: 6.0 nm) which achieves only 9% effective filtration into the urine with a serum half-life of 28 minutes [5, 14]. Additionally, clearance of antibody fragments is more rapid that that of the full intact antibody, which is not efficiently filtered and has a serum half-life up to many days.

Although the fate of globular proteins in the kidney provides an important framework for understanding general properties of renal clearance, the physical properties of nano-sizd agents differ from those of proteins in several ways. nano-sizd agent shape, surface chemistry, and interior charge is distinct and unlike proteins, which are heterogeneous and polydispersive, nano-sizd agents can be synthesized with near-spherical shape and identical surface chemistry [15]. Such differences may lead to distinct renal handling of nano-sizd agents compared to protein

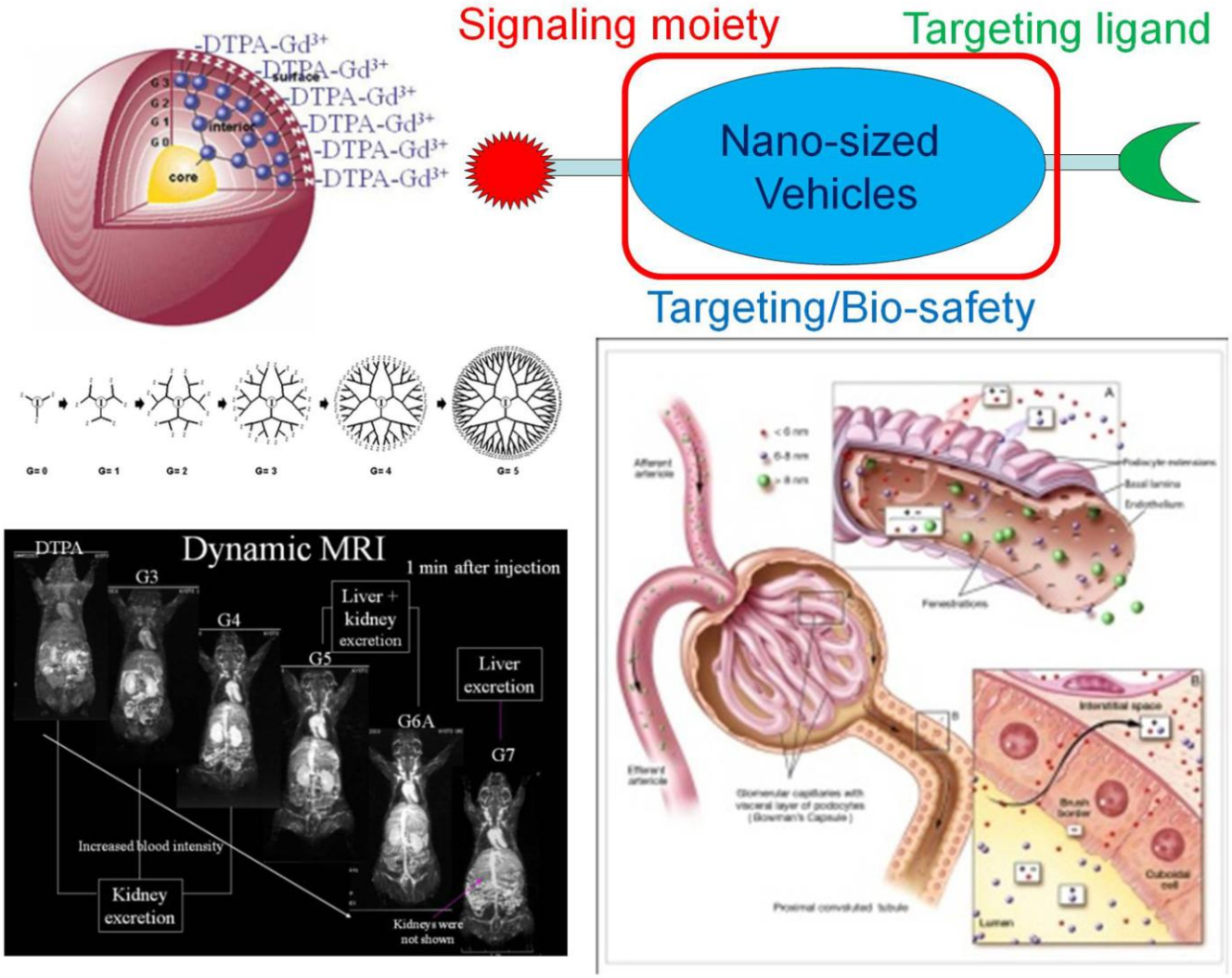

Fig. 1. Renal excretion of nano-sized molecules and paericles.

molecules. To evaluate the effect of these characteristics on filtration-size threshold, clearance studies of PAMAM dendrimer-based nano-sizd agents with homogenous chemical properties and near-spherical shape were conducted. Results showed that PAMAM dendrimers < 6 generations or about 5.4 nm in diameter demonstrated effective glomerular filtration [16, 17] (Fig. 1 left).

This filtration-size threshold is similar to that of conventional molecules. Additionally, renal clearance studies in mice using quantum dots revealed that renal excretion was observed for dots with HDs ranging form 4.36-5.52 nm [3]. Quantum dots > 8 nm (HD = 8.65 nm) did not demonstrate renal filtration but instead exhibited uptake in the reticuloendothelial system (RES) and lung [3]. The relationship between HD, renal clearance, and total body retention was determined to be sigmoidal with the 50% point for total body clearance at 4 h achieved with an HD of 5.5 nm [3]. Results of this study also suggests that the filtration-size threshold for of nano-sizd agents may comparable to proteins and other nano-sized molecules. With these data in mind it is reasonable to conclude that nano-sizd agents capable of being synthesized < 8 nm in diameter, such as dendrimers, fullerenes, carbon nanotubes, silica particles and quantum dots, can undergo renal clearance if other modifiable parameters (surface charge and chemistry) are optimized for this excretion pathway.

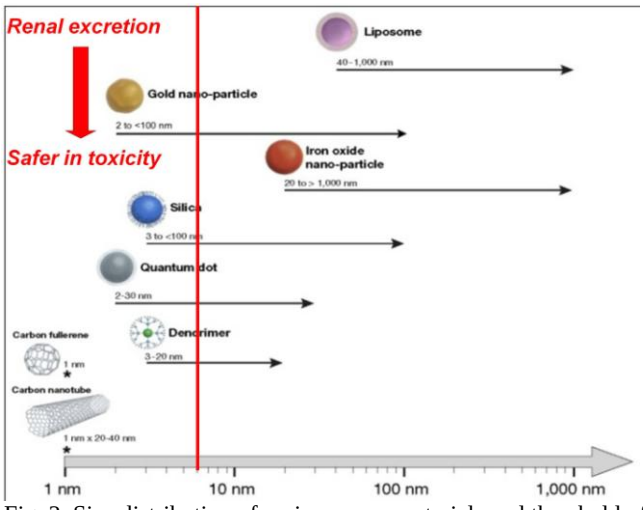

Fig. 2. Size distribution of various nano-materials and threshold of renal excretion.

In addition to size, surface charge is also an important determinant of the renal handling of nano-sized molecules. The effect of molecular charge on renal filtration is due to at least 2 factors: 1) potential interactions between charged molecules and serum proteins, resulting in increased HD due to particle adsorption [3], and 2) interactions between charged molecules and fixed charges within the glomerular capillary wall [10]. Studies using quantum dots as a model for *in vivo* nano-sizd agent clearance revealed that purely anionic or cationic charge was associated with agent adsorption by serum proteins [3]. Adsorption resulted in an increase in the HD to > 15 nm, dramatically reducing renal filterability [3]. Neutralization of the nano-sizd agent surface via PEGylation was shown to be effective in preventing serum protein adsorption; however, it was reported that synthesizing a PEGylated QD with an HD < 10 nm was not possible [3]. Therefore, PEGylation, also dramatically reduces particle renal filtration [3]. Interestingly, zwitterionic coatings were shown to prevent serum protein adsorption and were associated with the highest solubility and smallest HD [3] (Fig. 1 right lower).

Studies evaluating the effect of molecular charge on glomerular filtration of similarly sized molecules have shown that filtration is greatest for cationic molecules, followed by neutral molecules, while anionic molecules are least readily filtered through the glomerular capillary wall (Figure 2) [10, 11]. Direct comparison of differently charged molecules with respect to their glomerular filtration has been studied using a charge-modified Fab (HD: 6 nm), which was created by blocking branching amine residues with glycolate [18]. Weakly anionic Fab showed drastically less filtration compared to weakly cationic Fab fragments [18]. Charge-selective filtration is attributed to fixed negative charges within the capillary wall [10]. Molecular charge is of particular significance for the filtration of molecules within the 6-8 nm range, as these particles are not small enough to undergo charge-independent filtration, yet may still be filtered if molecular charge is favorable.

The final step in renal processing occurs at the proximal tubule where filtered molecules may be resorbed from the tubular fluid and previously unfiltered molecules may be actively secreted into proximal tubule lumen. Particle behavior at the proximal tubule is an important consideration because some molecules, such as glucose, achieve 100% resorption, negating the effects of glomerular filtration, while other agents, such as heavy metals, are highly toxic to proximal tubule cells and may potentially cause renal damage including acute tubule necrosis, interstitial nephritis and even renal failure. Research evaluating nano-sizd agent reabsorption at the proximal tubule is currently limited. Studies by Kobayashi et al indicate that some polyamine dendrimers may undergo proximal tubule reabsorption [19], but further investigation of this issue is needed to gain insight into the renal toxicity profile of nano-sized particles. Evaluation of agent behavior at each step in renal processing may lead to agents with optimal biocompatibility and clearance properties.

3 CONCLUSIONS

Nanoparticles represent a promising technology for the detection and treatment of human diseases such as inflammatory conditions and cancer. Yet this field poses many new questions regarding the pharmacokinetic behavior and safety of nanometer sized particles within living systems. In standard practice, the US Food and Drug Administration requires that agents administered for diagnostic purposes be cleared completely from the human

body within a reasonable time period [3]. This is a prudent approach given that the time required for total body clearance is directly related to total agent exposure. Although nanoparticles are known for their wide variability in shape, size, and chemistry, trends regarding particle clearance do seem to be generalizable despite particle differences. Given that renal filtration represents the ideal route for NP removal from the body, this review demonstrates particles may be optimized to increase renal clearance by modifications including size < 6 nm and zwitterionic or cationic surface charge [3]. Additionally, future research exploring alternative mechanisms for the removal of nanoparticles, such as by intracellular degradation may, lead to the utilization of currently unexploited pathways for particle clearance.

Clearance of metal-containing nanoparticles is an especially important consideration due to agent toxicity and potential for interference with other diagnostic imaging modalities. Metal nanoparticles may interfere with X-ray imaging due of changes in linear attenuation coefficient, magnetic resonance imaging because of proton-free voids, ultrasound because of increased echogenicity, and possibly even single photon emission computed tomography and positron emission tomography (PET) because of photon attenuation [3]. In addition, metal-containing nanoparticles require a solubilizing organic coating for biological compatibility, which increases HD and results in increased retention times [3].

Given that particle elimination continues to limit progress towards the clinical use of many of the emerging nanoparticles developed for imaging applications, a comprehensive understanding of the basic determinants of nanoparticle clearance from the human body would be of tremendous benefit as this would permit more expeditous development of particles capable of renal clearance as well as permit the optimization of agents excretion via the hepatobiliary pathway. With this said, however, given the wide variation in particle material, size, and surface coating, it is likely that agent specific clearance studies will also be required.

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