# Chapter 15
# The Absorption, Distribution, Metabolism, and Excretion Profile of Nanoparticles

**Yasuo Yoshioka, Kazuma Higashisaka, Shin-ichi Tsunoda, and Yasuo Tsutsumi**

**Abstract** Advances in nanotechnology have led to the recent development of many nanoparticles. With the growing commercialization of nanoparticles, opportunities for human exposure to nanoparticles will increase substantially. For the development of nanoparticles with efficacy and safety, a systematic and thorough analysis of the absorption, distribution, metabolism, and excretion (ADME) of nanoparticles is essential. In this chapter, we present the current understanding regarding the ADME profile of nanoparticles.

**Keywords** ADME • Biologic barriers • Protein corona • Safety

Y. Yoshioka, Ph.D. (✉) • K. Higashisaka
Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
e-mail: yasuo@phs.osaka-u.ac.jp

S.-i. Tsunoda
Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, 7-6-8 Saitoasagi, Ibaraki, Osaka 567-0085, Japan

The Center for Advanced Medical Engineering and Informatics, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

Y. Tsutsumi, Ph.D. (✉)
Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, 7-6-8 Saitoasagi, Ibaraki, Osaka 567-0085, Japan

The Center for Advanced Medical Engineering and Informatics, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
e-mail: ytsutsumi@phs.osaka-u.ac.jp



M. Akashi et al. (eds.), *Engineered Cell Manipulation for Biomedical Application*, Nanomedicine and Nanotoxicology,
DOI 10.1007/978-4-431-55139-3_15

## 15.1 Introduction

Nanotechnology makes it possible to design, characterize, and produce nanostructured materials by controlling their shape and size at the nanoscale. In general, nanoparticles are defined as materials whose structures have at least one dimension on the order of 100 nm or less, although there is no reason to assume that 100 nm would be an absolute threshold for changes in the physicochemical properties of these particles. Nanoparticles have various desirable properties, including enhanced electrical conductivity, tensile strength, and chemical reactivity, which are due to their increased surface area per unit weight compared with that of bulk-scale counterparts. Diverse nanoparticles such as silica nanoparticles and carbon nanotubes (CNTs) have become widespread in use through their applications in electronics, sunscreens, cosmetics, diagnostic medicines, and drug-delivery systems, among other products. In particular, the clinical applications of nanoparticles have been investigated for more than 30 years. For biomedical applications, nanoparticles including mesoporous silica nanoparticles, CNTs, quantum dots, and superparamagnetic nanoparticles have been evaluated as drug-delivery and diagnostic vehicles. For example, because of their unique mesostructural features, high drug-loading capacity, and sustained-release profiles, mesoporous silica nanoparticles are potential candidates for controllable drug-delivery agents, gene delivery vehicles, vaccine carriers, and many other biologic applications.

The increasing use of nanoparticles has prompted public concern regarding their potential toxicity. In particular, recent reports have indicated that CNTs cause mesothelioma-like lesions in mice, in a manner similar to that of asbestos-induced mesothelioma [1, 2]. Because nanoparticles have great potential to improve the quality of human life, it is essential to ensure the safety of nanoparticles for the development of safety-assessed products. The toxicity of nanoparticles is related to the dose, concentration, and duration of the exposure and their abundance and persistence in tissue. Accordingly, a systematic and thorough analysis of the absorption, distribution, metabolism, and excretion (ADME) of nanoparticles is essential as the basis for determining the potential for risk to human health. In addition, understanding of the ADME of nanoparticles is necessary not only in regard to their tissue toxicity but also their potential biomedical applications. In this chapter, we present the current understanding regarding the ADME profile of nanoparticles (particularly inorganic nanoparticles).

## 15.2 ADME via Several Exposure Routes

Various barriers prevent the entry of foreign substances, including viruses and bacteria, into the body. These same barriers modulate the access of nanoparticles and include the skin, gastrointestinal tract, and pulmonary system. Previously, only

small lipophilic molecules (<600 Da) and metallic ions (e.g., nickel and cobalt) have been able to penetrate the skin barrier. However, because of their small size, nanoparticles might be readily absorbed through the dermis of the skin and the pulmonary and gastrointestinal mucosa, thus positioning these compounds for distribution through the vascular circulation to all tissues in the body. With an average pore size of ~5 nm in mammals, the vascular endothelium presents another potential barrier to the absorption and delivery of nanoparticles, but nanoparticles smaller than this limit penetrate rapidly from blood across the endothelium and into tissue. In addition, nanoparticles potentially can translocate efficiently from blood into the liver, spleen, and bone marrow, because the discontinuous endothelium characteristic of these organs has pores of 50–100 nm in diameter. Therefore, methods for estimating the amount of the total external exposure, efficacy of absorption, and tissue biodistribution of nanoparticles are needed.

Many studies have reported that nanoparticles penetrate the biologic barriers after inhalation or oral or dermal exposure and have used qualitative and quantitative methods to assess the effect of the size and surface properties of nanoparticles on biologic behaviors. In the following sections, we discuss the absorption of nanoparticles via several exposure routes.

### 15.2.1 Dermal Exposure

Because clothing, drugs, cosmetics, and various skin care products contain nanoparticles, their contact with the skin occurs intentionally as well as accidentally. In particular, nanoparticles have been included in cosmetics and sunscreen to provide protection against ultraviolet radiation. Therefore, understanding the absorption rate of nanoparticles after exposure via the skin has garnered increasing attention during the past several years. However, whether nanoparticles actually penetrate the skin barrier in vivo is unclear, although many studies have assessed the skin penetration of nanoparticles after topical application of compounds. Several reports have stated that titanium dioxide, ZnO nanoparticles, and silver nanoparticles penetrate into the upper layers of the stratum corneum but not deeper into the viable epidermis and dermis [3–5]. In contrast, other studies showed that 40-nm, but not 750- or 1,500-nm, polystyrene nanoparticles and 40-nm silica nanoparticles can translocate to the viable epidermis in human skin explants with partially disrupted stratum corneum [6, 7]. In addition, Mortensen and colleagues showed that quantum dot nanoparticles penetrate deep into the epidermis and dermis of mice exposed to ultraviolet irradiation [8], which induces skin-barrier defects such as disruption of stratum corneum lipids and loosening of cell–cell junctions. Because consumers often apply sunscreen to sun-damaged skin, it is more important to examine the effect of ultraviolet radiation on the ability of nanoparticles to penetrate the skin.

### 15.2.2 Gastrointestinal Exposure

People in developed countries ingest an estimated $10^{12}$ to $10^{14}$ manufactured fine (diameter, 0.1–1 mm) to ultrafine (diameter, <100 nm) particles in food every day [9]. In particular, amorphous silica particles (including nanosize particles) are widely applied in food products and registered within the European Union as a food additive (E551). These particles are used mainly as thickening medium for pastes, as an anti-caking agent to maintain flow properties in powdered products, and as a carrier for fragrances and flavors in food and nonfood products [10]. However, little information about the absorption of nanosilica particles after oral exposure is available, mainly because there is no high-sensitivity method for detecting silicon in biologic tissues. One study showed the effect of size on the absorption of nanoparticles after oral administration in rats by using gold nanoparticles of different sizes (1.4–200 nm) [11]. The smallest particles had the highest absorption across intestinal membranes: after 24 h, 0.37 % of the applied 1.4-nm particles had reached the circulation. Surface charge was important in the absorption across intestinal membranes also, and more negatively charged gold nanoparticles were absorbed than were positively charged particles. Furthermore, the greatest accumulation of particles in the heart and brain after oral administration was associated with the 18-nm particles, which accumulated to even greater amounts than did the 1.4-nm particles; the precise mechanism underlying this effect is unknown. In summary, although small nanoparticles tend to be absorbed more readily than are large particles, no general assumption regarding tissue accumulation after particle absorption can yet be made.

### 15.2.3 Pulmonary Exposure

Nanoparticle-facilitated drug delivery through the lung is attractive because of the organ's large surface area. In addition, knowing the ADME profile of nanoparticles after lung exposure is particularly important, because the inhalation of nanoparticles increasingly is recognized as a major cause of adverse health effects. Gold nanoparticles 1.4 nm in diameter efficiently cross the air–blood barrier of the respiratory tract, whereas almost all 18-nm particles remain trapped in the lungs after intratracheal instillation in rats [12]. In addition, the biodistribution patterns (e.g., organ ratios) of 1.4-nm gold nanoparticles differed markedly after intravenous injection compared with intratracheal instillation. For example, the liver–blood ratio is 1.1 to 1 after instillation compared with 12.5 to 1 after intravenous injection, suggesting that 1.4-nm nanoparticles undergo some unknown chemical or biochemical transformation process during translocation through the lung. Another possibility is that the interaction of nanoparticles with alveolar fluid in the lungs during inhalation exposure or with blood proteins during intravenous injection has different effects on the surface properties of nanoparticles, leading to differences in tissue uptake. Therefore, data after intravenous injection might be unreliable for

predicting the biodistribution of nanoparticles after their passage through various barriers and may give misleading information about potential harmful effects.

Most of the biodistribution studies that have assessed nanoparticles to date occur over a relatively long time, from several hours to weeks. In contrast, Choi and colleagues used near-infrared fluorescent nanoparticles to examine biodistribution during the first hour after their administration [13]. In that study, nanoparticles with a hydrodynamic size less than 34 nm rapidly moved from the lung to mediastinal lymph nodes, and nanoparticles smaller than 6 nm rapidly moved from the lung to lymph nodes and the bloodstream, leading ultimately to renal clearance. This and other new experimental methodologies may provide new insights into the ADME profile of nanoparticles. In addition, future studies should minimize doses and examine biodistribution in more organs for prolonged periods to fully characterize the potential health effects of exposure to nanoparticles.

## 15.3 Translocation of Nanoparticles Across Internal Biologic Barriers

The blood–brain, blood–testis, and placental barriers protect particularly sensitive tissues from foreign chemicals. In this section, we discuss the translocation of nanoparticles across various internal barriers, especially the placenta.

Considerable evidence shows that, because of their physiologic immaturity, fetuses are more sensitive than are adults to numerous environmental toxins. Recurrent pregnancy loss affects 1–3 % of couples; many of these miscarriages undergo extensive—but ultimately uninformative—diagnostic testing. In addition, intrauterine growth restriction occurs in as many as 10 % of pregnancies and predisposes the child to a lifelong increased risk for hypertension, cardiovascular disorders, and renal disease, among others. Although many factors for miscarriage and intrauterine growth restriction have been suggested, the precise mechanism and mediators remain unknown. It is essential to assess the potential risk of nanoparticles to cause these pregnancy complications.

Normal placental development is required for successful embryonic growth, and placental dysfunction has been associated with miscarriage and fetal growth restriction. Some reports have warned about the potential adverse effects of nanoparticles on fetuses [14–17]. In one study, silica nanoparticles (diameter, 70 nm) and titanium dioxide nanoparticles induced miscarriage and fetal growth restriction in pregnant mice, whereas microscale silica particles did not induce these complications [15]. The observed pregnancy complications resulted from placental dysfunction, such as destruction of the placental vasculature. Notably, surface-modified silica nanoparticles (diameter, 70 nm) did not induce any pregnancy complications in mice. Furthermore, whereas maternal pulmonary exposure to carbon black during pregnancy had adverse effects on the offspring [18, 19], repeated oral administration of multi-walled CNTs during pregnancy did not cause fetal toxicities [20].

Several studies have addressed the biodistribution of nanoparticles to the fetus and placenta. For example, after intravenous injection of pregnant mice, silica nanoparticles (diameter, 70 nm) were detected in the maternal liver and placenta and the fetal liver and brain, although microscale silica particles were not noted in any of these tissues [15]. In addition, when administered by intravenous injection early during pregnancy, PEGylated single-walled CNTs reached the conceptus, whereas these nanotubes reached only the placenta and yolk sac—not the embryo—when injection occurred during later pregnancy stages [17]. Furthermore, gold nanoparticles (diameter, 13 nm) accumulated in fetuses more efficiently when pregnant mice were injected before embryonic day E11.5 than after E11.5, indicating that the stage of placental maturity influences the translocation of nanoparticles to murine fetuses [21]. This same study showed that modification of the surface of nanoparticles altered their translocation to fetuses. Therefore, the translocation of nanoparticles to fetuses during murine pregnancy is influenced both by the stage of placental maturity and by nanoparticle surface composition.

Mouse and human placentas differ in their modes of implantation, the relative importance of yolk sac placentation, and the structure (labyrinthine compared with villous) of the exchange area. For example, by using an ex vivo human placental perfusion model, Wick and colleagues showed that polystyrene nanoparticles smaller than 240 nm can cross human placental tissues to reach fetuses [22].

## 15.4 Translocation of Nanoparticles Across Cellular Barriers

Various pathways, including passive and active diffusion, enable nanoparticles to travel across external and internal barriers. For example, small nanoparticles might access the paracellular pathway by evading the tight junctions between epithelial and endothelial cells, which are considered to exclude molecules larger than 0.6–5 nm [23].

In the gastrointestinal tract, enterocytes (especially M cells) may facilitate the transport of nanoparticles through transcytosis. Chitosan nanoparticles enhance intestinal paracellular uptake by modulating tight junctions between cells [24]. Another potential mechanism for the translocation of nanoparticles in the gastrointestinal tract involves degrading enterocytes, which are shed in high numbers daily (mice, $2\times10^8$ cells; humans, $10^{11}$ cells) in the small intestine [25].

In human endothelial cells in vitro, iron particles induced the production of reactive oxygen species [3] and oxidative stress, consequently increasing microtubule remodeling and permeability in these cells [26]. In a particularly novel mechanism of nanoparticle transport, titanium dioxide nanoparticles have been shown to interact directly with the protein VE-cadherin at the inter-endothelial adherens junction niche to promote actin remodeling as well as internalization and degradation of VE-cadherin, thus increasing the leakiness of endothelial cells [27]. Therefore, the pathway that nanoparticles use to traverse cellular barriers likely varies depending on their material, size, and charge; these relationships need to be explored more thoroughly to fully understand the ADME profile of nanoparticles.

## 15.5 Metabolism, Degradation, Excretion, Clearance, and Biopersistence of Nanoparticles

The clearance or excretion of nanoparticles is important for assessing their long-term toxicity, because nanoparticles may be inherently stable. For example, quantum dots with the appropriate coating were retained in mice and remained fluorescent for at least 2 years [28]. In this way, the biopersistence of nanoparticles is a key factor in understanding their toxicity. The ability of cells to metabolize or degrade nanoparticles and to excrete or otherwise clear them from the body minimizes their potential toxicity. To this end, we need to learn the pathways that are responsible for breaking down nanoparticles and for removing them from the body. In addition, monitoring the concentration of nanoparticles in cells and tissues over long time periods (e.g., months) will inform us regarding the lifespan of these particles as well as their long-term biologic effects. In this section, we discuss the metabolism, degradation, excretion, clearance, and biopersistence of nanoparticles.

### *15.5.1 Excretion and Clearance of Nanoparticles*

There are two major routes for the excretion and clearance of nanoparticles: renal filtration, with excretion into the urine, and hepatobiliary processing, with excretion into the bile. In general, proteins with a hydrodynamic diameter of <5–6 nm are cleared rapidly from the body by renal filtration and urinary excretion. Choi and colleagues used quantum dots to show that the renal clearance of nanoparticles similarly is related to their hydrodynamic diameter: quantum dots smaller than 5.5 nm were excreted into urine rapidly and efficiently and eliminated from the body, but the renal clearance of quantum dots larger than 15 nm was relatively low [29]. In another study, the accumulation of 1.4-nm gold nanoparticles in the liver and spleen was significantly lower than that of 18-nm nanoparticles, and 1.4-nm gold nanoparticles were excreted by both the renal and hepatobiliary systems after intravenous injection [12]. In comparison, single-walled CNTs (average diameter, 1 nm; average length, 300–1,000 nm) and multi-walled CNTs (average diameter, 20–30 nm; average length, 500–2,000 nm) undergo rapid and effective renal clearance and urinary excretion after intravenous injection, although the precise mechanism is unknown [30].

In the reticuloendothelial system, hepatic Kupffer cells are the representative cells that have the ability to uptake particles. In general, the liver is the predominant target organ of nanoparticle accumulation as well as an important excretion route. Various studies have shown that about 4 % of the administered dose of polystyrene nanoparticles (diameter, 50 nm) was excreted as intact particles in bile within 24 h after intravenous injection, and about 30 % of the total dose accumulated in hepatocytes [31, 32].

Recent investigations have revealed a novel clearance mechanism in the immune system. Macrophages play an important role in the uptake of nanoparticles, and the biodistribution of nanoparticles (e.g., blood clearance) differed between strains of

mice with differences in global immune status [33]. In the cited study, Jones and colleagues used intravital microscopy to show that PEGylated particles (diameter, 300 nm) are cleared more slowly in Th1-prone mice than in Th2-prone mice. M2 macrophages, which are induced by Th2 cytokines and have high levels of endocytosis, were important in the enhanced clearance observed in Th2-prone mice. In addition, these results were observed in macrophages from humans, suggesting that global immune regulation might significantly affect nanoparticle clearance in humans. Furthermore, the study suggested that granulocytes, especially neutrophils, are also important in the clearance of nanoparticles, although this cell type is seldom addressed in this context [33]. In addition to their phagocytic capacity, neutrophils can release complexes of DNA and protein into the extracellular space, thus trapping pathogens at infection sites (neutrophil extracellular traps); neutrophils may trap nanoparticles in extracellular structures by a similar mechanism [34]. Clearly future studies need to address the excretion or clearance of nanoparticles targeted not only to the kidney and liver but also immune cells.

### *15.5.2 Metabolism and Degradation of Nanoparticles*

Nanoparticles had been thought to be resistant to metabolism and degradation under in vivo conditions, but several recent reports suggest that CNTs are degraded through natural enzymatic catalysis. In that regard, Kagan showed that myeloperoxidase (MPO), an abundant enzyme of inflammatory cells (neutrophils), played an important role in the oxidative biodegradation of single-walled CNTs [34]. In addition, single-walled CNTs that had been degraded by MPO in vitro failed to induce inflammatory and oxidative-stress responses after pharyngeal aspiration in mice, whereas intact nanotubes induced these responses. Consistent with these results, the clearance of single-walled CNTs from the lungs was much less effective in MPO-deficient than in wild-type mice after pharyngeal aspiration, whereas the inflammatory responses were much robust compared to wild-type mice [35]. In addition, single-walled CNTs are degraded by eosinophil peroxidase, a key oxidant-producing enzyme during inflammatory states [36]. Collectively, these findings suggest new ways to control the biopersistence of CNTs through genetic or pharmacologic manipulations. However, few studies have investigated the metabolism and degradation of nanoparticles in cells, because methods for monitoring a single particle in cells over time are currently unavailable. Given the numerous factors that might influence the metabolism and degradation of nanoparticles, methods for investigating these processes at the nanoscale are urgently needed.

### *15.5.3 Biopersistence of Nanoparticles*

The precise mechanism underlying the biopersistence of nanoparticles is not yet fully understood. To this end, Balasubramanian and coworkers found that gold nanoparticles were rapidly and consistently accumulated in the liver and spleen of

rats within 1 day after a single intravenous injection and remained at high levels at 2 months thereafter [37]. These findings are consistent with several studies showing that, regardless of their size, shape, dose, and material, intravenously injected nanoparticles accumulate in the liver and spleen and are retained in those organs for long periods of time. For example, regardless of the size of the silver nanoparticles (10 or 25 nm) to which rats were exposed orally for 28 days, the silver content in most tissues gradually decreased to control levels over the 4-month observation period [38]. However, the silver concentrations in the testes and brain did not decrease to control levels, suggesting reduced clearance of silver nanoparticles across biologic barriers such as the blood–brain and blood–testis barriers.

## 15.6 Protein Corona

When nanoparticles enter the body, they typically become coated with various proteins, thereby developing a "protein corona." The binding of proteins to nanoparticles depends on their various physical characteristics, such that the size and surface properties of nanoparticles play important roles in determining the protein corona. Some studies have suggested that these interactions with proteins actually define the biologic effects and biodistribution of nanoparticles. For example, a recent in vitro study suggested that the formation of coronas of blood proteins reduced undesirable cellular responses to and the cytotoxicity of CNTs [39]. In addition, protein coronas have proven to be a key factor in the recognition and phagocytosis of nanoparticles by macrophages. Deng and coworkers showed in vitro that negatively charged gold nanoparticles bind to and induce the unfolding of fibrinogen, consequently promoting its interaction with the integrin receptor Mac-1 [40].

Nanomaterials can be covered with biologic molecules other than proteins as well. For example, creating a "corona" of lipids has been suggested as a means to influence the cellular uptake and toxicity of nanoparticles [41, 42]. In this regard, Konduru and colleagues showed that the adsorption of phosphatidylserine onto the surface of single-walled CNTs enhanced their uptake by macrophages and dendritic cells in vitro [43]. In addition, single-walled CNTs were found to be coated with surfactant proteins and phospholipids after pharyngeal aspiration of the nanotubes in mice, and the presence of this surfactant coating enhanced the uptake of the nanotubes into macrophages in vitro [44].

The ADME of many nanoparticles in vivo likely is largely defined by the protein corona rather than the nanoparticle itself. However, little information is available that addresses relationships between differences in the ADME profiles of nanoparticles in vivo and the formation of protein coronas. In this context, one study revealed that attaching plasma proteins onto dextran-coated superparamagnetic iron oxide nanoparticles is unlikely to alter their clearance by the liver and spleen, because the plasma proteins do not mask the entire surface of the nanoparticle [45]. One key question is whether the corona present at the point of entry (e.g., blood, lung, or other) or that resulting from modification during subsequent translocation determines the biodistribution and effects of nanoparticles.

The detailed mechanism underlying the fate of the original corona as the coated nanoparticle passes through membranes and barriers and interacts with the extracellular matrix is unknown currently: the original corona may remain intact or be replaced by new biomolecules. Another important question is whether the exposure route influences the protein corona. For instance, the physicochemical changes in the protein corona that occur while nanoparticles reside in the lung or gastrointestinal tract may dramatically change their ability to cross various internal barriers. Whether the exposure route directs the formation of different coronas needs to be determined.

## 15.7 Manipulation of the ADME Profile for the Development of Nanomedicine

Foreign particles—including nanoparticles—are removed from the body by phagocytes, such as macrophages, which also recognize and avoid live, nonforeign cells. In medical applications, systemically administered nanoparticles should evade rapid clearance so that they achieve sufficient accumulation in targeted tissues and cells to yield effective local drug concentrations. One way to delay the clearance of nanoparticles is to conjugate them with polyethylene glycol (PEG) or another water-soluble polymeric modifier. The surface modification of nanoparticles with PEG decreases their uptake by macrophages and retards renal clearance, thereby prolonging the half-life of nanoparticles in vivo. The prolonged circulation of nanoparticles in the blood induces the enhanced permeability and retention effect, which is based on the leaky nature of tumor blood vessels and results in increased delivery of conjugates to tumor tissue [46, 47]. However, PEG modification of nanoparticles might hinder their uptake by and drug-delivery to diseased target cells. In this regard, Rodriguez and colleagues suggested another approach to prolonging the circulation time of nanoparticles. The membrane protein CD47, which is expressed on all cell membranes, is a marker of self that impedes the phagocytosis of nonforeign cells in mice. Nanoparticles carrying peptides designed from CD47 avoided macrophage-mediated clearance and were retained in the circulation, resulting in both enhanced tumor imaging and increased drug delivery [48]. Other homeostatic self factors might similarly be used to prevent the phagocytosis of nanoparticles and target them to specific tissues, thereby enhancing the delivery of therapeutics and imaging agents to these sites.

## 15.8 Conclusion

Engineered nanoparticles have remarkable structural diversity and adopt characteristic forms including tubes, dots, wires, fibers, and capsules. Several reports have shown that the shape of nanoparticles plays an important role in their biologic