Kinetic Behaviour of Nanoparticles Across the Biological Physiology

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Abstract. Nanotoxicokinetics is a subsection of the toxicology field that involves the study of kinetic displacement of nanoparticles (NPs) in an organism. Four different steps, namely absorption, distribution, metabolism and elimination (ADME), are involved in nanotoxicokinetics. However, only ADE will be covert in this mini review. Because of their size, NPs react differently than particulate matter larger than the nanometre unit in diameter. In the organism, a closer interaction between NPs and biological matrices, called nanotoxicodynamics, might increase the health effects. (Animals are usually in studies to evaluate the global interaction of NPs and biological matrices and to control and reduce the bias.) Understanding the different steps of kinetics is very important to increase the confidence of the amount of NP delivery in the target organ and to assess the level of risk. The objective of this work was to review the behaviour of the NPs interacting with the biological kinetic steps of the ADME and their limitations and constraints. Specifically, it was reviewed the impact of each of the four steps of nanotoxicokinetics, from exposure to elimination in the organism. Recent publications have provided some information on this issue, allowing for a better understanding on how the NPs behave across physiology; however, information is still lacking. We also systematically reviewed the ADME process, and supported our review with examples from the literature. We reviewed the two major factors that influence the absorption of NPs: enumerated biotransformation and elimination limitations. One of the focuses of this study was the interaction between NPs and biological matrices because the morphology and chemical properties may drive the potential for exposure. This paper present different examples of interactions find from literature. To study these interactions, we used a classical pharmacokinetic approach employed in the pharmaceutical industry and compared it to a dynamic predictive tool called the physiologically based pharmacokinetic model. This review would allow us to better interpret the behaviour of NPs. This review would also provide a better insight about the intake, site, and the disposition of NPs and would help identify the major consequences of the interaction of NPs with biological matrices. These interactions might have reversible or irreversible consequences for the integrity of the organism.
1. Introduction
The toxicology field is currently well positioned to understand the biological relevance between nanoparticles (NPs) and biological issues. Toxicological studies provide information on the defined disease mechanisms, based on dose–response relationships and related to the NPs characteristics that influence toxicity, including the size, surface area, chemistry or reactivity, solubility, and shape. Nanotoxicokinetics is a branch of toxicology and a new area in which this field evaluates NPs at the local and systemic levels. Nanotoxicokinetics is focused on understanding and predicting what is going to happen to the xenobiotic in the biota. Classically, we segment nanotoxicokinetics in four different steps, namely absorption, distribution, metabolism, and excretion (ADME). In the past, we did not imagine that particles might affect tissues at the systemic level. Toxicology studies aim to determine the biological plausibility of health effects from NPs and identify cascades of mechanisms that are causal for the gradual transition from the physiological status toward pathophysiological alterations, and eventually chronic diseases. In addition this field is considering the interaction between insoluble NPs and biological systems (e.g., body fluids, proteins, cells).

Many years later, we recognize that NPs range from 1 to 100 nanometres (nm), they can cross the biological membrane, and they can be translocated inside of cell organelles or entities such as the mitochondria, lysosome, nucleus, and others. Therefore, potential health risk will depend on the magnitude and nature of the exposures with NPs and on the physical behaviour of this particle related to dispersion, translocation and deposition. NPs have posed a new dilemma because they can migrate to body compartments away from the application site or deposition sites. In particular, because of their low uptake by macrophages, NPs are absorbed by endothelial cells, and they have access to cells in the epithelium, the interstitium, and the vascular walls [1]. Considering these challenges, it is important to understand the route taken by this new xenobiotic particulate structure.

The objectives of our study were to (1) highlight some limiting steps related to nanotoxicokinetics and (2) emphasise the difficulty of finding pertinent information because there is a lack of data in this field. To respond to these objectives our work focused on the ADME steps. The absorption step explored the different route by which NPs can reach the systemic circulation and then target tissue in the organism. The major exposure routes were oral, inhalation, and the cutaneous routes. Following absorption, NPs are distributed in the organism, are free bind to proteins, and are incorporated in specialised cells. Biotransformation will only barely be introduced because commercial NPs are not easily degraded unless they are functionalised with a long chain peptide or unstable group NPs. Section 4 will discuss excretion or externalisation of the NPs and the impact of this action.

2. Absorption
Absorption is the procedure by which a xenobiotic crosses the biological barrier from the environment into the organism. For NPs, absorption can be occurring via oral route from gastrointestinal tract (GIT), in the lung (inhalation), and across the skin (cutaneous). These routes have different properties and degrees of penetration depending on the characteristics of the NPs.

2.1. Oral absorption
According to the literature, mice, rats, sheep, pigs, and cows can absorb NPs from the GIT. Florence observed that the ability of intact microparticles and NPs to be absorbed through the gut walls was different [2]. A literature consensus mentioned that the absorption increases with decreasing particle diameter. NPs such as polystyrene latex have been recognised as a useful model because they do not easily degrade. This study confirmed that NPs ranging from 50 to 100 nm in diameter reached the maximal absorption rate. However, NPs greater than 1 micrometre (µm) are being trapped in the Peyer’s patches (PP) [3], [4]. It seems that at this size, NPs are not translocated to the systemic circulation. Several reviews on the oral uptake of NPs have been published.

Oral absorption is influenced by different characteristics related to the NPs (e.g., diameter, surface chemistry, surface ligands, shape and elasticity, physical and chemical stability) [5]. In general, smaller particles lead to a higher absorption rates below 1 µm. Particles greater than 3 µm are
phagocytes and stay sequestered in the GIT cells. This is what we should observe in theory, but not necessarily the experimental observation. For example, even for NPs, the surface charge might limit absorption compared to non-ionic NPs. Biological surfaces such as an epithelia-containing receptor at the surface can express higher absorption rates. Shape and elasticity facilitate the passage across the barrier [3].

In the GIT, it seems well established that the PP would be mostly implicated in the process of particle uptake via the specialised epithelial cells. However, these specialised cells represent only approximately 10% of the cells cover the dome of these patches. Florence mentioned that these immunologic cells have their analogues in the bronchus-, larynx-, and nose-associated lymphoid tissue regions (referred to as BALT, LALT, and NALT, respectively) [3]. It is not surprising that PP part of the gut-associated lymphoid tissue (GALT) structure of the GIT wall, is considering that the M cells, as part of the lymphoid tissue, adapted a large range of materials to phagocytes. The uptake of particles, micro-organisms, and macromolecules by M cells occurs through adsorptive endocytosis by an approach of clathrin-coated pits and vesicles formation through an endocytosis and phagocytosis process [6]. This route has a limitation by itself because M cells (lymphoid tissues) occupy a relatively small region of the total GIT surface area, which is not compensated for by enhanced affinities. It has been shown that particulate systems can gain entry through normal gut epithelial cells (enterocytes); however, this absorption rate is not significant compared to the M cells. In addition, gastric mucus covers the entire wall of the GIT. Some authors have reported that mucus might inhibit the uptake of NPs through a dense structure in the viscous glycoprotein gel. However, there is a thought that entrapment of NPs in mucus actually delays the transit of these NPs down the gut and brings them closer to the absorption sites [3].

In attempts to increase the GIT absorption rate, many researchers have tried to improve this rate by increasing the complexities of the NPs using different approaches such as functionalisation, with a group change regarding the surface properties, and increasing the propensity for absorption. The functionalisation approach may help increase the absorption rate, but it may also increase the size of the NPs, which reduced absorption based on our observations. An equilibrium needs to be reached because when the chemical surface of the NP is altered, the possible interaction of the NPs with the target organ and the toxicity of the NPs also change. In this scenario, even if the absorption rates increase; the new NP must be characterised. Other techniques have been proposed such as coating NPs with molecules or peptides such as lectins, invasin, or internalin fragments. Even the results from such manipulation of the surface properties are often clear in vitro, the adhesion to the cell increases, and there is some evidence that absorption is enhanced [3]. The attempt is to reach the target organ, cells (e.g., M cells or normal epithelial cells), or tissues. Thus, a lack of clarity of the outcome might result from the chemical and physical instabilities of the coated particles. The increased adhesion from the interaction of different receptors may lead to enhanced uptake into cells, but this does not necessarily translate into increased transport through and out of the cell.

In the situation in which the epithelium itself is not the target, movement of particles through the M cells or epithelial cells is only the first part of this voyage, which involves passage through the mesenteric lymph, filtration in the lymph nodes, and transfer to the blood and perhaps extravasation [3]. In a study by Carr and colleagues, the researchers said that estimation between the uptake and the volume of NP is not straightforward [7]. Other factors such as maceration may overestimate the uptake into the tissue. This observation was clearly demonstrated by microscopy showing that in the small intestine, most NPs are luminal or on the surface [7]. In this study, the researchers did not find a significant different in the uptake between particles; however, the sizes of the particles were greater than 1 µm.

Transcytosis of NPs across normal absorptive epithelium may be possible, even though the capacity is considered to be limited. The transcellular transport of NPs generally begins with the uptake by one of the following endocytotic mechanisms: pinocytosis, macropinocytosis, or endocytosis [4]. After the absorption, the amount of NPs can reach the blood circulation, and if enough of an amount is present, then this can induce a therapeutic or a toxic effect [4]. The amount of
NPs absorbed is variable and depends of the size and the surface chemistry of NPs. Several studies show that between 2% and 3% of NPs orally exposed are absorbed by the GIT tissue [2]. For researchers who want to increase the intestinal absorption, they need to understand and optimise the linkage between surface chemistry of the NPs and the M cells [4]. In general, the efficiency of the uptake for NPs is less than or equal to 100-nm in size and is approximately 15- to 250-fold higher compared to a larger size [8]. In addition, from the same region, PP tissue has 200-fold higher uptakes than a non-PP tissue from the same region.

In a histological study by Desai and colleagues, they demonstrated that NPs of 100 nm were diffused throughout the submucosal layers as opposed to the larger sizes, which were predominantly found in the tissue surface [8]. Jani and colleagues exposed rat GIT mucosa to different sizes of NP (i.e., 50 nm, 100 nm, 300 nm, 500 nm, 1 μm, 3 μm) every day for 10 days [9]. After 48 hours post-exposure, the researchers measured the amount of NPs in different regions. Several authors discovered that NPs less than 100 nm accumulated in GIT tissue, but if the sizes were greater than this cut off, then the accumulation rate was significantly lower [8, 9]. In another study, colloidal gold was administered in water for 7 days to mice; the results showed a large distribution across several organs, including kidney, spleen, liver, blood, and brain [10]. Even after 12 hours of post-exposure, NPs were still measured in the GIT which suggests sequestration in cells or at the surfaces.

2.2. Inhalation

As describe by the International Commission on Radiological Protection (ICRP) in its document, the Commission measured the distribution probability of particles related to their size [11]. The ICRP study showed that NPs with a diameter less than 10 μm have a greater probability of penetrating beyond the head airways. Particles less than 100 nm in aerodynamic diameter have a significant probability of reaching the alveolar region of the lungs. In fact, there is at least a 50% probability that particles less than 4 μm in aerodynamic diameter will reach this region [12]. Regarding the smaller diameter sizes of particles, another factor called inertia is secondary to Brownian diffusion in determining deposition, leading to particles penetrating deep into the lungs and diffusing to a large lung surface area presented in the alveolar region [12]. According to the ICRP, at the 1-nm-in-diameter size, almost everything will be deposited on the head and tracheobronchial region of the lung, which means that almost nothing will reach the alveolar region because the negligible mass significantly reduces the velocity of the NPs.

For NPs to deposit in the inner wall of the lungs, the particles may induce two types of toxicities (i.e., a local toxicity or a systemic toxicity), depending on their intrinsic properties. In addition to the deposition site, different factors will influence clinical observations. For instance, the deposition site will depend on the size and concentration of NPs, the durability of NPs (i.e., insoluble aqueous), and the stability of the NPs (stable NPs will have higher durability and defence immune system in the lung area). The last important factor focuses on mucociliary clearance (acting in the upper airways) and the macrophages’ immune system (acting in the lower airways and alveolar region), which tries to actively remove the deposition of NPs.

The local toxicities may cause inflammation, oxidative stress, tissue damage, and disease. Many of the biological mechanisms observed in the literature involved particle-related lung diseases (e.g., oxidative stress, inflammation, production of cytokines, chemokines, cell growth factors) [13]. Much of our understanding about the key factors that influence the biological reactivity and toxicity of airborne particulate matter has come from animal toxicological studies. These factors, including size, surface area, surface chemistry, solubility, and shape, will influence both the deposition of NPs in the lungs and the biological responses observed. Maynard and Kuempel reported that NPs, including carbon black (12 nm), elemental carbon (90 nm), and diesel exhaust particulate (120 nm), caused various cytoskeletal dysfunction [12]. These dysfunctions included impaired phagocytosis, inhibition of cell proliferation, and decreased cell viability in primary alveolar macrophages from dogs and mice from alveolar macrophage cell lines in 24 hours depending on the dosages [12]. Monteiro-Riviere and colleagues reported an important observation, saying that if the carbon nanotubes reach the lung tissue,
then they were more toxic than similarly chemically composed NPs such as carbon black or quartz dust (actually these two NPs are known for their lung toxicities) [14]. Monteiro-Riviere and colleagues argued that the observation was due to the tendency of carbon nanotubes to self-aggregate, and then remove themselves from the controlled condition [14]. These agglomerated NPs have a much higher residence time than dispersed NPs.

NPs that leave the lungs might enter the blood circulation, and then may cause endothelial cell injury (of the blood vessels) and prothrombotic effects [15]. Recent studies have indicated that NPs depositing in the nasal region may be transported to the olfactory bulb via the olfactory nerves [16]. The size of NPs may also allow cells and cellular organelles to enter. In a study of concentrated NPs from air pollution in human bronchial epithelial cells and mouse alveolar macrophages, the ultra-fine fraction (less than 100 nm) was found to penetrate into cells and localise in mitochondria, causing oxidative damage to mitochondrial membranes [17]. Unlike low molecular–weight drugs, which penetrate cells easily, the cellular uptake of polymeric pro-drugs is restricted to the endocytic route, which basically means that pro-drugs are delivered to the cellular lysosomes [1]. However, other studies of drug delivery across the blood–brain barrier further confirmed the importance of surface properties, showing that particle surface components may bind to the apolipoprotein E (ApoE) receptor, which mediates crossing of this highly complicated tight barrier [1]. The olfactory neuronal pathway represents a significant exposure route of Central Nervous System (CNS) tissue to inhaled solid manganese oxide NPs. In rats, which are obligatory nose breathers, translocation of inhaled NPs along neurons seems to be a more efficient pathway to the CNS, than via the blood circulation across the blood–brain barrier for humans. Given that this neuronal translocation pathway was also demonstrated in nonhuman primates, it is likely to be operative in humans as well [18].

2.3. Cutaneous route

The cutaneous route of exposure represents an important potential route of exposure for NPs because of the skin surfaces with workplace environment [14], [19]. The risk caused by NPs or every other chemical especially after an in vivo exposure would depend upon the skin absorption if no irritant NPs are present. The process of passage across the skin layer will depend on passive diffusion, and structural organisation will influence this diffusion. The stratum corneum provides a slow diffusion capability; however, dermis and epidermis have easier diffusion capabilities. In general, literature reported that normal skin diffusion is limited to NPs that are 100 nm in diameter. Intrinsic factors such as the chemistry surface shape of NPs can also influence the diffusion. However, more research should be conducted on this issue because there is no real consensus in existing literature. Kohli and Alpar conducted a study on percutaneous NPs and found that particles were able to permeate skin if they had a negative charge and were 50 and 500 nm in diameter [20]. This result suggests that negative particles with sufficient charges may be the ideal carriers for drugs. This is different from what we observe; usually the best particles for percutaneous permeation would be when they are lipophilic neutral and less than 100 nm in diameter [20]. The vehicle containing NPs is also a parameter that can influence the toxicity. However, at least one group of researchers (i.e., Schulz and colleagues) found that cutaneous absorption interaction was limited to the stratum corneum [21]. Multiwalled carbon nanotubes are capable of entering the keratinocytes and driving interleukin-releasing effects.

Diffusion across the stratum corneum is normally the rate-limiting step for percutaneous absorption. A pig is a good model because its structure is similar to a human, and the technique of porcine preparation has been validated by numerous investigators. The permeation of NPs throughout the models seems to be an accurate predictive tool for human extrapolation. Tan and colleagues conducted a human study on the percutaneous absorption of titanium dioxide (TiO$_2$) from sunscreens [22]. This study was the first one to present a direct linkage between the utilisation of sunscreen and the absorption of TiO$_2$ in humans.
3. Distribution
After the absorption from oral, inhalation, or cutaneous routes, these NPs are then translocated in the systemic circulation and delivery to different organs, including the liver, the spleen, the kidneys, the heart, and the brain, where they may be deposited [18]. Many target organs are being studied to measure the impact of these exposures; however, only a few groups are working on the kinetic distribution of NPs in these organs.

A study conducted by Kreyling and colleagues indicated that 1 week after inhalation, only a very small fraction of ultra-fine iridium particles (18 and 80 nm in diameter) had access to systemic circulation and extrapulmonary organs [23]. Chemical composition and physical structure of the NPs surface may be an important determinant in influencing systemic translocation of ultra-fine particles [23]. De Jong and colleagues exposed rats aged 6 to 8 weeks to a single oral exposure of polystyrene NPs from 10 to 250 nm in diameter at concentrations varying from 77 to 108 microgrammes per litre (µg/mL) [19]. NPs less than 100 nm were found mainly in the blood and liver 24 hours post-exposure. In addition, De Jong group’s found a lower amount of NPs expressed in percentage of doses in the spleen and kidney and only trace levels in the lungs, the testis, the heart, the brain, and the thymus [19].

These studies demonstrated that NPs, after deposition, are rapidly distributed across tissue compartments of the lung, and that these NPs may move between the tissue compartments. The NPs eventually reach the capillary lumen and may penetrate into circulating cells and constituents (e.g., erythrocytes, blood macrophages). Thereby, they may be distributed into other organs of the body, such as the liver, the heart, the kidneys, and even the brain [9, 19]. Nemmar and colleagues conducted an interesting human study in which they used the inhalation of radiolabelled technetium 99 carbon NPs less than 100 nm in diameter. The findings showed that radiolabelled technetium 99 carbon was detected in the blood 1 minute after inhalation, reached a maximum after 10 to 20 minutes, and remained for more than 60 minutes [24]. A gamma camera showed substantial radioactivity in the liver and other organs.

4. Excretion
In a study of rats by Peters and colleagues, a single intravenous dose of a fullerene labelled with technetium 99m showed a rapid distribution in different organs [18]. The researchers measured the biodistribution in different organs in mice and rabbits. They observed a decrease of the NPs in the organ tissues only between 1 and 3 hours post-exposures, except for liver at 3 hours, where it was higher than at 1 hour post-exposure. The elimination of the fullerene was mainly via urinary route [25]. In another comparison, Ogawara and colleagues administered a single intravenous dose of polystyrene microsphere (50 and 500 nm in diameter) into rats [26]. The distributions were independent of the size. The researchers measured the half-life (T1/2) for the alpha phase and terminal phase at approximately 1 and 53 hours respectively [26]. They believed that urinary elimination seemed to be the major elimination route. Singh and colleagues exposed mice to a single intravenous dose of single walled carbon nanotubes (1 nm in diameter and a length of 300 to 1,000 nm) [27]. Distribution was very quick across the organs. Excretion occurred via the urine pathway. In the same experiment, the researchers also studied the impact of the exposure route on the elimination half-life. Depending on the absorption route, the other local mechanism previously mentioned may also contribute to the elimination. In a study by Furumoto and colleagues, the researchers demonstrated that NPs can also be excreted via the biliary tract [28].

5. Discussion and conclusion
The interaction between NPs and the biological system is a huge challenge for toxicokinetic. The toxicokinetics discipline describes the absorption, the distribution the metabolism process, and the excretion of the xenobiotic (the NPs). At this point, our focus is to apply the approach that we have in place in case we need to use the same kinetic approaches or develop new tools. However, it is
important to point out that complementary approaches will be required to be able to adequately describe the experimental observation.

At the beginning of these experimental observations, we believed that inhalation was the major exposure route, with negligible contribution from the skin. However, observations such as those by Ryman-Rasmussen and colleagues [29] or results from Monteiro-Riviere and colleagues [14] clearly suggest that skin is surprisingly permeable to nanomaterials with diverse physicochemical properties and may serve as a portal of entry for localised, and possibly systemic, exposure of humans to quantum dots and other engineered nanoscale materials [29]. For skin absorption, these findings suggest that the toxicology of these structures must be assessed before widespread public exposure so that appropriate protective measures can be developed [14]. The status of this absorption will change with the arrival of new transformer NP (NP with exotic functionalisation) on the market.

This short review also points out that NPs can rapidly translocate from the lung into the cells, and then reach the systemic circulation. There is increasing evidence that the accumulation of NPs in different organs (e.g., heart, brain) shows sign of oxidative stress or other stress toxic effects. Even oxidative stress can occur for many other reasons, this significant addition may cause a long-term health effect. A long-term study is required to quantify the long-term risk. Given that this neuronal translocation pathway was also demonstrated in nonhuman primates, it is also likely to be operative in humans [30]. New technology approaches, such as a proteomic analysis profiling approach in which we detected much below the clinical sign effect of protein modification, will be useful to observe before the health effect is irreversible. However, these sophisticated approaches will only work if they are supported by a rigorous characterisation of the NPs in media.

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