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Review

Cellular elimination of nanoparticles

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ABSTRACT

Exposure of the general population to nanoparticles (NPs) occurs mainly by dermal and oral uptake of consumer products, food and pharmaceutical applications and by inhalation. While cellular uptake mechanisms have been intensely studied it is less well known how NPs are eliminated from the cells. Quantification of the amount of excreted particles is complicated by inherent limitations of the technologies that are suitable to study excretion. Among the mechanisms to decrease intracellular particle concentration active excretion by lysosomal exocytosis appears to be the most important. Lysosomal localization, small particle size and high intracellular and low extracellular particle levels facilitate exocytosis. Transporting epithelia, cells with secretory function and highly proliferative cells are expected to be able to decrease intracellular particle concentrations more efficiently than cells lacking these characteristics. As NPs can influence the extent of exocytosis it is possible that NPs can stimulate their excretion.

Contents

1. Introduction

Nanoparticles (NPs) can be taken up into the human body by exposure via the skin (cosmetics, sun protection, medical care products), the oral cavity (dental care products), the gastrointestinal tract (food, medications), and the lung (exposure at the work place, inhalation products). Cellular retention with release of the payload is intended for targeted delivery of drugs but usually unwanted for consumer products and at the workplace because NPs may cause adverse effects. Size around 50 nm, positive surface charge and functionalization with specific ligands for cellular uptake are used to increase cellular uptake in drug delivery (Chithrani, 2010; Nam et al., 2013). For cellular action the delivered active pharmaceutical ingredients usually have to be localized outside the lysosomes to avoid degradation. This phenomenon, termed endosomal escape, is achieved by different mechanisms, which have been described in various reviews (see for instance Shete et al. (2014); Varkouhi et al. (2011)). Similar size and surface parameters also favor the non-wanted uptake of NPs and was linked to cytotoxicity of NPs. Adverse effects include generation of reactive oxygen species, cytotoxicity, inflammation, etc. (Fröhlich, 2013).

In contrast to cellular uptake the elimination of NPs from the cells is less well studied and detection complicated by methodological limitations. Frequently used techniques for metallic and

Abbreviations: APCs, Antigen-presenting cells; CdSe/ZnS, cadmium selenide/zinc sulfide; NP, nanoparticle; PLGA, poly(DL-lactide-*co*-glycolide); Si, silicium; TiO_2, titanium dioxide.

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inorganic NPs, such as inductively coupled plasma spectrometry cannot be used for all materials. Furthermore, no differentiation between excretion of NPs and degradation of NPs can be made because the elements and not the physical appearance is determined. Fluorescence detection which works very well for particles with inherent fluorescence (e.g. quantum dots) is less suitable for other particles because the labeling can change the physicochemical properties of the NPs and the fluorescent moiety might be cleaved off. Imaging techniques, particle tracking by fluorescence and cell analysis by transmission electron microscopy can help in the discrimination between excretion of intact particles and ions but have other limitations. Single particle tracking, for instance, cannot be used when particles aggregate (Levi et al., 2005), while transmission electron microscopy cannot track individual particles to provide information on the mechanism of the extrusion (Chithrani and Chan, 2007). The study of cellular elimination, therefore, usually requires the combination of different techniques. Information on cellular particle elimination, on the other hand, is needed to estimate accumulation and develop techniques to influence particle excretion.

2. Mechanisms to reduce intracellular particle concentrations

In general, intracellular NP concentrations can be reduced through several mechanisms, such as i) cell death, ii) cell proliferation, iii) diffusion of NPs, iv) NP degradation/dissolution in lysosomes, v) uptake by a route avoiding intracellular residence (transcytosis), and vi) exocytosis.

2.1. Cell death and proliferation

When NPs induce cell death by necrosis or apoptosis the cell content is released into the extracellular space in free form or as apoptotic bodies. It is likely that phagocytes ingest these NPs together with residues of the cells but the fate of NPs released from dead cells is largely unclear. Another way to lower the intracellular concentration of NPs is cell division, where cellular content and cell organelles are distributed to the daughter cells. Both processes are not expected to be major mechanisms for cellular excretion of NPs because NPs usually do not reach cells in concentrations high enough to cause cell death. Dilution by proliferation, on the other hand, is unlikely because except of cells in bone marrow, intestines, epidermis and hair follicles the majority of human cells in an healthy body are rarely duplicating (Ramirez et al., 1997). NPs can disrupt cellular organelles, for instance lysosomes and reach the cytoplasm (Fig. 1).

2.2. Passive processes

Although active uptake is their main mechanism gold, titanium dioxide (TiO_2), polystyrene and silica NP can enter cells by diffusion. Red blood cells do not possess active uptake mechanisms and the presence of NPs in these cells proved the existence of passive uptake (Lin and Haynes, 2010; Mu et al., 2012; Rothen-Rutishauser et al., 2006; Slowing et al., 2009; Wang et al., 2012; Zhao et al., 2011). Theoretically, these NPs could also diffuse out of the cells. However, back-diffusion into the extracellular space is hindered by the physical nature of the cytoplasm, which is a complex gel with the cytoskeleton as structural obstacle (Florence and Attwood, 2016). Diffusion of very small (6 nm) dendrimers was 1000 times lower than diffusion in water. The plasma membrane separating the cytoplasm from the extracellular space is an additional hindrance and it is, therefore, not likely that passive mechanisms contribute much to cellular excretion of particles.

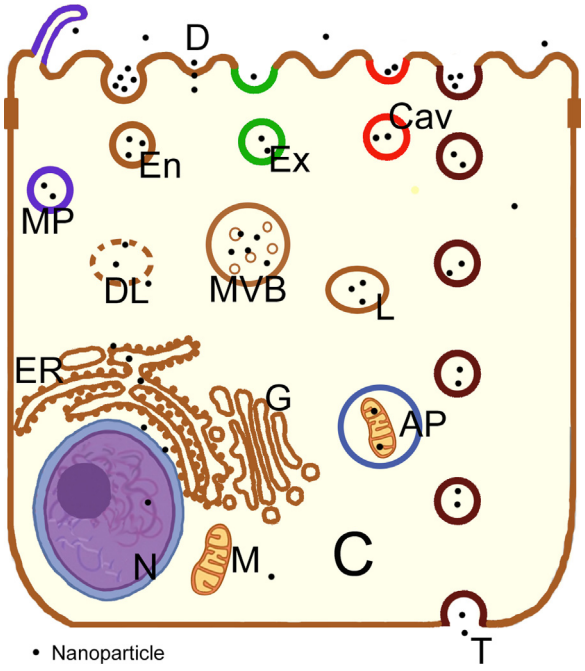

Fig. 1. Involvement of intracellular vesicles in the transport of nanoparticles (NPs). NPs can enter cells by macropinocytosis (MP) and other active uptake routes collectively indicated here as endosomes (En). They are delivered to lysosomes (L) via late endosomes or multivesicular bodies (MVB). When lysosomal membranes get destroyed (DL) NPs are released into the cytoplasm (C). Caveosomes (Cav) can deliver NPs to lysosomes, endoplasmic reticulum (ER) or Golgi apparatus (G) or transverse cell by transcytosis (T). Diffusion (D) is the passive entry of NP into cells, nucleus (N) and mitochondria (M). Export from the cells may occur via exocytotic vesicles (Ex) and by degradation and extrusion of autophagosomes (AP).

2.3. Intracellular degradation

Lysosomes play a central role in the cellular elimination of NPs either by dissolution or degradation or by exocytosis. Lysosomes are able to degrade not only biodegradable polymers, such poly(DL-lactide-co-glycolide) (PGLA) NPs and poly(amidoamine) dendrimers, but also metal oxide NPs such as iron oxide NPs, at least in part (Laskar et al., 2012; Lunov et al., 2010). The degradation can affect the polymeric shell or lysosomes can dissolve the core of the particles. Levels of released Silicium (Si) into the medium indicated that mesoporous silica particles were also partly degraded in lysosomes and that degradation was fast during the first two days and slower during the next days (Zhai et al., 2012). In total about 60% of total Si was released from the cells. Degradation of gold, silver, iron oxide and cadmium selenide/zinc sulfide (CdSe/ZnS) NPs in acidic pH with the prolonged intracellular release of the respective ions was postulated as main factor for cytotoxicity (Sabella et al., 2014).

2.4. Active mechanisms

Active uptake (endocytosis) is involved in cellular ingestion of NPs and can be roughly classified into clathrin-mediated uptake, caveolin-mediated, macropinocytosis, and clathrin- and caveolin independent uptake (for more detail see for instance Sahay et al. (2010)). With the exception of caveolin-mediated uptake all routes include lysosomes, the organelles that are involved in the degradation of macromolecules originating from the cell itself or taken up from the extracellular space. Only macromolecules and NPs that are taken up by the caveolin-mediated route can be delivered in addition to lysosomes to Golgi apparatus and to endoplasmic reticulum (Le and Nabi, 2003). Transcytosis is a specific form of caveolin-mediated uptake, where vesicles are transported across