Translocation and cellular entering mechanisms of nanoparticles in the respiratory tract

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Summary

Anthropogenic nano-sized particles (NSP), i.e., particles with a diameter of less than 100 nm, are generated with or without purpose as chemically and physically well-defined materials or as a consequence of combustion processes respectively. Inhalation of NSP occurs on a regular basis due to air pollution and is associated with an increase in respiratory and cardiovascular morbidity and mortality. Manufactured NSP may intentionally be inhaled as pharmaceuticals or unintentionally during production at the workplace. Hence the interactions of NSP with the respiratory tract are currently under intensive investigation. Due to special physicochemical features of NSP, its biological behaviour may differ from that of larger sized particles. Here we review two important themes of current research into the effects of NSP on the lungs: 1) The potential of NSP to cross the blood-air barrier of the lungs, thus gaining access to the circulation and extrapulmonary organs. It is currently accepted that a small fraction of inhaled NSP may translocate to the circulation. The significance of this translocation requires further research. 2) The entering mechanisms of NSP into different cell types. There is evidence that NSP are taken up by cells via well-known pathways of endocytosis but also via different mechanisms not well understood so far. Knowledge of the quantitative relationship between the different entering mechanisms and cellular responses is not yet available but is urgently needed in order to understand the effects of intentionally or unintentionally inhaled NSP on the respiratory tract.

Key words: nanoparticles; respiratory tract; lung; translocation; endocytosis; adhesive interactions

Introduction

Epidemiological studies have shown that exposure to airborne ambient particulate matter is correlated with adverse health effects, particularly increased respiratory and cardiovascular morbidity and mortality (see [1] for review). Further work has highlighted the fact that these correlations depend on particle size, the so-called ultrafine particles (UFP) contributing in significantly stronger fashion to the adverse effects [2]. UFP are particles with a diameter of 100 nm or less in all dimensions [3]. The most important anthropogenic sources of UFP are combustion processes of diesel oil, coal, heavy fuel oil or welding processes [3]. Apart from UFP, engineered nanoparticles (NP), defined as particles at least in one dimension smaller than 100 nm, have recently attracted attention. In contrast to UFP, which usually consist of an elemental carbon core with a shell of attached transition metals and/or organic carbon, NP are physically and chemically well-defined particles specifically designed for a certain use. For example, titanium dioxide NP are used in sun milk, carbon nanotubes are part of carbon fibre bicycles, silver NP have antibacterial properties in socks, etc. Particular interest has been focused on the biomedical applications of NP with respect to imaging and drug delivery [4]. The stability of NP as separate particles is obviously of critical importance for their nano-effects. Although the terms aggregation and agglomeration are frequently used interchangeably, there is a difference in their meaning [5]. Agglomerates of particles develop due to physical bonds (eg, van der Waals or electrostatic forces), whereas aggre-
gates are held together by chemical bonds. This is important because agglomerates can often be dispersed by ultrasonic treatment or in a certain biological environment, while the forces that hold aggregates together are more stable. The state of agglomeration or aggregation may therefore greatly influence the biological behaviour of particles (including translocation and cellular entering), an aspect which should be borne in mind in studies on NP and UFP.

While human inhalation exposure to UFP occurs on a regular basis, exposure to NP may be restricted to occupational or medicinal settings. Hence the interaction of both UFP and NP with the lungs requires thorough investigation. In the following, the term nano-sized particle (NSP) will be used whenever both UFP and NP are meant.

This review article focuses on the mechanisms by which NSP cross the air-blood barrier, thus entering the systemic circulation, and the mechanisms by which NSP enter cells. For a more detailed review on interactions of NSP with pulmonary structures the reader may refer to [6].

Fate of inhaled and deposited nano-sized particles – hypothetical routes

Since NSP effectively reach the alveolar region of the lungs, the size of the epithelial surface that may simultaneously come into contact with the particles amounts to approximately 140 m² [7]. Once NSP have reached the alveoli, they may be retained in the lungs for a long period and may be deposited, ie, come into physical contact with the surface structures. Larger sized particles are quickly displaced from the air-liquid interface to the watery hypophase under the surfactant [8], a process which probably holds true for NSP as well. In the hypophase, the particles may bind to surfactant proteins and glycoproteins or be taken up by macrophages. However, the uptake of NSP by alveolar macrophages seems to be on a far smaller scale than for larger sized particles [9]. The particles thus have a high probability of encountering the alveolar epithelium. Those particles that translocate over the alveolar epithelium may be deposited in the interstitium or translocate over the capillary endothelium and enter the circulation [10, 11]. Since all of the structures mentioned above may either influence the particle behaviour or may be influenced upon contact with NSP, the following paragraphs attempt to provide a mechanistic view of the phenomena of particle translocation and cell entering.

Translocation of nano-sized particles across the air-blood barrier

It having been proposed that adverse cardiovascular effects might be related to a translocation of NSP to the systemic circulation, several studies have addressed the question whether and to what extent NSP cross the air-blood barrier.

Animal models of NSP inhalation and instillation have provided evidence that a small fraction of the particles applied translocate to the circulation and can reach extrapulmonary organs via the bloodstream. These findings were observed for titanium dioxide NP in the rat [10, 11] as well as for diesel exhaust particles in the hamster [12]. In the human lung one study reported a rapid and significant translocation of inhaled carbonaceous NP to the systemic circulation [13]. In contrast, other subsequent studies failed to confirm this finding and detected only a low degree of translocation for iridium [14] or carbon NSP [15–17]. The studies of Nemmar et al. [13] and Mills et al. [16] had a very similar design and Mills et al. [16] have argued convincingly that the strong translocation of 99mTc labeled particles observed by Nemmar et al. [13] was mainly related to the translocation of soluble 99mTc-pertechnetate or pertechnegas which was cleaved from the carbonaceous particles. Based on these studies, it is reasonable to conclude that a small fraction of NSP translocates across the air-blood barrier to the circulation, but the significance of this translocation for human health requires further study.

A very specific property that has been assigned to inhaled NSP refers to translocation from the nasal epithelium to the brain. Elder et al. [18] showed that ultrafine manganese oxide particles translocate to the olfactory bulb and other regions of the central nervous system, although the question whether entire particles were translocated or solubilised manganese was transported to the central nervous system [19] warrants further research. However, additional support for translocation of ultrafine particles via olfactory axons comes from a study by Oberdörster et al. [20], who showed that inhaled radioactive carbon particles were significantly enhanced in the olfactory bulb after exposure, in contrast to other brain regions that showed only inconsistent increases in carbon particles.
Cellular entering mechanisms

There is little evidence for paracellular transport of NSP in the healthy lung, but this route may be enhanced when additional air- or blood-borne substances, such as ozone, hydrogen peroxide or histamine, increase the permeability of epithelial or endothelial barriers [21]. Most particles crossing the alveolar epithelium will however use a transcellular route for which endocytosis has been observed as a key mode of cellular particle uptake in several studies: after inhalation exposure, single and agglomerated gold NSP were observed in membrane-bound vesicles in AM and alveolar type I cells of rat lungs [22]. In a macrophage cell line, gold NP were found in pinocytic vesicles and lysosomes [23]. Similarly, titanium dioxide NP were localised inside vesicular structures, including multivesicular and lamellar bodies, in an immortalised alveolar epithelial cell line [24]. Further support for an active energy-dependent transport process comes from the observation that low temperature and metabolic inhibitors inhibit the uptake of magnetic silica-coated NP [25]. Receptor-mediated endocytosis of particles via scavenger receptors was shown to be involved in macrophages [26, 27].

Apart from endocytosis, however, other routes of cellular entry are likely. Very few titanium dioxide NP were observed inside red blood cells after inhalation exposure in rats [10], suggesting passive movement of NP across the plasma membrane. This mechanism was confirmed by the presence of polystyrene NP as well as titanium dioxide NP in red blood cell culture [28]. Ambient UFP were observed in mitochondria of macrophage and epithelial cell lines [29] indicating direct access to the enzymes of the respiratory chain. C60 fullerenes were found inside the nucleus and the free cytoplasm of human monocyte-derived macrophages [30], and 1.4 nm gold particles were bound to the nuclear DNA of eleven different normal or cancer cell lines [31]. The latter finding raises the possibility that some particles entering the nucleus may directly interact with nuclear DNA. The particle entering...
processes different from endocytosis have been related to adhesive interactions due to electrostatic forces, Van der Waals or steric interactions [32]. Little is known, however, about the quantitative relationship between active endocytotic routes and passive routes of entry. We have recently established a quantitative approach to guarantee an unbiased way of investigating this relationship in tissues and cells [33]. In addition, the significance of a specific intracellular localization needs to be analyzed in quantitative terms to distinguish between sporadic and scientifically relevant particle distributions. The different mechanisms of cellular entering and intracellular trafficking are summarized in figure 1. Some examples of intracellular NP localization visualized by transmission electron microscopy are shown in figures 2 and 3.

Composition and surface characteristics of the particles obviously influence the entering mechanism and the intracellular trafficking of particles [34–36]. In particular, protein adsorption may determine the mechanism of NSP trafficking as evidenced for the translocation of poly(ethylene glycol-co-hexadecyl)cyanoacrylate NP into rat brain endothelial cells [37]. Future studies therefore need to define the experimental setting of particle exposure both from the particle and from the biological side in great detail.

**Open questions**

1. Although it is accepted that a small fraction of inhaled particles translocates across the air-blood barrier, there is currently no rationale for relating these particles to the adverse health effects associated with particle exposure. Therefore, future studies are warranted addressing the following questions: Do particles need to translocate to the circulation to induce adverse cardiovascular health effects? What are the cumulative effects of particle translocation? What is the fate of the translocated particles?

2. Similarly, there is evidence that most nano-sized particles entering cells are taken up by endocytosis but other routes of cellular entering also exist. Systematic studies are needed investigating the mechanisms of cellular particle entering and the effects of endogenous and exogenous factors on these mechanisms. Quantitative studies have to be performed to relate the cellular reactions such as the generation of reactive oxygen species and inflammatory proteins to a specific intracellular localization.

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