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On the contribution of the phagocytosis and the solubilization to the iron oxide nanoparticles retention in and elimination from lungs under long-term inhalation exposure

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ABSTRACT

The aim of our study was to test a hypothesis according to which the pulmonary clearance *vs.* retention of metal oxide nanoparticles (NPs) is controlled not only by physiological mechanisms but also by their solubilization which in some cases may even prevail.

Airborne Fe_2O_3 NPs with the mean diameter of 14 ± 4 nm produced by sparking from 99.99% pure iron rods were fed into a nose-only exposure tower. Rats were exposed to these NPs for 4 h a day, 5 days a week during 3, 6 or 10 months at the mean concentration of 1.14 ± 0.01 mg/m^3. NPs collected from the air exhausted from the exposure tower proved insoluble in water but dissolved markedly in the cell free broncho-alveolar lavage fluid supernatant and in the sterile bovine blood serum. The Fe_2O_3 content of the lungs and lung-associated lymph nodes was measured by the Electron Paramagnetic Resonance (EPR) spectroscopy.

We found a relatively low but significant pulmonary accumulation of Fe_2O_3, gradually increasing with time. Besides, we obtained TEM-images of nanoparticles within alveolocytes and the myelin sheaths of brain fibers associated with ultrastructural damage.

We have developed a multicompartmental system model describing the toxicokinetics of inhaled nanoparticles after their deposition in the lower airways as a process controlled by their (a) high ability to penetrate through the alveolar membrane; (b) active endocytosis; (c) *in vivo* dissolution.

To conclude, both experimental data and the identification of the system model confirmed our initial hypothesis and demonstrated that, as concerns iron oxide NPs of the dimensions used, the dissolution-depending mechanisms proved to be dominant.

1. Introduction

One of the physico-chemical properties of metal and, in particular, metal-oxide nano-particles (Me-NPs) is that, being virtually insoluble in de-ionized water, they do (depending on chemical composition) get more or less solubilized *in vitro* in biological milieus. This suggests that Me-NPs are most likely to be similarly solubilized *in vivo*.

The toxicological importance of such dissolution is not only to be expected *a priori* (Utembe et al., 2015) but is also supported by experimental findings, including our own data obtained under subchronic intra-peritoneal exposure of rats repeatedly to metallic silver NPs in comparison with much less soluble NPs of metallic gold (Katsnelson et al., 2013), and especially to nanoparticles of copper oxide (Privalova et al., 2014) and manganese oxide (Minigalieva et al., 2015). At the same time, the predominant accumulation of NPs in the RES-cell rich organs (liver and spleen) which has been well-established in numerous experiments including our own studies involving the above-mentioned and other Me-NPs (Katsnelson et al., 2010, 2015), suggests the essential toxicokinetic role of NP phagocytosis by resident macrophages.

Of special interest is, however, the comparison of the impacts produced by solubility and phagocytosis on the fate of NPs deposited onto the free surface of the so called 'pulmonary region'

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of the lungs under long-term inhalation exposure. This type of exposure is particularly important for just the metal-oxide NPs because, along with so-called engineered NPs widely manufactured for different technical, scientific and medical usages, there exists a lot of respective nanoscale by-products, namely a substantial fraction of nanoscale ("ultrafine") particles of the above-mentioned chemical composition within the particle size distribution of condensation aerosols generated by arc-welding and various metallurgical technologies and thus polluting the workplace air and ambient air of respective industries. Specifically, Fe_2O_3-NPs, along with other chemical species of iron and other metals as well as with micro-scale particles of the same iron oxide, constitute a higher or lower proportion of this pollution depending on different technological parameters (International Institute of Welding, 2009; Lehnert et al., 2012; Ennan et al., 2013; Lewinski et al., 2013).

The aerodynamic mechanisms defining the primary deposition of inhaled NPs predominantly in the pulmonary region of the lungs and in the nasal passages have been long established in science and included into the well-known Lung Deposition Model of the International Commission on Radiological Protection (ICRP, 1994). Subsequently updated quantitative estimates of this deposition based on various mathematical models cannot be regarded as fundamentally different (e.g. Kolanjiyil, 2013; Kreyling et al., 2013). In the meantime, differences of opinion concerning the role of the physiological and physico-chemical mechanisms controlling the further destiny of deposited NPs should just be characterized as fundamental. Up to the present time, there are not enough specific experimental and modelling estimates of the Me-NP dissolution's contribution for any generalizations to be made, and even if this role was experimentally estimated as low in relation to TiO_2-NPs (Creutzenberg, 2013), it is most likely to be a special case rather than a general pattern. For example, Adamcakova-Dodd et al. (2014) exposed mice sub-acutely and sub-chronically to ZnO-NPs in an inhalation experiment and found a transient initial phase of substantial increase in the concentration of Zn^{2+} ions in the BALF, which indirectly points to the dissolution of these NPs even before their penetration into the pulmonary interstice. Dumkova et al. (2016) who subjected mice to the round-the-clock inhalation exposure to 12 nm CdO-NPs during 6 weeks registered the accumulation of persistent NPs not only in lungs but in different other organs but did not discussed a possible toxicokineric role of their dissolution.

Kolanjiyil (2013) developed and described in detail in his thesis a very interesting multicompartmental system model for the kinetics of deposition and retention of inhaled NPs in the lungs the parameters of which he estimated based on several published experimental studies involving various Me-NPs under short-term inhalation exposures, in low concentrations as a rule. In this model he assumed the insolubility of these NPs as one of the basic premises. On the contrary, Katsnelson et al. (2010) suggested that the multicompartmental system model of Me-NPs toxicokinetics they proposed should include additional hypothetical flows of solubilized NPs eliminated from the lungs and lung-associated lymph nodes into the blood and lymph, emphasizing however that they did not yet have any data for quantitative identification of these flows.

As for modeling the role of pulmonary phagocytosis, the author of the above-mentioned thesis noted that "Katsnelson et al., 1992 published a multi-compartmental retention model of bio persistent particles in the pulmonary region of the lung. They included [*into the model*] neutrophils which also are engaged in phagocytosis and contribute to alveolar clearance by transfer with their particle load onto the mucociliary escalator". Nevertheless, the model suggested by Kolanjiyil (2013) himself does not allow for this mechanism and presents the alveolar macrophage (AM), in full correspondence with the prevailing tradition, as not the main but the only phagocytal effecter of the nanoparticle pulmonary clearance. This rather essential issue will be considered in more detail in the Discussion Section.

It should be also noted that it is not only for Me-NPs but for micrometric mineral dust particles as well that the role of differences in the low solubility is not always negligibly small not to exert any influence on the pulmonary toxicokinetics. Thus, there is evidence of faster clearance of rat lungs from silicon dioxide (accompanied with involution of silicotic nodules) a few months after discontinuing a long-term exposure of rats to an industrial condensation aerosol of this material compared with a similar exposure to the same concentration of quartz dust (Petin, 1978). Also, silicon dioxide was observed to accumulate at a slower rate in the pulmonary tissue as a result of inhalation exposure to particles of fused silica and, particularly, of dry colloidal silicic acid in comparison with crystalline silica despite equal mass concentrations and similar particle size distributions of respective aerosols in the inhalation chambers (Podgayko et al., 1982; Katsnelson et al., 1984a,b). Moreover, the same structural and surface features of those amorphous silica particles that render them *more soluble* as compared with quartz have also been observed to determine their *higher cytotoxicity* for macrophages, which should have caused *less* efficient elimination but did not. In other words, in these comparative experiments, the role of dissolution in the process of particle elimination proved to be rather noticeable. All this has a direct bearing on the analysis of Me-NP pulmonary toxicokinetics considering the fact that their *in vivo* solubilization and toxicity vary (both uni- and contradirectionally) in a rather wide range.

Starting from the above theoretical premises and previously gathered research experience, we chose for the present study an aerosol of iron oxide (Fe_2O_3) particles in the lower nanoscale range. The choice was made partly because we expected these NPs to be of a non-negligible *in vivo* solubility while not featuring too high a toxicity. Another reason for this choice was practical: as stated above, Fe_2O_3 particles (nanoscale included) constitute a major proportion of welding fumes and steel metallurgy emissions, and thus many thousands of workers all over the world are exposed to their inhalation impact (Lewinski et al., 2013).

Thus, the aim of this study was to test (taking the pulmonary toxicokinetics of iron oxide NPs as a case study) the consistency of a working hypothesis according to which the pulmonary clearance *vs.* retention of metal oxide nanoparticles (NPs) is controlled not only by phagocytosis-dependent physiological mechanisms but also by the NP solubilization which in some cases may even prevail.

2. Materials and methods

2.1. Experimental techniques

Airborne iron oxide NPs were obtained by sparking from 99.99% pure iron rods using the Palas DNP-3000 generator and fed into a nose-only exposure chamber/tower (CH Technologies, USA) for 60 rats. An analogous chamber was used for a sham exposure of control rats.

As can be shown by the SEM imaging (Fig. 1) NPs collected on the polycarbonate filter have spherical form and either are singlet or form small aggregates. The latter, when being rather compact were measured as one particle, and even with such approach, the particle size distribution (Fig. 2) proved rather clean-cut and restricted to the nanometric range with mean (±s.d.) diameter 14 ± 4 nm.

The chemical identity of the NPs sampled on the filters was confirmed by the Raman spectroscopy to be Fe_2O_3 (Fig. 3).