Assessment of Comparative Toxicity of Nano- and Microparticles Magnetite

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ABSTRACT

The key mechanism of pulmonary region's clearance from deposited particles is recruitment of phagocytizing cells on its free surface. Phagocytosis hinders penetration of these particles into pulmonary interstices and thus promotes mucociliary clearance[1]. The alveolar macrophage (AM) is the main cell effector of phagocytosis-mediated pulmonary clearance, the neutrophile leukocyte (NL) being the most important auxiliary one. NLs recruitment is controlled by the number of destroyed AMs. That's why the intensity of NLs recruitment toward deposited particles depends quantitatively on their cytotoxicity for macrophages[1-5].

Thus NL/AM ratio may be used as a criterion of comparative cytotoxicity of particles.

Another nano-toxicological problem of theoretical and practical importance is the size-dependence of the comparative resorptive toxicity of particles of substances that are deemed innocuous in bulk.

Keywords: nanoparticles, magnetite, subchronic toxicity.

1 METHODS

We prepared 3 batches of magnetite (Fe_3O_4) particles of 10nm, 50nm and 1μm nominal sizes. Aqueous suspensions were prepared with the help of ultrasonication and instilled intratracheally into the lungs of rats in a 2 mg dose in 1 ml sterile deionized water. 24 hours later cells in the broncho-alveolar lavage fluid (BALF) were counted, centrifuged and studied by optical, transmission electron (TEM) and semi-contact atomic force microscopy (sc-AFM). Iron content was determined photometrically in the homogenized tissue of non-lavaged lungs.

In another experiment aqueous suspensions of the same particles were injected i.p. to rats at a dose of 500 mg/kg in 4 ml of sterile deionized water 3 times a week during 5 weeks. Following exposure, functional and biochemeical indices and histopathological changes in spleen and liver tissues of exposed rats were evaluated for signs of toxicity. The iron content of the blood was measured photometrically, and that of the liver and the spleen by AAS method.

2 RESULTS

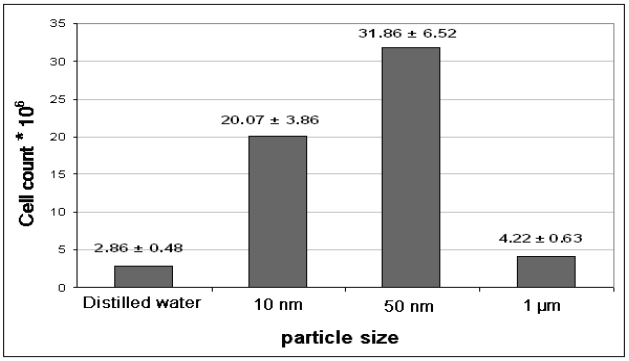


Figure 1: The increase in total cell number in BALF of rats 24 hours after instillation of Fe_3O_4 particles of different sizes in dose 2 mg in 1 ml sterile distilled water.

As can be seen from Fig. 1, instillation of magnetite nanoparticles of both sizes caused a higher increase in cell count of the BALF than instillation of microparticles did, but response to 10 nm particles was a bit weaker than to 50 nm particles. The last fact is probably due to a faster liberation of lungs during elapsed 24 hrs from minute particles because of both their more significant dissolution and more avid phagocytosis (see below). Indeed, it was found that iron content of the pulmonary tissue 24 hrs after instillation of 10 nm particles was significantly lower than after instillation of the 50 nm ones.

Nevertheless, judging by NL/AM ratio (Figure 2), cytotoxicity of nanoparticles with diameter 10 nm is a bit higher than that of 50 nm nanoparticles, while they both are **much more cytotoxic than micrometric particles** of the same substance.

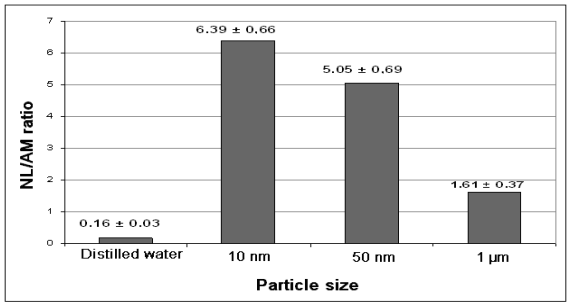
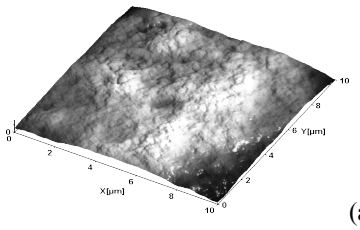


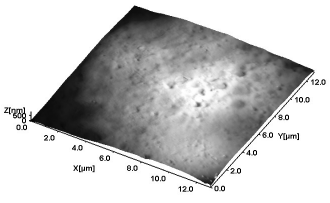
Figure 2: The ratio of neutrophile leucocytes to alveolar macrophages in the same BALF.

Apparently, the smallest size (and thus the greatest specific surface) of nanoparticles, as well as peculiarities of their surface's structure, along with making them especially biologically aggressive, may cause enhanced solubility of particles deposited in lungs and, in the final count, accelerate pulmonary clearance and mitigate development of pathologic changes. (Such dual role of solubility is well-known concerning ultrafine aerosols of silicon dioxide as compared with quartz [1]).

Phagocytic activity of AM as well as of NL is demonstrated by the count of particles visible within cells under optical microscopy with magnification x1000. «Low loaded» AMs and NLs compose 1,7-2 times fewer percentage of total number of corresponding cells in BALF of lungs instilled with 10 nm particles as compared with those instilled with 50 nm particles, while nanoparticles of both sizes were engulfed far **more actively than microparticles**. It should be qualified however that particles seen as separate ones under the said magnification were not primary single nanoparticles but rather their small aggregates.



(a)



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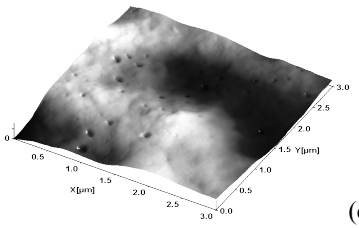

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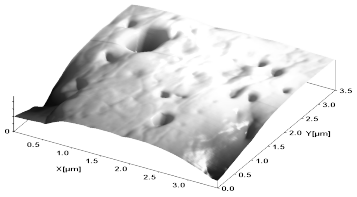
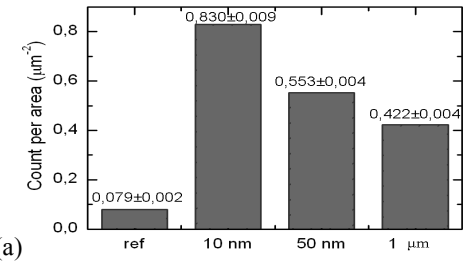


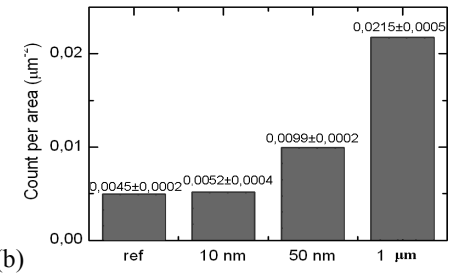
(d)

Figure 3: Typical cell surface topography measured by semi-contact AFM: (a), controls; (b) after instillation of 10 nm magnetite; (c) 50 nm magnetite; (d) 1 μm magnetite.

Data obtained with the sc-AFM (Figures 3 and 4) are in a good accordance with those of the optical microscopy, if one assumes that micro-pits on the cell surface are traces of the plasma membrane's invagination in the act of particles' phagocytosis.



(a)



(b)

Figure 4: Average surface concentration of micro-pits (a) of all transverse dimensions, and (b) with transverse dimensions of >1 μm detected on the surfaces of cells of each group ($x \pm S_x$).

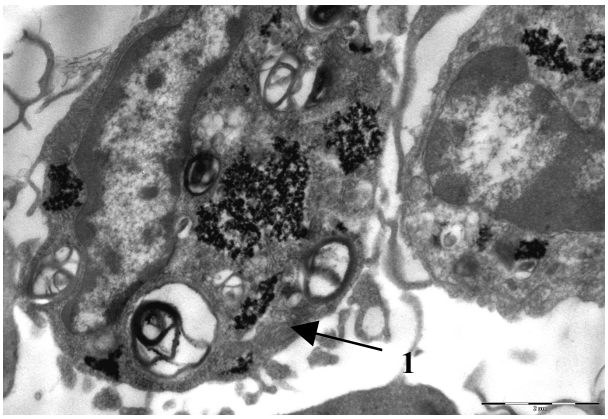


Figure 5: 10 nm magnetite particles in an AM gathered within endosomes (arrow 1). TEM, magnification *8900

It is known that products of macrophage disintegration stimulate attraction of AM and especially of NL, as well as their phagocytic activity[6]. This mechanism allows to explain positive rank correlation between the cytotoxicity of different magnetite particles and the avidity of their engulfment by viable phagocytes.

Transmission electron microscopy shows that internalized nanoparticles are primarily situated within the smallest phagosomes, which then merge into bigger phagosomes-endosomes (Figure 5).

The destruction of some of these phagosomes' membrane results in formation of "free" clusters of nanoparticles, often in close contacts with mitochondrial membranes and cristae (Figure 6) as well as with nuclear membranes (Figure 7). In such cases it is possible to see loss on the normal structure of those membranes.

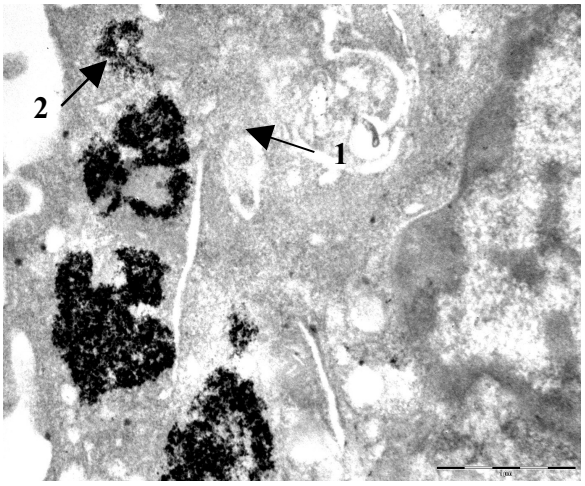


Figure 6: Contact of clustered 10 nm magnetite particles with membranes (arrow 1) and cristae (arrow 2) of AM's mitochondria. TEM, magnification *22 000

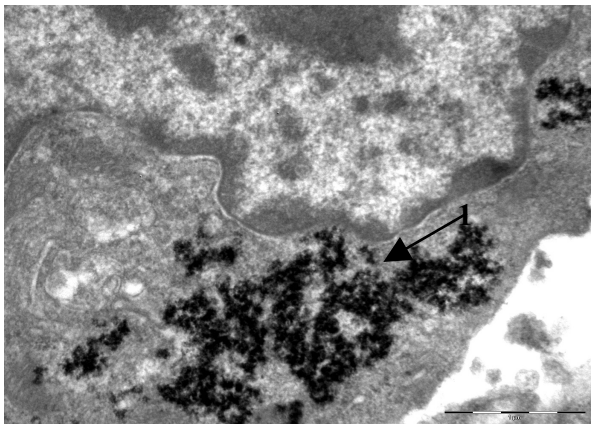


Figure 7: Contact (arrow 1) of clustered 10 nm magnetite particles with the damaged nuclear membrane of an AM. TEM, magnification *22 000

An extremely high cytotoxicity of nanoparticles is confirmed not only by comparative testing of nano- and micrometric particles of magnetite but also by comparing response to a suspension of 10 nm particles of this material with that to suspensions of other mineral particles with size distributions characteristic of ordinary industrial dusts (disintegration aerosols).

As can be seen from Figure 8, iron oxide, which is of low biological aggressiveness as a bulk material and as a micrometer particle, proved to be much more aggressive in the nano-range (judging by the NL/AM ratio) in comparison not only with another rather "inert" material such as titanium dioxide but also with highly cytotoxic quartz dust.

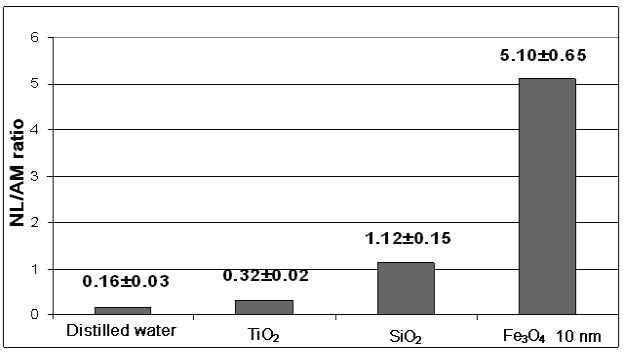


Figure 8: The ratio of the number of neutrophil leucocytes (NL) to the number of alveolar macrophages (AM) in the BALF of rats 24 hours after the instillation of magnetite nanoparticles (10 nm) and polydisperse suspensions of TiO_2 and SiO_2 (at a dose of 2 mg in 1 ml of distilled water) ($x \pm S_x$)

The results of our study with repeated i.p. injections of the suspensions under study show that sub-chronic resorptive

toxicity of iron oxide Fe_3O_4, which proved negligible under the action of even finest particles in the micrometer range, is substantial for nanoparticles.

It is more difficult to give a single unqualified answer to the question whether the toxicity of this substance increases with a decrease in the size of its particles *within the nanometric range*. Our results suggest that this dependence on nanoparticle's size (for a given mass dose) is different for essentially different effects, and is not unique due to complex and often opposing relationships between the intrinsic bioaggressivity of nanoparticles, on the one hand, and complex mechanisms that control their biokinetics, on the other hand.

As an example of resulting toxicokinetics differences we give in the Figure 9 data on accumulation of total iron (as estimated with the AAS) in the liver and the spleen of rats.

Accordingly, the histo-pathological changes were maximal in livers and spleens of rats injected with 50 nm particles. Meantime, judging by many systemic indices, the 10 nm particles are equally or even more toxic as compared with 50 nm ones.

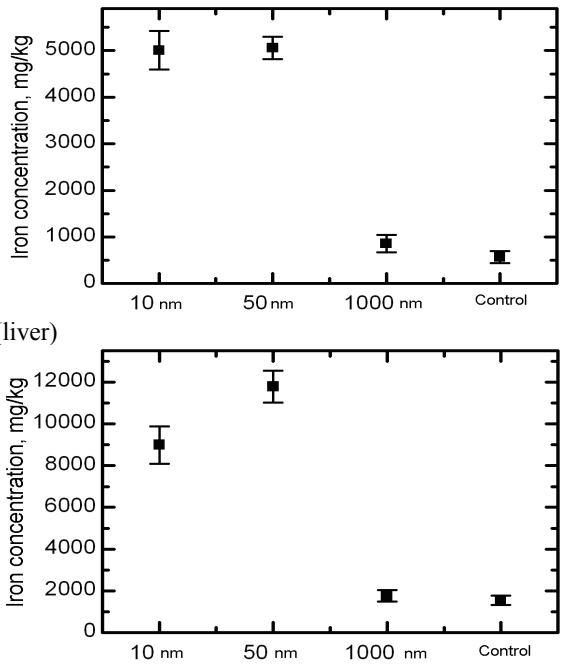


Figure 9: Mean value (±s.d.) of total iron concentration in the rat tissues of liver and spleen by groups of rats exposed to magnetite particles of different size. AAS method.

3 CONCLUSION

The results of this study demonstrate that the widespread concept of quasi-defenselessness of organism against nanoparticles should be critically re-evaluated, as one of the key host's defense mechanisms: phagocytosis of nanoparticles deposited in the respiratory tract, – is even more active than in the case of microparticles. At the same time, our results confirm that different size nanoparticles of a substance can be indeed much more biologically aggressive than their micrometric counterparts as concerns both local and resorptive toxicity.

Based on comparative toxicity data and taking into account air quality standards established in Russia for different mineral particulates, we proposed as the Tentative Safe Exposure Levels of nano-magnetite:

- in the workroom air 0.1mg/m^3,
- in the ambient air 3 μg/m^3.

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