COMPARISON OF RESPIRATORY TOXICITY OF TiO$_2$ AND Fe$_3$O$_4$ NANOPARTICLES AFTER INTRAVENOUS INSTILLATION: AN EXPERIMENTAL STUDY

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SUMMARY

Objective: Nanomaterials consist of particles smaller than 100 nm – nanoparticles (NPs). Their nano dimensions allow them to penetrate through various membranes and enter into the bloodstream and disseminate into different body organs. Massive expansion of nanotechnologies together with production of new nanoparticles which have not yet been in contact with living organisms may pose a potential health problem. It is therefore necessary to investigate the health impact of NPs after experimental exposure. Comparison of the effect of TiO$_2$ and NPs Fe$_3$O$_4$ in Wistar rats at time intervals 1, 7, 14 and 28 days was performed by studying the cytotoxic effect in the isolated inflammatory cells from bronchoalveolar lavage (BAL).

Methods: Wistar rats were intravenously (i.v.) given a suspension of NPs TiO$_2$, or Fe$_3$O$_4$ (coated by sodium oleate) via the tail vein. After time intervals of 1, 7, 14 and 28 days, we sacrificed the animals under anaesthesia, performed BAL and isolated the cells. The number of animals in the individual groups was 7–8. We examined the differential count of BAL cells (alveolar macrophages – AM, polymorphonuclear leukocytes – PMN, lymphocytes – Ly); viability and phagocytic activity of AM; the proportion of immature and multinuclear cells and enzymes – cathepsin D – CAT D, lactate dehydrogenase – LDH and acid phosphatase – ACP.

Results: We found that TiO$_2$ NPs are relatively inert – without induction of inflammatory and cytotoxic response. Exposure to nanoparticles Fe$_3$O$_4$ induced – under the same experimental conditions – in comparison with the control and TiO$_2$ a more extensive inflammatory and cytotoxic response, albeit only at 1, 7 and 14 days after injection.

Conclusions: The results suggest that TiO$_2$ and Fe$_3$O$_4$ nanoparticles used in our study were transferred from the bloodstream to the respiratory tract, but this effect was not observed at 28 days after i.v. injection, probably due to their removal from the respiratory tract.

Key words: TiO$_2$ and Fe$_3$O$_4$ nanoparticles, intravenous administration, bronchoalveolar lavage, inflammatory cells, cytotoxic parameters

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INTRODUCTION

Nanotechnology is defined as the understanding and control of matter at dimensions of roughly 1–100 nm, where unique physical properties make novel applications possible. Nanoparticles are particles that are less than 100 nm in size in more than one dimension. The European Commission defines nanomaterial as a natural, incidental or manufactured material containing particles in an unbound state or as an aggregate or agglomerate and where, generally for 50% or more of the particles in the number size distribution, one or more external dimensions range from 1–100 nm in size. They can be spherical, tubular, or irregularly shaped and can exist in fused, aggregated or agglomerated forms (1–3).

Nanoparticles are used in many areas, such as electronics, optics, engineering, construction, textiles, chemicals, food, automobiles, aerospace, military industries and have considerable applications in medicine especially in diagnostic and therapy.

In the medical field, NPs TiO$_2$ and Fe$_3$O$_4$ are currently considered to be relatively inert carriers for therapeutic and diagnostic pharmaceuticals, and they are also presumed for i.v. administration. Because of their nano dimensions they can probably penetrate through various membranes and pass from the bloodstream to different organs in the body (4).

In addition to engineered and newly created nanomaterials, naturally existing NPs are found in the environment as result of active volcanoes, fire, exhaust gases from cars, etc. These have an impact on the environment.

Nanoparticles of different sizes can be characterized with other effects and different reactivity. Substances consisting as NPs (nano-size) have different properties than larger particles from the same material, e.g. microparticles of micrometre size. Nanoparticles cause inflammation and other changes in the organism in contrast to the same mass of fine respirable particles, in the 2–3 micron range, composed of identical materials (5–7).

Nanoparticles may present new health risks for humans and other animal species or plants. Normal human defence mechanisms may not be able to respond adequately to the newly created nanoparticles. In addition, NPs can be released into the environment and influence it. Massive expansion of nanotechnologies together with production of new nanoparticles, which have not
yet been in contact with living organisms, may present a potential problem for public health (4, 5, 7).

Nanoparticles of titanium dioxide cause genetic damage in mice, suggesting that humans may be at risk of cancer or genetic disorders resulting from exposure (8–10). Titanium dioxide dust, when inhaled, has recently been classified by the International Agency for Research on Cancer (IARC) as Group 2B – potentially carcinogenic to humans. The findings of the IARC are based on the discovery that high concentrations of powdered and ultrafine titanium dioxide dust caused respiratory tract cancer in rats exposed by inhalation and intratracheal instillation (8).

Magnetite – Fe₃O₄ nanoparticles – has been recently classified by the IARC as Group 3 (the agent is not classifiable as to its carcinogenicity to humans). Iron oxide nanoparticles – Fe₃O₄ are new materials used in biotechnology and nanotechnology, food and drugs administration, etc. They are actually being used in biomedical applications such as magnetic resonance imaging contrast agents, thermotherapy agents and drug carriers for cancer treatments, amongst others.

All these applications are risky on different levels: single exposure, workplace exposure, incidental and environmental release, and potential long-life exposure. In this regard, the toxic effect of iron oxide nanoparticles is not well known and needs to be assessed (11). In the medical field, like NPs TiO₂, are NPs Fe₃O₄ currently also considered to be a relatively inert carrier for therapeutic and diagnostic pharmaceuticals, and there is also presumption for their intravenous (i.v.) application. Because they have nano dimensions, they can probably penetrate through various membranes and pass from the bloodstream to other organs in the body (5).

Therefore the aim of our study was to discern the impact of NPs Fe₃O₄, or TiO₂ injected intravenously into the tail vein of experimental animals on the selected BAL parameters, to compare the results of examined BAL parameters influenced by TiO₂ or Fe₃O₄ against control groups and each other (Fe₃O₄ to TiO₂); and to find out changes of selected BAL parameters at 1, 7, 14 and 28 days after i.v. injection of NPs TiO₂ and Fe₃O₄.

**MATERIALS AND METHODS**

Wistar rats have been i.v. applied (to a tail vein of an animal) a suspension of NPs TiO₂ or Fe₃O₄. The rats were killed under anesthesia at 1, 7, 14 and 28 day intervals, bronchoalveolar lavage (BAL) was performed and the cells were isolated from the lavage.

We used suspensions of nanoparticles in the following dosages:
- TiO₂ – 1.0% of LD₅₀ = 0.592 mg/kg of body weight of the animal and
- Fe₃O₄ – 1.0% of LD₅₀ = 0.364 mg/kg of body weight of the animal.

Control animals were intravenously given saline solution (into the tail vein) in the same volume as NP suspension. Size of NPs TiO₂ were in the range of 15–60 nm and NPs Fe₃O₄ – magnetite (coated with sodium oleate) in the range of 14–51 nm.

We studied the differential count of BAL cells (alveolar macrophages – AM, polymorphonuclear leukocytes – PMN, lymphocytes – Ly); viability and phagocytic activity of AM, as well as the proportion of immature and polynuclear cells and enzymes CAT-D, LDH and ACP.

The BAL fluid cells were counted in a Bürker’s chamber and differential counts of alveolar macrophages – AM, polymorphonuclear leukocytes – PMN, lymphocytes – LY and immature cells in the BAL fluid were performed on May-Grünwald Giemsa-Romanowski stained preparations counting 200 cells. Prior to that, the cells were cytocentrifuged on the slide using Cytospin (Shandon) centrifuge.

The phagocytic activity of AM was investigated via the modified method by Fornusek et al. using 2-hydroxyethylmetacrylate particles (MSHP) from Neosys, Prague. Fifty millilitre of particles in phosphate buffer (PBS) was added to 100 ml of BAL fluid and incubated for 60 min at 37°C and shaken at short intervals. Staining was performed by the May-Grünwald Giemsa-Romanowski method. Cells were considered positive (normal phagocytizing activity) when they phagocytized three or more particles (12).

To determine the viability of AM, 200 µl of 0.25% erythrosine solution was added to 200 µl aliquots of the cell suspension. The number of viable and non-viable cells was counted using Bürker’s chamber.

The activities of LDH were measured photometrically with an Eppendorf Geratebau photometer at 366 nm, using LDH-UV (lactate dehydrogenase-ultraviolet) kits.

The ACP measurements were carried out photometrically with a Specol Zeiss 1 (Jena, Germany) at 420 nm using acid phosphatase kits.

For determination of CAT-D levels the cell suspension was diluted with Triton X 100 in PBS (final concentration of Triton: 0.1%), mixed, three times frozen and thawed, centrifuged at 14,000 x g for 20 min. The activity of CAT-D was measured spectrophotometrically. A detailed description of the mentioned methodology can be found in papers by Hurbankova et al. (13–15) and Dziedzic et al. (16).

For statistical analysis (Mann-Whitney test), we have used v. 19 SPSS software. Because all groups contained a small number of samples (7 to 8 rats) and the samples were not normally distributed, the Kruskal-Wallis test was used for statistical processing of obtained results. All tests were done at a significance level of α = 0.05, if the calculated p-value was less than α, the result was considered as statistically significant.

**RESULTS**

A statistically significant decrease was observed in the AM percentage from the differential BAL cell count comparing NPs Fe₃O₄ to the control – on days 1 and 7 after the injection; and NPs Fe₃O₄ as compared to TiO₂, 7 days after the injection (Tables 1 and 2).

The results of the PMN percentage showed statistically significant increase in the differential BAL cell count on comparison of NPs Fe₃O₄ with the control (days 1 and 7 after injection); and Fe₃O₄ with TiO₂ – 7 days after the injection (Tables 1 and 2).

Ly percentage showed a statistically significant increase in the results of the differential BAL cell count comparison of NPs Fe₃O₄ with the control – days 1, 7, and 14 after instillation; and NPs Fe₃O₄ and TiO₂ – 7 days after the injection (Tables 1–3).

An increase in immature cells was statistically significant only in the case of NPs Fe₃O₄ against the control, on the first day after injection (Table 1).
A statistically significant increase was observed in multinucleated cells only in the comparison of NPs Fe₃O₄ to TiO₂ – 1 day after injection (Table 1).

The viability of AM was decreased with a statistical significance on comparing of NP TiO₂ to the control – 1 and 14 days after instillation; and NPs Fe₃O₄ to the control – 1, 7, and 14 days after injection (Tables 1–3).

Phagocytic activity was reduced with a statistical significance at 7 days after instillation of NPs TiO₂ and Fe₃O₄ in comparison with the control; and 14 days after the injection when comparing NPs Fe₃O₄ to TiO₂ (Tables 2 and 3).

The level of the enzyme CAT-D was statistically significantly increased comparing NPs TiO₂ to the control after day 1 of instillation; and when comparing NPs Fe₃O₄ to the control after 1, 7 and 14 days after i.v. injection (Tables 1–3).

The lactate dehydrogenase and ACP levels were not statistically significantly affected after exposure to NPs Fe₃O₄ and not even after exposure to TiO₂ (Tables 1–4).

After 28 days of nanoparticle installation the parameters did not show statistically significant changes (Table 4).

**DISCUSSION**

The impact of nanoparticles on health is becoming a public health issue, resulting in an important requirement for research. In the medical field, nanoparticles (NP) Fe₃O₄ and TiO₂ are currently considered to be relatively inert carriers for therapeutic and diagnostic pharmaceuticals, and they are also presumed for i.v. administration (17).

**Table 1. Inflammatory and cytotoxic BAL parameters one day after i.v. injection**

<table>
<thead>
<tr>
<th>BAL parameters</th>
<th>Control group mean (SD)</th>
<th>TiO₂ mean (SD)</th>
<th>Fe₃O₄ mean (SD)</th>
<th>TiO₂ in comparison with control</th>
<th>Fe₃O₄ in comparison with control</th>
<th>Fe₃O₄ in comparison with TiO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential count cells</td>
<td>AM (%)</td>
<td>96.380 (1.300)</td>
<td>94.380 (2.880)</td>
<td>80.870 (7.570)</td>
<td>↓ n.s.</td>
<td>↓ &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>PMN (%)</td>
<td>0.500 (0.756)</td>
<td>2.130 (2.031)</td>
<td>10.750 (6.042)</td>
<td>↑ n.s.</td>
<td>↑ 0.001</td>
</tr>
<tr>
<td></td>
<td>Ly (%)</td>
<td>1.130 (0.835)</td>
<td>3.500 (2.138)</td>
<td>8.380 (5.605)</td>
<td>↑ n.s.</td>
<td>↑ &lt; 0.001</td>
</tr>
<tr>
<td>Immature forms of AM (%)</td>
<td>13.500 (3.505)</td>
<td>17.380 (7.386)</td>
<td>22.130 (4.518)</td>
<td>↑ n.s.</td>
<td>↑ 0.013</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>Polynuclear cells – AM (%)</td>
<td>0.750 (0.886)</td>
<td>0.500 (0.756)</td>
<td>2.000 (1.069)</td>
<td>↓ n.s.</td>
<td>↑ n.s.</td>
<td>↑ 0.018</td>
</tr>
<tr>
<td>Viability of AM – % of living cells</td>
<td>79.500 (5.732)</td>
<td>62.880 (6.334)</td>
<td>63.250 (9.468)</td>
<td>↓ 0.007</td>
<td>↓ 0.006</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>Phagocytic activity of AM (%)</td>
<td>50.130 (3.137)</td>
<td>47.500 (2.726)</td>
<td>43.630 (7.210)</td>
<td>↓ n.s.</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>LDH µkat.g proteins⁻¹</td>
<td>2.611 (1.319)</td>
<td>3.191 (1.361)</td>
<td>2.489 (1.396)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>AcP nkat.g proteins⁻¹</td>
<td>37.630 (20.126)</td>
<td>55.817 (18.207)</td>
<td>64.565 (26.115)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>CAT D Utyr.mg proteins⁻¹</td>
<td>31.571 (14.345)</td>
<td>71.930 (21.532)</td>
<td>105.029 (39.477)</td>
<td>↑ 0.042</td>
<td>↑ 0.005</td>
<td>↑ n.s.</td>
</tr>
</tbody>
</table>

n.s. – statistically nonsignificant; ↓ – decrease of values; ↑ – increase of values; AM – alveolar macrophages; PMN – polymorphonuclear cells; Ly – lymphocytes; LDH – lactate dehydrogenase; ACP – acid phosphatase; CAT D – cathepsin D

**Table 2. Inflammatory and cytotoxic BAL parameters 7 days after i.v. injection**

<table>
<thead>
<tr>
<th>BAL parameters</th>
<th>Control group mean (SD)</th>
<th>TiO₂ mean (SD)</th>
<th>Fe₃O₄ mean (SD)</th>
<th>TiO₂ in comparison with control</th>
<th>Fe₃O₄ in comparison with control</th>
<th>Fe₃O₄ in comparison with TiO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential count cells</td>
<td>AM (%)</td>
<td>97.750 (0.707)</td>
<td>95.380 (2.504)</td>
<td>82.710 (5.057)</td>
<td>↓ n.s.</td>
<td>↓ &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>PMN (%)</td>
<td>0.880 (0.354)</td>
<td>1.250 (0.707)</td>
<td>3.710 (1.380)</td>
<td>↑ n.s.</td>
<td>↑ 0.001</td>
</tr>
<tr>
<td></td>
<td>Ly (%)</td>
<td>1.380 (1.061)</td>
<td>3.380 (2.875)</td>
<td>13.570 (3.867)</td>
<td>↑ n.s.</td>
<td>↑ &lt; 0.001</td>
</tr>
<tr>
<td>Immature forms of AM (%)</td>
<td>12.130 (2.588)</td>
<td>14.000 (5.529)</td>
<td>15.570 (4.791)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>Polynuclear cells – AM (%)</td>
<td>0.630 (0.518 )</td>
<td>1.000 (0.926)</td>
<td>0.710 (0.486)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>Viability of AM – % of living cells</td>
<td>79.500 (2.268)</td>
<td>68.750 (8.049)</td>
<td>51.290 (11.982)</td>
<td>↓ n.s.</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>Phagocytic activity of AM (%)</td>
<td>46.130 (2.416)</td>
<td>28.880 (9.717)</td>
<td>22.000 (9.574)</td>
<td>↓ 0.010</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>LDH µkat.g proteins⁻¹</td>
<td>3.661 (1.369)</td>
<td>4.521 (1.419)</td>
<td>4.275 (1.660)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>AcP nkat.g proteins⁻¹</td>
<td>50.049 (8.406)</td>
<td>57.660 (13.544)</td>
<td>68.171 (8.382)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td>CAT D Utyr.mg proteins⁻¹</td>
<td>48.451 (21.462)</td>
<td>91.086 (20.016)</td>
<td>93.710 (11.493)</td>
<td>↑ n.s.</td>
<td>↑ 0.010</td>
<td>↑ n.s.</td>
</tr>
</tbody>
</table>

n.s. – statistically nonsignificant; ↓ – decrease of values; ↑ – increase of values; AM – alveolar macrophages; PMN – polymorphonuclear cells; Ly – lymphocytes; LDH – lactate dehydrogenase; ACP – acid phosphatase; CAT D – cathepsin D
NPs TiO$_2$ have been considered as inert materials and have been used as control group in experimental work for decades, but their inert character has recently been questioned (18, 19).

With exception of airborne particles reaching the lungs, information on the behaviour of nanoparticles in the body is still minimal. Currently, inhalation is the main route of human exposure to nanoparticles (5). From the bloodstream nanoparticles are probably transferred to other organs such as the brain, liver, heart, respiratory tract, spleen, and possibly the foetus in pregnant women. The effects of long-term exposure to nanoparticles on human health have not yet been sufficiently demonstrated. Besides occupational exposure, epidemiological studies have shown that environmental exposure to air pollution (including nanoparticles) is associated with detrimental effects – with disorders related to oxidative stress (11).

Oxidative stress is probably the main important factor in the mechanism of action, which has significantly contributed to the understanding of the development of many lung diseases in experimental and epidemiological studies after exposure to solid particles and nanoparticles. When particle size becomes progressively smaller, the particle surface in turn becomes progressively bigger and in the interaction of this surface with the environment, oxidative stress is induced. Further experimental and human studies are needed in order to truly understand the health effects of nanoparticles (10, 20–22).

The lung is often the major target organ for toxic effects of many atmospheric pollutants, both gaseous and particulate in nature. The lung responds continually to chemical and physical stimuli. Not all the responses evoked by substances are injurious. Many responses and changes in the lung are not clearly definable as lung disease or damage but can be discerned by bronchoalveolar lavage (BAL). In many cases, the cellular constituents obtained in the lavage provide a good indication of lung injury.

Examination of the number and the type of the cells obtained via BAL, as well as of their viability and state of activation, enables us to understand the extent of the harmful effects caused to the lung by inhaled noxious substances (15, 23).

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**Table 3. Inflammatory and cytotoxic BAL parameters 14 days after i.v. injection**

<table>
<thead>
<tr>
<th>BAL parameters</th>
<th>Control group mean (SD)</th>
<th>TiO$_2$ mean (SD)</th>
<th>Fe$_3$O$_4$ mean (SD)</th>
<th>TiO$_2$ in comparison with control</th>
<th>Fe$_3$O$_4$ in comparison with control</th>
<th>Fe$_3$O$_4$ in comparison with TiO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential count cells</td>
<td>AM (%)</td>
<td>99.250 (1.035)</td>
<td>97.500 (1.773)</td>
<td>96.630 (1.302)</td>
<td>↓ n.s.</td>
<td>↓ n.s.</td>
</tr>
<tr>
<td></td>
<td>PMN (%)</td>
<td>0.380 (0.744)</td>
<td>0.630 (0.744)</td>
<td>0.500 (0.756)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td></td>
<td>Ly (%)</td>
<td>0.380 (0.518)</td>
<td>1.870 (1.808)</td>
<td>1.880 (0.835)</td>
<td>↓ n.s.</td>
<td>↑ 0.014</td>
</tr>
<tr>
<td></td>
<td>Viability of AM – % of living cells</td>
<td>81.130 (5.194)</td>
<td>69.500 (7.559)</td>
<td>69.630 (8.516)</td>
<td>↓ 0.026</td>
<td>↓ 0.029</td>
</tr>
<tr>
<td></td>
<td>Phagocytic activity of AM (%)</td>
<td>30.370 (7.051)</td>
<td>34.75 (6.692)</td>
<td>24.630 (3.815)</td>
<td>↑ n.s.</td>
<td>↓ 0.005</td>
</tr>
<tr>
<td></td>
<td>LDH µkat.g proteins$^{-1}$</td>
<td>5.701 (2.663)</td>
<td>5.820 (2.139)</td>
<td>7.135 (2.096)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td></td>
<td>AcP nkat.g proteins$^{-1}$</td>
<td>53.244 (24.546)</td>
<td>74.313 (9.686)</td>
<td>94.505 (26.541)</td>
<td>↑ n.s.</td>
<td>↓ n.s.</td>
</tr>
<tr>
<td></td>
<td>CAT D Utyr.mg proteins$^{-1}$</td>
<td>54.866 (15.195)</td>
<td>91.106 (31.945)</td>
<td>98.824 (17.861)</td>
<td>↑ n.s.</td>
<td>↑ 0.002</td>
</tr>
</tbody>
</table>

n.s. – statistically nonsignificant; ↓ – decrease of values; ↑ – increase of values; AM – alveolar macrophages; PMN – polymorphonuclear cells; Ly – lymphocytes; LDH – lactate dehydrogenase; AcP – acid phosphatase; CAT D – cathepsin D

**Table 4. Inflammatory and cytotoxic BAL parameters 28 days after i.v. injection**

<table>
<thead>
<tr>
<th>BAL parameters</th>
<th>Control group mean (SD)</th>
<th>TiO$_2$ mean (SD)</th>
<th>Fe$_3$O$_4$ mean (SD)</th>
<th>TiO$_2$ in comparison with control</th>
<th>Fe$_3$O$_4$ in comparison with control</th>
<th>Fe$_3$O$_4$ in comparison with TiO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential count cells</td>
<td>AM (%)</td>
<td>98.430 (2.149)</td>
<td>96.430 (2.370)</td>
<td>96.130 (3.271)</td>
<td>↓ n.s.</td>
<td>↓ n.s.</td>
</tr>
<tr>
<td></td>
<td>PMN (%)</td>
<td>0.140 (0.378)</td>
<td>0.710 (0.756)</td>
<td>0.750 (1.035)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td></td>
<td>Ly (%)</td>
<td>1.430 (1.813)</td>
<td>2.860 (1.676)</td>
<td>3.130 (2.850)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td></td>
<td>Viability of AM – % of living cells</td>
<td>68.570 (11.133)</td>
<td>70.290 (4.271)</td>
<td>70.250 (8.447)</td>
<td>↑ n.s.</td>
<td>↓ n.s.</td>
</tr>
<tr>
<td></td>
<td>LDH µkat.g proteins$^{-1}$</td>
<td>4.833 (1.468)</td>
<td>6.158 (2.020)</td>
<td>5.842 (1.724)</td>
<td>↑ n.s.</td>
<td>↓ n.s.</td>
</tr>
<tr>
<td></td>
<td>AcP nkat.g proteins$^{-1}$</td>
<td>69.995 (9.765)</td>
<td>95.733 (26.211)</td>
<td>99.494 (27.260)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
<tr>
<td></td>
<td>CAT D Utyr.mg proteins$^{-1}$</td>
<td>67.234 (13.538)</td>
<td>89.361 (42.010)</td>
<td>92.559 (21.903)</td>
<td>↑ n.s.</td>
<td>↑ n.s.</td>
</tr>
</tbody>
</table>

n.s. – statistically nonsignificant; ↓ – decrease of values; ↑ – increase of values; AM – alveolar macrophages; PMN – polymorphonuclear cells; Ly – lymphocytes; LDH – lactate dehydrogenase; AcP – acid phosphatase; CAT D – cathepsin D
to Dziedzic et al., long-term recruitment of PMN might be an important factor in prediction of lung metaplastic processes (16).

NPs deposited in the alveolar region are digested by specialized defence cells – alveolar macrophages which are located in the alveoli. Pulmonary toxicity studies in rats demonstrate that NPs produce enhanced inflammatory responses when compared to larger-sized particles of identical chemical composition at equivalent mass concentrations.

Inflammation is the common factor that binds together these adverse effects and the ability of NPs to cause inflammation can be seen as an important property. Pulmonary inflammation, which results in changes in membrane permeability, may in turn allow distribution of particles beyond the lung. The NPs have shown significant translocation from the lung into the bloodstream, but also vice versa, as demonstrated in our studies as well as in others (4, 6, 15).

AM constitute important and frequently utilized tools for examination of cytotoxicity. AM are the predominant cells present in BAL and changes in their number or function are the factors determining lung injury and characterizing the pathogenesis of such a response. During the migration of monocytes into tissues they differentiate until they become multifunctional tissue macrophages. They may at this stage be regarded as “immature macrophages”. During the immune process, the number of “immature macrophages” significantly increases. Decrease of the macrophage count, the viability or the phagocytic capacity may result in an impaired clearance of the inhaled materials (16, 24).

Multinucleated cells (MNC) were observed in some chronic inflammation processes of the lung. It has been recognized that these processes may arise by fusion of macrophages engaged into cleaning foreign material from tissue and have been named as “foreign body cells”. The experimental evidence for this view derives from the finding that if more than one macrophage is attached simultaneously to the same foreign material in vitro, fusion to multinuclear cells may occur. An increasing number of MNC after 14 days of exposure to TiO₂ in our experiment was detected (25).

CAT-D is a lysosomal enzyme that is released into the BAL in case of lung tissue damage. BAL cells significantly contribute to the increasing activity of lysosomal enzymes in the BAL – especially macrophages. The increase of activity of lysosome enzymes is a good indicator of cell activation (especially PM), and simultaneously reflects the degree of defence capability of the lung. Cathepsin D is a major enzyme involved in the degradation of proteins in lysosomes. It is essential for proteolysis of proteins which regulate cell growth and tissue homeostasis (26).

CONCLUSION

We found that in our experiments NPs TiO₂ are relatively inert – without induction of greater inflammatory response and cytotoxic damage, suggesting their low biopersistence, respiratory toxicity and relatively rapid elimination from the lungs – under the conditions provided by us (dose and time dependence).

Based on our results, exposure to nanoparticles Fe₃O₄ (coated by sodium oleate) induced, in comparison with the control and TiO₂, higher extensive inflammatory response, cytotoxic changes, but only 1, 7 and 14 days after injection.

On the basis of our results we surmise that the TiO₂ and Fe₃O₄ nanoparticles used in our study were transferred from the bloodstream to the respiratory tract. However, this effect was no longer seen 28 days after i.v. injection, probably due to removal of these NPs from the respiratory tract.

Newly, our experimental knowledge could be used in the field of i.v. injection of nanoparticles (in therapy and diagnostics), in the prevention of pulmonary diseases developed as a result of inhaled particle aerosols and nanoparticles in working and living environments and furthermore help in improving the health status of the population. Our results are also important for strategic and legislative activity.

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Conflict of Interests

None declared

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