Investigation of hematotoxic effect of nano ZnO, nano Fe₃O₄ and nano SiO₂ in vitro

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ARTICLE INFO

Article History:
Received 11 March 2017
Accepted 3 April 2017
Published 11 May 2017

Keywords:
Hematotoxic
Nano ZnO
Nano Fe₃O₄
Cristobalit
Red blood cells

ABSTRACT

Objective(s): Evaluation of nanomaterials interaction with blood ingredients is a part of preclinical risk assessment of newly-synthesized materials, especially for nano-sized pharmaceuticals which are intravenously administrated. The red blood cells (RBCs) are susceptible to oxidative stress damage. This study was designed to evaluate induced oxidative hematotoxic effect of nano ZnO, Fe₃O₄ and nano SiO₂ on human red blood cells in vitro.

Methods: Blood samples were collected from healthy male volunteers. RBCs were exposed to different concentrations (50, 100, 250mg/ml) of nano ZnO, nano Fe₃O₄, and nano SiO₂, at 4°C for 24 hours. Lipid peroxidation and intracellular Glutathione (GSH) level were studied as the biomarkers of oxidative stress.

Results: The results showed that the lipid peroxidation had significantly increased. However, after exposure to nanoparticles, the GSH level of RBCs considerably decreased compared to the controls (p<0.05).

Conclusions: This study suggests that nanoparticles could induce oxidative stress that eventually leads to cell toxicity and hemolysis of RBCs at certain dosages. This study provides important experimental information for safety assessment and pharmacokinetics of high-doses intravenous administration of nanoparticles for design of clinical trials.

How to cite this article:

INTRODUCTION

Nanomaterials are defined as a new class of materials having unique physical and chemical properties. There is great interest in applying nanomaterials in different fields of medicine and industry. The potential benefit of nanomaterials in medicinal devices or drug delivery proposes new methods in diagnosis, medication, and treatment of complicated diseases such as cancers. Any nanomaterials applied as pharmaceuticals or medical devices must be assessed for potential risk for consumers before approval for prescription.

Evaluation of nanomaterials interaction with blood ingredients is a part of preclinical risk assessment of newly-synthesized materials, especially for nano-sized pharmaceuticals which are intravenously administrated [1]. Several studies have shown that the unique structure and size of the nanoparticles could lead to oxidative injury and cell toxicity in different cell cultures and also in blood cells [2-3]. Formation of reactive oxygen and nitrogen species (RONS) at levels higher than the body’s antioxidant capacity has been known as the oxidative stress. Reactive oxygen and nitrogen species are generated in the body as the result of normal metabolism.
and either as the result of exposure to a variety of chemicals, drugs, and pollutants. Nucleic acids, proteins, lipids are cellular macromolecules that may be harmed by RONS. Oxidative stress also involves in pathogenesis of different diseases including cancer, neurodegenerative diseases, atherosclerosis, kidney and liver damage, rheumatoid arthritis, immunological disorder, and aging [4-5].

Lipid peroxidation of polyunsaturated fatty acids leads to production of malondialdehyde (MDA) that is a useful biomarker of oxidative stress. MDA is commonly measured by the thiobarbituric acid-reactive-substances (TBARS) assay. Small thiols including tripeptide glutathione (GSH) are considered as the protective sulphhydryl antioxidants and radical scavengers. Glutathione protects sensitive thiol groups (—SH) of proteins against oxidative stress [6].

The RBCs are intrinsically susceptible to oxidative stress damage because of exposing to high oxygen pressure and the presence of polyunsaturated fatty acid in their cell membrane. Furthermore, RBCs do not have endoplasmic reticulum and nucleus to replace damaged proteins [7]. Some studies have investigated the hematotoxic effects of nanoparticles on the blood cells in vivo and in vitro [8-13].

Nano Fe₂O₃, nano ZnO, and nano SiO₂ are well-known nanoparticles that have promising biomedical applications and their oxidative stress effects have been reported in different studies [9, 14-15]. Zinc nanoparticles are used in cosmetics ointment, sunscreens, food additive, antimicrobial, fungicide, UV protection coatings, and as a catalyst [15]. SiO₂ nanoparticles have found extensive applications in chemical mechanical polishing and as the additives to drugs, cosmetics, printer toners, varnishes, and food. In recent years, the use of SiO₂ nanoparticles has been extended to biomedical and biotechnological fields [16]. Fe₂O₃ nanoparticles have been used as the contrast agents in Magnetic resonance imaging (MRI) angiography and perfusion imaging [17].

In this study, the induced oxidative hematotoxic effects of different doses of nano ZnO, nano Fe₂O₃, and nano SiO₂ have been studied on human RBCs.

MATERIALS AND METHODS
Nano particles characterization
Silicon Dioxide (SiO₂) Nanopowder (99+%, 20-30 nm, amorphous) was purchased from Nanosany Corporation, Iranian Nanomaterials Pioneers Company, Mashhad, Iran. Figure 1 and Figure 2 represent transmission electron microscope (TEM) and scanning electron microscope (SEM) images of nano SiO₂.
Iron Oxide (Fe₂O₃) Nanopowder (99+%, 20-40 nm, spherical) was purchased from Nanosany Corporation, Iranian Nanomaterials Pioneers Company, Mashhad, Iran. Figure 3 represent the TEM image of nano Fe₂O₃.
The nano-zinc metal powder (99.99+, 20-40 nm) was purchased from the Intelligent Materials Pvt. Ltd., Nanoshel LLC, Wilmington, DE, USA. Figure 4 represent transmission electron the TEM image of nano ZnO.
All the other chemicals used in this experiment were analytical grade with the highest purity.
Blood samples preparation

Blood samples were collected from healthy male volunteers in heparinized tubes and centrifuged at 3000 rpm for 25 min. Plasma and the buffy coats of blood were discarded. RBCs were washed three times with phosphate-buffered normal saline and then exposed to different concentrations (50, 100, 250 mg/ml) of nano ZnO, nano Fe₃O₄, and nano SiO₂ before incubation at 4°C for 24 h. Each experiment was performed in triplicate.

Lipid peroxidation assay

Lipid peroxidation in human erythrocytes was quantified by measuring the formation of TBARS. Erythrocytes were mixed with trichloroacetic acid 20% (1:1). Samples were centrifuged (600g×10 min) and then thiobarbituric acid 15% was added to the supernatants. Finally, the samples were heated at temperature 100°C for 15 min and the absorbance of the supernatant was measured at 532 nm. The quantities of TBARS were presented as the percentage of TBARS production over the control.

Quantification of intracellular GSH levels

Cellular level of reduced GSH was determined using the GSH colorimetric assay kit. This method is based on a chemical reaction between GSH and 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) that results in generating glutathione disulfide (GSSG) and-nitro-5-thiobenzoic acid, a yellow colored product. Thus, GSH concentration in a sample solution can be determined by the absorbance measurement at 412 nm [18].

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the results. P value greater than 0.05 was considered insignificant.

Figure 5 indicates schematic of the quantification of oxidative stress induction assay.

RESULTS AND DISCUSSION

Effect of Zn, Fe₃O₄ and SiO₂ nanoparticles on lipid peroxidation

TBARS assay is a sensitive and simple method for detecting lipid peroxidation which is commonly used as an important biomarker of oxidative stress. As showed in Table 1, the percentage of lipid peroxidation biomarker, malondialdehyde level, was significantly increased in treated red blood compared to the control groups after incubation with different concentrations of all nanoparticles.

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Nano ZnO</th>
<th>Nano Fe₃O₄</th>
<th>Nano SiO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/ml (control)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>151±9.3*</td>
<td>176±6.4*</td>
<td>167±15.1*</td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>157±7.4*</td>
<td>182±4.3*</td>
<td>152±3.6*</td>
</tr>
<tr>
<td>250 mg/ml</td>
<td>183±11*  **</td>
<td>190±9.4*</td>
<td>171±15.1*</td>
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</tbody>
</table>
(p<0.05) and at higher doses of nano ZnO and Fe$_3$O$_4$ nanoparticles more significant effects were seen (p<0.05). Furthermore, exposure to the different doses of Fe$_3$O$_4$ nanoparticles has led to high lipid peroxidation level compared to controls. However, dose dependency of lipid peroxidation was not significant. In SiO$_2$ treated groups, significant increase in lipid peroxidation level was showed (p<0.05). Nanoparticle-free RBCs were used as the control groups. The significant data are indicated by* versus control p<0.05. ** shows significant data compared to other doses of that nanoparticle p<0.05.

**Effect of ZnO, Fe$_3$O$_4$, and SiO$_2$ nanoparticles on intracellular GSH levels**

GSH is a special sulhydryl-contained molecule in cells that maintains homeostasis of cellular oxidation-reduction. Alterations in GSH homeostasis can be considered as the indication of functional damage to the cells. As can be seen in Table 2, compared to the controls, the level of GSH in RBCs significantly decreased after exposure to nanoparticles in all treated groups (p<0.05). Nanoparticle-free RBCs were used as the control groups. The significant data are indicated by* (p<0.05) versus control.

The data showed a significant decrease in GSH

<table>
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<th>Table 2. GSH levels of RBCs after 24h exposure to ZnO, Fe$_3$O$_4$, SiO$_2$ nanoparticles</th>
</tr>
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<tbody>
<tr>
<td>Concentration (mg/ml)</td>
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<tr>
<td>------------------------</td>
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<tr>
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<tr>
<td>0 mg/ml (control)</td>
</tr>
<tr>
<td>50 mg/ml</td>
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<tr>
<td>100 mg/ml</td>
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<tr>
<td>250 mg/ml</td>
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</table>
CONCLUSIONS
In conclusion, this study suggested that nanoparticles could induce oxidative stress, cell toxicity, and RBC hemolysis at certain doses. This study provides important experimental information for the safety assessment and pharmacokinetics of high doses intravenous administration of nanoparticles and for the design of clinical trials.

CONFLICTS OF INTEREST
The authors declare that there are no conflicts of interest.

REFERENCES
Mohammad Kazem Koohi et al. / Investigation of hematotoxic effect


