Nanomed Res J 5(4):364-368, Autumn 2020

RESEARCH ARTICLE

The Investigation of the Cytotoxicity of Copper Oxide Nanoparticles on Peripheral Blood Mononuclear Cells

Maryam Zivari Fard [1], Marjan Fatholahi [1], Morteza Abyadeh [2], Azam Bakhtiarian[1], Seyyedeh Elaheh Mousavi [1,*], Mojtaba Falahati [3,*]

[1] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[2] Department of Molecular Systems Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

[3] Department of Nanotechnology, Faculty of Advanced Sciences and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

ARTICLE INFO

Article History:
Received 20 Aug 2020
Accepted 13 Oct 2020
Published 01 Nov 2020

Keywords:
Copper Oxide Nanoparticles
Peripheral Blood Mononuclear Cells
Toxicity

ABSTRACT

Copper (II) oxide nanoparticle (CuO-NP) is extensively used in a wide variety of industrial and biomedical applications. Reduced size of Copper oxide (CuO) particles from micron to nanoscale caused superior physical characteristics on one side while making them cytotoxic on another side. Therefore there is an urgent need to examine the cytotoxicity of CuO NP. The present study evaluated the toxic effects of CuO-NPs exposure on peripheral blood cells (PBMCs). PBMCs were exposed to different concentrations of CuO-NPs (0, 1, 10, 50, 100, 200 µg/ml), and cytotoxicity was investigated using MTT assay for cell viability, LDH Assay for cell membrane damage and Acridine orange/propidium iodide staining for cell apoptosis. The results of this study indicated that CuO-NPs exert concentration-dependent toxicity on peripheral blood cells. While low concentration of CuO-NPs (1 µ g / ml) did not affected cells viability, higher concentrations (≥10µg / ml) reduced cell viability (up to %27.01 for 200 µg / ml). Moreover, LDH leakage at ≥1µg / ml showed a cell's membrane damage and also cell apoptosis was observed. In conclusion, the results of this study showed the concentration-dependent toxicity of CuO-NPs on PBMCs.

How to cite this article
Zivari Fard M, Fatholahi M, Abyadeh M, Bakhtiarian A, Mousavi SE, Falahati M. The Investigation of the Cytotoxicity of Copper Oxide Nanoparticles on Peripheral Blood Mononuclear Cells. Nanomed Res J, 2020; 5(4): 364-368.
DOI: 10.22034/nmrj.2020.04.008

INTRODUCTION

Nanoparticles refer to a group of particles that have a size between 1 and 100 nanometers and are used in a wide variety of fields because of their unique physicochemical properties [1-3]. Metal-based nanoparticles, including metal oxides, are among the most widely used nanomaterials [4, 5], One of the most common members of these materials is Copper (II) oxide nanoparticle (CuO NP), which is broadly used in various industrial and commercial applications including; catalysts, photovoltaic cells, gas sensors, agrochemicals, paints, and antimicrobial products, due to their superior physicochemical, electrochemical, photovoltaic and photoconductive properties [6, 7]. The fast pace of using CuO-NPs and consequently releasing of these NPs into the environment raised concerns from the public and government globally about the toxicological effects of these NPs, and studies on the health effects of these nanoparticles have gained a great deal of attention. Two important routes of exposure to CuO-NP are inhalation and skin exposure [8, 9]. Several studies have shown that a part of CuO-NPs cytotoxicity is resulting from the release of copper ions [10, 11]. Rapid dissolution of CuO-NPs and the role of copper ions in toxicity is a major concern to assess

* Corresponding Authors Emails: *semousavi@sina.tums.ac.ir*
mojtaba.falahati@alumni.ut.ac.ir

NP-mediated toxicity [12, 13].

During the last decades, numerous evidence indicating the cytotoxicity of CuO-NPs on several cell models [14, 15] and showed that these NPs cause oxidative stress, DNA damage and cell death [10, 16], moreover toxicological studies on animal models showed the association of CuO-NPs exposure with inflammation and liver damage [17]. A limited number of studies have assessed the cytotoxicity of CuO-NPs on the immune system [18], But no study has been done on the effects of CuO-NPs on peripheral blood cells (PBMCs). PBMCs derived from hematopoietic stem cells (HSCs) and are critical components of the innate and adaptive immune system therefore their dysfunction has a grave impact on the entire immune system [19]. We herein performed the present study to investigate the toxicity of CuO-NPs on PBMCs.

MATERIALS AND METHODS

Material

Copper oxide nanoparticles were purchased from US Nano, as a powder with a particle size of 25-50 nm, with a purity of 99.95% and nearly spherical morphology. All other used materials and their companies are as follow; RPMI1640 (biosera), Fetal Bovine Serum (Gibco BRL), NaCl (merck), NaOH (merck), penicillin/streptomycin solution 100X, PBS tablet, MTT (3-(4, 5-dimethylthiazol-2-thiazol-2-tl)-2, 5-diphenyl tetrazolium bromide) (Sigma), dimethyl sulfoxide (DMSO), phytohemagglutinin (Gibco), Trypan Blue in 0/9% NaCl solution 0/4% (wt/vol) (Gibco),), dimethyl sulfoxide (DMSO), Lactate dehydrogenase kit (sigma), Acridine orange (sigma), Ethidium Bromide (sigma).

MTT Assay

Isolation of peripheral blood mononuclear cell was done using Ficoll-Hypaque according to the standard method. MTT test was used to evaluate the cytotoxicity of CuO-NPs. 1×10^4 cells per well seeded in 96-well plate, then exposed to different concentrations of CuO-NPs (0, 1, 10, 50, 100, 200 µg / ml) for 24 hours. The control group received only a culture medium with a solvent. After 24 hours, the culture medium containing nanoparticles was removed with one wash. Then 20 µl of MTT solution (5 mg/ml) was added and incubated for 4 hours and then 200 µl of DMSO was added. After 10-20 minutes, the optical density was measured by a spectrophotometer plate reader.

LDH Assay

The permeation of LDH in PBMCs was evaluated by using an LDH test. PBMCs was seeded into the 96-well plates then exposed to different concentration of CuO-NPs (0, 1, 10, 20, 50, 100, 200 µgmL-1) for 24h. Finally, 50 µl cell medium was used for LDH leakage analysis, and the absorption was measured by ELISA reader (at 490 nm).

Apoptosis Assay

Apoptosis induced by CuO-NPs in PBMCs was investigated using a fluorescence microscope after dyeing PBMCs by two DNA binding stain; ethidium bromide and Acridine orange. The cells were exposed to IC50 concentrations of CuO-NPs (47.5 µg/ml) for 24 h. The cells was fixed with formaldehyde. Then stained with two dyes AO (100 µg/mL) and EB (100 µg/mL) at room temperature for 20 minutes. DNA fragmentations of PBMCs was observed by a fluorescence microscope.

Statistical analysis

Statistical review of results was done with one-way ANOVA, also the statistical differences were considered significant at the level of $P<0.05$.

RESULTS AND DISCUSSION

High concentrations of CuO-NPs reduced cell survival

To evaluate the effect of CuO-NPs on PBMCs viability, cell cultures were exposed to CuO-NPs for about 24 hr, results of MTT assay showed a significant reduction in cell survival (Fig. 1). Looking more closely at Fig. 1, CuO-NPs with a concentration of 1 µ g / ml did not cause significant cytotoxicity. However, higher concentrations of CuO-NPs led to increased cytotoxicity. Intriguingly, the survival rate of PBMCs after treatment with CuO-NPs was reduced in a dose-dependent manner; moreover, adverse effects of Co-NPs on PBMCs started at a concentration of 10µg / ml ($P< 0.05$). And by increasing the concentration up to the level of 200 µg / ml, the cell viability was reduced by %27.01($p <0.001$) (Fig. 1). In agreement with our results, several lines of evidence have indicated the cytotoxic potential of CO-NPs on different cell lines. For instance; CuO-NPs caused a decrease in HepG2 cells' viability [20], human lung epithelial cells (A549) [21], human HEp-2 airway epithelial cells [22], and human cardiac micro-vascular endothelial cells [23].

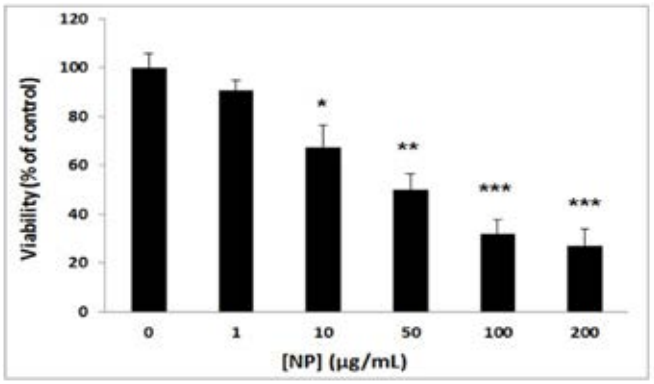

Fig. 1. Evaluation of cytotoxicity of CuO-NPs using MTT method. Different concentrations of CuO-NPs (0, 1, 10, 50, 100, 200 μg/mL).

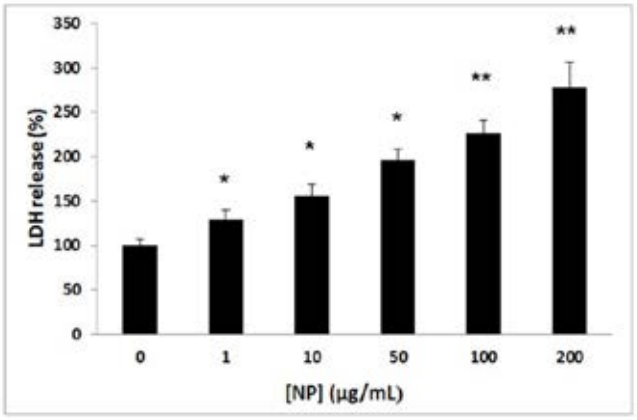

Fig. 2. Evaluation of the effect of CuO-NPs on release of LDH in cell culture medium.

Karlsson et al, compared the toxicity of different nanoparticles including; CuO, Fe_2O_4, Fe_3O_4, TiO_2, on human A549 lung epithelial cells, and identified CO-NPs as the most toxic nanoparticles, which caused DNA damage [24]. Furthermore, dose-dependent cytotoxicity of CuO NPs has been reported previously on different cell lines; Assadian et al, reported cytotoxicity in a concentration-dependent manner on blood lymphocytes [25]. Jing et al, also observed the cytotoxicity effects of CuO NPs on lung epithelial cells through a concentration-dependent manner [26]. The exact mechanism of CuO-NP toxicity is not yet known, however, the main accept mechanism is due to the release of copper ions, lysosomal dysfunction and subsequently induced severe oxidative stress [10, 11].

CuO-NPs caused cell membrane damage

To assess the impact of CuO-NPs on cell membrane LDH leakage assay was employed. The release of LDH, a cytoplasmic enzyme, is resulted in damage to the cell membrane [27]. Exposure of PBMCs to CuO-NPs (1–200 mg/ml) for 24h caused a notable ($P < 0.01$) increase in LDH release into the media. The release of the cytoplasmic LDH enzyme started at a concentration of 1 µg / ml ($P < 0.05$) and this release increased in a dose-dependent manner (Fig. 2). The outcome of the present study showed that CuO-NPs caused cell death and membrane damage and consequently led to LDH leakage (Fig. 2). In agreement with our results, recently Narsimha et al, Showed that exposure of human embryonic kidney cells to CuO-NPs not only increased cytotoxicity in a concentration-dependent manner but also caused increasing LDH levels and cell membrane damage [28]. Furthermore, an investigation of CuO-NPs toxicity on human lung epithelial cells showed cell death and DNA damage by affecting cell membrane integrity [29].

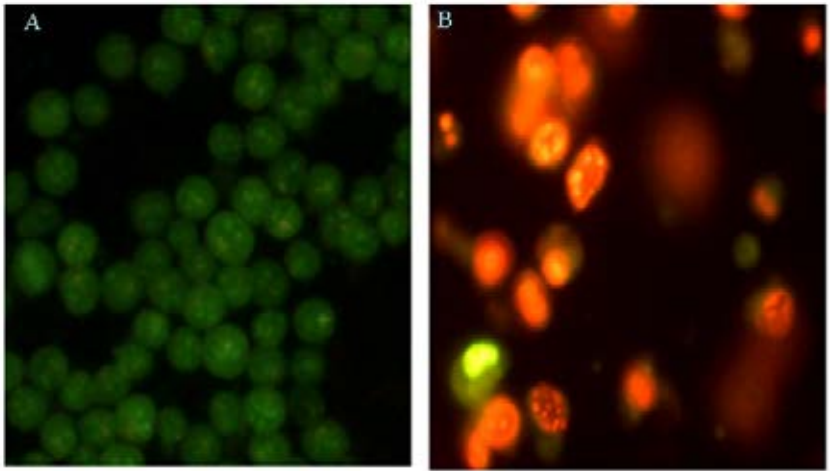

Fig. 3. The effect of CuO-NPs on PBMCs determined by Acridine orange/ethidium bromide assay. A) Control group, B) 47.5 **µg**/mL CuO-NPs group.

CuO-NPs induced apoptosis in cells

Apoptosis is induced by cytotoxic agents through death signaling pathways in vulnerable cells [30]. To ensure the induction of apoptotic death by CuO-NPs, we used Acridine orange/ethidium bromide staining to determine the type of induced death. Treatment of PBMCs with CuO-NPs (the concentration at which the cells were treated for 24 h was the same as the IC50 concentration determined by the MTT assay, 47.5 µ g / ml). As shown in Fig. 3, exposure to CuO-NPs with a concentration of 47.5 µg / ml for 24 hours, lead to condensation of the nucleus of these cells and thus the visibility of these cells (characteristic of apoptotic cells). As it's depicted in Fig. (3-A), the control cells are shown in green, while the apoptotic cells are orange Fig. (3-B). This test confirms the cytotoxicity effect of CuO-NPs on PBMCs. In line with our result, Shafagh et al, observed that CuO-NPs induced apoptosis in chronic myeloid leukemia (CML) K562 cell line, in addition, they reported up regulation of P53 and elevated Bax/Bcl-2 ratio, therefore suggested a key role for mitochondria-mediated pathway in cell apoptosis caused by CuO-NPs [31]. Furthermore, Kukia et al showed that CuO-NPs caused apoptosis in glial cancer (B92) cell line, even a low concentration (5µg/ml) of CuO-NPs caused significant cell apoptosis (32%) [32].

CONCLUSION

The results of this study showed the toxicity of CuO-NPs on PBMCs, which reduced cell viability, enhanced cell membrane disruption and apoptosis; moreover, a dose dependent cytotoxicity of CuO-NPs on PBMCs was observed. While our findings showed the toxicity of CuO-NPs on PBMCs, further studies are needed to reveal the mechanisms underlying the immune toxicity of CuO-NPs.

FUNDING

This research was supported by the Tehran University of Medical Sciences.

DISCLOSURE STATEMENT

None.

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