

Original Article



Cell Membrane Damage by Iron Nanoparticles: An in Vitro Study

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Abstract

Application of nanotechnology in medicinal and biological fields has attracted a great interest in recent years. In this paper, the cell membrane leakage induced by iron nanoparticles (Fe-NP) against PC12 cell line which is known as a model of nervous system cell line, was investigated by the lactate dehydrogenase (LDH) test. Therefore, PC12 cells were incubated with different concentrations of Fe-NP and the test has been performed after 48h of incubation of the cells with Fe-NP. The resulting data showed that Fe-NP induced the damage of PC12 cell membrane in a concentration-dependent manner. Hence, it may be concluded that the different cytotoxicity effect of NPs may be referred to the concentration of NPs, type of the NPs and the cells. Indeed, the kind of cytotoxic impacts of NPs on the cells can be reduced by considering the mentioned parameters.

Keywords: Cell, cytotoxicity, LDH, Nanoparticle, membrane

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Introduction

Nanoparticles (NPs) have opened a great interest in recent years in nanomedicine to optimize and target conventional drug delivery systems (Formoso et al., 2016). NPs, due to their distinctive features such as small size, high surface volume, unique physicochemical properties, and surface functionalization have attracted a great interest in drug delivery and treatment system covering wider applications including disorders of central nervous system (Sobczak-Kupiec et al., 2016, Choi et al., 2016). NPs can be used to improve the function of drugs which are susceptible to degradation and/or destabilization. NPs also can be functionalized with different moieties which make them excellent candidates with favorable properties of targeted and controlled release potential (Pathak et al., 2015, Mukherjee et al., 2015, Hola et al., 2015). However, to design these foreseeable advantages proposed by nano delivery systems, challenges which develop the safe system, remarkable biocompatibility, high drug loading, targeted drug delivery, and release control should be certified (Kesharwani et al., 2015, Jayaraman et al., 2015). Biocompatibility plays a key role to design a proper agent in drug system for in vitro and in vivo study (Shah et al., 2015). However, with modification of drug system via NPs, some noteworthy side effects are induced. Therefore, the evaluation of the cytotoxic effect of NPs is the most important stage before any medicinal application (Jain et al., 2015, Valdiglesias et al., 2015). Hence, nanotoxicity is referred to the examination of the potential toxic effects of NPs on biological systems (Valdiglesias et al., 2015). The field arose due to growing application of nanotechnology and the corresponding health impacts of nanomaterials, especially on human beings.

Iron nanoparticles (Fe-NPs) have attracted a potential interest in all medicinal fields such as drug delivery, cancer treatments, and imaging (Valdiglesias et al., 2015). However, the cytotoxic effect of these NPs on the nervous system has remained unknown.

In this study, the potential cytotoxic effect of Fe-NPs on the PC-12 cell as a nervous system model cell was assayed by lactate dehydrogenase (LDH) test. Indeed, this test can reveal the integrity disruption of the cell membrane.

Materials and methods

Materials

The cell culture medium Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin, and fetal bovine serum (FBS) were purchased from Gibco BRL (Life technology, Paisley, Scotland). Fe-NP was purchased from Sigma-Aldrich Company (USA).

Methods

Cell culture

The PC12 cell lines were purchased from Pasteur Institute of Tehran, Iran. The cells were incubated at 37°C in a 90% humidified atmosphere with 5% CO₂. The cells were cultured in DMEM with 10% (v/v) FBS, and (100 units/ml penicillin and 100 µg/ml streptomycin. The concentration of Fe-NP in DMEM was 0.1, 1, 5, 10 and 100 µg/ml. Afterward, PC12 cells with a density of 10000 per well in a 96 well plate were cultured and various concentrations of Fe-NPs were added to the wells for LDH assay.

LDH assay

The integrity of cell membrane was determined using an LDH kit (Parsazmoon, Tehran, Iran). The cells were seeded at a density of 10000 cells/well in 96-well plates in 100 µl of fresh medium. Afterward, the cells were incubated with different concentrations of Fe-NP (0.1, 1, 5, 10 and 100 µg/ml) for 48 h. The supernatant was collected for lactate LDH assay. Data were reported as a percentage of the control samples. Cell death was examined by leakage of LDH from cells. The LDH activity was measured spectrophotometrically according to the manufacturer's protocols.

Results and discussion:

Damage to the plasma membrane integrity, resulted from the formation of reactive oxygen species (ROS), is one of the key mechanisms by which NPs induce the reduction of viability of the neuronal cells. The leakage of the cytoplasmic enzyme like LDH into culture medium is known as a marker for cell membrane damage. As shown in Figure 1, PC12 cells exposure

to 100 $\mu\text{g}/\text{mL}$ of Fe-NP results in the release of LDH to the cell culture medium by about fivefold, compared to the control.

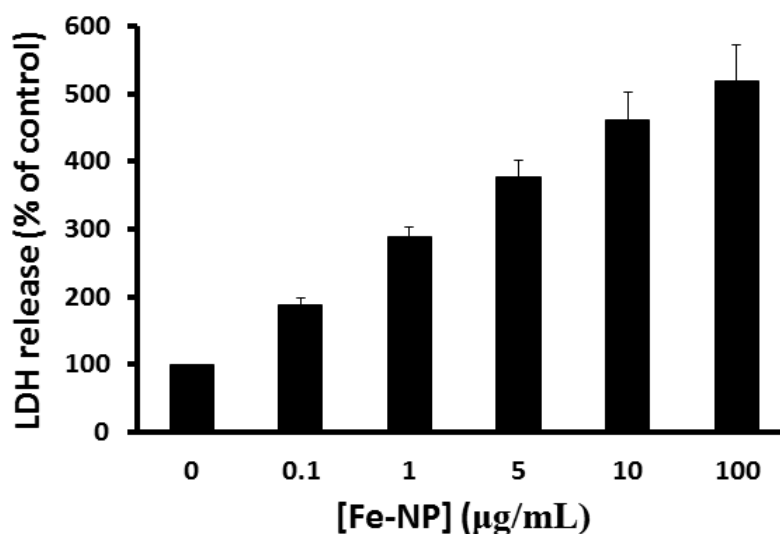


Figure 1. The various concentrations of Fe-NP induced LDH release of PC12 cells

The increase in LDH release was possibly the result of the high concentration of neurotoxic Fe-NP in the treated samples. This data may reveal that Fe-NP can bind to the membrane by means of hydrophilic bonds. Indeed, hydrophilic Fe-NP can establish new bonds with hydrophilic patches of membrane proteins and induce the conformational changes of membrane proteins. This event probably results in damage to the membrane integrity and following LDH release.

Conclusion

The concentration-dependent cytotoxic effect of NP against nervous system cells is providing them considerable physicochemical and biological characteristics that make them potentially different from their following larger counterpart, especially in medicinal applications. In vitro cell membrane leakage assays was nominated for demonstrating dose-dependent cytotoxicity of Fe-NPs on PC12 cells. This technique demonstrated the effect of particles concentration on the chosen cells, where the concentration of 100 $\mu\text{g}/\text{mL}$ showed the highest toxicity. The LDH assay looks relatively simple and cheap. However, the data obtained could serve as potential information in predicting possible in vivo cytotoxicity.

Acknowledgement

The financial support of Islamic Azad University of Pharmaceutical Sciences Branch, (IAUPS), Tehran, Iran is gratefully acknowledged.

Conflicts of Interest

None of the authors have any conflict of interest associated with this study.

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