Original Article

The Cytotoxicity of Dextran-coated Iron Oxide Nanoparticles on Hela and MCF-7 Cancerous Cell Lines

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Received: 09.05.2017 *Accepted: 13.06.2017*

ABSTRACT

Background: Recently, iron oxide nanoparticles have attracted attention in various diagnosis and treatment fields. The aim of the present study was to investigate the cytotoxicity of various concentrations and incubation times of dextran-coated iron oxide nanoparticles (DIONPs) on HeLa and MCF-7 cancerous cell lines.

Methods: This in-vitro study was conducted at Pharmaceutical Sciences Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran in 2016. The dextran-coated iron oxide nanoparticles (DIONPs) uptake and cytotoxicity at different concentrations (10, 40 and 80 µg/ml) and different incubation times (6, 12 and 24 h) were assessed on HeLa and MCF-7 cell lines. The viability of the cells was measured by MTT assay.

Results: DIONPs entered into the HeLa and MCF-7 cells. After 6, 12 and 24 h incubation times and in all concentrations, the viability of HeLa cells was more than 94%. For MCF-7 cell line, increasing incubation time from 6 to 24 h at a concentration of 10 µg/ml decreased the cells viability from 98% to 95%. When the cells were exposed to concentrations of 40 and 80 µg/ml of the nanoparticles, significant reductions in the cells viability was observed from 98% to 91.6% and from 95% to 88%, respectively.

Conclusion: DIONPs cytotoxicity increased by increasing the incubation time from 6 to 24 h and also increased with increasing the nanoparticles concentration from 0 to 80 µg/ml. In general, DIONPs did not cause considerable toxicity in both cell lines especially at lower concentrations. Therefore, these nanoparticles are good candidates for use in biomedical and cancer research studies.

Keywords: Cytotoxicity, Dextran, HeLa Cells, Iron Oxide Nanoparticles, MCF-7 Cells.

IJT 2017 (5): 31-36

INTRODUCTION

Nowadays, due to advancements in nanotechnology, the applications of various nanoparticles such as gold, silver, gadolinium and metal oxide nanoparticles have increased in biomedical research fields [1]. Because of great stability, biocompatibility and easy preparation prosses of iron oxide nanoparticles ($IONP_S$), they have attracted considerable attention [2]. Since nanoparticles have the potential to evade the immune system, they can be internalized into tumor cells via enhanced permeation and retention (EPR) mechanism [3].

One problem with the nanoparticles is their accumulation and agglomeration in aqueous suspensions [4]. Due to Van-der Waals forces, nanostructures can adhere to each other and consequently, their surface to volume ratio reduces, resulting in the loss of some properties [5]. To reduce the cytotoxicity of naked nanoparticles and preserve their nanoscale

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properties, the surface of metal nanoparticles is usually coated with certain agents such as polymers or biomolecules [6]. In addition, coating the nanoparticles increases their stability, half-life in blood circulation, and decreases their tendency for agglomeration [7].

Former studies regarding naked IONPs toxicity demonstrated significant toxic effects in biological systems [5]. To reduce the cytotoxicity of metal nanoparticles, some substances such as dextran, polyvinyl alcohol, and polyethylene glycol have been used as effective coatings [8]. In a study, the cytotoxicity of chitosan-coated IONPs on human hepatocellular carcinoma cells (SMMC-7721) was assessed [9]; as the results, the nanoparticles at 123.52 µg/ml concentration induced a significant reduction in cells viability after 12 h of incubation time (killing 90% of cells). The cytotoxic effects of naked IONPs and polyvinyl alcohol (PVA)-coated IONPs were compared on L929 cells [5]. Naked nanoparticles interact with cells medium protein and ions and consequently lead to increase cytotoxicity, but the coated nanoparticles displayed more biocompatibility due to reduced interaction with proteins. Moreover, in another in-vitro study, A-549 cells viability decreased to 84%, 72% and 56% after 24 h incubation with concentrations of 10, 25 and 50 µg/ml of IONPs, respectively [8]. Although, different coatings have been investigated for IONPs, there has been no comprehensive in-vitro study in relation to cytotoxic effects of dextran-coated IONPs (DIONPs), yet. Dextran ($C_6H_{10}O_5$), a branched polysaccharide, with a low molecular weight is widely used to coat nanoparticles [10,11].

The aim of present study was to investigate the cytotoxicity of various concentrations and incubation times of DIONPs on HeLa and MCF-7 cancerous cell lines.

MATERIAL AND METHODS

Preparation of Dextran-coated Iron Oxide Nanoparticles (DIONPs)

$DIONP_S$ (Dextran-Fe_3O_4) that was synthesized by coprecipitation method [8] was purchased from NanolabInc (Tehran, Iran). Chemical structure and transmission electron microscopy image of the nanoparticles are shown in Figure 1.

Cell Culture

This study was conducted at Pharmaceutical Sciences Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran in 2016. HeLa and MCF-7 cell lines were obtained from Pasteur Institute (Tehran, Iran). The cells were cultured in T75 cm^2 BD Falcon™ cell culture flasks (Franklin Lakes, NJ, USA) and maintained at 37 °C in 95% CO_2 humidified incubator using Dulbecco's modified Eagle's medium (DMEM) with 10% (v/v) heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin (Sigma-Aldrich, MO, USA). When the cells reached to 80%–90% confluency in cultures, they were detached using trypsin-EDTA (0.25%) and then were plated (5×10^3 cells/well) in 96-well plates.

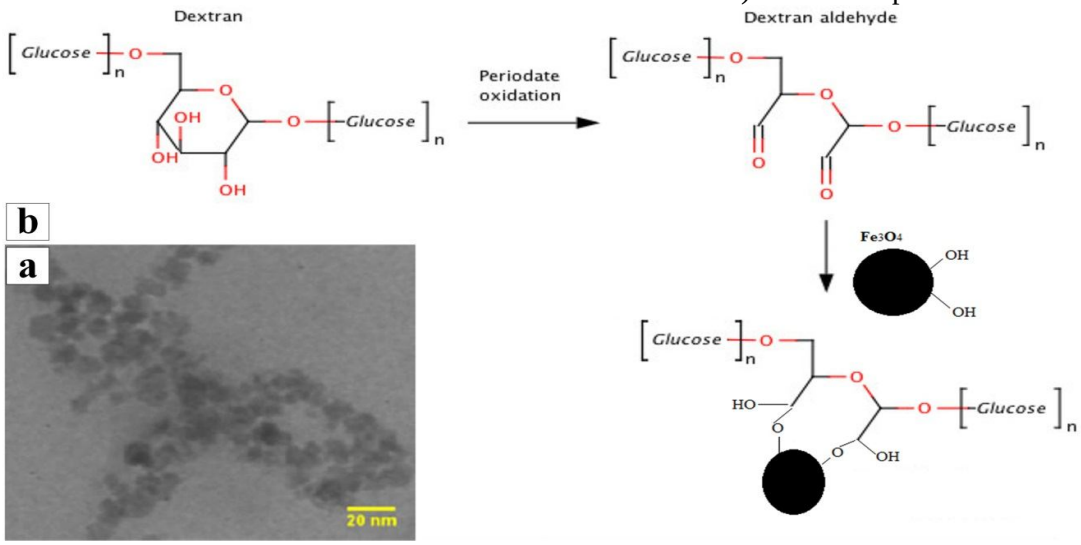

Figure 1. (a) Transmission electron microscope (TEM) image of synthesized DIONPs. (b) Chemical structure and schematic illustration of dextran-coated iron oxide nanoparticles.

Prussian Blue Staining

Prussian blue staining was carried out to indicate $DIONP_S$ internalization into HeLa and MCF-7 cells at different concentrations. About 5×10^4 cells of each cell line were separately plated in six-well plates and incubated with the nanoparticles at concentrations of 10, 40 and 80 µg/ml. After 24 h of incubation, the media was removed and the cells were rinsed 3 times with phosphate buffer solution (PBS, pH = 7.47± 0.1); then the cells were fixed for 30 min with PBS containing 4% formaldehyde. Subsequently, the cells were rinsed again with PBS and were evaluated by Prussian blue staining [12]; briefly, a ratio of 1:1 mixture of 4% potassium ferrocyanide (II) trihydrate (Sigma-Aldrich) and 4% HCl (Sigma-Aldrich) solution was added to the wells, and then the cells were incubated at room temperature. The Prussian blue staining results were assessed using an Olympus BX50 light microscope (Olympus Optical Co., Tokyo, Japan).

Cytotoxicity of the DIONPs

MTT assay was carried out to investigate cytotoxicity of the nanoparticles at different concentrations on HeLa and MCF-7 cell lines. About 5×10^3 cells of both cell lines were separately plated in 96-well plates and were maintained at 37 °C in 95% CO_2 humidified incubator. After two days of plating and reaching cells to more than 60% confluency, they were exposed to various concentrations (0, 10, 40 and 80 µg/ml) of the nanoparticles for different incubation times (0, 6, 12, 24 h). In MTT assay, the supernatant of the cells in wells was removed and replaced by 200 µl of 0.5 mg/ml MTT solution, and then the plates were incubated at 37 °C for 4 h. Then, the medium was carefully aspirated, and the purple formazan crystals were dissolved in DMSO (100 µl/well). The absorbance of the wells was determined using an ELISA plate reader (Biotek H1 M) with a test wavelength of 570 nm and a reference wavelength of 630 nm.

Statistical Analysis

All experiments were repeated at least three times and each experiment was done in triplicates. The cells viability values were presented as mean ± standard deviation. The cells viability among different groups were compared using one-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests, with a confidence interval of 95%.

RESULTS

Prussian Blue Staining

The results of Prussian blue staining (Figure 2) showed that the DIONPs at various concentrations (10, 40 and 80 µg/ml) could become internalized into the cells in both HeLa and MCF-7 cell lines.

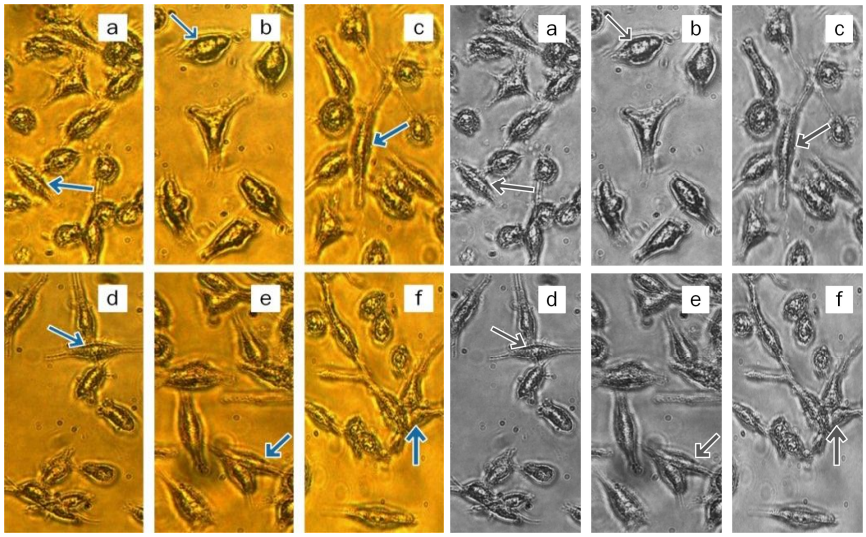

Figure 2. Identifying the intracellular iron oxide nanoparticles using Prussian blue staining. (a), (b) and (c) HeLa cells incubated at 10 µg/ml, 40 µg/ml and 80 µg/ml concentrations of DIONPs, respectively. (d), (e) and (f) MCF-7 cells incubated at 10 µg/ml, 40 µg/ml and 80 µg/ml concentrations of the DIONPs, respectively.

Cytotoxicity of the Nanoparticles

The results of the nanoparticles' cytotoxicity tests at different incubation time periods (6, 12 and 24 h) and with different concentrations on the HeLa and MCF-7cell lines are shown in Figure 3 and 4.

The viability of HeLa cells was more than 94% at all concentrations after 6, 12 and 24 h of incubation. For MCF-7 cell line, increasing incubation time from 6 to 24 h, at nanoparticles concentration of 10 µg/ml decreased the cells viability from 98% to 95%. Increasing the incubation time from 6 to 24 h, when these cells were exposed to concentrations of 40 and 80 µg/ml of the nanoparticles, cells viability reduced from 98% to 91.6% and from 95% to 88%, respectively.

After 6 h of incubation time, there were no significant differences in cells viabilities between the groups treated with different concentrations of the nanoparticles and their control groups (P>0.05); however, after 12 and 24 h of incubation, significant differences were noted (P<0.05). In other words, after 12 h of incubating the cells with DIONPs, some cytotoxicity was observed in both cell lines. The comparison of the cell viability between HeLa and MCF-7 cells lines revealed that the cytotoxicity level in MCF-7 cells was significantly more than in HeLa cells, especially at higher concentrations (40 and 80 µg/ml) (P<0.05).

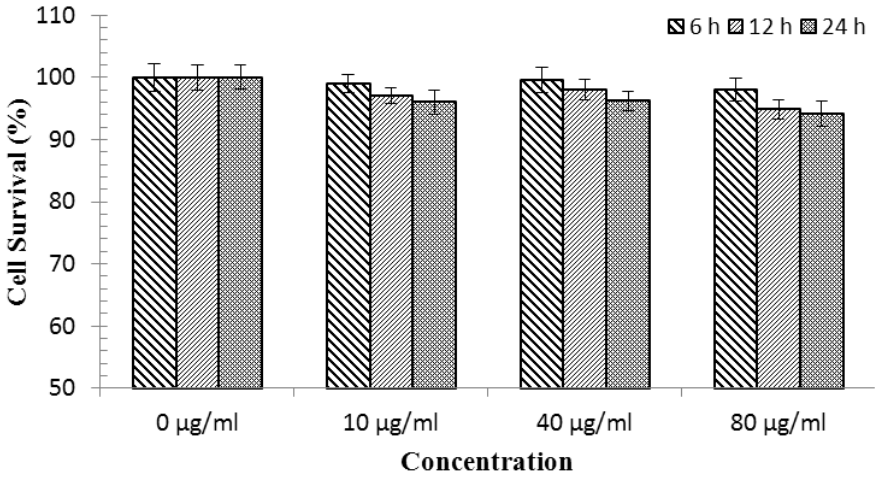

Figure 3. HeLa cell's viability after incubating with different concentrations of DIONPs and various incubation times.

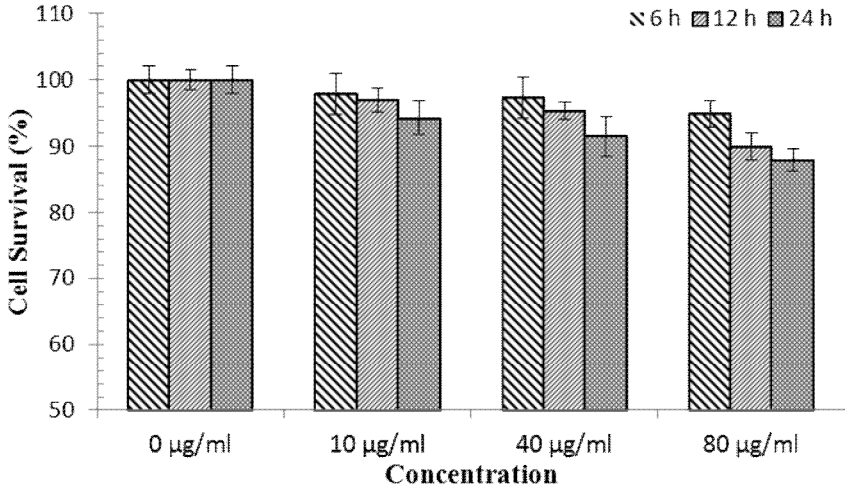

Figure 4. MCF-7 cell's viability at incubating with different concentrations of DIONPs and various incubation times.

DISCUSSION

The cytotoxicity induced by nanoparticles depends on various factors such as type, size, concentration, incubation time, and the type of cell line [13-15]. The cytotoxicity of several metal oxide nanoparticles on A549 cell line has been investigated; as the results, $IONP_S$ at concentrations of less than 100 µg/ml, did not cause significant cytotoxicity [16]. Also, dextran-amino coated $IONP_S$ at 0.05, 0.1 and 0.5 mg/ml concentrations had no significant cytotoxicity in HeLa cells [17]. The cytotoxicity of $IONP_S$ coated with a bipolar surfactant, tetramethylammonium 11-aminoundecanoate, on normal cells and breast cancer cells [18] depends on concentration of these nanoparticles; so that at concentrations of 0.1 to 10 µg/ml no cytotoxicity was observed. The results of the mentioned studies are in agreement with results of the present study.

Due to the small size, nanoparticles have the ability to interact with cells and subcellular structures such as DNA, but there is not adequate information concerning the mechanism of these interactions [16]. Exposing cells to nanoparticles can cause electrical imbalance in the cell membrane and disturbance may accrue in cell membrane integrity [19]. The cytotoxicity of iron oxide nanoparticles is mainly due to reactive oxygen species (ROS) in the cells. These products can oxidize lipids and damage the cell membrane, resulting in eventual cell death [20]. In addition, the presence of nanoparticles within the cell can instigate alterations in protein steric structure that could be irreversible [10]. Coating of nanoparticles not only leads to their biocompatibility but also results in a lower affinity for connecting to proteins and ions and reduced cytotoxicity [21]. Dextran-coated $IONP_S$ at concentrations of more than 2 mg/ml have been toxic for human prostate cells (DU145 cells) [12]. The cytotoxicity of $IONP_S$ (50 µg/ml) coated with various substances such as glucose and polyvinyl chloride has been investigated on BxPC3 cells [22]. One, three and six hours of incubation with 50 µg/ml of $IONP_S$ coated with glucose or polyvinyl chloride did not cause significant cytotoxicity; however, after 24 h of incubation, the cells viability decreased to about 28% and 22% when the cells incubated with $IONP_S$ coated with polyvinyl chloride and glucose, respectively. These findings are in line with our results at 6 h incubation time; however, our coating agent was different (dextran). In our study, the average HeLa and MCF-7 cells viabilities decreased to 94% and 88%, respectively after 24 h of incubation with the highest concentration (80µg/ml) of DIONPs. The cytotoxicity induced by dextran-coated nanoparticles is due to detachment of dextran from the nanoparticles, which lead to naked $IONP_S$ aggregation [23].

The cytotoxic effects of $IONP_S$ were coated with protein has been assessed on mice fibroblast cell lines [24]; the authors have used MTT assay to evaluate the cells viability after 24, 48 and 72 h of incubation with the nanoparticles and reported a significant reduction in cells viability after incubation with commercial naked $IONP_S$ (46% cell death after 24 h of incubation) as compared to cells viability after incubation with protein-coated $IONP_S$ (less than 5% cell death after 24 h of incubation). In our study, the results of 24 h incubation period at the highest tested concentration of nanoparticles (80 µg/ml) with the HeLa and MCF-7 cells, showed average reductions of 6% and 12% in cells viabilities, respectively. Since the reduction in the cells viability was relatively low, dextran-coated $IONP_S$ at tested concentrations can be used for biomedical researches and applications. Based on former studies, we know that nanoparticles can improve some properties of tissues and cells, but it is important for them to be non-toxic for use in different applications in medicine and biology. Coating the nanoparticles with some appropriate agents such as dextran can reduce their cytotoxicity, and making them valuable in the diagnosis and treatment of cancers.

CONCLUSION

Cytotoxicity of dextran-coated $IONP_S$ in HeLa and MCF-7 cell lines mainly depend on incubation time and the concentration of these nanoparticles. In this study, the cytotoxicity of DIONPs increased by longer incubation times as well as increasing concentrations. Generally, dextran-coated IONPs do not cause significant cytotoxicity in both cell lines especially at low incubation time periods and with concentrations of less than 40 µg/ml. Therefore, these nanoparticles are good candidates for use in biomedical and cancer research studies.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences for the financial support. The authors declare that there is no conflict of interests.

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