Cytotoxic Effect of Iron Oxide Nanoparticles on Mouse Embryonic Stem Cells by MTT Assay

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ABSTARCT

Background: Despite the wide range of applications, there is a serious lack of information on the impact of the nanoparticles on human health and the environment. The present study was done to determine the range of dangerous concentrations of iron oxide nanoparticle and their effects on mouse embryonic stem cells.

Methods: Iron oxide nanoparticles with less than 20 nanometers diameter were encapsulated by a PEG-phospholipid. The suspension of iron oxide nanoparticles was prepared using the culture media and cell viability was determined by MTT assay.

Results: MTT assay was used to examine the cytotoxicity of iron oxide nanoparticle s. Royan B1 cells were treated with medium containing different concentrations (10, 20, 30, 40, 50, and 60µg/ml) of the iron oxide nanoparticle. Cell viability was determined at 12 and 24 hours after treatment which showed significant decreases when concentration and time period increased.

Conclusion: The main mechanism of nanoparticles action is still unknown, but *in vivo* and *in vitro* studies in different environments suggest that they are capable of producing reactive oxygen species (ROS). Therefore, they may have an effect on the concentration of intracellular calcium, activation of transcription factors, and changes in cytokine. The results of this study show that the higher concentration and duration of treatment of cells with iron oxide nanoparticles increase the rate of cell death.

Keywords: Cytotoxicity, Iron Oxide Nanoparticle, MTT Assay.

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INTRODUCTION

Nanoparticles can show unique biological behavior [1]. These particles have features, such as large surface area (increasing physical, chemical, and biological) and higher solubility and mobility [2, 3]. There are increasing concerns about development and production route of nano-materials and nanotechnology as well as their wide ranges of applications. The new physical and chemical properties of novel engineered nanoparticles make them extremely attractive for use in applications e.g. medical sciences [4]. Despite the wide range of applications, there is a serious lack of information on the impact of nanoparticles on human health and the environment [5,6]. Iron oxide nanoparticles can serve as a vehicle to deliver drugs to a target organ and/or tissue across the blood brain barrier (BBB) [7]; however, little is known about the influence of iron oxide nanoparticles on the intracellular or molecular level inside cells. Many studies have been conducted to evaluate the potential toxicity of iron oxide nanoparticles [8]. The latest entry in evaluating hazardous drugs, chemicals and environmental stressors is that of the stem cells, which have the potential to evaluate the chemicals and environmental stressors in a dynamic fashion [9,10]. This study investigated the cytotoxic effects of iron oxide nanoparticles on mouse embryonic stem cells using MTT assay for measuring cell viability**.**

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MATERIALS AND METHODS

Preparation of nanoparticles

Iron oxide nanoparticles less than 20 nanometers in diameter were prepared by Zist Shimy Azma Company. The nanoparticles were encapsulated by a PEG-phospholipid shell to increase their biocompatibility and water dispersibility. Here, the iron oxide nanoparticles were suspended in Kno DMEM cell culture medium and dispersed by an ultrasonic bath for 10 minutes.

Cell culture and nanoparticle suspension preparation

Mouse embryonic stem cells (Royan B1) were obtained from Royan Institute. The cells were cultured in Kno DMEM supplemented with 15% FBS (fetal bovine serum), L-glutamine (2 mM), nonessential amino acids (1×), LIF, β-mercaptoethanol, Pen/Strp 1% on the mouse embryonic fibroblast (MEF). The suspension of iron oxide nanoparticles was prepared using the culture media and dispersed for 10 minutes by an ultrasonicator bath to prevent aggregation. From the suspension, different final concentrations of the iron oxide nanoparticles were prepared in cell growth medium. Concentration ranges were 10, 20, 30, 40, 50, and 60 µgr/ml.

Cell viability assay

Cell viability was assessed by using the MTT assay, which was based on the reduction of the dye MTT to formazan crystals, an insoluble intracellular blue product, by cellular dehydrogenases. The cells were seeded on 96-well plates with 1×10^4 cells in 50µl medium per well and cultured for 2 hours for stabilization. The cells were cultured in the medium containing different concentrations of the iron oxide nanoparticles for 12- and 24-hour periods. Culture medium without the iron oxide nanoparticles served as the control in each experiment. At the end of the exposure, 20µl MTT was added to each well to a final concentration of 2mg/ml and afterwards the cells were cultured for 2h at 37◦C. The medium was then removed carefully and 150µl DMSO was added in and mixed with the cells thoroughly until formazan crystals were completely dissolved. This mixture was measured in an ELISA reader with a wave length of 570nm. Cell viability was expressed as a percentage of the viability of the control culture.

RESULTS

MTT assay was used to examine the cytotoxicity of the iron oxide nanoparticles. Royan B1 cells were treated on a medium containing different concentrations (10, 20, 30, 40, 50, and 60µg/ml) of the iron oxide nanoparticle. Cell viability was determined at 12 and 24 hours after treatment. As it is shown in Figure 1, cell viability decreased when the concentration and time period.

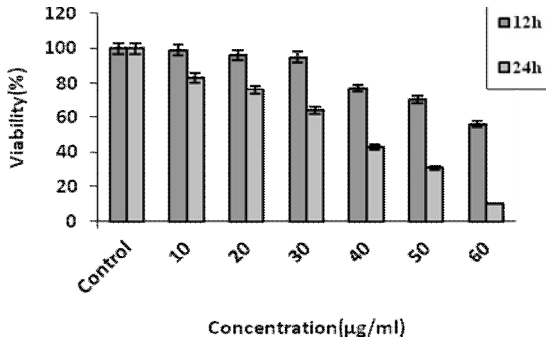

Figure 1. Effect of different concentration of iron oxide nanoparticle on viability.

DISCUSSION

The main mechanism of nanoparticles action is still unknown; however, *in vivo* and *in vitro* studies in different environments suggest that they are capable of producing reactive oxygen species (ROS). Therefore, they may have an effect on the concentration of intracellular calcium, activation of transcription factors, and creating changes in cytokine. ROS can damage cells in various ways including damage to DNA, interfering with cell signaling pathways, and inducing changes in gene transcription [11,12]. Mitochondrial DNA is highly vulnerable to oxidative attack dramatically [13,14]. In 2003, Agarwal *et al.* showed that high levels of ROS cause break-down in mitochondrial inner and outer membrane and, therefore, the protein cytochrome c release from

mitochondria, resulting in trigger cascade events and activation of apoptotic. Hence, ROS acts as a mediator in the process of apoptosis [15].

Reactive oxygen species or free radicals of oxygen have the short lifetime of the intermediate chemical compounds with one or more unpaired electrons in the last electron shell. Therefore, they are highly reactive molecules that for obtaining an electron invade adjacent stable molecules and cause oxidation. Molecules lose their electrons to become a free radical and continue the cycle [16-18]. The extent of damage caused by ROS depends not only on the type and amount but also the time and duration of exposure to ROS and external factors, such as temperature, pressure, oxygen, and deployment environment consisting of ions, proteins, and the amount removed on the ROS [19]. ROS production in the presence of nanoparticles can cause serious damage and inheritance to the DNA [20,21], for example, chemical modification of histones or other proteins, that are involved in shaping the structure of DNA, open helical structure of DNA and subject DNA to any changes [1,22].

ROS that are most common in biological systems include superoxide anion (O2), hydrogen peroxide (H2O2), full Aksyl radicals (ROO-), and reactive hydroxyl radicals (OH-)[23]. To measure ROS levels, there are two methods: direct and indirect. In the direct method, resonance spectroscopy-electron spin is used [24,25]. Indirect methods include chemiluminescence technique (chemiluminescnce) [26], thiobarbituric acid reaction (C4H4N2O2S) [27], nitro blue tetrazolium staining [28], and flow cytometry [29]. In 2009, Fen Wang *et al.* examined the effect of oxidative stress-induced silica dioxide nanoparticles (sio2) on human embryonic kidney cells (HEK293) [13]. Cellular oxidative stress characterized by higher levels of ROS, reduced the expression of GSH and increased lipid peroxidation. Furthermore, the presence of the cytoplasmic enzyme lactate dehydrohenase series of specific mitochondrial enzymes can also be considered as an indicator of necrosis. In this study, the levels of these enzymes in the cytoplasm of cells treated with silica nanoparticles increased dramatically. In addition, most of the cells that were exposed to silica nanoparticles were dried which had condensed nuclei indicating signs of apoptosis.

In 2011, Wang *et al.* investigated impact of single-walled carbon nanotubes on pc12 cell lines. These cells differentiate to sympathetic ganglion cells in response to NGF. The experimental observations show that cells that had been exposed show a decrease in the amount of biological capacity (viability). Pc12 cells block the cell cycle in the phase of G2/M and apoptosis is affected in a dose-dependent manner and decreased mitochondrial membrane potential [30].

The results of this study show that the higher the concentration and duration of treatment of cells with iron oxide nanoparticles increases the rate of cell death. This can be due to increased production of oxygen free radicals.

CONCLUSION

The results of this study showed the presence of a direct relationship between the duration of treatment and increasing concentrations of iron oxide nanoparticles and cell death in mouse embryonic stem cells According to previous studies, it can be concluded that high concentrations of iron oxide nanoparticles and the duration of treatment increase the production of oxygen free radicals within the cell.

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