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Comprehensive cytotoxicity studies of superparamagnetic iron oxide nanoparticles

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ABSTRACT

Recently lots of efforts have been taken to develop superparamagnetic iron oxide nanoparticles (SPIONs) for biomedical applications. So it is utmost necessary to have in depth knowledge of the toxicity occurred by this material. This article is designed in such way that it covers all the associated toxicity issues of SPIONs. It mainly emphasis on toxicity occurred at different levels including cellular alterations in the form of damage to nucleic acids due to oxidative stress and altered cellular response. In addition focus is been devoted for in vitro and in vivo toxicity of SPIONs, so that a better therapeutics can be designed. At the end the time dependent nature of toxicity and its ultimate faith inside the body is being discussed.

1. Introduction

Superparamagnetic iron oxide nanoparticles (SPIONs) have been found promising candidate in nanobiotechnology for wide range of applications such as magnetic separation, drug delivery, magnetic resonance imaging (MRI) and magnetic hyperthermia (MH) [1–4]. Most importantly the site-specific drug and diagnostics agent delivery by using SPIONs is the most exciting applications in cancer theranostics [5,6]. The wide ranges of potential bio-applications of SPIONs are influenced by its physical, chemical, and magnetic properties along with its shape and size. The toxicity of SPIONs towards normal cells are hindering its successful implication as therapeutic agent. High degree of nonspecific binding to cell components and biological fluids by SPIONs as well as colloidal instability of SPIONs during their delivery into biological media are the main cause of the toxicity [7]. The response of these particles to living system both in terms of acute and chronic toxicity is main concern in terms of clinical activity [8]. Moreover the degradation and it's accumulation inside the body of this nanoparticles following administration is very important point of study. Currently the most trusted and easiest approach to study the In vitro cytotoxicity studies of nanoparticle is by using different cell lines varying their incubation times and evaluating by colorimetric assays [9,10]. This approach has gained lots of publicity. However, the main drawbacks of these studies include a wide range of nanoparticle concentrations and exposure time [11,12].

In addition, various researchers used different cell lines with varying culturing conditions which made things more difficult, as direct comparisons between the available studies and their own results are not validated. It is to be note that while working on SPIONs, the reported toxicity taken into consideration includes, inflammation, diminished mitochondrial activity, the cellular stress mediated generation of reactive oxygen species (ROS) and chromosome condensation [13–18].

This article is designed in such way that it covers all the associated toxicity issues of SPIONs. SPIONs are manufactured in higher quantities in order to meet the demands for rapidly growing field of nanomedicine for biomedical applications. But exposure to human body and ecosystem needs to address. This review mainly aims to collect the toxicological in vitro and in vivo data along with major adverse effects of SPIONs [19]

2. Why toxicity study of SPIONs?

SPIONs are the most preferred candidate in biomedical applications for diagnostics and therapeutics. Many in vivo toxicity appliances of SPIONs are needed in most of biomedical applications. Hence it is important to study the overall toxicity associated with them. SPIONs are

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very small in size, comparable with the biomolecules. Such a small size can cause sequestration of these moieties into various body systems and can interfere with their normal functioning. They might cross blood-brain barrier and damage neural functions, also can cross nuclear membrane and cause mutations. The bare SPIONs have very low solubility which can lead to agglomeration which can obstruct blood vessels [11].

SPION are coated with a suitable biocompatible material for increase in stability, water dispersibility and biocompatibility.

3. In vitro toxicity studies of SPIONs

In order to confirm the toxicity, different assays are available. Each assay is based on some different principle, for more accurate results it is recommended to carry multiple assay for same samples. Some of the widely used assay are lactate dehydrogenases assay (LDH), Sulphorhodamine B (SRB) assay, protein assay, neutral red, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

3.1. In vitro assays for cytotoxicity studies of SPIONs

MTT assay is a widely accepted, non-radioactive, colorimetric based assay [20,21]. MTT is derivative of a tetrazolium salt, which is converted into purple formazan insoluble complex by enzyme within the mitochondrial dehydrogenases [22]. Recent reports suggest that that reduction of MTT can also be facilitated by NADH or NADPH within the cells and also outside of mitochondria [22]. Therefore further modification of the initial protocol by Mossmann was proposed [23,24] in order to increase the repeatability and the sensitivity of the assay. Only active mitochondria contain these enzymes; therefore, the reaction only occurs in living cells [25].

The neutral red uptake assay is based on the ability of viable cells to incorporate and bind the supra vital dye neutral red. This assay is widely used cytotoxicity assay used for biomedical and environmental applications. The principle behind this is the weak cationic dye penetrates cell membranes by the mechanism of nonionic passive diffusion and concentrates in the lysosomes.

The dye binds to lysosomal matrix by electrostatic interaction, which is then extracted from the viable cells by using an acidified ethanol solution, and the absorbance of the solubilized dye is quantified using a spectrophotometer [26].

Another important assay commonly used is, LDH leakage assay which is based on the measurement of lactate dehydrogenase activity in the extracellular medium. The silent features like reliability, speed, and simple evaluation are the major strengths of this assay [27].

The most widely used assay for viability study is the trypan blue. The assay is simple method of determining cellular viability [28]. In this the cells are sedimented onto slides and fixed in a mixture of trypan blue and paraformaldehyde. The nonviable cells a stain with dark blue color, whereas viable cells exclude the dye [29]. The major concern with trypan blue assay is its difficulty to interpret because of staining artefacts.

A number of techniques for detecting DNA damage (e.g. micronuclei, mutations, structural chromosomal aberrations) have been used to identify substances with genotoxic activity. The comet assay, also known as single-cell gel electrophoresis (SCGE), is so named because damaged cells form a comet-shaped pattern after electrophoresis. It is a sensitive method to measure genotoxicity and cytotoxicity of chemical and physical agents. The comet assay has also been used to analyse the capacity of cellular DNA repair [30].

Continues metabolic process produces reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. ROS generation is normally counterbalanced by the action of antioxidant enzymes and other redox molecules. However, higher levels of ROS can lead to cellular injury and may damage biomolecules such as DNA, lipids and proteins [31]. This excess reactive oxygen species should be eliminated from the cell. The cellular antioxidant enzymes and other redox molecules take care of excessive ROS and counterbalance ROS generated in the cell [32].

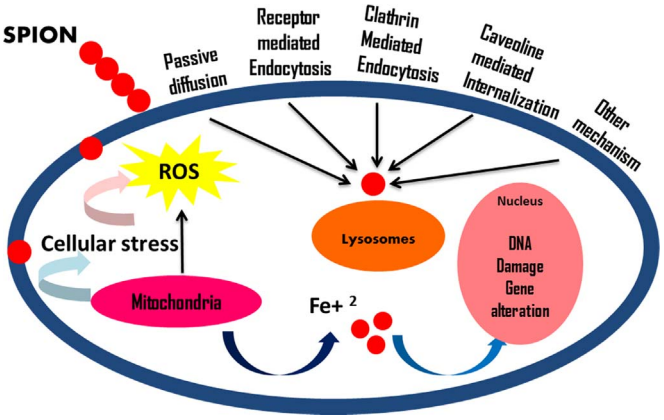

Fig. 1. Schematic representation of possible mechanism of SPIONs interaction and SPIONs-induced toxicity at cellular level.

3.2. Mechanism associated with in vitro toxicity of SPIONs

The most beautiful features of SPIONs is they can be easily attracted and manipulated by using external magnetic field and in addition the superparamagnetic properties, enables them to work as magnetic switches. In addition the least toxic effect shown on human body has attracted researcher to explore this system for maximum biomedical applications [33,34].

Fig. 1 represents the possible mechanism of SPIONs interaction with cell and toxicity at cellular level. The figure suggests that SPION can interact with cell by different mechanisms. The prominent one are, a) passive diffusion b) Receptor mediated endocytosis c) clatharin mediated endocytosis d) and caveoline mediated endocytosis. After entering inside the cell SPION are degraded by enzymes present in lysosomes and breaks the assembly to form ions. This Fe + 2 ions generates reactive oxygen species (ROS) by altering mitochondrial and other organelle functions and induction of cell signalling pathways which leads to activation of inflammatory tells [35,36]. Possible mechanism of SPIONs interaction and SPIONs-induced toxicity at cellular level is shown in Fig. 1.

3.2.1. SPION associated plasma membrane toxicity

The SPION also shows toxicity by damaging the plasma membrane and proteins. In addition to induction of cell signalling pathways, SPION can stimulates the redox reactions and up regulate plasma membrane proteins which results in the generation of cellular stress and ultimately cell death [37,38].

It is observed that the toxicity assay based upon mitochondrial functionality (e.g., MTT and XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide)), which are based upon reductase enzyme may show large errors [39]. The reason behind this is the redox active surface of SPIONs could widely impact electron flow and change the mitochondrial functionality [40–42]. The study done by Jeng and Swanson [16] showed that SPIONs had a major effect upon mitochondrial function and maximum concentration tested was ([Fe] ≈ 2.5 mM) at this concentration there was statistically significant change in the mitochondrial function. In another study done by Au et al. [40] similar results were observed and the authors have concluded that SPION alters mitochondrial function as well as decreased cell viability.

The study lead by the Stroh et al. [14] confirmed that citrate-coated SPIONs results in a substantial increase in protein oxidation and oxidative stress [14]. The study also concluded that iron was the source to