In vitro & in vivo toxicity of CoFe₂O₄ for application to magnetic hyperthermia

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ABSTRACT

Magnetic nanoparticles offer some attractive possibilities in biomedicine because it has the special physical properties. Magnetic hyperthermia using magnetic nanoparticles requires that the magnetic particles have not only high heating ability in low magnetic field but also to be non-toxic for biomedical use. Although the reports of bulk magnetic materials or Fe₃O₄ nanoparticles have known for hyperthermia, it has not reported about application to hyperthermia and the biological responses of CoFe₂O₄ nanoparticles. Therefore, we investigated the in vitro & in vivo toxicity as well as heating ability under magnetic field for application of CoFe₂O₄ nanoparticles to hyperthermia.

Keywords: CoFe₂O₄, magnetic nanoparticles, toxicity, hyperthermia

1 Introduction

Nanomaterials are receiving increasing attention for their promise as engineering and biomedical miracles. In fact, nanomaterials help in overcoming the limitations of size and can change the outlook of the world regarding science. For examples, magnetic nanoparticles in gene delivery, biosensors, drug delivery and hyperthermia had attracted increasing fundamental and technological interests. However, their use may ultimately be limited, because the lack of information regarding the toxicity of synthesized magnetic nanoparticles poses serious problems [1]. Therefore, the toxicity of magnetic nanoparticles has raised a critical factor on evaluating their applications, recently.

Hyperthermia using magnetic particles has been recognized as a useful therapeutic modality in the treatment of malignant tumors. The magnetic nanoparticles with alternating magnetic field are expected useful thermoseed in hyperthermic cancer treatment since they can be targeted and confined to the cancer site without damaging normal cell. However, for the successful bio-application of magnetic nanoparticles in hyperthermia, it is essential to understand the biological fate and potential toxicity of nanoparticles. The production of localized hyperthermia for tumor treatment has been hindered by a number of physical and biologic factors. Therefore, the magnetic nanoparticles for magnetic hyperthermia require not only optimistic magnetic properties and narrow particle size distribution but also to be non-toxic and biocompatibility. The magnetic nanoparticles are completely foreign to the biological environment; therefore it is important that they must be tested for any harmful effects on the cells. In this study, we focused the magnetic hyperthermia using CoFe₂O₄ nanoparticles among various biomedical applications. We had studied CoFe₂O₄ nanoparticles for high effective hyperthermic thermoseed [2]. Iron oxide particles already known as nontoxic magnetic materials and also MRI contrast agents have already been approved for human use [3]. However, it was not reported about the toxicity of CoFe₂O₄ in hyperthermia or biomedical uses in detail. Therefore, we investigated the biocompatibility as well as the heating ability of CoFe₂O₄ particles in vitro and in vivo.

2 Materials and Methods

CoFe₂O₄ nanoparticles were synthesized by sol-gel route. Their magnetic and structural properties were determined by XRD, VSM and SEM, respectively. The exothermic energy from the CoFe₂O₄ suspensions was monitored for 300 sec under alternating magnetic field (150 KHz, 7kW). The cytotoxicity in various doses CoFe₂O₄ was examined using the MTT assay. The acute systematic toxicity in vivo test was investigated with ten of ICR mice. Animals were checked clinical signs and weight of animals were also measured for 7 weeks after injection. And histological analysis was performed on stained organs with Haematoxylin and Eosin or Prussian blue.

2.1 Preparation and Characterization

Cobalt acetate tetrahydrate [Co(CH₃CO₂)₂•4H₂O], and iron nitrate nonahydrate [Fe(NO₃)₃•9H₂O] were selected as precursors of CoFe₂O₄ nanoparticles. They were dissolved in acetic acid, methanol and water for 1 h. This solution was refluxed at room temperature for 24 h to allow gel formation and dried at 100 °C for 24 h. Then, it was fired at 800 °C for 6 h in air. The structures of samples were determined by X-ray diffraction (XRD; D/MAX Rint2000,
Rigaku, Japan) using Ni-filtered Cu-ka X-rays with the phase being identified by a comparison with the JCPDS database. A vibrating sample magnetometer (VSM7300, Lakeshore, USA) was used to measure the saturation magnetization (Ms) and the coercive force (Hc) of each sample. The average size, size distribution and morphology were estimated using transmission electron microscopy (JEM4010, JEOL, Japan). An external alternating magnetic field was applied to the magnetic particles using a heating system (SPM78, YoungChang, Korea). The exothermic energy from the samples was monitored by a thermometer [alcohol] under an alternating magnetic field (frequency: 150 KHz, 3kW). The SAR (specific absorption rate) of the samples was determined by time-dependent temperature measurements. The concentration of all samples was 10 mg/ml in phosphate buffered solution (PBS; Gibco, USA) and treated with ultrasound for 10 min before the measurement.

2.2  

2.2 In vitro cell viability/cytotoxicity studies

The established L929 cell line (fibroblast connective tissue) from the Korean Cell Line Bank (KCLB, Korea) was used in these experiments. The cytotoxicity of cobalt ferrite suspensions in different concentrations was examined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyloxazole) method. The MTT assay is a simple non-radioactive colorimetric assay to investigate the level of cell cytotoxicity, proliferation or viability through measuring the succinate dehydrogenase mitochondrial activity. MTT is a yellow, water-soluble, tetrazolium salt. Metabolically active cells can convert this dye into a water-insoluble dark blue formazan by the reductive cleavage of the tetrazolium ring [4]. Formazan crystals can then be dissolved in an organic solvent such as dimethylsulphoxide (DMSO) and quantified by measuring the absorbance of the solution (570 nm). The spectrophotometer was calibrated to zero absorbance using a culture medium without the cells. The relative cell viability (%) related to the control wells containing the cell culture medium without the magnetic particles suspension was calculated using the following formula: \[ \text{Relative Cell Viability} = \frac{[A]_{\text{test}}}{[A]_{\text{control}}} \times 100 \].

2.3  

2.3 In vivo Acute systematic toxicity test (Intravenous injection)

Animal selection and management, surgical protocol, and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. For the acute systematic test, ICR mice, 6 weeks of age weighing 22 ~ 25 g, were used. Ten male ICR mice were assigned for each test groups. All test procedure was followed by ISO10993-11. We selected 1 ml of CoFe2O4 particle suspension (10 mg/ml). The sample was performed by autoclaving 121 °C for 1 hour. Each mouse received a single intravenous injection of magnetic suspension by 24 gauge needle syringe for 5 min. Animals were checked clinical signs such as diarrhea, piloerection, and mortality and body weight of animals were also measured for 7 weeks after injection. For the histological examination, test animals were anesthetized after acute systematic toxicity test and vital internal organs of each mouse were excised. Samples of these organs were fixed in 8% formalin, embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin (H&E). For the detection of iron particles, Prussian blue was stained with section of each organ. Detailed microscopic examination was carried out on organs of animals.

3 RESULTS

CoFe2O4 nanoparticles were synthesized by sol-gel process. Their magnetic and structural properties of the particles were determined using an x-ray diffractometer and vibrating sample magnetometer, respectively.

<table>
<thead>
<tr>
<th>Particle size (nm)</th>
<th>Ms (emu/g)</th>
<th>Hc (kOe)</th>
<th>Crystal structure</th>
<th>ΔT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoFe2O4</td>
<td>68</td>
<td>72</td>
<td>23.9</td>
<td>Spinel</td>
</tr>
</tbody>
</table>

Table 1: Characterization of prepared sample.

The XRD diffraction patterns of the phase evolution of the CoFe2O4 nanoparticles exhibit a spinel structure (Table 1). The magnetic properties of the prepared CoFe2O4 nanoparticles showed the saturation magnetization, 72 emu/g and the coercivity, 23.9 kOe. The temperature change of the 5 ml of CoFe2O4 suspension (10 mg/ml) under alternating magnetic field (150 kHz and 3 kW) was ΔT=23.5 °C for 10 min. And the saturation of increasing rate in temperature change was represented at 46 °C after 8 min, approximately.
Cell viability of CoFe$_2$O$_4$ nanoparticles are shown in Fig. 1. There was no decrease of cell viability as increasing the concentration of CoFe$_2$O$_4$ nanoparticles suspension. The cytotoxicity of CoFe$_2$O$_4$ was not seen at concentration below 10 mg/ml. The cell viability at concentration of ≤ 10 mg/ml was 100-76% which is mild toxicity. However, the cell viability of CoFe$_2$O$_4$ magnetic suspension was 48.2 % at the concentration of 2000 μg/ml.

The cytotoxicity of CoFe$_2$O$_4$ nanoparticles was determined by the number and the surface area because cytotoxicity is represented by exposure of cells to toxicants. Therefore, the number and surface area showed in Fig. 2. The large surface area of CoFe$_2$O$_4$ nanoparticles increases a contact with cell layer. Cell damages will be affected by surface area rather than the concentration of particles [6]. Cell reproduction can be measured by several parameters including cell count, DNA content, protein content, or enzyme activity. Although a morphological alteration can’t observe in the cell layer and/or cell shape, MTT assay in this study is a simple non-radioactive colorimetric assay to evaluate the level of cell cytotoxicity, proliferation or viability through measuring the dehydrogenase mitochondrial activity.

As preliminary study of hyperthermic treatment in vivo, the acceptable amount of CoFe$_2$O$_4$ nanoparticles suspension in vivo should be determined with injecting the CoFe$_2$O$_4$ nanoparticles suspension. Thus, the amount of injecting CoFe$_2$O$_4$ nanoparticles suspension was determined by the in vitro test and other references [7]. The 1 ml of CoFe$_2$O$_4$ particle suspension (10 mg/ml) was selected for in vitro toxicity test. In vivo toxicity test, there was no significant difference in body weight compared to control group in Fig. 2. During the study period, injected with CoFe$_2$O$_4$ nanoparticles suspension for 7 weeks did not cause any adverse effects on growth. In addition, abnormal clinical signs or behaviors were not detected in both groups. In histopathologic evaluation, Fig. 3 showed that CoFe$_2$O$_4$ nanoparticles are distributed in each organ. As shown in Fig. 3, CoFe$_2$O$_4$ nanoparticles were found in almost all organs such as heart, kidney, liver, spleen and stomach [8]. The highest amount of CoFe$_2$O$_4$ nanoparticles were taken up by the liver, but not seen in the lung. The reticuloendothelial system (RES) is the liver, spleen and lung and non-RES organs is heart, kindeny and stomach. In our study, the localization of CoFe$_2$O$_4$ nanoparticles was not consistent with the RES system. And most of the intravenously injected particles are recognized by the body as being the foreign and subsequently removed from the blood circulation by the macrophages of the mononuclear phagocytic system (MPS). This so called “MPS clearance” leads to a high uptake of particles in the Kupffer cells of the liver, and about 80-90% of the injected dose accumulate in this organ within 5 minutes after injection. A lower percentage of about 2-5% is taken up by the macrophages of the spleen, and only little is found in other organs such as lungs, kidneys, heart and bone marrow [9]. These are well matched with our results. The CoFe$_2$O$_4$ nanoparticles might give more damages to liver in over dose than other organs. Fig. 4 showed histopathological evaluation of the organs from the mice injected CoFe$_2$O$_4$ nanoparticles suspension. From the Fig. 4, abnormal histopathological findings were not observed. The liver from CoFe$_2$O$_4$ injected mouse reveals no remarkable changes in the dose of 20 mg CoFe$_2$O$_4$ nanoparticles, such as cytoplasmic vacuolization and karyorrhexis. And, no atypical tubules and necrosis were also observed in kidney of test group.

![Figure 1: (a) Cell viability of CoFe$_2$O$_4$ nanoparticle in different concentration (10, 40, 100, 500, 1000 and 2000 μg/ml) and (b) Total surface area and number per well according to the concentration of CoFe$_2$O$_4$ nanoparticles.](image1)

![Figure 2: Weight changes of (a) control and (b) CoFe$_2$O$_4$ injected mice group for acute systemic toxicity.](image2)

![Figure 3: Light microscope images of tissue from test group (Prussian Blue staining).](image3)
CONCLUSION

The biologic factors were important for clinical application. The important factors, which determine the biocompatibility and toxicity of ferrite, are the magnetically responsive components such as magnetite, iron, nickel-zinc-copper, lithium, nickel, cobalt ferrite [10]. In order to minimize cytotoxic problems in the body before biomedical applications, the materials must be assessed for compatibility with biological environment. CoFe$_2$O$_4$ nanoparticles were synthesized by sol-gel route. The temperature change under alternating magnetic field and saturation magnetization (Ms) was $\Delta T=23.5$ °C, Ms=72 emu/g. The cell viability at concentration of $\leq$ 10 mg/ml was 100-76% which is mild toxicity. The cytotoxicity of CoFe$_2$O$_4$ nanoparticles would be determined by the number and the surface area because the cytotoxicity is raised by exposure of cell layer to toxicants. Therefore, cell damages will be affected by surface area rather than the concentration of particles. In vivo toxicity test, during the study period, injected with CoFe$_2$O$_4$ nanoparticles suspension for 7 weeks did not cause any adverse effects on growth because no significant differences in the body weight gain or loss between control and test group. In addition, abnormal clinical signs or behaviors were not detected in both groups. In histopathologic evaluation, CoFe$_2$O$_4$ nanoparticles were found in almost all organs such as heart, kidney, liver, spleen and stomach. The tissue images of the CoFe$_2$O$_4$ injected group reveals no remarkable changes compared with control. Finally, CoFe$_2$O$_4$ could be expected as bio-materials for magnetic hyperthermia through the results of heat generation and in vitro & in vivo toxicity test.

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REFERENCES