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Biological effects of magnetic fluids: toxicity studies

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Abstract

Toxicity of ionic and citrate-based magnetic fluids administrated intraperitoneally to mice was investigated through cytogenetic analysis, evaluation of mitotic index and morphological and cytometric alterations. Both magnetic fluid samples cause severe inflammatory reactions, being very toxic and thus not biocompatible. Peritoneal cells and tissues studies may provide a useful strategy to investigate the in vivo biological effects of magnetic nanoparticles.

Keywords: Ionic magnetic fluid; Citrate-based magnetic fluid; Toxicity; Macrophage; Biocompatible material

1. Introduction

Magnetic fluids (MF) are stable colloidal suspensions composed of monodomain ferrite-based (MFe_2O_4) magnetic nanoparticles dispersed in organic or inorganic liquid carriers [1]. Magnetic nanoparticles are quickly synthesized through chemical condensation reactions in aqueous medium [2]. Conventional MF are organic-based colloidal suspensions stabilized by steric repulsion after coating the magnetic nanoparticles with surfactant agents. Ionic MF (IMF) are water-based colloidal suspensions stabilized by coulombic repulsion after adding an electric surface charge density at the magnetic nanoparticles [3]. Low-pH IMF presents a positive surface charge density whereas high-pH IMF presents a negative surface charge density. The new MF known as biocompatible MF, highly stable in water medium at neutral pH and physiological salinity, may present a variety of biological effectors chemisorbed at the magnetic nanoparticle surface. The colloidal stability of biocompatible MF upon clustering may depend on both steric and coulombic repulsion. To fit a particular interest, nucleotides, oligonucleotides, peptides, vitamins and antibiotics can be bounded at the magnetic nanoparticle. There are many possibilities of applications of biocompatible MF in biology and medical diagnosis and therapy, as for instance separation and purification of cells [4],

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MRI contrast agents [5] and magneto-thermocytolisis [6,7].

Biological effects of biocompatible MF must be evaluated before medical and clinical applications can be put forward. DNA damage is the primary lesion mediating many cytotoxic and mutagenic events and it was observed in macrophages adhering to the surfaces of foreign bodies [8]. Macrophages play an important role in immune system and every alteration in their nuclear material can alter all the biological responses [9]. Therefore, macrophages constitute an adequate model for in vivo studies. On the other hand, IMF are usually toxic materials and a protective coating using a biological molecule, such as citrate, would provide some protection against toxicity. Thus, the purpose of this work was to investigate the toxicity of IMF and citrate-based MF (CMF) through different biological approaches using peritoneal cells and tissues of mice.

2. Materials and methods

IMF based on $MnFe_2O_4$ magnetic nanoparticles (10 nm diameter) has been chemically synthesized by condensation reaction of Fe^{3+} and Mn^{2+} ions in alkaline aqueous solution [2]. Though the isoelectric points were not experimentally obtained the surface charge density of the 0.5 M uncoated MF was estimated to be on the order of 10^{-4} C/m^2. Throughout this work molarity refers to the total cation (Fe^{3+} plus Mn^{2+}) concentration. CMF ($MnFe_2O_4$) was obtained by chemisorption of citrate anions at the nanoparticle surface. We estimated the citrate concentration on the order of 3×10^{-11} mol/cm^2 at the nanoparticle surface. The samples were intraperitoneally injected in Swiss mice. Five different aspects were investigated in this work. First, the LD-50 was investigated after intraperitoneal injection of 50–500 μl, 0.5–0.0005 M IMF or CMF. Death and clinical observations were made during a period of ten days. Second, the number of cells in division between 3000 total cells in cytogenetic preparations was used as the mitotic index of animals. Third, peritoneal macrophages and bone marrow cells were harvested 1, 7 and 21 days after the administration of the MF samples and prepared for cytogenetic analysis [10]. Metaphases were analyzed and the cells with structural or numerical chromosome aberrations were recorded. Alternatively, cytogenetic studies were made after induction of peritoneal macrophages mitosis by hyperimmunization of mice with ovalbumin [11]. Mice were twice administrated intraperitoneally with 100 μl of 0.05 M IMF, CMF and 2×10^{-2} M citrate, 24 and 48 h before cell collection. Peritoneal and bone marrow cells were collected and prepared for cytogenetic analysis [10]. Fourth, imprints of peritoneal cells were made in glass slides, stained by Giemsa and analyzed for cytometric and morphological alterations. One thousand cells per animal were scored for the cytometry. Fifth, the spleen and the kidneys of animals were histologically prepared and examined.

3. Results and discussion

Table 1 summarizes the main results obtained in our investigation. Citrate alone and the control show no significant difference in all the biological tests performed. The intraperitoneal administration of CMF resulted in death and in diarrhea, showing very high toxic action. The ability of macrophages to proliferate is gradually lost [12]. However, the administration of CMF increased several times the mitotic index of peritoneal macrophages, similarly to the foreign bodies reactions [8]. Proliferation of mononuclear phagocytic cells has been reported as a signature of a variety of inflammatory reactions [13] and thus our data suggest that CMF are more toxic than the IMF. Cytogenetic analysis of the metaphases induced 7 or 21 days after administration of IMF or CMF, has not showed structural chromosome aberrations (SCA), though it shows a high frequency of hypodiploidy in some animals. In addition, we found that some cells showed very atypical chromatin patterns. The frequency of cells with SCA for hyperimmune animals treated with IMF increased from about 1% (normal index) up to 6.3% and in the mice treated with citrate-based the metaphases showed no SCA. About 25% of peritoneal resient macrophages metaphases analyzed after the immune stimulation displayed

Table 1
Effects of ionic MF and citrate-based MF on peritoneal cells and tissues of mice. Note: M ∅: macrophage; SCA: structural chromosome aberration; BMC: bone marrow cells; Ly: lymphocyte; Np: neutrophil

Effects	Citrate/Control	Ionic MF	Citrate-based MF
LD 50	No death in 30 days	No death in 30 days	Death of 2/6 mice in 48 h
Clinical symptoms	Normal	Diarrhoea	Diarrhoea
Mitogenic activity on M ∅	1–3/3000 cells	1–3/3000 cells	38/3000 cells
Cytogenetic analysis of M ∅:			
1. MF-induced mitosis	No metaphases	No metaphases	Hypodiploidies and polyploidies; No SCA
2. MF and ovalbumin-induced mitosis	SCA = 1.0%	SCA = 6.3%	SCA = 1.0%
3. BMC	Normal (< 1.0%)	Normal (< 1.0%)	Normal (< 1.0%)
Cytometry	> 80% Macrophage	Migration of Ly	Migration of Np and Ly
Morphology of peritoneal cells	Normal	Apoptosis of M ∅; damage of nuclear material of Ly	Apoptosis of M ∅; damage of nuclear material of Ly and Np
Morphology of spleen and kidneys: 1. parenchyma	Normal	Normal	Normal
2. hilum	Normal	Inflamatory reaction	Severe inflamatory reaction

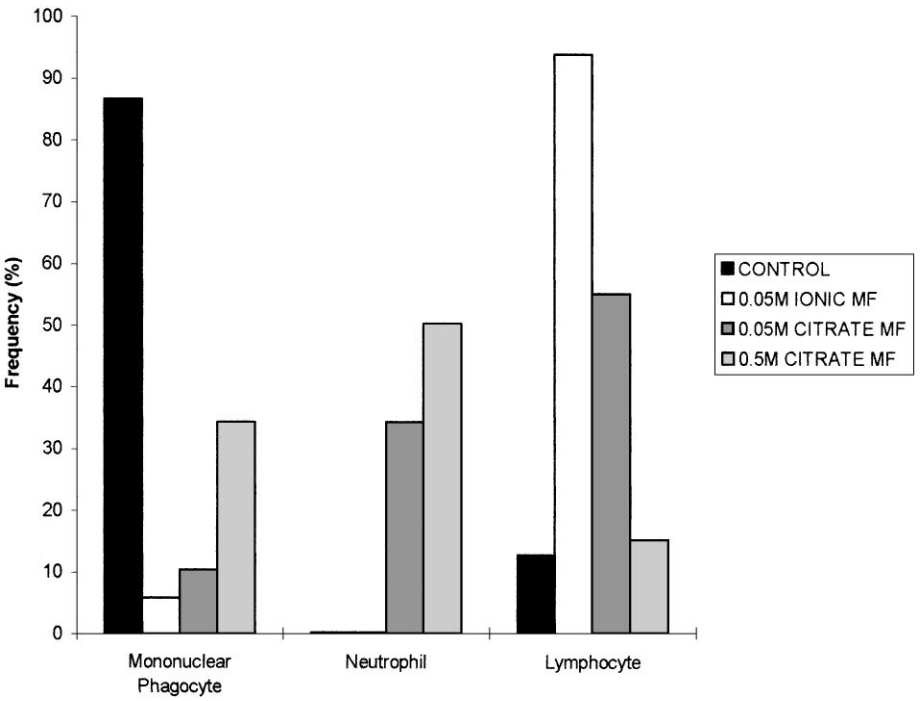

Fig. 1. Frequency of mononuclear phagocytes, neutrophils and lymphocytes 24 h after administration of 100 μl of ionic and citrate-based MF.

magnetic particles between the spreaded chromosomes.

According to the literature the predominant cell in the Swiss mice peritoneal cavity is the macrophage [14]. However, we found that treatment with both MF samples for 24 h changes the peritoneal cell population frequencies. As shown in Fig. 1, MF increases enormously the lymphocyte frequency while CMF alters the lymphocyte as well as the neutrophil populations. Such a frequency shift was also observed in hystological preparations made with the hilum of spleen and kidneys (data not shown). It is interesting to note that for higher MF doses the nuclei material of neutrophils and lymphocytes is severely damaged. The damage was proportional to the number of magnetic particles phagocyted by macrophages. All the MF effects observed are time and dose dependent. The huge increase on the frequency of neutrophils and lymphocytes in the peritoneal cavity after MF treatment is an evidence of an inflammatory process. Macrophages secrete interleukin-I, an important factor in inflammatory reactions that is responsible for the events that change the peritoneal cavity population [15,16]. In the inflammatory or in the phagocytosis process, mice peritoneal macrophages have been shown to produce reactive oxygen radicals and nitric oxide that can induce cellular DNA damage and citotoxicity [17] and might be responsible for the peritoneal cells abnormalities observed in this study.

In summary, the data obtained in this work show that the IMF and CMF used in our experiments have caused severe inflammatory reactions in the peritoneal cavity of mice, indicating that both samples are very toxic and can not be seen as biocompatible agents. As far as the biocompatibility feature is concerned our results indicate that adsorption of carboxilic acids at the nanoparticle surface do not necessarilly turns a MF biocompatible, even at neutral pH. Indeed, it is demonstrated for the first time in this work that peritoneal cells and tissues provide a useful model for the in vivo study of biological effects of magnetic nanoparticles.

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