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Toxic effects of ionic magnetic fluids in mice

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Abstract

Toxicity of ionic and tartrate-based magnetic fluids administered intraperitoneally to mice was investigated through morphological and cytometric alterations and cytogenetic analysis. Both magnetic fluids cause cellular death, mutagenicity and severe inflammatory reactions, being very toxic and thus not biocompatible. Peritoneal cell and tissue studies may provide a useful strategy to investigate the in vivo biological effects of magnetic nanoparticles.

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

Magnetic fluids (MF) are highly stable material systems composed of magnetic nanoparticles (MNP) with typical diameter below 15 nm dispersed in a nonmagnetic matrix. From the practical point of view the current interest in MNP spans from the development of high-density storage media, where spontaneous magnetization reversal determines the efficiency of the stored information [1], to the development of biocompatible materials for active targeting of cancer cells with both diagnostic and therapeutic values [2]. The high stability of a MF is achieved through a combination of particle thermal motion and particle–particle repulsion, both working against Van der Waals and magnetic dipole interactions that tend to stick particles together. Steric repulsion prevents particle agglomeration in surfactant containing MF while coulombic repulsion accounts for the stability in ionic MF [3,4]. In biocompatible MF, however, both steric and coulombic repulsion play a very important role in the fluid stability. Actually, in the first step of the synthesis of a biologically active MF, an ionic MF is used as chemical precursor to which a buffer-like compound is added [5]. The biological specificity of a biocompatible MF is then achieved by adding different biological effectors, for instance antibodies, at the previously coated MNP in order to obtain the final structure. Besides specificity, toxicity tests need to be performed, especially with the intermediate product, before medical and

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clinical applications can be put forward. We showed previously that peritoneal cells and tissues provide a useful model for the in vivo study of the biological effects of MNP [6]. In this work, the toxicity of manganese ferrite nanoparticles (10 nm diameter) uncoated and coated with tartrate anions in aqueous medium is investigated after intraperitoneal administration to female Swiss mice.

The strategy we used to coat the MNP surface is based into two aspects: adsorption ability and biocompatibility. Carboxylic group is expected to act as a chelant agent for the metallic ions (Fe^{3+} and Mn^{2+}) at the nanoparticle surface. While citrate, already tested [6], is a biological molecule tartrate is not found in biological systems. Thus, tests involving both ends, i.e., biological and non-biological coating would be of interest as far as toxicity is concerned. Whether carboxylic groups are physiosorbed or chemisorbed at the particle surface has not been tested in this work. However, we estimated the tartrate concentration at the MNP surface to be on the order of 10^{-11}mol $\times$ cm^{-2}.

The manganese ferrite nanoparticles were prepared using a slight variation of the Massart condensation method [3]. The preparation process starts with a chemical reaction among aqueous solutions of Mn^{2+} and Fe^{3+} salts in alkaline medium followed by peptization in acid medium [4]. The tartrate-based nanoparticles are obtained after treating the acid ionic MF with aqueous solution of sodium tartrate. Five different aspects were investigated in this work, namely, the determination of the LD-50, evaluation of mitotic index, cytogenetic analysis, morphological and cytometric observations. First, the LD-50 was investigated after intraperitoneal injection of 50–500 μl of 0.5–0.0005 M ionic MF or tartrate-based MF. Death and clinical observations were made during a period of at least 10 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 studies [7]. Briefly, 20 μg of colchicine in 1 ml of phosphate-buffered saline (PBS) was injected intraperitoneally in order to achieve interruption of cell division. Two hours later, the cells were collected with PBS containing 5 IU heparin per ml. Metaphases were analyzed and the cells with structural or numerical chromosome aberrations were recorded. Blind analysis of samples was carried out in these cytogenetic studies. Alternatively, cytogenetic studies were made after induction of peritoneal macrophage mitosis by immunization of mice with 0.2 ml of a 1 mg/ml solution of crude ovalbumin emulsified in Freund's complete adjuvant (1 : 1 v/v) [8]. Three weeks after immunization animals were challenged with 0.2 ml of the same solution of ovalbumin. Mice were twice administered intraperitoneally with 100 μl of 0.05 M ionic or tartrate-based MF, 24 and 48 h before cell collection. Peritoneal and bone marrow cells were collected 30 h after challenge and prepared for cytogenetic analysis as described above. Fourth, the spleen and the kidneys of two different animals in each experimental group were treated with Bouin fixative for 8 h, dehydrated, embedded in a block of paraffin, sectioned at 5 μm and stained with hematoxylin and eosin. The sections were histologically examined and photographed in an Olympus BX50 microscope. Fifth, imprints of peritoneal cells were made in glass slides, stained by Giemsa and analyzed for both morphological and cytometric alterations. One thousand cells per each animal were scored for the cytometry.

Table 1 summarizes the main results obtained. The intraperitoneal administration of large quantities of tartrate-based MF, specifically 0.5 ml of a 0.5 M solution did not result in death for a period of 30 days, thus the LD-50 determination was not possible. The treatment with tartrate-based MF did not cause diarrhea in the animals, which was different from the results observed with the same dose of ionic MF. In general, the effects of tartrate-MF were very similar to that of ionic-MF (Table 1). The spontaneous mitotic index of peritoneal macrophages in the control mice has been shown to be of the order of 0.1%, as expected from the fact that the ability of the macrophages to proliferate is gradually lost [9]. The mitotic index did not change 7, 21 and even 37 days after treatment of the animals with ionic MF or tartrate-based MF, in the concentration range of 0.0005 M to 0.5 M. Such a result was not expected since the literature reports that

Table 1
Effects of ionic MF and tartrate-based MF on peritoneal cells and tissues of mice

Effects	Control	Ionic MF	Tartrate-based MF
LD 50	No death in 30 days	No death in 30 days	No death in 30 days
Clinical symptoms	Normal	Diarrhea	No diarrhea
Mitogenic activity on M ∅	Normal (0.1%)	Normal (0.1%)	Normal (0.1%)
Cytogenetic analysis of M ∅:			
1. MF-induced mitosis	No metaphases	No metaphases	No metaphases
2. MF and ovalbumin-induced mitosis	SCA = 1.0%	SCA = 6.3%	SCA = 6.0%
3. BMC	Normal (< 1.0%)	Normal (< 1.0%)	Normal (< 1.0%)
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	Inflammatory reaction	Severe inflammatory reaction
Cytometry	> 80% macrophage	Migration of Ly	Migration of Np and Ly

Note: M ∅, macrophage; SCA, structural chromosome aberration; BMC, bone marrow cells; Ly, lymphocyte; Np, neutrophil.

proliferation of mononuclear phagocytic cells is a feature of inflammatory reactions [10] caused by foreign bodies [11]. In order to observe the effect of the MF in peritoneal resident macrophage chromosomes, the mitosis of cells was immunologically induced with ovalbumin injection. The frequency of cells showing structural chromosome aberrations in the case of the animals treated with 100 μl of ionic or tartrate-based 0.05 M MF, 24 and 48 h before cell collection, was increased from the normal index of 1% up to 6.3% for ionic MF and 6.0% for tartrate-based MF, demonstrating their mutagenic activity. A significant fraction of the peritoneal-resident macrophages metaphases analyzed after the immune stimulation showed magnetic particles between the spreaded chromosomes, an evidence that cells under division in the peritoneal cavity after the immune stimulus are resident macrophages [8]. Differently from macrophages bone marrow cells chromosomes were not affected by the ionic or tartrate-based MF treatment. Morphological observations of peritoneal imprints prepared 24 h after tartrate-based MF treatment showed many neutrophils and the majority of macrophages with internalized MNP. It has been also observed blebbing of the cytoplasmic membrane, cell contraction and marked condensation of the macrophage nucleus, features considered indicative of apoptosis [12]. Although the predominant cell in the Swiss mice peritoneal cavity is the macrophage [8], the treatment with MF shows that after 24 h the peritoneal cell population frequencies have been changed. As shown in Fig. 1, ionic MF increases enormously the lymphocyte frequency while tartrate-based MF alters the lymphocytes as well as the neutrophil population. Our data indicate that the neutrophil frequency increases when the concentration of the administered tartrate-based MF is increased. Such a frequency shift was also observed in histological preparations of spleen and kidneys tissues. Though the parenchyma of the organs was not affected 24 h after the treatment with ionic or tartrate-based MF, as compared to the control-untreated animals, the hilum of both organs showed a significant increase in neutrophil and lymphocyte populations (see Fig. 2). The nucleus material of neutrophils and lymphocytes is severely damaged after administration of higher MF doses. It seems that all the cellular damage observed here was proportional to the number of MNP internalized by peritoneal macrophages through the phagocytic process. The MNP not

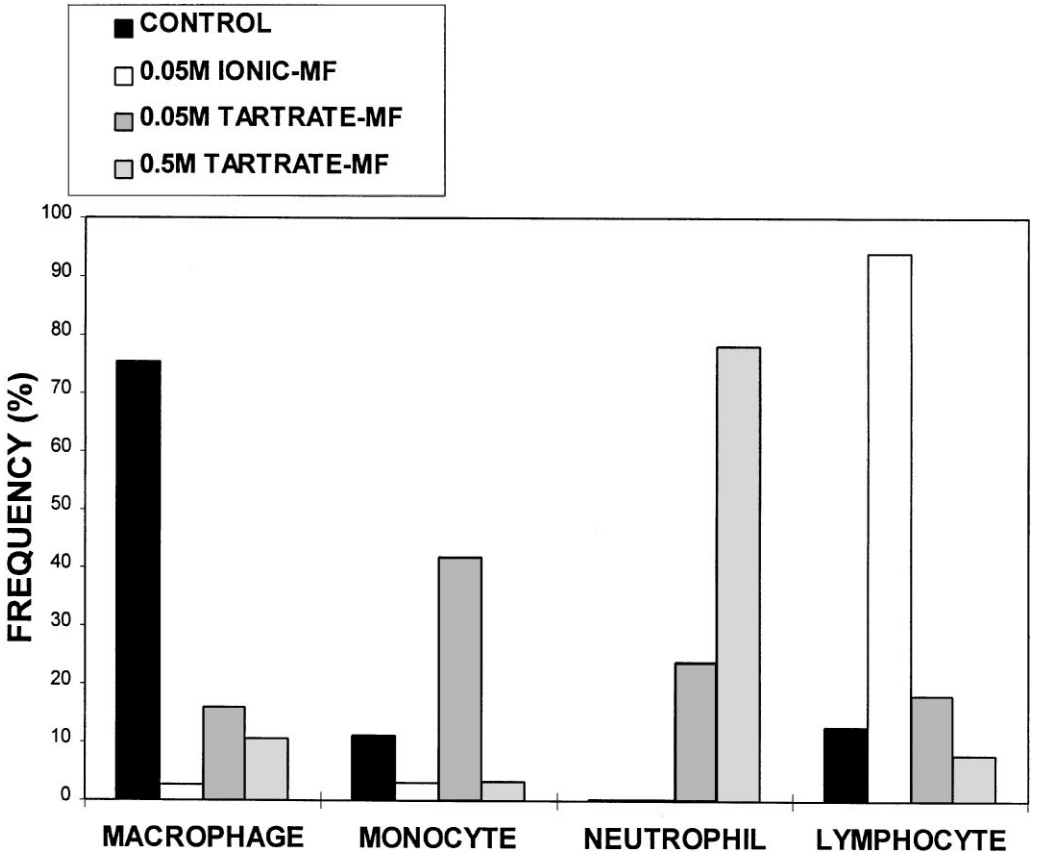

Fig. 1. Frequency of macrophages, monocytes, neutrophils and lymphocytes 24 h after administration of 100 μl of ionic or tartrate-based magnetic fluids.

internalized by cells are progressively agglomerated in clusters, which are larger than what macrophages are able to phagocyte. Consequently, 21 days after the MF injection, neutrophil and lymphocyte damage was found to be very low. All the MF-effects observed in the present study are time and dose dependent. In response to inflammation or in the process of phagocytosis of foreign particles, macrophages produce reactive oxygen radicals that can injure even normal tissues in the immediate vicinity and induce cellular DNA damage. Such reactive species might be responsible for the peritoneal cell and chromosome abnormalities observed in this study [13].

Tartrate-based MF behave as citrate-based MF [6] in that both cause inflammatory reactions in the peritoneal cavity as observed in morphological preparations of spleen and kidneys. In addition, we found very similar neutrophil frequency changes for both samples. However, the two MF differ in their toxicity characteristics: tartrate-based MF has not caused death or diarrhea in mice, has not increased the mitotic index of macrophages and was the only coated sample that has caused structural chromosome aberrations in the peritoneal immune-stimulated macrophages.

In summary, the data obtained in this work show that the ionic and tartrate-based MF used in our experiments cause severe inflammatory reactions in the peritoneal cavity of mice, indicating that both samples are very toxic and cannot be seen as biocompatible agents. As far as the toxicity testes are concerned the low grafting coefficient (10^{-11}mol $\times$ cm^{-2}) would explain the little differences observed

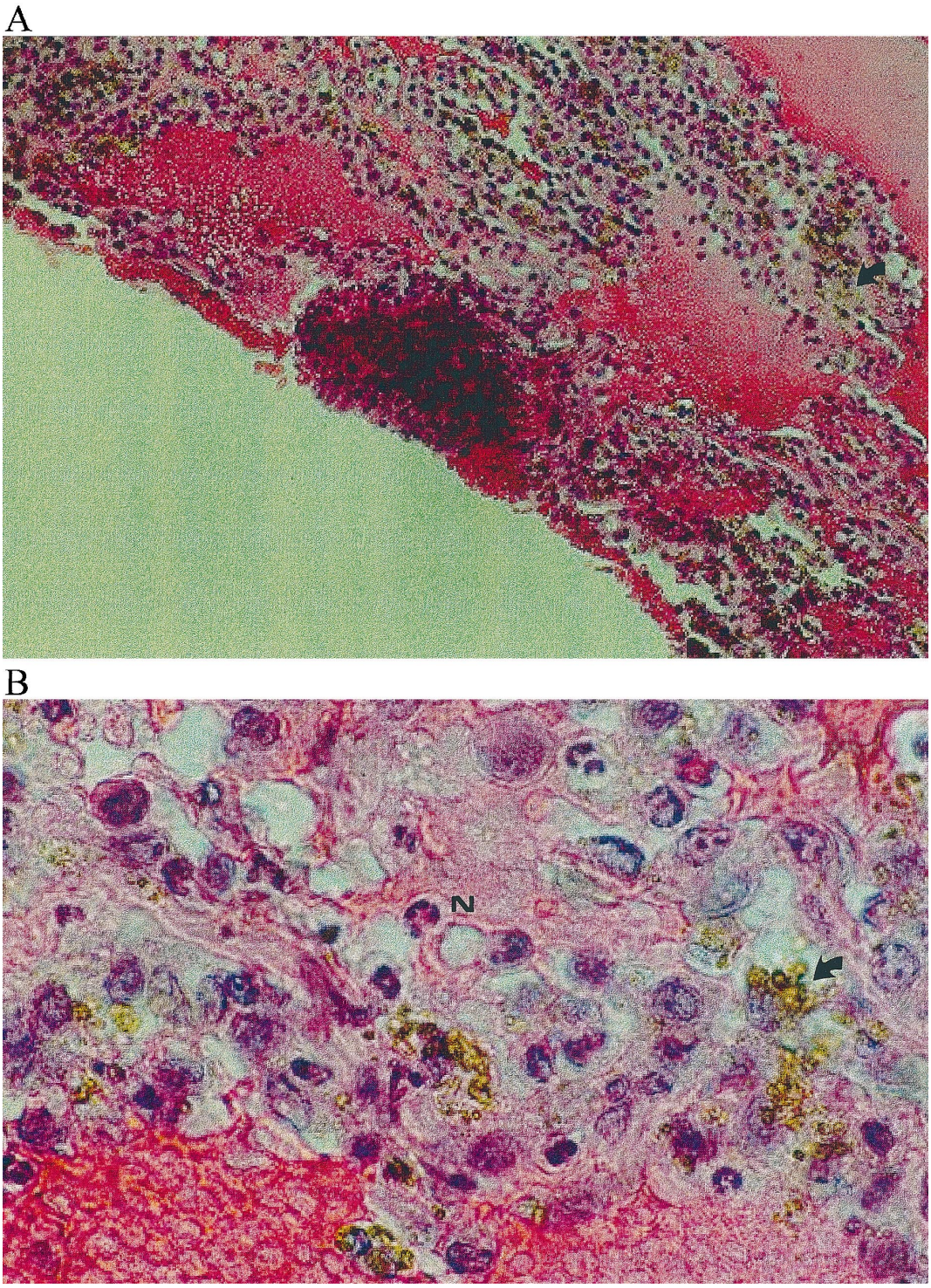

Fig. 2. Blood vessel in the spleen hilum infiltrated with macrophages and neutrophils (N), showing magnetic particles (arrow) 24 h after administration of 100 μl of ionic or tartrate-based 0.05 M magnetic fluids. A: X 200; B: X1,000.

between coated and uncoated nanoparticles. Our results indicate that adsorption of carboxylic acids at the nanoparticle surface does not necessarily turn a MF biocompatible, even at neutral pH and physiological salinity. In addition, we found that tartrate-based MF is less toxic than citrate-based MF [6]. Indeed, it is confirmed in this work that peritoneal cell and tissue provide a useful model for the in vivo study of biological effects of MNP.

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