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Effects of AC magnetic field and carboxymethyldextran-coated magnetite nanoparticles on mice peritoneal cells

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Abstract

A portable apparatus was developed to perform magnetohyperthermia (MHT) assays. In order to investigate its efficiency on cell lysis, biological effects of the AC magnetic field exposure after carboxymethyldextran-coated magnetite-nanoparticles (CMDC) treatment were investigated. Phagocyte capacity, cell viability, and morphology data evidenced that the CMDC sample and the apparatus are useful to further investigate MHT in cancer therapy.

Keywords: Magnetic hyperthermia; Magnetic fluid; Cell viability; AC magnetic field; Phagocytosis; Hyperthermia; Carboxymethyldextran; Nanoparticles; Toxicity; Peritoneal mouse cells

1. Introduction

In the last years, hyperthermia has been used as an alternative cancer therapy to minimize severe adverse effects of conventional treatments. Hyperthermia of tumors can be achieved using different strategies, such as application of radio-frequency fields and microwaves or introduction of ferromagnetic needles in the cancer site. Although hyperthermia has been shown to be an extremely powerful anti-cancer agent and also a technique capable of intensifying the efficacy of radiation and chemotherapy, the full potential of this therapy is hindered by a number of limitations, as for instance the non-homogeneous distribution of temperature over the cancer site, low specificity, patient discomfort, and thermo-tolerance development [1]. The above-mentioned limitations could be overcome by the use of biocompatible magnetic nanoparticles as a material basis to support the

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development of hyperthermia [2–4]. Magnetic fluids (MF) are stable colloidal suspensions usually containing ferrite-based (MFe_2O_4) magnetic nanoparticles dispersed in organic or inorganic liquid carriers. Biocompatible MF, highly stable in water medium at neutral pH and physiological salinity [5,6], may present a good thermal potential [7]. In the magnetohyperthermia (MHT) process (also known as magnetothermocytolysis [8]), the tumor cell lysis is obtained by thermal dissipation when the magnetic nanoparticles are exposed to an AC magnetic field. In order to perform the MHT experiments, we developed a portable apparatus operating at 1 MHz with 40 Oe of field amplitude [9]. In this equipment, a metallic core with a cylindrical (10.7 mm diameter) cross section closely wound by a coil of wire concentrates the alternating magnetic flux. The solenoid core is linked to an adjustable support to apply the magnetic field in the targeted region. As one important question concerning the use of the MHT technology is the possible adverse effects of magnetic field exposure to the organism [10,11], biological tests related to the AC magnetic field genotoxic and cytotoxic actions and also to its inflammatory effects were performed [9] and showed that the damage induced by the AC field to normal cells is related to the exposure time. Using the conditions where no adverse biological effects were induced, the aim of this work was to investigate the efficiency of the developed apparatus on the cell lysis through analysis of biological effects of the AC magnetic field exposure after carboxymethyldextran-coated magnetite-nanoparticles (CMDC) treatment.

2. Materials

The MF sample used to carry out the experiments was obtained by chemical co-precipitation of Fe(II) and Fe(III) ions in alkaline medium to produce 5.0 nm average-diameter magnetite particles, following surface coating with a single layer of carboxymethyldextran (CMDC). After surface coating the magnetite nanoparticles, the MF sample was diluted in water and stabilized at neutral pH, in a concentration of about 4×10^{16} particle/mL. The study was performed in four experimental groups: (1) In the control group (C) animals were not treated. (2) In the MF group 100 μL of CMDC sample containing about 4×10^{15} particles was intraperitoneally administrated to the animals. Peritoneal cells were collected at 0, 5, 10, 15, 30 min, 1, 2, 6, 12, 24, 48 h and 7 days after CMDC treatment. (3) In the AC group the animal's abdomen was exposed to AC magnetic field for 3 min. The peritoneal cells were collected as described for the MF group. (4) In the MFAC group, animals were first treated with CMDC as described for the MF group and subsequently exposed to AC magnetic field for 3 min. The peritoneal cells were collected in two different ways: (a) for shorter experimental times (0 up to 30 min) the cells were collected immediately after exposure to the AC magnetic field and (b) for longer experimental times (1 h up to 7 days) the animal's abdomen was exposed to the AC magnetic field 30 min after CMDC treatment. Exposure to the AC magnetic field was never longer than 3 min.

In order to evaluate phagocyte capacity, the peritoneal cells were spread on glass slides subsequently stained by Wrigth–Giemsa. Two hundred macrophages with or without internalized magnetic nanoparticles were scored per slide. The macrophage phagocyte capacity of both control and AC groups was assessed by inclusion of Indian ink. The data were analyzed by the statistical Scheffe test (ANOVA, $p < 0.05$). The glass slides were also morphologically analyzed to evaluate the efficacy of the peritoneal cell lysis.

The cell viability test was performed by Nigrosin exclusion of peritoneal cells, as described elsewhere [12]. Briefly, peritoneal cells were collected with cold PBS and diluted with Nigrosin 0.05%. The cells were immediately scored in a Neubauer chamber. In this method, viable cells are stained in a brilliant and slightly green color while dead cells are colored in black. The data were also analyzed by the statistical Scheffe test (ANOVA, $p < 0.05$).

3. Results and discussion

Clinical observations did not reveal any adherence of peritoneal organs or tissue burnt in the peritoneum, apathy or irritability at any researched time.

The phagocyte capacity was determined by the frequency of cells with internalized particles and increased from zero to 30 min in all experimental groups (Fig. 1). Interestingly, AC magnetic field exposure did not decrease the macrophage phagocytic activity. Indeed, it was sometimes higher, especially in the presence of CMDC sample. The absence of peritoneal cell population frequency changes at the same experimental conditions was previously shown [9]. As the macrophage is a phagocytic cell and is the predominant population in the peritoneum of Swiss mice [13], this increase in phagocytosis process was expected. The phagocyte capacity alterations in the subsequent times is probably a result of renewal of the cells by migration of blood cells to the peritoneum and/or migration or even death of the macrophages with internalized particles. Seven days after the initial treatment, the peritoneal macrophages still contain magnetic nanoparticles in MF and MFAC groups and carbon particles (Indian ink) in the AC group.

The CMDC treatment induced a decrease of the viable cells frequency (Fig. 2) from 10 min until 48 h after injection, showing that the magnetic nanoparticles by themselves can induce the cell death. This observation is in accordance with some data obtained after intraperitoneal injection of nanoparticles of magnetite coated with dextran,

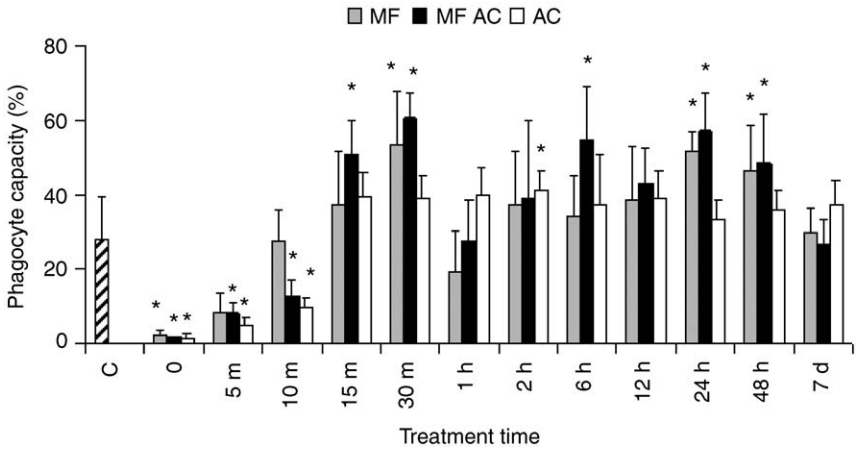

Fig. 1. Effects of (1) CMDC sample treatment (MF group), (2) AC magnetic field exposure (AC group), and (3) MF and AC (MFAC group) on peritoneal macrophages phagocyte capacity.

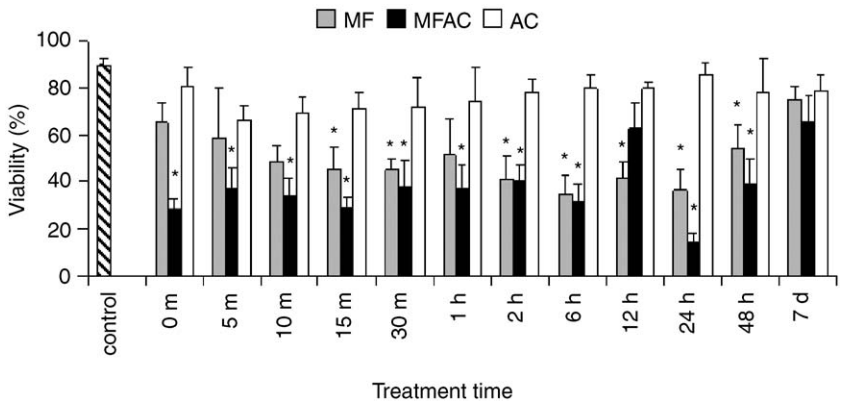

Fig. 2. Effects of (1) CMDC sample treatment (MF group), (2) AC magnetic field exposure (AC group), and (3) MF and AC (MFAC group) on peritoneal macrophages viability.

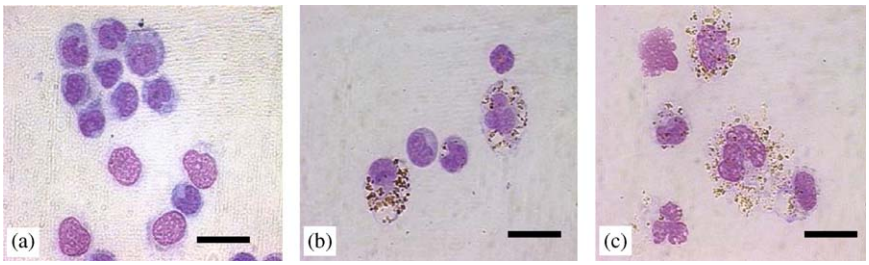

Fig. 3. Effects of CMDC sample and strong AC magnetic field on peritoneal cells. The photomicrographies show peritoneal cells of (a) control animal, (b) mice treated with an intraperitoneal injection of CMDC (100 μL of a 4×10^{16} particle/mL sample), and (c) mice treated with an intraperitoneal injection of CMDC (100 μL of a 4×10^{16} particle/mL sample) and AC magnetic field of 3 min. Size bars = 8 μm.

dimercaptosuccinic acid (DMSA), or citric acid (data not shown). On the other hand, exposure to AC magnetic field for 3 min did not show any significant alteration in the macrophages viability frequency (Fig. 2), in accordance with previous cytotoxicity tests [9]. Nevertheless, exposure to AC magnetic field 30 min after the CMDC treatment (group MFAC) induced a pronounced decrease in the viability frequency, mainly at 24 h after the CMDC injection (Fig. 2). The decrease of viable cells frequency, as evidenced by morphology analyses (Fig. 3), is obtained by cell lyses both in the MF and MFAC groups (Fig. 3). Although magnetic nanoparticles and carbon particles are still present seven days after the treatment as shown by morphology and phagocytic capacity tests, at this time the macrophage viability of all investigated groups was not significantly different from the control frequency.

The phagocytosis process and subsequent lyses were registered by morphological analysis. At 5 min after CMDC treatment magnetic nanoparticle clusters were observed internalized in macrophages. The phagocytosis process achieved its maximum at 30 min after CMDC treatment (Fig. 3b). Fig. 3c was obtained 30 min after CMDC injection and immediately after exposure to AC magnetic field, and shows the initiation of the lyses process.

We showed previously that the exposure of peritoneum cells to AC magnetic field produced by the new proposed apparatus by 3 min does not induce any significant inflammatory or genotoxic effect [9]. In this work, we show that this magnetic field can improve the phagocytosis process and induce the cell lysis. The data suggest that the CMDC sample and the developed apparatus are very useful to further investigate the MHT for cancer therapy.

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