Investigation of a magnetohyperthermia system efficacy

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This study reports on the successful use of magnetic albumin nanosphere (MAN) with *in vivo* magnetohyperthermia (MHT) in a mouse Ehrlich tumor. Maghemite nanoparticles (8.9 nm average diameter) were encapsulated within MAN (73.0 nm average diameter). Ehrlich tumor obtained after implantation of tumor cells in the subcutaneous tissue of mice was used as a model throughout this study. MHT was performed with MAN (40 μL) containing 1.2×10^{15} particle/mL and 40 Oe amplitude ac magnetic field oscillating at 1 MHz. Animals not treated, treated with MAN, or exposed to the ac field were used as controls. Histopathological analysis was carried out after 2, 5, or 11 days of tumor implantation. We found that the MHT most efficient condition was obtained while applying the ac field protocol twice a day during three consecutive days. Further, in this ac field-treated group no proliferation cells were detected. © *2011 American Institute of Physics*. [doi:10.1063/1.3559498]

I. INTRODUCTION

Magnetohyperthermia (MHT) represents a novel and promising therapy for cancer treatment.[1–3] This statement comes from the fact that MHT may achieve the specific lysis of tumor cells, improve the patient outcomes while minimizing the subsequent toxicity effects.[4] The local heating of biological tissues, which depends on both the ac applied field characteristics and the magnetic sample's properties,[5] induces several cellular element and pathway changes, including proteins denaturation.[6] As a consequence, hyperthermia induces damage to the cytoskeleton, the cytoplasmic, and organelles membrane leading to the cell death by apoptosis or necrosis. MHT may be performed using biocompatible nanosized magnetic samples and an appropriate ac magnetic field-based therapeutic protocol. To operate as a versatile MHT promoter a new magnetic albumin-based nanosphere was developed.[7] As mandatory, magnetic albumin nanosphere (MAN) nanotoxicity was previously assessed by several *in vivo* tests able to reveal specific characteristics of magnetic nanoparticle–cell interaction.[8] MAN tests, including cytometry, genotoxicity, viability, and morphology were performed from 30 min up to 30 days, showing impressive levels of biocompatibility. This study was developed with the aim to probe the use of MAN in the investigation of the therapeutic outcomes of the MHT procedure applied to the treatment of tumors. Ehrlich-tumor-bearing mice were used as the animal model and received three or six cycles of the treatment protocol: intratumoral injection of MAN followed by ac magnetic field exposure.[1]

II. MATERIALS AND METHODS

The MAN (73.0 ± 3.0 nm in average diameter) used in this study encapsulates maghemite (γ-Fe_2O_3) nanoparticles (8.9 ± 0.1 nm in average diameter), the latter previously suspended as ionic magnetic fluid and stabilized at low-*p*H value. Production of MAN was performed while dispersing the magnetic fluid sample in aqueous medium containing bovine serum albumin (BSA).[7] The maghemite content in the MAN sample used in the present study was about 25% in mass. At this maghemite particle content (about 7% in volume fraction) the particle–particle interaction is quite effective and the magnetocrystalline anisotropy energy is around 1.119 eV.[9] All animal handling and procedures were approved by the Animal Ethics Committee of the University of Brasilia, Brazil. Animals were anesthetized and subcutaneously injected with Ehrlich ascites tumor cells. Group C (control) received no treatment. Group MN received intratumoral injection of MAN (40 μL) containing 1.2×10^{15} particle/mL without any further treatment. Group MF was submitted to the ac magnetic field (40 Oe amplitude ac magnetic field oscillating at 1 MHz). By its turn, group MHT received both the MAN injection and the ac magnetic field exposure. Treatments were performed (a) once a day for three consecutive days; (b) twice a day for three consecutive days; and (c) once a day each 3 days up to 9 days. Tumor section/area was collected 2, 5, or 11 days after the tumor

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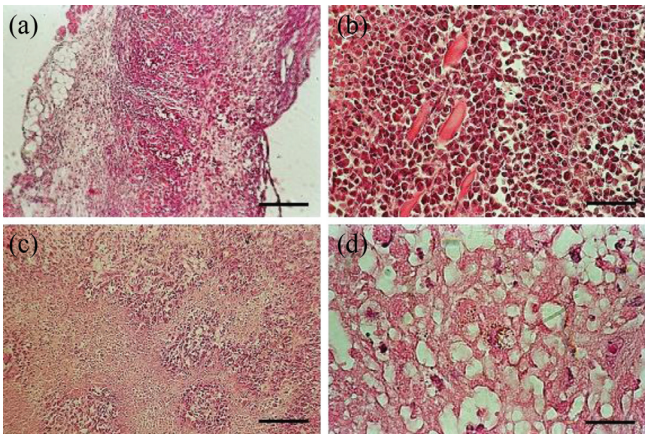

FIG. 1. (Color online) Photomicrographs of tumor tissues of Ehrlich-tumor bearing animals: (a), (b) areas of viable tumor cells; (c), (d) areas of necrosis. Size bar = 20 μm.

injection, stained with hematoxylin-eosin, and investigated by morphological analysis (light microscopy).[10]

III. RESULTS AND DISCUSSION

The morphological analysis allowed one to determine the intensity of necrosis in the experimental mice (Fig. 1). The necrosis degree was classified as (−), (+), (++), (+++), (++++) corresponding, respectively, to the tumor area presenting 0%, 1%−25%, 26%−50%, 51%−75%, and 76%−100% of necrosis (see Table I). As expected, control mice presented increasing necrosis areas directly related to the tumor development. Exposure to ac magnetic field promotes no change on the typical necrosis patterns of control animals at the respective time windows (Table I, MF1−MF9). On the other hand, the intratumoral treatment with MAN accelerated the necrosis process. Necrosis was particularly intense after six times MAN treatment (MN3, MN4), although this group still shows areas of cell proliferation. Morphological analysis showed that most of the MHT treatments did not induce necrosis areas that could be identified as different from the respective control animals. However, MHT procedure performed twice, for three consecutive days (Table I, MHT4−MHT6), resulted in the most intense necrosis pattern observed in this study. Indeed, areas of cell proliferation were not seen in this group, evidencing the efficacy of the MHT treatment while in the appropriate condition. One animal presented necrosis even in some surrounding muscle cells, evidencing the effectiveness of the treatment. Ehrlich tumor is a very aggressive tumor and spontaneously presented intense necrosis process 11 days after the subcutaneous implantation in mice. Then, 11 days may be considered too long, not providing the adequate circumstances to investigate the MHT efficacy. Nevertheless, 5 days after MAN injection the natural necrosis is still at a low level, providing reasonable condition to carry on the present investigation. Finally, variation on the tumor size is expected, as we are using nonisogenic animals. In our experiment we found the tumor size varying depending on the particular treatment we employed. For instance, for treatments MHT1 × and MHT2 × (see Table I) we found the average values for the tumor volumes equals to 85 and 44 mL, respectively.

IV. CONCLUSIONS

In this study we report on the successful use of biocompatible MAN to perform magnetohiperthermia and subsequent *in vivo* lysis of Ehrlich tumor cells. While ac magnetic fields have no effect on the necrosis of the Ehrlich tumor, the intratumoral injection of MAN increased the necrosis process. However, we found the total lysis of tumor cells was obtained only after the exposition of MAN treated tumor to

TABLE I. Effects of MHT treatment performed with MAN on necrosis of Ehrlich tumor bearing mice.

	Without treatment	Treatment			
2 days[a]	**Control**			...	...
	C1 (+)				
	C2 (−)			...	...
	C3 (++)			...	...
5 days[a]			**Control MN**	**Control MF**	**MHT (MAN+MF)**
		1×	MN1 (+++)	MF1 (+)	MHT1 (++)
			MN2 (−)	MF2 (−)	MHT2 (++)
				MF3 (++)	MHT3 (++)
5 days[a]	C4 (+)				
	C5 (++)		MN3 (++++)	MF4 (+++)	MHT4 (++++)
	C6 (+++)	**2×**	MN4 (++++)	MF5(−)	MHT5 (+++)
				MF6 (++)	MHT6 (++++)
11 days[a]	C7 (++++)		MN5 (+++)	MF7 (++++)	MHT7 (++++)
	C8 (++++)	**3/3**	MA6 (+++)	MF8 (++++)	MHT8 (++++)
	C9 (+++)			MF9 (++++)	MHT9 (++++)

[a]Days for cell collecting after tumor implantation; All data refer to Ehrlich tumor bearing mice: Group Control (C1–C9) = without treatment; Group MN (MN1–MN6) = animals treated with the sample MAN; Group MF = (MF1–MF9) = animals exposed to ac magnetic field; Group MHT (MHT1–MHT9) = animals treated with MAN and exposed to ac magnetic field; 1 ×, 2 ×, and 3/3, correspond, respectively, to treatments once a day for three consecutive days; twice a day for three consecutive days, once each three days up to nine days. Symbols (−), (+), (++), (+++), (++++) correspond, respectively, to the tumor areas with 0%, 1%−25%, 26%−50%, 51%−75%, and 76%−100% of necrosis.

the ac magnetic field under particular condition: two cycles during three consecutive days. This fact emphasizes that further investigation of the MHT process is required to improve patient outcomes. MAN was originally designed to function as a magnetic drug delivery system (DDS) and our finding evidence its potential to be simultaneously used in MHT and DDS applications.

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