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MRI-Adaptive Magneto-Thermo-Chemotherapy for Improved Cancer Treatment

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Abstract. Dextran-ferrite (DF) has been synthesized and tested as magnetic resonance imaging (MRI) negative contrast agent for tumors, invasions and metastases. MRI-adaptive Magneto-thermo-chemotherapy (MTCT) by cisplatin (CP), melphalan (MP) and DF led to improved cancer treatment. MTCT by using AC magnetic field (0.88 MHz, 7.3 kA/m and 0.15 kW) was performed at early stages of oncogenesis at +46ºC for 30 min using DF at a dose of 60 mg Fe/kg containing CP or MP. MTCT led to regression of adenocarcinoma Ca-755 tumor ~45 mm^3 before metastases in female mice up to 40% and increasing of life span up to 280%. As for tumor ~300 mm^3, the use of MTCT with slime aspiration and the invasions of cyclophosphamide into metastases led to 200% increased life span.

Keywords: Nanoparticles; Dextran-ferrite; Tumors; Invasions; Metastases; MRI-adaptive magneto-thermo-chemotherapy.
PACS: 75.50.-y; 78.67.Bf; 82.70.Dd; 85.75.Ss**;** 87.64.-t; 87.80.-y.

INTRODUCTION

Early detection of invasions and metastases by magnetic resonance imaging (MRI) is an important problem in revealing of malignant tumors. During investigation of early stage oncogenesis and metastases by 7 Tesla MR-scanner "BioSpec 70/30" (Bruker), we have found that weak proton signals from small sites of pathogenic cells are neutralized by strong signals from normal tissues. To reveal the tumors by contrast-enhanced MRI, we have synthesized and tested Dextran-ferrite (DF) as the first step of our research. DF sol (DFS) was compared with some other currently used contrast agents. The second step was treating the tumors by combination of several procedures and drugs. Firstly, we used cisplatin (CP) and melphalan (MP), which are well-known chemotherapeutic drugs for treatment of the breast, lung, ovarian and others cancer. Besides, CP's and MP's activities can be increased by combining them with DFS [1,2]. The complex treatment that combines the magnetically controlled anticancer drugs, targeting them to the tumor by a gradient of permanent magnetic field (MF); heating tumor by AC MF with necrotic slime aspiration is well-known as magnetohydrodynamic thermochemotherapy [1].

This paper is devoted to MRI-adaptive MTCT. We aimed to apply this method on the murine mammary Ca-755 adenocarcinoma, Lewis lung carcinoma and B16 melanoma and compare with our previous results [1-6]. Besides we investigated MRI contrast enhancement by magnetic agents used for drug targeting and tumor hyperthermia [7]. The goal of such research is optimization of the treatment on oncogenesis by dynamic MRI that controls MTCT.

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It should be noted that MRI-enhancement and targeting of the drugs in the tumor tissues was studied in [1,8,9], the DF saturation magnetization and other physical, chemical, and biological properties were described in [4,10-12].

MATERIAL AND METHODS

The specific power absorption rate (SAR) of the magnetic materials was calculated from the time-temperature dependencies as the amount of power converted into the heat per gram of Fe, zeta-potential (ζ) and Gaussian/Nicomp distribution analysis of particle diameters of DF, chemotherapeutic drugs CP and MP combined with DF were studied as described in [1-6,10-12]. DF elemental analysis was studied using plasma atomic emission spectrometry with focus on the determination of Fe as described in Ref. [6,10]. Detection of superparamagnetic materials in DF antitumor drugs, in tumors and tissues *in vitro* and *in vivo* in mice were carried out by the "BioMag" device [7].

Superparamagnetic particles of Fe_3O_4 were synthesized by chemical co-precipitation method. Dextran-ferrite was synthesized from Fe_3O_4 nanoparticles by covering them with Dextran T10 obtained from "Sigma" company. The coated nanoparticles were separated magnetically. DF nanoparticles cores had diameters between 3 and 10 nm, with the hydrodynamic diameter of the coated nanoparticles being between 24 and 40 nm.

Targeting of the drugs in hypodermic and skin tumors tissues was performed by $SmCo_5$ bandages according to references [8,9] or by superconducting 7 Tesla magnet. The magnetic drug targeting and the DF concentration were monitored by the "BioMag" device [7].

We tested a 0.1% DFS in 7% dextran as MRI contrast agent for MTCT. For this purpose, the DF at the doses of 0.01, 0.05, and 0.08 mmol Fe/kg were injected into mice with mammary Ca-755 adenocarcinoma, Lewis lungs carcinoma and B16 melanoma.

To prepare tumor-bearing animals, the Ca-755 adenocarcinoma was implanted subcutaneously into the right axillary region in 150 female C57Bl/6j mice that were 6 – 10 weeks old by injection of 10^6 viable tumor cells in 0.2 ml of saline, pH 7.4. The B16 melanoma was implanted similarly in 60 female C57Bl/6j mice. The Lewis lungs carcinoma was implanted intramuscular into the right femoral region in 60 female C57Bl/6j mice. The cisplatin and melphalan containing 40% dextran-ferrite sol were used as chemotherapeutic drugs having anticancer properties for MTCT and cyclophosphamide (CPA) for the invasions and metastases systemic treatment.

Initially, we obtained native (no contrast agent) MR images of 60 mice with Ca-755 adenocarcinoma, 60 mice with Lewis lungs carcinoma and 60 mice with B16 melanoma. We used following parameters of scanning (TR/TE) to receive T1-, T2-, T2*-weighted images: (500/15 msec, spin-echo), (1900/80 msec, spin-echo) and (500/15 msec, gradient echo with 30^0 flip angle).

Then 0.01–1.0 ml DF (dose up to 12 mg Fe/kg) was injected into mice caudal veins. Hereafter DF-enhanced T_2-weighted MR-images were received within 1-24 h. In this case we detected MR-signal decrease in zones of accumulation of contrast agent.

The initial MTCT was performed in mice with Ca-755 separated into two sets of four groups (1,1`,2,2`,3,3` and 4,4`) depending on the mouse tumor volume. In the first set (groups 1-4), the tumor volumes V reached 45 mm^3 while in the second set (groups 1`-4`) V ranged from 30 to 300 mm^3. Volumes were calculated as $V = 0.5 \cdot (l \cdot n^2)$, where l was the length and n the width of the tumor. The control group 1 consisting of 20 mice was injected by 300 μl saline buffer. Ten mice from each of the 2nd, 3rd and 4th group were injected by 300 μl of CP and MP containing DF (net Fe_3O_4 weight: 83 mg; CP 40 μg; MP 4 μg; pH 7.4; ζ +13 mV; Ms 8.2 kA/m; SAR 260 W/g Fe). Simultaneously with the injection, a non-uniform MF was applied by $SmCo_5$ magnetic bandages (0.2 T induction and gradient 15 mT/cm) in order to keep and concentrate the nanoparticles in the tumor tissue [1,2]. Within 30 minutes mice were placed inside a 60x200 mm air-cooled inductor with RF power for MTCT. Using similar MTCTs for the tumors of ~300 mm^3 and treatment of metastases by injection into mice caudal veins CPA from 0.2 to 2.0 mg in dependence of its volume was performed. Interstitial CP, MP and CPA concentrations were determined by thin layer chromatography as described earlier [1-4]. Statistical analysis was done as described in Ref. [13].

RESULTS AND DISCUSSION

DF accumulation in the normal tissues was visualized by MRI and detected by the "BioMag" device. At invasion tubular adenocarcinoma Ca-755 cancer cells look as small channels which were distributed for limits of a lacteal channel or lobular. The tubes leave on border of a healthy tissue through cracks in a membrane of a capsule of a mature tumor. Having encountered on resistance of a membrane of a healthy tissue some tubes twist together as a spiral which will penetrate through a membrane and enters into a healthy tissue. In a healthy tissue the spiral is

untwisted and tubes are distributed on all volume, disseminating tumor cells (Fig. 1). The measurements have shown that DF concentration in normal tissues is 6 times higher compared with Ca-755 tissues. The DF appears to be a promising MRI-negative contrast agent for detection of early stage tubular form invasion and metastases (Fig. 1a), lobular form invasion and metastases (Fig. 1b), and mixed form invasions and metastases (Fig. 2) of Ca-755. It also might be used for the oncogenesis check at MTCT. Thus, experiments showed the 0.1-1.0% DFSs are MR-negative contrast agents that enhanced MRI visualization of the invasions and metastases. It should be emphasized that such early stages of the invasions and metastases were not detected by non-enhanced DF or magnevist. The plasma elimination half-life of DF observed by the "BioMag" instruments at 0.05 mmol Fe/kg was 20 min.

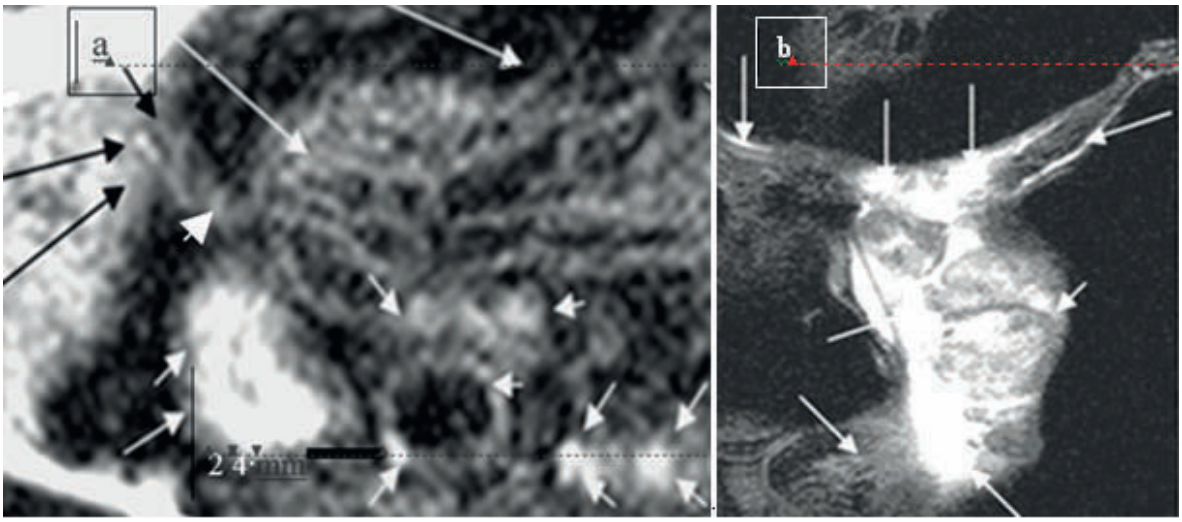

FIGURE 1. MRI of tubular form Ca-755 after DFS intravenous injections. An invasion of the mature tumor tubs in normal muscle tissues in group 2` mice: (a) a place in a membrane of a capsule of a tumor of the tubes leave on border of a healthy tissue (black arrows); a place in a membrane of a healthy tissue some tubes twist together as a spiral (the end of a white arrow), a place of an inlet, untwisting of the spiral and invasion of the tubes in normal tissues (major white arrows), metastases (short white arrows); (b) MRI of lobular form Ca-755: an invasions of cells in lymphatic system and other normal tissues (white arrows).

From 20 min to 24 h after intravenous injection of DF, a contrast MRI enhancement because of the invasions and metastases was clearly detected: in tumors - as clear spots on dark background (Fig. 1, 2). The histological, pathological and morphological analyses of lymph nodes and other organs revealed the presence of the invasions and metastases. DF-enhanced MRI has allowed to distinguish benign and malignant lymph nodes and to detect the invasions and metastases. Besides, with DF injection, it was possible to detect invasions and metastases of the Luis lungs carcinoma in the lungs and urine bladder in 40 mice of 50 with tumors from 120 to 300 mm^3, and B16 melanoma in the lungs and kidney in 42 mice of 50 with tumors from 150 to 300 mm^3.

TABLE 1. MTCT effect on small Ca-755 tumor (group 1-4) 36 days after the start of the treatment. The increase in the life span (ILS) corresponds to the number of treatments.

Group	Number of treatments	Average tumor volume±SD (mm^3)	Relative tumor volume	Complete regression (%)	(ILS) (%)
1	0	45.0 ± 14.0	1.00	0	0
2	3	32.4 ± 11.0	0.75	0	150
3	3	16.2 ± 6.0	0.36	20	180
4	4	4.4 ± 2.0	0.10	40	280

Relative tumor volume = (average tumor volume of each group)/(average tumor volume of group 1).

From 40% to 80% of DF, from 46% to 65% of CP and from 22% to 36% of MP were detected in the tumor after the first injection in groups 2, 2`, 3, 3`, 4 and 4`. Accumulation of DF in tumor was increased by magnetic bandage

targeting and revealed by the "BioMag" and MRI data. Besides ferrite aggregates accumulated in tumors were visible in Prussian blue stained sections.

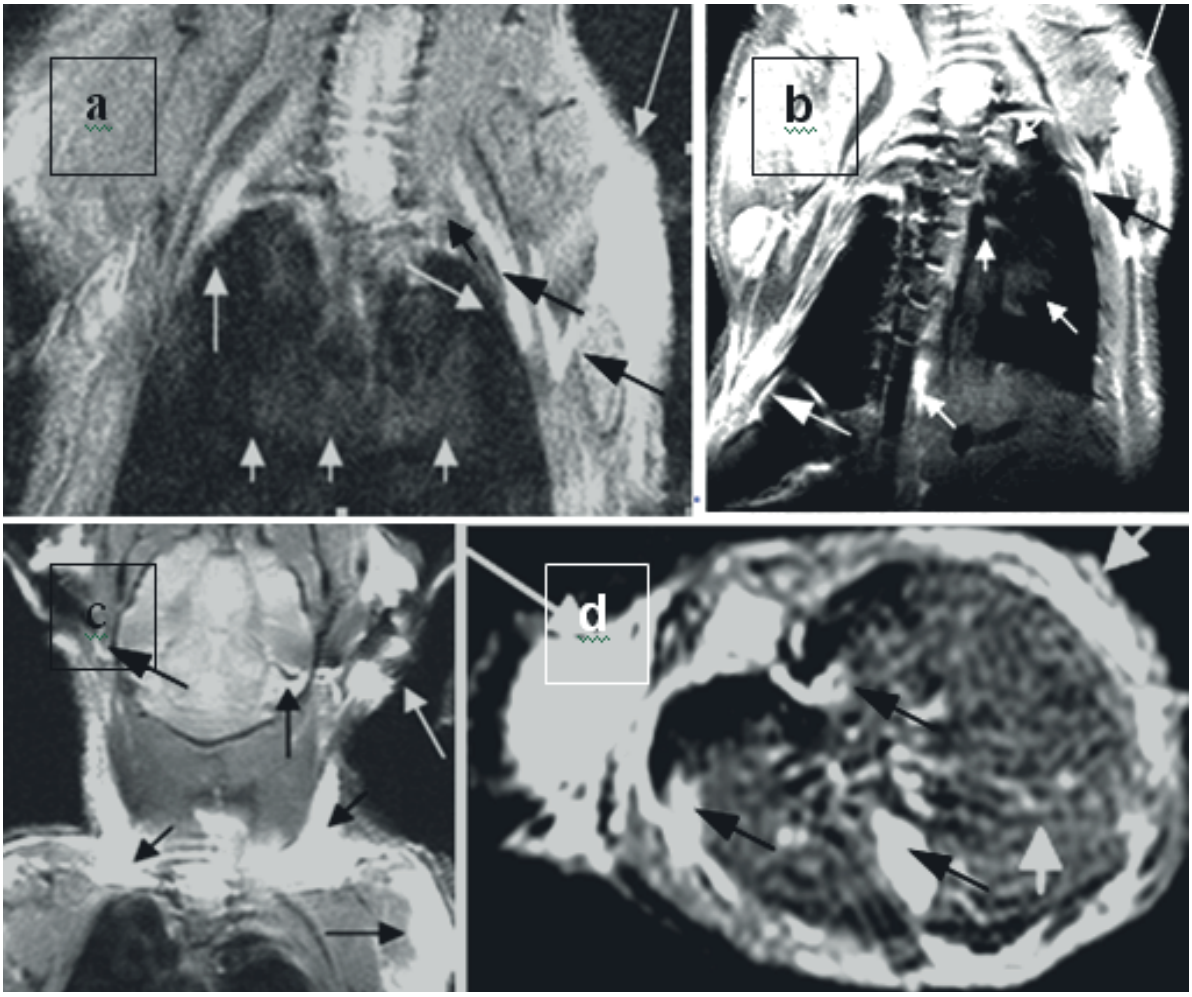

FIGURE 2. MR-imaging of oncogenesis of tubular, lobular and mixed forms of the Ca-755 that were implanted similarly: (a) lobular form of tumor relapse (outside long arrow) after 3 procedures of MTCTs and its tubular form invasion in the lungs (long arrows) and metastases (short arrows) after 39 days after Ca-755 implantation in group 3` mice; (b) invasion in the lungs (black arrow and short white arrows) and metastasis in the lungs and backbone after 10 days after MTCTs (short arrows) in group 2` mice; (c) lobular form of tumor relapse after 6 procedures of MTCTs (white arrow) and it invasion in the lymphatic system and brain after 29 days after MTCTs (black arrows) in group 4` mice; (d) lobular form tumor relapse (outside long arrow) after 3 procedures of MTCTs and its tubular form invasion in the lymphatic system and liver (short white arrow and black arrows), after 29 days after tumor MTCTs in group 4` mice.

TABLE 2. MTCT effect on large Ca-755 tumor (group 1`-4`) 36 days after the start of the treatment.

Group	Number of treatments	Average tumor volume±SD (mm^3)	Relative tumor volume	(ILS) (%)
1`	0	480.0 ± 150.0	1.00	0
2`	3	290.4 ± 91.0	0.60	94
3`	3	130.2 ± 42.0	0.27	158
4`	4	45.0±14.0	0.09	200

After the beginning of the invasions and metastases, 3 and 4 times of MTCTs demonstrated significant tumor responses over 36 days in groups 3, 3` and 4, 4` comparatively to control (groups 1 and 1`) and to groups 2 and 2`

(Tables 1, 2). Temperature of 43ºC for groups 2–4 and 2`–4` was reached after 6 min of MTCT when the AC MF (frequency of 0.88 MHz, amplitude 7.3 kA/m and power 0.15 kW) was used with CPA for the invasions and metastases systemic treatment. The temperature at the outer skin covering the tumor increased to about 45ºC and was maintained with an accuracy of at least ± 1ºC.

MTCT of ~45 mm^3 Ca-755 in C57Bl/6j mice by placement of the tumors in AC MF led to hyperthermia at 46ºC for 30 min. As the result such treatment led to tumor regression before metastases by 40%, and 280% increase of life span has been achieved (Table 1). The same treatment of ~300 mm^3 metastases tumors increased the animal's life span by 200% (Table 2). Using the similar MTCTs for B16 melanoma of ~30 mm^3 the tumor regression was 30% and extension of life span was 150%, while for Lewis lungs carcinoma ~30 mm^3 the tumor regression was 30% and the extension of life span was 160%.

CONCLUSIONS

MRI enhanced with the dextran-ferrite sol gives possibility to visualize the invasions and metastases of Ca-755 in the lymph system, lungs, brain and liver which were not palpable upon physical examination and could not be visualized using the non-enhanced MRI and Magnevist. This system is thus useful for treatment planning. Accumulation of DF in adenocarcinoma can be significantly increased by magnetic bandage targeting as revealed by "BioMag" instruments and MRI data. MRI-adaptive magneto-thermo-chemotherapy promoted an increase in the life spans of mice with Ca-755 adenocarcinoma, Lewis lungs carcinoma and B16 melanoma.

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