MAGNETOLIPOSOME MEDIATED LOCAL ELECTROMAGNETIC TUMOR HYPERTHERMIA

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Abstract

Magnetoliposomes prepared by enwrapping 8 nm sized superparamagnetic magnetite grains with phospholipid bilayer were evaluated as possible new material for local electromagnetic hyperthermia both in vitro and in vivo after their injection into implanted BP-6 tumor in rats. As has been found the center of tumor is heated in 10 minutes from 35°C to 44.1°C using magnetic field with induction 1.5 mT and frequency 3.5 MHz.

Keywords

magnetoliposomes, high-frequency magnetic field, ferrofluid, hyperthermia, cancer treatment

1. Introduction

It has been recognized that, when heat is applied to areas of animal tissue containing both normal and malignant cells, increase of temperature in these areas to the range 42-45°C lead to preferential destruction of the malignant cells. Examinations of tumors subjected to such heat treatments (known as hyperthermia) have revealed that the tumors undergo specific destruction with no substantial damage to adjacent normal cells such as fibroplasts and endothelial cells [1,2]. Heating to higher temperatures up to 56 °C, yielding widespread necrosis, coagulation or carbonization is called thermo-ablation. The combined effect of radiation and hyperthermia takes place at the cellular level and is mainly due to the heat induced malfunction of repair processes after radiation induced DNA damage. Moreover, heat treated cancer cells may be better recognized by the host immune system due to the alterations of some cell surface receptor molecules.

Hyperthermia is therefore a very promising approach for cancer therapy. Local heating in well-defined regions is a challenging point to be achieved in hyperthermia [3].

This goal was accomplished partially by the physical phenomena of hysteresis heat loss of ferromagnetic materials placed within the cancer tissue and then heated in a low frequency alternating electromagnetic field. The ferromagnetic microparticles are delivered into the tumor either by transarterial catheter injection of its specific blood supply, or by direct injection into the tumor body [4,5].

In our recent studies we have shown that liposomes (lipid vesicles) with encapsulated sub-monodomain ferrofluid particles (magnetoliposomes-MLs) may be effectively targeted to desired site in the rat body using strong external magnetic field [6]. Encapsulated ferrofluids are suspensions of magnetic particles of size much smaller than a magnetic domain (1-100 nm). A carrier liquid (coatings) prevents the particles from aggregation. These subdomain superparamagnetic particles produces substantially more heat per unit mass than the 1000 times larger multidomain ferrite particles of similar composition [7], when exposed to a high-frequency magnetic field. The mechanism of heating is based on Brownian relaxation (rotation of the particle as a whole according to external magnetic field) and Neél effect (reorientation of the magnetisation vector inside the magnetic core against an energy barrier). Due to their promising properties, we have analysed the possibility of magnetoliposomes as mediators in electromagnetic hyperthermia.

2. Material and methods

For the preparation of MLs we have used oleic acid stabilized superparamagnetic magnetic fluid with the following parameters: average particle diameter 8 nm and saturation magnetization $4\pi M$=40 kA/m [8], kindly supplied by Dr. V. Badescu (Iasi, Romania). These coated magnetite particles were encapsulated into liposomes prepared from soybean phosphatidylcholine (Sigma, USA) according to the procedure described in [9-12].

System for evaluation of the thermal property of magnetoliposomes is shown in Fig. 1. The system consisted of a high-frequency producing unit (GV6A, ZEZ Rychnov, Czech Republic) with 6 kW generator, giving a high-frequency magnetic field with frequency 3.5 MHz and induction 1.5 mT (measured according to the Faraday law in the middle of the irradiation cell using small coil connected to the high-frequency voltmeter, positioned in the direction perpendicular to the magnetic field [13]) in three-turn pancake coil, thermometry system consisting from copper-constantane thermocouple connected to voltmeter and reference stabilized thermal bath.

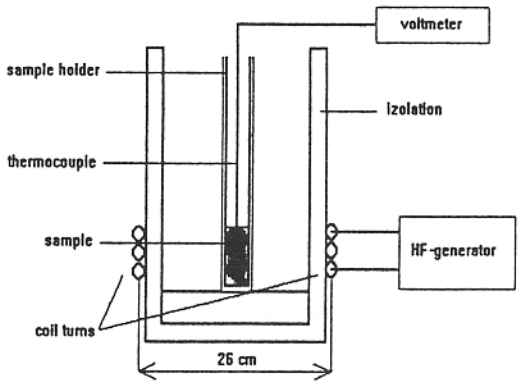

Fig.1 Experimental setup for the evaluation of magnetoliposome heating property in a high-frequency magnetic field.

3. Results and discussion

Fig. 2 shows the heating of MLs suspension in alternating magnetic field for various contents of encapsulated magnetite (spectrophotometrically determined according to iron content).

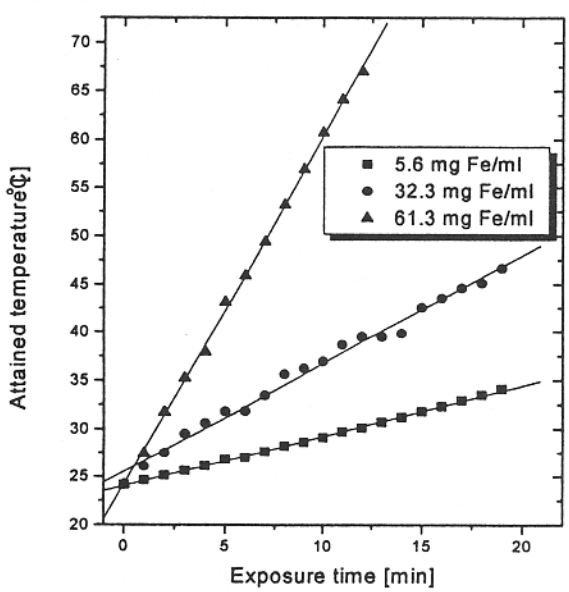

Fig. 2. Heating of magnetoliposome suspension in a high-frequency magnetic field

We have performed also preliminary *in vivo* experiments to evaluate heating capabilities of MLs in the living organisms. For these purposes BP-6 cells derived from a rat sarcoma induced by 3,4-benzpyrene were used. Three adult female Sprague-Dawley rats 200 g in weight were inoculated with $2x10^6$ cells in 0.5 ml of saline subcutaneously in the right and left posterior flanks. Tumors were allowed to grow for 27 days when the average size in length and wide was 1.5 cm. Before the hyperthermic treatment the rats were anaesthetized by administration of 0.1 mg/kg Atropin s.c., 10 mg/kg of Rometar i.m. and 90 mg/kg of Narkamon i.p. and then 1 ml of MLs suspension in saline buffer with total Fe concentration 61.3 mg/ml was injected into the center of tumor using a 24-gauge needle. Rats with injected MLs were subsequently exposed to the high-frequency magnetic field. Temperature in the center of tumor was measured after the treatment by inserting thermocouple fiber into the desired site. Optimal increase of temperature to the 44.1°C was achieved after 10 min exposure. We have measured also surface temperature in other parts of the body and also temperature in the center of tumor without the injected MLs using the same method, and as we have found that the initial rat temperature ~35°C increased at most by 2°C.

These our results therefore represents MLs as a promising material suitable for localized tumor treatment.

The suggested method may be further improved by covalent coupling of antibodies against neoplastic cells to the surface of magnetoliposomes which allow more specific delivery of heat to malignant cells. Moreover the presented approach may be useful also for the treatment of AIDS, via the thermal inactivation of HIV viruses, after their binding to the HIV-specific CD4 receptors reconstituted on the MLs surface [14,15].

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