

## Effect of Functional Magnetic Particles on Radiofrequency Capacitive Heating: An *in vivo* Study

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**Specific heating of magnetic particles in radiofrequency (RF) capacitive hyperthermia and its hyperthermic effect were investigated in an *in vivo* study. Magnetite cationic liposomes (MCLs) were injected into a rat tumor on the femur and 8 MHz-RF capacitive heating was applied to the rat under ‘mild heating’ conditions. Although the input power of RF capacitive heating was low under the same power conditions, the MCLs-injected tumor was heated over 43°C, whereas it was only heated to 41°C in the case of the rats not injected with MCLs. A necrotic area in the tumor was observed in the heated rats. From the results of histological observation of the removed tissue, the necrotic area in the MCLs-injected tumor was wider than that in MCLs-free tumor. Complete tumor suppression was observed in 71% (5/7) of MCLs-injected rats, and the hyperthermic effect was greatly improved by the MCLs.**

Key words: RF capacitive heating — Magnetic particle — Regional hyperthermia

Hyperthermia is a promising approach in cancer therapy and various methods are employed to achieve it.<sup>1,2)</sup> However, the inevitable technical problem with hyperthermia is the difficulty of uniform heating of only the tumor region to the required temperature without damaging normal tissue. Therefore, some researchers have proposed inductive heating methods using submicron magnetic particles for hyperthermia.<sup>3–5)</sup> We have also developed ‘magnetite cationic liposomes’ (MCLs) for intracellular hyperthermia.<sup>6–8)</sup> MCLs were developed to improve adsorption and accumulation into the tumor cells and showed ten-fold higher affinity for the tumor cells than neutrally charged magnetoliposomes.<sup>6)</sup> This is enabled by the electrostatic interaction with the negatively charged cell membrane.<sup>9,10)</sup> The hyperthermic effect of the MCLs was examined *in vivo*.<sup>8)</sup> MCLs were directly injected into solid tumors formed subcutaneously in F344 rats and the rats were irradiated three times for 30 min with an alternating magnetic field. An alternating magnetic field with relative low frequency of the order of 100 kHz heated only the tumor containing the MCLs. As a result, complete tumor regression was observed in many rats after the irradiation.

Inductive heating using submicron magnetic particles is a superior method as stated above, but it cannot yet be practiced in Japan since magnetic field generators are still in an experimental stage. In Japan, capacitive heating of tumors using a radiofrequency (RF) electric field is a popular heating method and has been clinically used.<sup>11,12)</sup> Capacitive heating is, however, not suitable for site-spe-

cific hyperthermia, because it is unable to heat tumors specifically. The specific adsorption rate (SAR) of electric field energy depends on the electrical properties of each tissue, such as permittivity and electric resistance. Since the difference in the electrical properties of tumors and normal tissues is not high, it is difficult to heat only the tumor specifically. In order to prevent excessive heating of normal tissue, ‘mild’ heating conditions are often applied. Heating of the tumor is then often insufficient and complete suppression of the tumor is rather difficult. Against this background, we demonstrated that magnetic particles could be used as a good heating generator for RF capacitive heating,<sup>13)</sup> like inductive heating using an alternating magnetic field in an *in vitro* experiment. The magnetite-containing agar phantom was heated higher than the magnetite-free phantom under the same heating conditions, and SAR in the phantom was improved 1.5 times by the magnetite particles.<sup>13)</sup> The rate of temperature increase was approximately proportional to the magnetite concentration to the power 0.8. We concluded that the magnetite particle is a promising heating mediator for capacitive heating.

In the present paper, we simulated a case of ‘mild heating’ in hyperthermia treatment and investigated the enhancement of hyperthermic effect by magnetic particles and the temperature profiles in RF capacitive heating *in vivo*.

### MATERIALS AND METHODS

**Materials** Dilauroylphosphatidylcholine (DLPC) and dioleoylphosphatidylethanolamine (DOPE) were purchased from Sigma Chemical Co. (St. Louis, MO), and *N*-( $\alpha$ -

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trimethylammonioacetyl)didodecyl-D-glutamate chloride (TMAG) was from Sogo Pharmaceutical Co. (Tokyo). Mouse anti-rat monoclonal antibody and goat anti-mouse monoclonal antibody were purchased from Dainippon Pharmaceutical Co. (Suita). All other chemicals were obtained from Wako Pure Chemical Industries Co. (Osaka).

**Preparation of the MCLs** Magnetite ( $\text{Fe}_3\text{O}_4$ ; average particle size: 10 nm), used as the core of the MCLs, was a kind gift from Toda Kogyo Co. (Hiroshima). MCLs were prepared by the sonication method, as described previously.<sup>6</sup> All MCL concentration values are expressed as the net magnetite concentration.

**Cell culture and tumor-bearing animals** The rat glioma cell line T9 was used in order to compare the results with those in the case of the induction method used in our previous paper.<sup>8</sup> The cells were maintained at 37°C in a 5%  $\text{CO}_2$  atmosphere in Eagle's minimum essential medium (Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum, 5 mM nonessential amino acids, and antibiotics (100 U/ml penicillin, and 100  $\mu\text{g}/\text{ml}$  streptomycin).

Female Fischer 344 (F344) rats, 6 weeks old, were purchased from Japan SLC (Hamamatsu). To prepare tumor-bearing animals, rat glioma T9 cell suspensions (approximately  $1 \times 10^7$  cells in 0.1 ml of phosphate buffer (0.05 M sodium phosphate and 0.15 M NaCl, pH 7.4)) were injected subcutaneously into the left femur of F344 rats under short-term anesthesia by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The rats were then separated into three groups. Animals in group I, the control group, were not injected with the MCLs or subjected to RF field irradiation, while group II was not injected with the MCLs, but was subjected to RF field irradiation, and group III was injected with the MCLs and subjected to RF field irradiation. Each group consisted of 10–13 rats. Five to ten animals in each group were used for monitoring tumor growth; the others were employed for the observation of histological features of various tissues. Tumor sizes were measured every 2 days. The volume was determined by using the following formula<sup>14</sup>:

$$\text{tumor volume} = 0.5 \times (\text{length} \times \text{width}^2)$$

where the unit of length and width is the millimeter. The rats for histological observation were sacrificed by injection of an excess volume of pentobarbital sodium solution and the tumor tissue was removed and fixed with 10% formaldehyde solution when the hyperthermia treatment was finished. The removed tissue was observed histologically. Animal experiments were performed according to the principles laid down in the "Guide for the Care and Use of Laboratory Animals" prepared under the directions of the Office of the Prime Minister of Japan.

**In vivo hyperthermia** On the 11th day after transplanta-

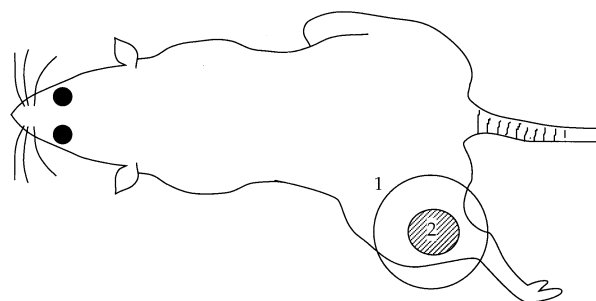


Fig. 1. A schematic illustration of the configuration in the rat experiment. 1, outline of RF electrode and water pad; 2, tumor.

tion, when ellipsoidal tumors 13–18 mm in length had formed, hyperthermia treatment was started. First, the power of the electrodes was decided for each rat. The rat tumor was set between a pair of electrodes, which were covered with water pads in order to prevent skin burning, as shown in Fig. 1. In this study, an 8 MHz RF capacitive heating device (Thermotron RF-IV, Yamamoto Vinyter Co., Osaka) was used. A 20-mm electrode (upper) and a 30-mm electrode (lower) were used. A salt solution (34°C) was perfused into the water pads for electrode temperature control. The power of electrodes was set so as to heat the tumor center to 41°C. Normally, it was set at 60 W for 5 min and then at 40 W thereafter. The pattern of power input was modified for each rat, and the temperature of the tumor center usually became stable within 10 min. When the power level had been chosen, the power was turned off, then the needle (needle size: 25 G) of a syringe containing MCL solution was inserted in the longitudinal direction subcutaneously from the tumor edge for group III rats and MCLs were injected. After 1 h, the power was turned on again. For group II rats, the same procedure was done without injection of MCLs. Temperature was measured using a Teflon-coated micro thermocouple that was inserted into the tumor through a 21-gauge angiocatheter. The angiocatheter was inserted from the same point to the same depth used for the MCLs injection. Other micro thermocouples were set between the skin covering the tumor and the water pad. The micro thermocouples were connected to a Thermotron RF-IV or a recorder (Yokogawa Electronics Co., Tokyo) and monitored by these devices.

## RESULTS

### Effect of magnetic particles on heat generation *in vivo*

In this experiment, about 3 mg of MCLs was injected at the center of the tumor, and approximately 2.4 mg of MCLs was accumulated in the tumor. The MCLs were highly localized around the point where the needle was inserted. Fig. 2 shows typical profiles of temperature

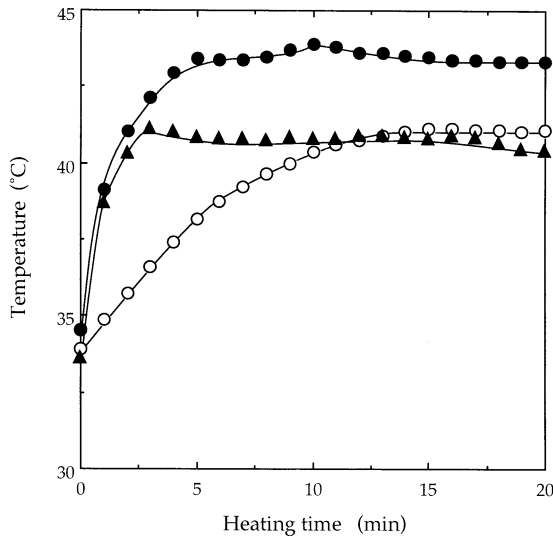


Fig. 2. Typical temperature rise of the tumor with or without MCL. Closed and open circles, tumor center and tumor surface contacting the water pad in group III (RF field irradiation with MCL); closed triangles, tumor center of group II (RF field irradiation alone).

increase in group II and III rats. In the case of group II, the temperature was controlled at about 41°C from the time after 3 min by applying a predetermined power to the RF heating device, which corresponds to ‘mild’ heating condition. The heat balance between input power and heat dissipation by blood flow reached a steady state. In the case of group III with MCLs in the tumor, the temperature increased rapidly over 43°C after 5 min even though the power input was the same as in the case of group II. At and after 5 min from the start, the temperature was kept at 43–44°C. The temperature of the tumor surface, which is the interface between the tumor and the water pad, gradually increased and reached 41°C at 10 min after the start. This pattern was the same as in the case of group II.

**Heat generation in an *in vivo* experiment** Fig. 3 shows the time courses of tumor growth in rats of each group. The tumor volume in group I animals steadily increased with no evidence of regression. In the case of group II animals, tumor growth was partially suppressed, but all tumors except one grew again. In contrast, complete tumor suppression was observed in many group III animals. All these data are summarized in Table I. Complete tumor suppression was observed in 71% (5/7) of group III animals, whereas no tumor suppression was observed in group II animals.

Fig. 4 shows HE-stained specimens from each group. Histological observation of the removed tissue showed that the necrotic area in the group III specimen was wider than that in the group II specimen. In the group II speci-

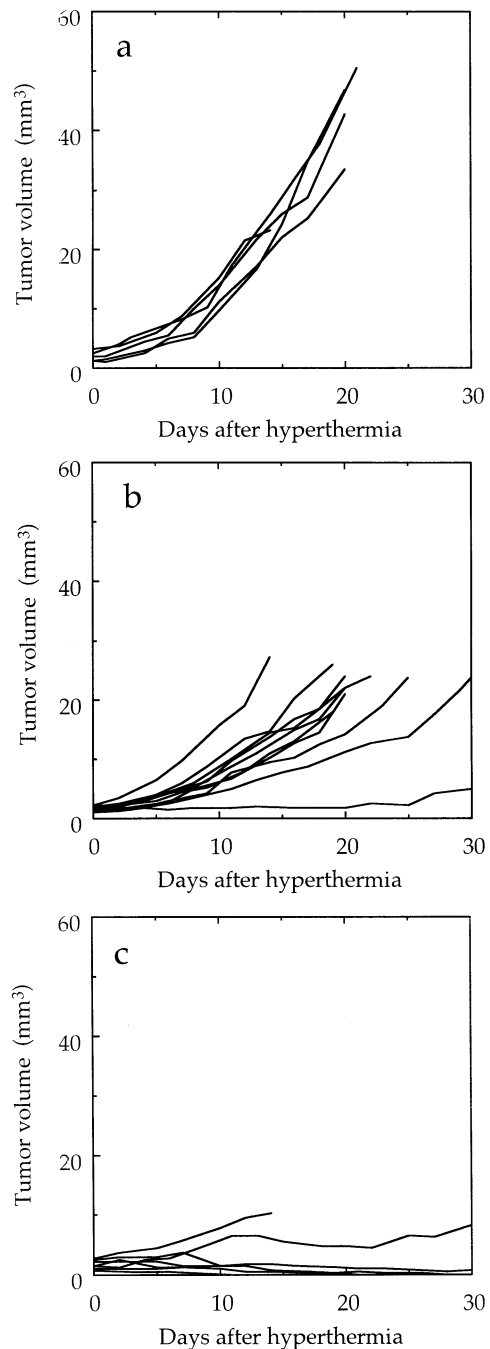


Fig. 3. Time course of tumor growth. a, group I (control); b, group II (RF field irradiation alone); c, group III (RF field irradiation with MCL).

men, the necrotic area spread towards the body side of the tumor, because the electrode side of the tumor was cooled by the water pad on the electrode. In spite of the same power condition, the whole tumor in the group III animals

was heated. Therefore, the hyperthermic effect was greatly improved by the MCLs.

**DISCUSSION**

Based on these results, it was concluded that magnetite particles could effectively enhance a hyperthermic effect under a ‘mild’ condition of capacitive RF heating. In the present study, we examined mild or insufficient heating conditions, as well as severe heating conditions. For rats without MCL injection, the temperature of the tumor was kept at 43–44°C for 20 min. Very severe damage to normal tissue was observed; burnt skin was observed in many rats and some rats died unexpectedly after hyperthermia in our experimental conditions. Therefore, we had to decrease the tumor temperature from 43–44°C to 41°C.

However, mild or insufficient heating leads to potential treatment failure. In regional hyperthermia including capacitive RF heating, the heating process is sometimes stopped owing to problems such as overheating of normal tissue or algia of skin. As shown in Fig. 2, the presence of the magnetite particles in tumor tissue could improve the heating, and the terminal temperature reached 44°C from 41°C. This may be due to the localization of electric current to the tumor region or heat generation from magnetite particles. In hyperthermia, a difference of 3°C can be critical. When heating at 41°C for 40 min was applied to T9 cells *in vitro*, 15% of the cells survived.<sup>6)</sup> However, at 3°C higher temperature, T9 cells were completely killed.<sup>6)</sup>

In the previous study of inductive heating using MCLs, three successive heatings led to complete regression of the tumor, since the MCLs diffused throughout the necrotic

Table I. Hyperthermic Effect on Solid Glioma Tissue Formed Subcutaneously

Group	Number of rats tested	Treatment	Tumor take	Average tumor volume <sup>a)</sup> (cm <sup>3</sup> )	Complete regression <sup>b)</sup> (%)
I	5	No irradiation	5/5	25.5±3.8	0
II	10	RF heating alone	10/10	12.1±6.6	0
III	7	RF heating+MCL	2/7	2.6±3.8	71

a) Tumor volume = 0.5 × (length × width<sup>2</sup>) at day 14 after hyperthermia.

b) Data at day 30 after hyperthermia.

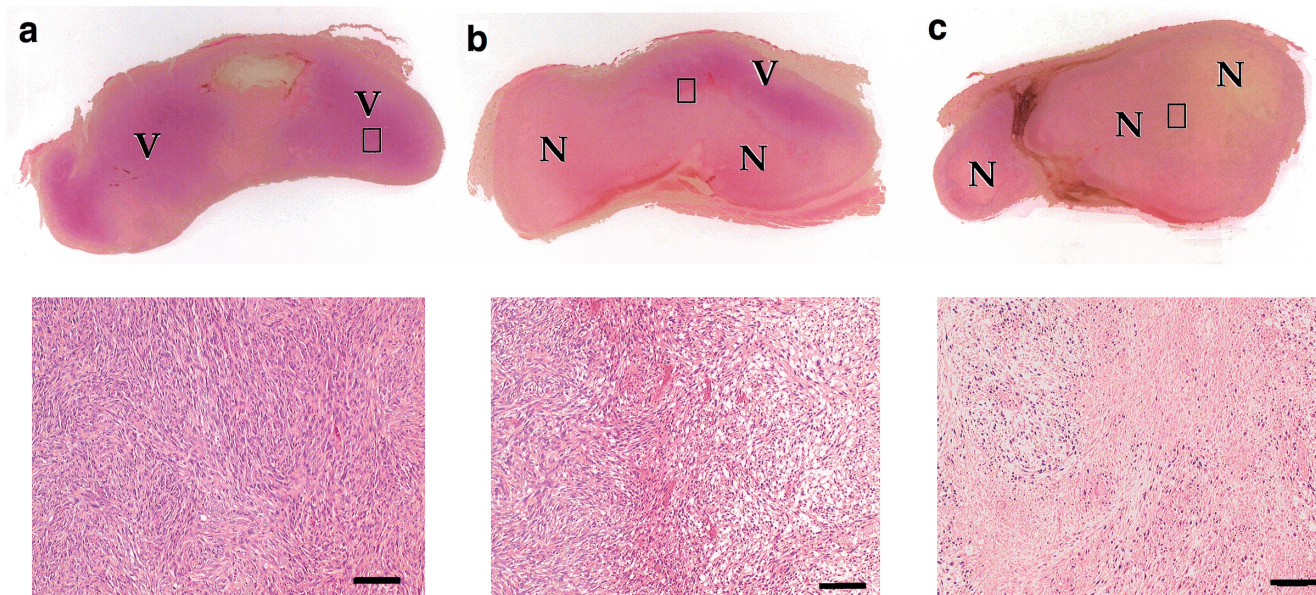


Fig. 4. Photographs of tumor specimens and light microscopic sections histologically stained with HE. Tissues removed from rats at the 4th day after the MCL injection. Upper, section of whole tumor; lower, microscopic section. a, group I (control); b, group II (RF field irradiation alone); c, group III (RF field irradiation with MCL). V, N and rectangles in the upper photos indicate viable tissue, necrotic tissue, and the sites of the lower photos, respectively. Scale bars in the lower photos indicate 100 μm.

tissue and the MCL area became wider with the repeated irradiation.<sup>8)</sup> In order to heat the tumor throughout with one heating, multiple injections of MCLs should be investigated for the inductive heating. On the other hand, in the case of RF capacitive heating, SAR of the magnetite in RF heating is lower than that in inductive heating.<sup>13)</sup> However, RF capacitive heating could heat all of the tumor area and almost all the tumor tissue became necrotic, as shown in Fig. 4, even in a single heating. Therefore, it was concluded that the MCLs helped to heat the tumor to an 'effective' temperature (Fig. 4). RF capacitive heating alone could heat at least half of the tumor (group II). The MCLs simultaneously heated the whole of the tumor at the same electrode power in the group III rats. As a result, almost of the tumors regressed with only one heating.

A major problem for capacitive RF heating is excess heating of subcutaneous fat, and unexpected heating owing to edge effects.<sup>15)</sup> Applying a surface cooling system using water pads covering the electrodes solves these problems.<sup>12)</sup> In the present study, we also used water pads to avoid excess heating of skin covering the tumor. However, such a cooling system makes it difficult to control temperature, and effective heating could not be achieved around the tumor area near the skin, as shown in Fig. 4b. Although a viable tumor region was observed, the severe burn region spread to normal tissue and bleeding was also observed. In order to solve this problem, temperature monitoring using many temperature sensors will be needed. On the other hand, with MCLs, effective heating was observed even around the tumor area near the skin. The terminal temperature at the surface of the tumor (open circle in Fig. 2) was 41°C, but the tumor was wholly

necrosed by hyperthermia (Fig. 4c). It means that the combination of RF heating and the MCLs can uniformly heat the tumor.

The delivery method of magnetic particles to the tumor will be a major problem. In the present study, the MCLs were injected directly into the tumor, but this method is limited to easily accessible tumors. We have demonstrated specific delivery to mouse renal cell carcinoma using G250 antibody-conjugated magnetoliposomes (G250-FML).<sup>16)</sup> The G250-FMLs were specifically delivered to the cancer by intravascular injection and only the tumor was heated under alternating magnetic field irradiation. If good antibodies are developed for other types of tumor, similar specific delivery of magnetic particles could be applied and selective heating of the tumor will be possible.

In conclusion, a selective heating method for RF hyperthermia was presented. It was shown that magnetic particles can work to concentrate RF energy in a specific part of the body. By tumor-specific heating, an effective hyperthermic effect was obtained in spite of the condition of mild hyperthermia.

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