Effect of Functional Magnetic Particles on Radiofrequency Capacitive Heating

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Magnetic particles (magnetite) were used to make radiofrequency (RF) capacitive hyperthermia effective to a specific site. In an agar phantom experiment, a magnetite-containing agar piece was buried in a large agar phantom and heated by an 8 MHz-RF capacitive heating device. The magnetite-containing agar piece was heated more than the magnetite-free agar phantom, and the specific adsorption rate in the phantom was increased 1.5 times by the magnetite particles. The temperature distribution in the large agar phantom showed that the highest temperature was obtained at the center of the magnetite-containing piece. The rate of temperature increase was approximately proportional to the magnetite concentration to the power 0.8. This method was applied to an in vivo experiment using a pig. Magnetite was prepared as a colloidal material dispersed in a carboxymethylcellulose solution (CMC-Mag) and intramuscularly injected in the pig femur. As a result of 8 MHz-RF heating, the temperature at the CMC-Mag-injected point increased to over 43°C after 7 min, while the temperature at a point without magnetite was under 40°C at the same time. The specific adsorption rate in the magnetite-containing tissue was twice that of the magnetite-free tissue. In addition, the time required to reach a temperature of over 43°C was only 7 min, while it was over 15 min in the case without the CMC-Mag.

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

Capacitive heating of tumors using a radiofrequency (RF) electric field was first reported by LeVeen et al.1 However, their method caused excessive heating of fat layers, and has been largely replaced by induction heating in the USA and Europe. In Japan, capacitive heating has become a popular heating method and has been clinically used.2, 3 The method can be used to heat large- and deep-seated tumors. A great advantage of capacitive heating is that it is non-invasive. On the other hand, the capacitive heating method is not suitable for site-specific hyperthermia, because it is unable to heat tumors only. The specific adsorption rate (SAR) of electric field energy depends on the electrical properties of each tissue, such as permittivity and electric resistance, and the difference in the electrical properties of tumors and normal tissues is not large. Therefore, additional heating mediators or implantable heating applicators are needed to concentrate the electric field to a specific tissue.4, 5 However, only a few mediators have been proposed and those that have been developed have been insufficiently effective.

For induction heating, which is an alternative heating method for generating hyperthermia, many heating mediators have been proposed, such as ferromagnetic needles,6, 7) glass-ceramic particles8) and colloidal magnetic iron oxides.9,10) In particular, colloidal magnetic iron oxide can be metabolized and excreted from the body without surgical removal, so that this material is superior to the others. We have also reported antibody-conjugated magnetoliposomes (MLs) and magnetite cationic liposomes (MCLs) as induction heating mediators and demonstrated their hyperthermic effect in in vitro and in vivo studies.12-14) The MCLs showed a ten-fold higher affinity for rat glioma cells than neutrally charged MLs, because their positive surface charge can promote the adhesion of MCLs to the cells.

In the present paper, we investigated the possibility of using magnetic particles (magnetite) as a heating mediator for RF capacitive heating in agar phantoms. Furthermore, the profiles of the temperature increase were investigated in an in vivo experiment.

MATERIALS AND METHODS

Magnetite Magnetite (Fe3O4) particles (average size, 25 nm) were kindly provided by Toda Kogyo Co., Ltd. (Hiroshima). Colloidal magnetite dispersed in a carboxymethylcellulose solution (CMC-Mag) was prepared as follows. Carboxymethyl cellulose (CMC) sodium salt was purchased from Wako Pure Chemical Co., Ltd. (Osaka). Magnetite (0.1 g) was added to 10 ml of 4% CMC at 60°C while stirring with a glass impeller. After mixing for 30

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min, the suspension was sonicated for 15 min by a probe-
type sonicator (Ohtake Works, Tokyo) at 40 W, and well-
dispersed CMC-Mag was obtained. The magnetite con-
centration was measured by the potassium thiocyanate
method.\textsuperscript{15}

Preparation of phantom A 4\% agar gel was used for
preparation of the phantom. Agar was purchased from
Wako Pure Chemicals Co. A small cylindrical agar piece
containing the magnetite (magnetite piece) was prepared
by a modification of the method of Jordan \textit{et al}.
\textsuperscript{11} Briefly, the magnetite was added to liquid agar at 60°C while stir-
ing with a glass impeller. After mixing for 30 min, the
suspension was sonicated for 15 min by a probe-type soni-
cator at 40 W. The sonicated mixture (20 ml) was poured
into a polypropylene cylinder (28 mm in diameter) and
was rapidly cooled by placing the cylinder in ice water.
Fig. 1A shows a schematic illustration of the phantom. A
large phantom piece (315 mm $\times$ 122 mm $\times$ 93 mm) was
bored with a cork borer, and the cylindrical magnetite
piece was inserted in the large phantom piece. The remain-
ing space was filled with 4\% agar. An agar piece without
magnetite for control experiments (control piece) was also
inserted in a symmetric position with respect to the center
axis of the electrode (see Fig. 1B).

In \textit{vitro} RF hyperthermia An 8 MHz-RF capacitive
heating device (Thermotron RF-8, Yamamoto Vinyter Co.,
Osaka) was used. The diameter of the pair of electrodes
for the phantom was 25 cm. The electrode was covered
with a water pad but cooling water was not circulated.
The configuration of the phantom and the electrodes is shown
in Fig. 1B. Power control of the electrodes was performed
by the standard method used in practical clinical situations
in the Department of Urology, Nagoya City University
Medical School. SAR (W/g) was calculated by use of the
following equation:

$$\text{SAR} = c \frac{dT}{dt}$$

where $c$ is the specific heat capacity (J/°C/g), $T$ is the tem-
perature increase (°C) and $t$ is the time (s). The value of $c$
was 4.18 for the agar phantom and 3.85 for muscle of pig.
$\frac{dT}{dt}$ was calculated from the slope of the temperature
increase when the input power was constant (e.g., 7–9 min
from the start of heating as shown in Fig. 2).

Temperature was measured using a Teflon-coated
microthermocouple that was inserted into the phantom
through a 21-gauge angiocatheter. The microthermocou-
bles were connected to the Thermotron RF-8 or a recorder
(Yokogawa Electric Co., Ltd., Tokyo).

In \textit{vivo} RF hyperthermia A female pig (10 weeks old,
30 kg) was obtained from Morishita Farm (Mie). The pig
was premedicated with pentobarbital sodium (15 mg/kg)
intraperitoneally and atropine sulfate (0.5 mg) intramuscu-
larly. Anesthesia was maintained by additional intravenous
administration of ketamine chloride (1 mg/ml). Ten millili-
ters of CMC-Mag (10 mg/ml) was injected intramuscu-
larly into the right femur as shown in Fig. 3. The needle
for injection was inserted to 50 mm below the skin sur-
face. For setting of the microthermocouple, a 21-gauge
angiocatheter was inserted from the same point until the
same depth as used for the magnetite injection. Another
angiocatheter was inserted at a point 50 mm distant from
the magnetite-injected point as a control. The microther-
mocouples were connected to the Thermotron RF-8 and
monitored. A pair of 30-cm electrodes was used for \textit{in}

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Fig. 1. Schematic illustration of the phantom (A) and the configuration of the phantom and the electrodes of the RF capacitive heating device (B). 1, phantom; 2, control piece; 3, magnetite piece; 4, RF electrode; 5, water pad.
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vivo hyperthermia. A salt solution for the electrode temperature control was perfused into the water pad. Power control of the electrodes was performed by the method described above. When the experiment was finished, the pig was killed by injection of a large volume of pentobarbital sodium solution and the muscle tissue around the CMC-Mag-injected point was removed and fixed with 10% formalin solution. The removed tissue was observed histologically.

RESULTS

Heat generation in the phantom Fig. 2 shows the temperature increases of the magnetite piece and the control piece in the agar phantom. The concentration of the magnetite was 10 mg/ml, and the room and initial agar temperatures were 20°C. The power of the electrodes was raised step by step as shown in the upper graph of Fig. 2. The temperature of the magnetite piece increased by 9°C at 5 min and by 24°C at 9 min. On the other hand, the temperature in the control piece increased by only 6°C at 5 min and by 16°C at 9 min. The temperature difference between the two agar pieces reached about 8°C at 9 min. SAR values were 265 mW/g-agar for the magnetite piece and 181 mW/g-agar for the control piece. Therefore, SAR of the phantom was improved 1.5 times by the magnetite particles.

Fig. 4 shows the temperature distribution in the magnetite piece containing 10 mg/ml of magnetite. All values represent the temperature increase after 10 min treatment. When only agar (control piece) was inserted in the phantom, the highest temperature was observed at the center axis of the electrode. The temperature increase then gradually declined from the center of the phantom toward the edge of the electrode (data not shown). On the other hand, when the magnetite piece was inserted in the phantom,
the temperature reached its highest value (26.8°C) at the center of the magnetite piece, and the temperature difference between this point and the control piece reached 8.3°C at 10 min after the start of heating. Furthermore, the highest temperature point moved to the site of the magnetite piece from the center of the phantom and reached a temperature that was 6.0°C higher than that at the center of the phantom.

Fig. 5 shows the time courses of the temperature difference between the magnetite piece and the control piece for various concentrations of magnetite. The temperature difference increased with the magnetite concentration. For example at 9 min, the increase was 1.3°C at 1 mg/ml (net 20 mg magnetite), 5.1°C at 5 mg/ml (net 100 mg magnetite) and 7.7°C at 10 mg/ml (net 200 mg magnetite).

**Heat generation in an in vivo experiment** Fig. 6 shows the temperature profiles during the RF heating in the *in vivo* experiment. Ten milliliters of CMC-Mag (10 mg/ml) was injected intramuscularly. The power of the electrodes was raised step by step as shown in the upper graph. The temperature at the point of the CMC-Mag reached over 45.0°C at 10 min, while the temperature at the control point rose from 38.0°C at the start of the experiment to only 40.7°C at 10 min. It is clear that heating was specifically enhanced at the CMC-Mag site. It should be noted that when the temperature at the CMC-Mag site reached over 43°C, the temperature at the control point was still under 39.8°C. This means that damage to normal tissue by excess heating can be avoided by decreasing the input power once the temperature at the CMC-Mag site reaches 43°C.

Histological observation of the removed tissue confirmed that the CMC-Mag existed intracellularly. In addition, the tissue was not damaged by the hyperthermia, because the heating time was short (15 min). Unfortunately, we could not observe the magnetite distribution, because the magnetite particles were washed out from the tissue section during the preparation of the histological specimen.

**DISCUSSION**

Based on these results, we concluded that magnetite can effectively heat the region in which the magnetite particles

| Magnetite concentration [mg/ml] | SAR value [mW/g] | Net SAR value [mW/g] |
|--------------------------------|-----------------|---------------------|
| 0                              | 181             | 0                   |
| 1                              | 195             | 14                  |
| 5                              | 240             | 59                  |
| 10                             | 265             | 84                  |

Net SAR value = (SAR of magnetite piece) − (SAR of control piece).
are present. Furthermore, this effect depended on the magnetite concentration. Table I shows the SAR value for each magnetite concentration. From the calculated value of net SAR, it was found that the relationship was approximately proportional to the magnetite concentration to the power 0.8. The reason for this is unclear. However, the SAR of the phantom was clearly increased by the presence of the magnetite. The reason why the SAR increased is also unknown, but the change of dielectric loss in the phantom by the magnetite is one possibility. Another is hysteresis loss in the magnetite owing to the induced magnetic field accompanying the current induced by the RF electric field.

In the case of the in vivo experiment, the temperature difference between the CMC-Mag and the control increased immediately after the heating was started, and after 9 min, the temperature increase rate at the CMC-Mag site decreased and became similar to that at the control point. Since both points were in normal tissue, an increase of blood flow may have cooled the tissue. The difference was significantly greater than that in the in vitro experiment (Fig. 2). SAR values at CMC-Mag and the control sites were 61 mW/g-tissue and 26 mW/g-tissue at 8 min, respectively. These values were lower than those in the agar phantom, because total injected magnetite was half that of the phantom experiment. However, the difference in the SAR values was about 2.4 times greater, which was higher than that in the in vitro experiment. It seems that the electrical properties of tissues were more affected by the magnetite injection than those of the agar phantom. In the present study, the magnetite distribution in the tissue could not be measured. A high concentration region of magnetite particles might locally exist in the tissue. Further investigation is needed to explain these phenomena.

Several problems in regional deep heating with capacitive RF at the early stage include excess heating of subcutaneous fat, unexpected heating by edge effects, and insufficient penetration depth. Hiraoka et al. overcame these difficulties by applying a surface cooling system, using electrodes covered with a water pad, and a pair of large electrodes. These improvements led to clinical application of RF capacitive heating in Japan. However, the time required to heat the tissue to the effective temperature, the so-called temperature accelerating period, was occasionally extended to over 30 min. Additionally, a heating period of more than 30 min is preferred for killing tumor cells. For patients, such a long heating period is a severe burden. Furthermore, a long temperature accelerating period may result in the malignant tissue becoming heat-tolerant. Hasegawa et al. have reported that quick heating results in a greater hyperthermic effect than does slow heating. In the present study, we demonstrated that quick heating is possible, as shown in Fig. 6. Because of the use of magnetite, the temperature accelerating time required to heat the tissue to over 43°C was only 7 min, while it was over 15 min in the case without the CMC-Mag. Furthermore, specific heating was possible as shown in Figs. 4 and 6. Therefore, our proposed method should be effective in terms of both selectivity and speed.

In conclusion, a selective heating method for RF hyperthermia was developed. It was shown that magnetic particles can work to concentrate RF energy to a specific part of the body and to shorten the temperature accelerating period.

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REFERENCES

1) LeVeen, H. H., Ahmed, N., Piccone, V. A., Shugaar, S. and Falk, G. Radiofrequency therapy: clinical experience. Ann. NY Acad. Sci., 335, 362–371 (1980).

2) Hiraoka, M., Jo, S., Akuta, K., Nishimura, Y., Takahashi, M. and Abe, M. Radiofrequency capacitive hyperthermia for deep-seated tumors. Cancer, 60, 121–127 (1987).

3) Hiraoka, M., Nishimura, Y., Nagata, Y., Mitsumori, M., Okuno, Y., Li, P. Y., Abe, M., Takahashi, M., Masunaga, S., Akuta, K. and Koishi, M. Site-specific phase I: II trials of hyperthermia at Kyoto University. Int. J. Hyperthermia, 10, 403–410 (1994).

4) Kamiyama, Y., Nakagawa, A., Matsu, Y., Nakagawa, M., Takai, S., Ututsuji, S., Kubo, N. and Nakase, Y. Selective thermocoagulation of unresectable malignant tumors using radiofrequency. Hyperthermic Oncol., 2, 195–196 (1996).

5) Ohmoto, Y., Fujisawa, H., Ishikawa, T., Koizumi, H., Matsuda, T. and Ito, H. Sequential change in cerebral blood flow, early neuropathological consequences and blood-brain barrier disruption following radiofrequency-induced localized hyperthermia. Int. J. Hyperthermia, 12, 321–334 (1996).

6) Stauffer, P. R., Cetas, T. C. and Jones, R. C. System for producing localized hyperthermia in tumors through magnetic induction heating of ferromagnetic implants. Natl. Cancer Inst. Monogr., 61, 483–488 (1982).

7) Kobayashi, T., Kida, Y., Tanaka, T., Kageyama, N., Kobayashi, H. and Amemiya, Y. Magnetic induction hyperthermia for brain tumor using ferromagnetic implant
with low Curie point. *J. Neurooncol.*, 4, 175–181 (1986).

8) Luderer, A. A., Borreli, N. F., Panzarino, J. N., Mansfield, G. R., Hess, D. M., Brown, J. L. and Barnett, E. H. Glass-ceramic-mediated, magnetic-field-induced localized hyperthermia: response of murine mammary carcinoma. *Radiat. Res.*, 94, 190–198 (1983).

9) Tazawa, K., Nagase, T., Kasagi, T., Maeda, M., Sawadaishi, M., Odagiri, H., Shinbo, T., Karaki, Y., Fujimaki, M. and Honda, T. Intracellular hyperthermia for the treatment of cancer (I): raising the high temperature with exciting submicron particles. In “Hyperthermia Cancer Therapy,” pp. 276–277 (1986). Mag. Bros. Inc.

10) Chan, D. C. F., Kirpotin, D. B. and Bunn, P. A., Jr. Synthesis and evaluation of colloidal magnetic iron oxides for the site-specific radiofrequency-induced hyperthermia of cancer. *J. Magn. Magn. Mater.*, 122, 374–378 (1993).

11) Jordan, A., Wust, P., Fähling, H., John, W., Hinz, A. and Felix, R. Inductive heating of ferromagnetic particles and magnetic fluids: physical evaluation of their potential for hyperthermia. *Int. J. Hyperthermia*, 9, 51–68 (1993).

12) Shinkai, M., Matsui, M. and Kobayashi, T. Heat properties of magnetoliposomes for local hyperthermia. *Jpn. J. Hyperthermic Oncol.*, 10, 168–177 (1994).

13) Shinkai, M., Yanase, M., Honda, H., Wakabayashi, T., Yoshida, J. and Kobayashi, T. Intracellular hyperthermia for cancer using magnetite cationic liposomes: *in vitro* study. *Jpn. J. Cancer Res.*, 87, 1179–1183 (1996).

14) Yanase, M., Shinkai, M., Honda, H., Wakabayashi, T., Yoshida, J. and Kobayashi, T. Intracellular hyperthermia for cancer using magnetite cationic liposomes: an *in vivo* study. *Jpn. J. Cancer Res.*, 89, 463–469 (1998).

15) Owen, C. S. and Sykes, N. L. Magnetic labeling and cell sorting. *J. Immunol. Methods*, 73, 41–48 (1984).

16) Harn, G. M., Kernahan, P., Martinez, A., Pounds, D. and Prionas, S. Some heat transfer problems associated with heating by ultrasound, microwaves, or radio frequency. *Ann. NY Acad. Sci.*, 335, 327–346 (1980).

17) Kato, H., Horaoka, M., Nakajima, T. and Ishida, T. Deep-heating characteristics of an RF capacitive heating device. *Int. J. Hyperthermia*, 1, 15–28 (1985).

18) Hasegawa, T., Hara, K., Asoh, H., Soejima, A. and Yamamoto, I. *Jpn. J. Hyperthermic Oncol.*, 13, 175 (1997) (in Japanese).