RAPID COMMUNICATION Jpn. J. Cancer Res. 88, 630–632, July 1997

Intracellular Hyperthermia for Cancer Using Magnetite Cationic Liposomes: *Ex vivo* Study

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Heating properties of magnetite cationic liposomes (MCL) were investigated in *ex vivo* experiments using implanted cell pellets. The cell pellets, which consisted of rat glioma T9 cells into which MCL had been incorporated in a petri dish, were implanted subcutaneously in the left femoral region of female F344 rats. The rats were placed in a magnetic field generating coil and irradiated with an alternating magnetic field (384 Oe, 118 kHz) for 60 min. The cell pellets were heated to over 43°C by MCL in the magnetic field, but other body parts of the rats were not heated. After 3 cycles of magnetic heating, all glioma cells were killed and no tumor take was observed.

Key words: Cationic liposome — Magnetite — Magnetoliposome — Intracellular hyperthermia — Glioma cell

We have developed magnetite cationic liposomes (MCL) as an intracellular heating mediator.[1] The MCL show ten times higher affinity for glioma cells than that of the magnetoliposomes with neutral charge, because of the positive charge on the surface. Due to this high affinity for the cells, the MCL incorporated in cells were able to heat a glioma cell pellet of 80 μl in volume (5.4 mm in diameter) to above 43°C in an alternating magnetic field (384 Oe, 118 kHz). The viable cell number decreased to about one-thirtieth of that of the control after 20 min of irradiation, and after 40 min of irradiation, no viable cells remained in *in vitro* experiments. But cells which did not incorporate the MCL showed no decrease of the viable cell number under 60-min irradiation. In the present paper, we report the heating properties of the MCL in the body. We implanted rat glioma cell pellets, which contained MCL,[1] subcutaneously in the left femoral region of the rats and applied an alternating magnetic field. We observed the effect of intracellular heating of the glioma cells on tumor take.

We examined the hyperthermic effect of the MCL using female F344 rats (7–8 weeks old) and rat glioma T9 cell pellets. The MCL were prepared with colloidal magnetite[2] and a lipid mixture of *N*-(α-trimethylammonioacetyl)didodecyl-D-glutamate chloride (Sogo Pharmaceutical Co., Ltd., Tokyo), dilauroylphosphatidylcholine and dioleoylphosphatidylethanolamine (Sigma Chemical Co., St. Louis, MO) of 1 : 2 : 2 molar ratio according to our previous method.[1] The cell pellets were prepared as reported.[1] Rat glioma T9 cells (1×10^6) were incubated for 8 h with MCL-containing medium (20 μg/ml). The cells were maintained at 37°C in a 5% CO_2 atmosphere in Eagle's minimum essential medium (Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum, 5 m*M* nonessential amino acids, and antibiotics (100 U/ml of penicillin and 100 μg/ml of streptomycin sulfate). All pellets were implanted subcutaneously in the left femoral region of the rats under general anesthesia with Nembutal. The volume of the pellets was about 150 μl. The magnetic field irradiation was performed by using a horizontal coil (inner diameter: 7 cm, length: 7 cm) with a transistor inverter (LTG-100-05, 5.0 kW, 118 kHz, Dai-ichi High Frequency Co., Ltd., Tokyo). The magnetic field is theoretically almost homogeneous (384 $\pm$20 Oe) within the range of 90% of the radius (3.2 cm from the center) from the center and within the range of 42% of the coil length (3 cm) from the center of our coil.[3] The rats were laid inside the coil and the region of implanted cell pellets was placed at the center of the coil. Fig. 1 shows temperature profiles at the center of the implanted cell pellet and in the rectum. The temperature at the center of a cell pellet under irradiation was measured with an optical fiber thermometer inserted under the skin. The temperature at the pellet was elevated quickly by magnetic heating and reached over 43°C after 20 min. This result agreed with the *in vitro* data.[1] In contrast, the temperature in the rectum remained between 35 and 36.5°C during the magnetic heating. It was confirmed that the magnetic field generated by our equipment did not affect regions without MCL. Fig. 2 shows

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percent tumor take after the magnetic heating. A tumor take was defined as a palpable lump over 3 mm in thickness. Each group consisted of 5 rats. In the control experiment with no magnetic field exposure (group I), tumor take was observed in 80% of rats at 5 days after and in all rats at 6 days after implantation. Tumor take was observed in only one of the group II rats irradiated once for 60 min after implantation. Cell pellets were implanted in subcutaneous space via a syringe. Therefore, it is likely that the shape of the implanted pellet was different in each rat. Since the temperature increase upon irradiation of the MCL was influenced by the shape of the implanted pellet, it seems that there was an inhomogeneity of the temperature increase. However, multiple exposure to the magnetic field was effective. In the case of group III irradiated three times for 60 min at 12 h intervals, no tumor take was observed up to 90 days in

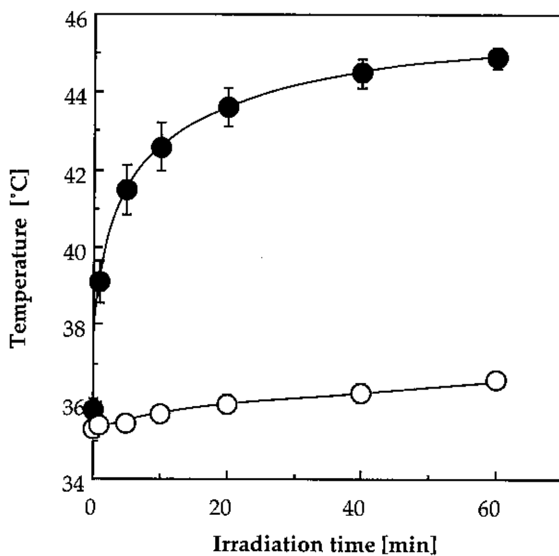

Fig. 1. Temperature increase of cell pellet and the body in an alternating magnetic field. Symbols: ●, in the cell pellet; ○, in the rectum.

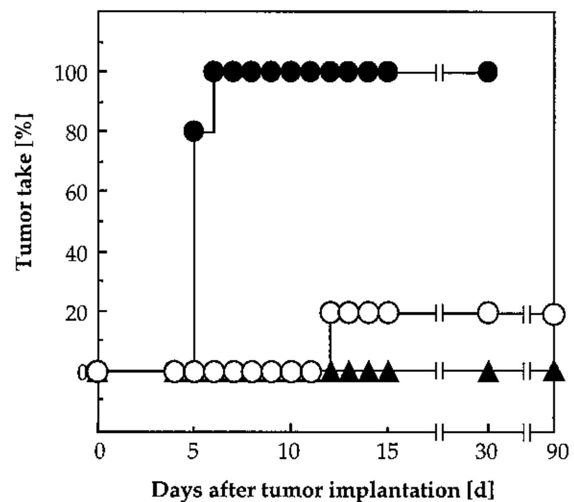

Fig. 2. Tumor take of implanted T9 cell pellets. Symbols: ●, group I (control); ○, group II (rats were irradiated for 60 min once); ▲, group III (rats were irradiated for 60 min three times every 12 h). All of the group I rats and one of the group II rats which showed tumor take were sacrificed at the 30th day after the start of the experiment.

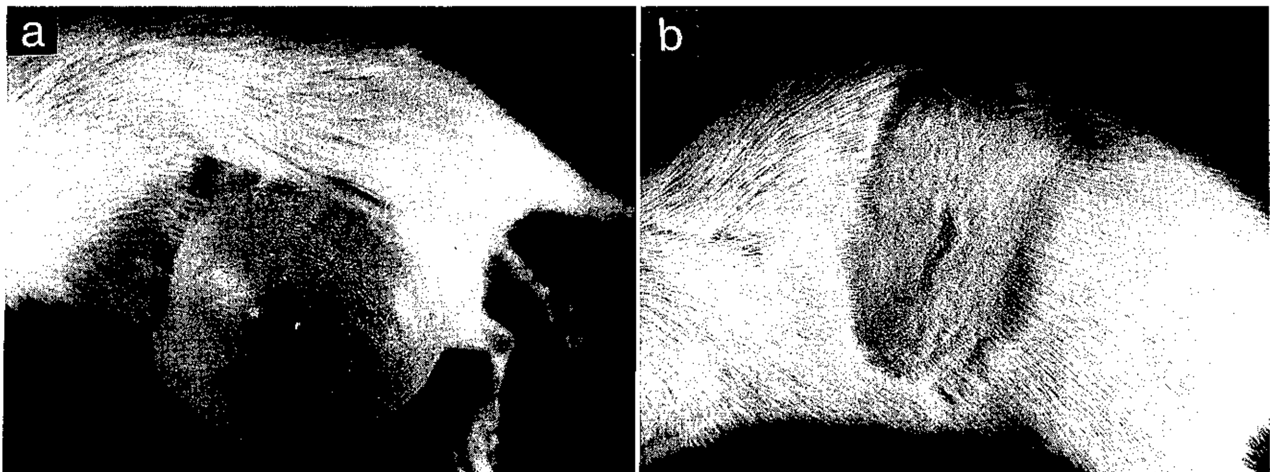

Fig. 3. The rats at the 28th day after the start of the experiment; a and b show one of the group I rats and one of the group III rats, respectively.

all rats. The difference of the result from group I was clear, as shown in Fig. 3, a and b. Fig. 3 shows the rats at the 28th day after the start of the experiment. In the animal shown in Fig. 3b (one of group III), a small burn was observed on the skin (black spot). No burn region was found on the muscle under the cell pellet. The skin burn is not due to eddy current heating, because in another experiment without MCL, temperature increases at the skin and the cell pellet were almost equal to that in the rectum (data not shown). The blood flow rate in muscle has been reported to be about half that in the skin,[4] but the absolute blood volume in the muscle is larger than that in the skin, so that the muscle in contact with the cell pellet was considered to be more efficiently cooled than the skin. In the future, when MCL is applied to brain tumor, temperature control will be necessary during heating, taking account of the blood flow rate, MCL concentration, tumor mass and tissue mass. Computer simulation and control are important for safety during hyperthermia operations, as discussed previously.[5]

In the present study, we could completely suppress tumor cell growth by multiple exposures to the alternating magnetic field. This may be an optimum result, because all the tumor cells uniformly incorporated the MCL. Next we intend to study the effect of the MCL against real tumors. If the MCL can be incorporated in almost all the tumor cells, it should prove to be highly effective.

(Received April 8, 1997/Accepted May 30, 1997)

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