Original Contribution

Magnetite Needle as Heating Mediator for Intracellular Hyperthermia of Tumor

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Abstract : Magnetite needle, in which magnetite particles are molded into needle-shape, was developed and we applied it as a heating mediator for intracellular hyperthermia. We investigated the hyperthermic effect on solid subcutaneous gliomas in the femoral region of female F344 rats. Magnetite needles were inserted into the solid tumors, which were then irradiated by an alternating magnetic field (118 kHz, 384 Oe). The rats were divided into a control group that was not irradiated (control group), or a group that was irradiated for 30 min three times (treatment group), then we evaluated the hyperthermic effect on tumor growth. Tumors completely regressed in 75% of the treated rats. Histological observations showed that magnetite particles in the control group were localized only around the point of the tumor where the needle was inserted. However, the particles in the treatment group were widespread, and necrotic cells were observed throughout almost the entire tumor tissue. The magnetite particles were flowed away by blood flow as the tumor regressed, and so the needles did not require removal from the femoral region. Moreover, the administration of two magnetite needles required only three minutes, although the magnetite cationic liposomes were injected over at least 120 min to prevent backflow. These results suggest that magnetite needles are simple and effective therapeutic particulate heating mediators that can be clinically applied to treat tumors.

Key Words : glioma cells, heating mediator, induction heating, intracellular hyperthermia, magnetite needle

Introduction

Hyperthermia using heating mediators is a promising approach to uniform heating of only tumor areas to a desired temperature without invading normal tissue ^{1) 2)}. Inductive heating methods have been investigated that use sub-micron magnetic particles as heating mediators ³⁻⁵⁾. We have also developed 'magnetite cationic liposomes'(MCLs) applicable for intracellular hyperthermia ⁶⁻⁸⁾. These liposomes improve adsorption and accumulation into the tumor cells and have ten-fold higher affinity for tumor cells than neutrally charged magnetoliposomes ⁶⁾. Because of this feature, MCLs can be superior heating mediators in hyperthermia treatment. We demonstrated a significant effect of MCLs in the rat tumor

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model. However, when tumors are located in organs with high blood flow such as the brain, the temperature cannot rise sufficiently to kill tumor cells, since heat is dissipated by the blood flow.

On the other hand, needle-type heating mediators have been developed for use in hyperthermic therapy ^{1) 2) 9) 10)}. Such needle-type mediators can generate heat and increase temperature in organs with high blood flow. However, needles should be removed from the body after hyperthermia ^{2) 10)}.

The present paper describes the new magnetite particles molded into needle shape and their application as a heating mediator to perform intracellular hyperthermia. This product has a characteristic of needle-type heating mediator, and temperature can be increased in organs with high blood flow. Furthermore, the magnetite needles revert into magnetite particles, disperse in the organ after hyperthermic treatment, and are gradually removed from the organ. Therefore, the magnetite needles are not necessary to be removed from the body after hyperthermia. The magnetite needles have several features, such as ease of manipulation, improved target accuracy and a higher concentration of magnetite (10 mg of $\text{Fe}_3\text{O}_4/\text{ml}$).

Materials and Methods

Materials

Carboxymethyl cellulose (CMC) and all other chemicals were obtained from Wako Pure Chemical Industries Co. (Osaka). Magnetite particles (average particle size : 25 nm) were a gift from Toda Kogyo Co. (Hiroshima).

Preparation of magnetite needles

Magnetite particles (640 g) and CMC (160 g) were suspended in 340 ml of water for 90 min, and thin sticks were formed by an extruder. These sticks were air-dried at room temperature for 2 days and heated at 80-100°C for 9 h. The resultant magnetite needles were approximately 0.6 mm in diameter and contained 1.83 grams of magnetite per milliliter. This preparation was performed by Tokai Carbon Co. (Tokyo).

Cell culture

The rat glioma cell line T-9 was 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 mM nonessential amino acids, and antibiotics (100 U/ml penicillin, and 100 mg/ml streptomycin).

Preparation of tumor-bearing rats

Rat glioma T-9 cell suspensions (approximately 1×10^7 cells in 100 ml phosphate buffer (0.05 M Na phosphate and 0.15 M NaCl, pH 7.4)) were subcutaneously injected into the left femoral regions of female F344 rats (6-7 weeks old) under short-term intraperitoneal Nembutal anesthesia. The rats were then divided into control and treatment groups ($n = 7-10$ each group). Animals in the control group were not exposed to a magnetic field, whereas the treatment group was exposed three times. Tumor growth was monitored in five to eight rats in each group and histological features of various tissues were examined in the remainder. Tumor volume was measured every two days using the following formula ¹¹⁾ :

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

where the units of length and width are in centimeters.

Hyperthermia for subcutaneous tumor

Fig. 1. shows that magnetite needles can be delivered via a syringe through an indwelling catheter with 20G $\times$ 32mm (Venura syringe, Top Co., Tokyo) into tumor tissue (Fig. 1B).

Eleven days after injecting T-9 cell suspensions, ellipsoidal tumors had reached 10 - 15 mm in length. A catheter was inserted in the longitudinal direction subcutaneously from the tumor edge, then two magnetite needles (each 1 cm in length ; total magnetite weight, 11.5 mg) were inserted into the tumor and positioned at a distance 5 mm apart. Twenty-four hours later, the rats were anesthetized and exposed to a magnetic field (frequency and intensity, 118 kHz and 30.6 kA/m (384 Oe), respectively) established by use of 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., Tokyo). The magnetic field within the coil was expected to be almost homogeneous (384 ± 20 Oe) within a range of 90% of the radius (3.2 cm from the center) and 43% of the coil length (3 cm from the center)⁷⁾. Anesthetized rats were positioned inside the coil such that the tumor region was at the center. The magnetic field was applied for 30 min and repeated three times at 24-h intervals. Tumor and rectal temperatures were measured using an optical fiber probe (FX-9020 ; Anritsu Meter Co., Tokyo).

Fig. 1. Photographs of magnetite needles. For injection into tumors, the magnetite needle is inserted through a syringe and catheter. Photographs are before (A) and after (B) assembly.

Preparation of specimens for histological observation

Tumors were removed 24 h after needle insertion or after three exposures to hyperthermia. Blood was replaced with phosphate buffer, then the tumor was extracted, fixed with 10% formalin, and longitudinally sectioned. Pathological specimens were cut into sections of 6 mm thickness, and stained with hematoxylin and eosin.

Animal experiments proceeded according to the "Guide for the Care and Use of Laboratory Animals" prepared under the directions of the Office of the Prime Minister of Japan.

Results

Temperature increase by magnetite needles in alternating magnetic field

We measured the temperature at the outside of the tumor by use of the inserted magnetite needles. The hyperthermic effect at the peripheral area of the tumor would be weaker than that at the inside of the tumor. Fig. 2. shows the temperature increase outside the skin covering the tumor caused by the first magnetic field exposure. The temperature of the tumor was rapidly elevated and reached over 43°C after 5 min. Compared with MCLs, the temperature increase was rapid in the case of the magnetite needle if the same power was applied since the concentration of magnetite in the magnetite needle was much higher than that of MCLs. When magnetic field exposure was extended, the tumor temperature at the outside of the skin continued to increase up to 50°C. Therefore, the power of the magnetic field was manually controlled to maintain the tumor temperature at around 44°C. The profile of temperature increase in the second or third exposure was similar to that during the first exposure to magnetic field by controlling the power of the magnetic field.

Fig. 2. Temperature increases at tumor surface (open circles) and rectum (closed circles) during magnetic field exposure.

Monitoring tumor growth after hyperthermia

Fig. 3. shows the time courses of tumor growth in each group. The tumor volume in the control rats steadily increased with no evidence of regression. In contrast, tumors completely regressed in many of the exposed rats. In most instances of complete regression, the tumor volume continued to increase in the first five days, then began to decrease and finally disappeared. Tumors did not reappear after complete regression for over a period of 3 months (data not shown).

The data from Fig. 3 are summarized in Table I. The incidence of complete tumor regression in the treated rats was 75%. The rats were also weighed for 90 days. The gains of rats with completely regressed glioma tumors were similar to those of control rats without tumors (data not shown), and severe

Fig. 3. Time courses of tumor growth after magnetite needle insertion. Control group was not exposed to magnetic field. Treatment group was exposed to a magnetic field three times at 24-h intervals.

Table 1. Hyperthermic effect on subcutaneous tumors 16 days after magnetite needle insertion.

Group	n	Average tumor volume ^a (cm ³)	Relative tumor volume ^b	Complete regression (%)
Control	4	11.1	1	0
Treatment	8	1.69	0.15	75

a: Tumor volume = $0.5 \times (\text{length} \times \text{width}^2)$

b: Relative tumor volume = (average tumor volume of each group) / (average tumor volume of control)

side effects such as unusual behavior or hematuria were absent.

Histological observation revealed that the magnetite needles existed at the position where the needles were inserted before the first hyperthermic treatment, reverted into particles and dispersed widely in the tumor tissue after three hyperthermic treatments, which was similar in the case of MCLs ⁷⁾. The areas where the particles had spread closely coincided with the necrotic area in the treatment group. The necrotic area spread with repeated treatments, and it never surpassed the corresponding areas containing magnetite particles. In contrast, particles were localized in the control group only around the areas where magnetite needles were inserted.

Discussion

The effect of hyperthermia depends on whether the tumor tissue is heated uniformly to over 43°C so that the tumor tissue is killed by necrosis. Therefore, a sufficient quantity of heat mediator must be accumulated in the tumor. Wada *et al.* reported that the terminal temperature depended on the dose of dextran magnetite complex (DM) in the normal tongue of the golden hamster ¹²⁾. In the previous paper ⁷⁾, we reported that a total of 3 mg of MCLs could heat 1 ml of subcutaneous tumor to temperatures above 43°C. Heat is generated by the magnetite particles when the magnetic field is applied, and is

removed by the blood flow around the tumor. In fact, 3.5 mg/ml of MCLs could not heat 0.1 ml of brain tumor in a mouse since blood flow in the brain is much faster than that in subcutaneous tumors in the femoral area. When the diameter of the affected region is halved at the same concentration of the mediator, heat evolution per volume drops to 1/8, but heat transfer via the surface of the affected area drops to only 1/4. Therefore, concentrated magnetite solution should be administered to double the concentration in the tumor. Compared with DM and MCLs, our heat mediator can be prepared at a higher concentration (1.83 grams of magnetite per milliliter), which means that more magnetite can be delivered to the tumor tissue. The present study used two pieces of the magnetite needles to heat tumors. The total quantity of magnetite particles was 11.5 mg, which generated a very high temperature, since this amount of particles correspond to 1.6 ml of MCLs. This is an advantage of the needle-type magnetite heating mediators since injecting 1.6 ml of the MCLs into 1 ml of tumor is obviously difficult.

To inject 1.6 ml of MCLs into a tumor, the time required would be at least two hours since backflow should be prevented. On the other hand, two magnetite needles were easily inserted within 3 min without inducing the cerebral pressure changes by liquid injections. This presents another advantage of magnetite needles in a clinical therapeutic environment.

Many needle-type heating mediators such as ferromagnetic tumor implants have been proposed ^{1) 2) 9) 10)}. Prediction of the temperature distribution by means of computer simulation is not hard if the blood flow rate around the tumor tissue is known. However, these protocols have some downsides, such as accidental shifting in the affected part or troublesome removal from the body. The magnetite needles proposed here are made of magnetite particles with about 25 nm and carboxymethyl cellulose. As binding ability of carboxymethyl cellulose is not so strong, the magnetite needles became individual magnetite particles where they were inserted in tissues, and dispersed gradually in tissues after repeated heatings. As described above, the profile of temperature increase in the second or third exposure was similar to that during the first exposure to magnetic field. Therefore, sufficient magnetite particles that can heat tumor still remained before the third exposure. We found here that the magnetite needles or particles disappeared completely after tumors had regressed. They may be eventually flowed away by blood flow or removed by phagocytic cells. In this way, it is not necessary to remove the magnetite needles after therapeutic treatment. This is not a feature of the conventional needle-type heat mediators and is therefore the third advantage of magnetite needles. The magnetite particles removed from the tumor tissue were deposited in liver, but they were disappeared from liver within a month. We could not detect the magnetite in other organs except spleen, but almost magnetites were also disappeared from spleen after a month. No severe side effects such as unusual behavior or hematuria were observed.

We used CMC as the bulking agent for the magnetite needles. Water-soluble CMC has no known toxicity and is widely used in drugs and foods. Oligosaccharides, polysaccharides, collagen or gelatin should also be effective as a CMC substitute.

Conclusion

Our new magnetite needles were used to treat solid tumors by hyperthermia *in vivo*. The tumor tissue completely disappeared in many rats exposed to a magnetic field, and the needles were simply administered through a syringe. The magnetite needle will be a novel and potentially effective mediator of hyperthermia that can be used to treat other malignant solid tumors without severe side effects.

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References

- 1) Stauffer P.R., Cetas T.C., Jones R.C. System for producing localized hyperthermia in tumors through magnetic induction heating of ferromagnetic implants. Natl. Cancer Inst. Monogr., 61 : 483-487, 1982.
- 2) Brezovich I.A., Atkinson W.J., Lilly M.B. Local hyperthermia with interstitial techniques. Cancer Res., 44 (Suppl. 10) : 4752s-4756s, 1984.
- 3) Tazawa K., Nagase T., Kasagi T., Maeda M., Sawadaishi M., Odagiri H., Shinbo T., Karaki Y., Fujimaki M., Honda T. Intracellular hyperthermia for the treatment of cancer (I) : Raising the high temperature with exciting submicron particles. Hyperthermia in Cancer Therapy, Mag. Bros. Inc., 276-277, 1986.
- 4) Jordan A., Wust P., Fahling H., John W., Hinz A., Felix R. Inductive heating of ferromagnetic particles and magnetic fluids : physical evaluation of their potential for hyperthermia. Int. J. Hyperthermia, 9 : 51-68, 1993.
- 5) Mitsumori M., Hiraoka M., Shibata T., Okuno Y., Masumaga S., Koishi M., Okajima K., Nagata Y., Nishimura Y., Abe M., Ohura K., Hasegawa, M., Nagae H., Ebisawa Y. Development of intra-arterial hyperthermia using a dextran-magnetite complex. Int. J. Hyperthermia, 10 : 785-793, 1994.
- 6) Shinkai M., Yanase M., Honda H., Wakabayashi T., Yoshida J., Kobayashi T. Intracellular hyperthermia for cancer using magnetite cationic liposome : *in vitro* study. Jpn. J. Cancer. Res., 87 : 1179-1183, 1996.
- 7) Yanase M., Shinkai M., Honda H., Wakabayashi T., Yoshida J., Kobayashi T. Intracellular hyperthermia for cancer using magnetite cationic liposomes : an *in vivo* study. Jpn. J. Cancer Res., 89 : 463-469, 1998.
- 8) Shinkai M., Yanase M., Suzuki M., Honda H., Wakabayashi T., Yoshida J., Kobayashi T. Intracellular hyperthermia for cancer using magnetic cationic liposomes. J. Magn. Magn. Mater., 194 : 176-184, 1999.
- 9) Kobayashi T., Kida Y., Ohta M., Tanaka T., Kageyama N., Kobayashi H., Amemiya Y. Magnetic induction hyperthermia for brain tumor using ferromagnetic implant with low curie temperature. Neurol. Med. Chir., 26 : 116-121, 1986. (in Japanese)
- 10) Stauffer P.R., Cetas T.C., Fletcher A.M., DeYoung D.W., Dewhirst M.W., Oleson J.R., Roemer R.B. Observation on the use of ferromagnetic implants for inducing hyperthermia. IEEE Trans. Biomed. Eng., BME-31 : 76-90, 1984.
- 11) Yoshida J., Mizuno M., Yagi, K. Secretion of human β -interferon into the cystic fluid of glioma transfected with the interferon gene. J. Clin. Biochem. Nutr., 11 : 123-128, 1991.
- 12) Wada S., Tazawa K., Furuta I., Takemori S., Minamimura T., Nagae H. Application of hyperthermia using dextran magnetite complex (DM) for head and neck cancer. Jpn. J. Hyperthermic Oncol., 14 : 197-205, 1998.

ガン組織内温熱療法用発熱体としての 針状成形マグネタイト

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要 旨: 針状成形マグネタイトを開発し, 細胞内温熱療法における加温材として使用した. 雌の F344 ラットの大腿部皮下に移植されたグリオーマ固形組織に対する温熱療法の効果を検討した. 針状成形マグネタイトを皮下腫瘍に挿入し, 118kHz, 384 Oe の交番磁場を照射した. ラットは照射を行わなかった対照群と 3 回照射された治療群とに分けられ, 腫瘍成長に対する温熱療法の効果を評価した. 腫瘍の完全退縮は, 治療群の 75% で観察された. 組織学的観察の結果, 針状成形マグネタイトは, 対照群では挿入されたポイントのまわりにのみ存在していた. 一方, 治療群の場合, 磁性微粒子が広範囲に拡がり, ほとんど腫瘍組織全体にわたって壊死細胞が観察された. マグネタイト微粒子は腫瘍の退縮と共に血流によって除去された. そのため, ラット大腿部から針状成形マグネタイトを取り出す必要はなかった. さらに, マグネタイト微粒子の注入時間がマグネタイトカチオニックリポソーム (MCL) の場合, 注入時の逆流を防ぐために少なくとも 120 分かかるのに対し, 2 本の針状成形マグネタイトを腫瘍に挿入する時間はわずか 3 分だった. これらの結果は, 針状成形マグネタイトが温熱療法用の微粒子状発熱体を注入するための単純かつ効果的な方法であることを示唆している.