Original Contribution

Development of a Portable Inductive Heating System Using Dextran Magnetite Complex (DM) — heating Characteristics in Vitro and the Antitumor Effect in VX-2 Tumor-bearing Rabbits —

KENJI TAZAWA ^{1*}, SHIGEHITO WADA ², MIKI YATSUZUKA ¹,
TOMOHIRO SAITO ³, KENICHI TAZAWA ³, ISAMU NAGANO ⁴,
IGARASHI KOUICHI ⁵, ISAO FURUTA ², HIDEO NAGAE ⁶

¹ School of Nursing, Faculty of Medicine, Toyama Medical and Pharmaceutical University,
Toyama, Japan.

² Department of Dentistry and Oral Surgery, Faculty of Medicine, Toyama Medical and
Pharmaceutical University, Toyama, Japan.

³ Second Department of Surgery, Faculty of Medicine, Toyama Medical and Pharmaceutical
University, Toyama, Japan.

⁴ Department of Radio Wave Engineering, Faculty of Engineering, Kanazawa University,
Ishikawa, Japan.

⁵ Komatsu Power Tron Corp. Ltd., Ishikawa, Japan.

⁶ Nagoya Research, Meito Sangyo Corp. Ltd., Aichi, Japan.

Abstract: Dextran magnetite complex (DM) is a colloidal suspension of subdomain particles which efficiently generate heat under an alternating current (AC) magnetic field. Inductive heating using DM has attracted attention as a new method to overcome various problems in existing local hyperthermia. To make the system more convenient for clinical application, we developed a new portable device.

The device can use an alternating current (AC) of 200V (maximum output 3kW), and it is extremely small, about 0.4×0.4×0.4 meters long. The frequency can be adjusted continuously within the range of 100-500kHz. DM suspension containing 14mg Fe/ml (0.25M), 28mg Fe/ml (0.5M) or 56mg Fe/ml (1.0M) was used in an in vitro study, referring to our previous studies. The results obtained were as follows. 1) The temperature of DM rose in proportion to the iron concentration of DM. 2) The temperature of DM rose in proportion to the square of the magnetic field intensity. The temperature elevations were about 8°C/min and 14°C/min at frequencies of 299kHz and 418kHz, respectively, using a DM of 28mg Fe/ml and a magnetic flux density of 5.2mT. These results were identical to the results of the heating characteristics in the previous device. Moreover, this device was evaluated for its anti-tumor effect in VX-2 tumor-bearing rabbits. By using hyperthermia treatment with this device, the inhibition of the tumor growth in the treated group (n = 8) was significantly greater than in the control group (n = 3), and the frequency of lung metastasis was decreased, too. Thus, this study confirmed the

Received 24 March 2003, Accepted 23 June 2003. *Corresponding author, Tel : +81-76-434-7426, Fax : +81-76-434-5181,
E-mail : tzwa@ms.toyama-mpu.ac.jp

usefulness of this portable hyperthermic device for local cancer therapy.

Key words: Dextran magnetite complex, inductive heating, portable device, VX-2 tumor

Introduction

In recent years, local hyperthermia has been performed using radiofrequency (RF) heating, microwave heating, or ultrasonic heating. RF heating is divided into capacitive heating and inductive heating. Capacitive heating is still the most commonly used in local hyperthermia but has disadvantages such as unclear borders between the heated and non-heated areas and difficulty in heating deep areas¹⁾. Inductive heating, which utilizes the heating effects of various magnetic materials inserted or injected into the tissue in an induced magnetic field, has attracted attention as a method that can overcome the various problems of capacitive heating²⁻⁵⁾. Our research group previously reported a new inductive system using dextran magnetite complex (DM) that generates the efficient heat generation at a low concentration in vitro and in vivo¹⁾⁶⁻¹⁰⁾. However, this device is extremely large and heavy ($0.8 \times 0.8 \times 2.0\text{m}$, over 300kg) as it is equipped with vacuum tubes. To make it more convenient for clinical application, we developed a new portable device with a high-frequency inverter for inductive heating, and then evaluated the possible clinical application of this device in vitro and in experimental tumor-bearing animals.

Materials and Methods

The portable AC magnetic field generator

We developed a portable AC magnetic field generator with semiconductors which is similar in performance to a conventional generator with vacuum tubes (the model TY-1 generator at Toyama Medical and Pharmaceutical University, Toyama, Japan). 200V/60Hz AC power is first converted to DC voltage and then to AC (100k-400kHz) current by high-frequency inverters. Using a solenoid type induction coil, we can generate a AC magnetic field as intense as 6mT at the center of the coil. The old

device is very large and heavy, about $0.8 \times 0.8 \times 2.0\text{m}$, over 300kg whereas, the new device is small and light, $0.4 \times 0.4 \times 0.4\text{m}$, about 25kg (Fig. 1). The temperature was measured using photosensor-type thermometer (model 3000, Luxtron, CA, USA) and a thermography (Thermo Tracer 6T66, Sanei, Tokyo, Japan).

Magnetic fluid

DM was purchased from Meito Sangyo, and a suspension with an iron concentration of 335mg/ml (6M) at manufacturing was diluted with sterile distilled water for use in our experiment. DM is a complex

Fig. 1. Transition of inductive heating system using DM
(A) old device, $0.8 \times 0.8 \times 2.0\text{m}$, over 300kg
(B) new device, $0.4 \times 0.4 \times 0.4\text{m}$, about 25kg

of dextran and superfine iron oxide particles, and not a mere suspension of the two. As shown in the assumed structural diagram in Fig. 2, dextran chemically binds to the circumference of superfine iron oxide particles, and this complex is stable as a colloid without aggregation or deposition in various solvents or serum. We compared the temperature characteristics in inductive heating at a frequency zone of 500kHz between DM and iron oxide, pure iron, or ferrite, and found more efficient heat generation at a low concentration using DM ¹⁾⁶⁾. In addition, the toxicity is extremely low ¹¹⁾.

Animals and VX-2 cell transplantation

Twenty-week-old male Japanese white rabbits (n = 13) were purchased from Sankyo Laboratory (Toyama, Japan) and housed in a specific pathogen-free animal facility in the Animal Center of Toyama Medical and Pharmaceutical University. The VX-2 cells were obtained from the Cancer Cell Repository (Tohoku University, Sendai, Japan) and maintained subcutaneously in the femoral region of rabbits in our University. A small piece of the growing tumor tissue was transplanted to the femoral region of each rabbit, and the animals were used in the experiments when the long diameter of the tumor reached 1 cm or more.

Assessment of antitumor effect

The animals were arbitrarily divided into a control group and a treated group, and a DM suspension of 56mg Fe/ml was infused into the center of the tumor over about 3 minutes using a 23G needle in the latter group. DM was injected as 15mg Fe/mm³ in proportion to the tumor volume of each animal. Under AC magnetic field (frequency : 256kHz, magnetic flux density : about 5mT), the temperatures of the target area and the surrounding normal tissue were measured by optical thermometers and thermography. After the treatment, the tumor volumes were calculated with the following equation.

Fig. 2. Structure and physical characteristics of DM

$$V = (\pi/6) \times a \times b^2 \quad V : \text{Tumor volume (mm}^3\text{)}$$

a : Long diameter of tumor (mm) b : Short diameter of tumor (mm)

To assess the antitumor effect, the tumor growth and the animal survival were observed for four weeks, and then all animals were sacrificed to evaluate of lung metastasis.

Statistical Analysis

The difference in tumor growth inhibition between the treated group and the control group was analyzed statistically by Student's t-test. Survival curves for both groups were drawn using the Kaplan-

Fig. 3. Relation between the DM concentration and the temperature rise

Fig. 4. Relation between the magnetic flux intensity and the temperature rise

Meier method, and the difference in the survival between these groups was assayed by a long rank test. In both analyses, a p value less than 0.05 was considered significant.

Results

We measured the heating characteristics of DM which was placed directly at the center of the inductive coil. Fig. 3 shows the relation between the DM concentration and the temperature rise for 10 seconds (ΔT) in vitro. A magnetic flux density of 5.9mT was set in this experiment at a frequency of 460kHz or 335kHz. At a frequency of 335kHz, there were temperature rises of approximately 2.4°C, 4.9°C, and 8.9°C by inductive heating for 10 seconds at concentrations of 14, 28, and 56mg Fe/ml, respectively. Moreover, at the higher frequency of 460kHz, the temperature rise of approximately 13.8°C during 10sec-inductive heating was observed at a concentration of 56mg Fe/ml. As the DM concentration increased, more efficient heat was generated and the temperature of DM rose in proportion to the iron concentration of DM. Fig. 4 shows the ΔT at each frequency versus the magnetic flux intensity at a DM concentration of 28mg Fe/ml. As the magnetic flux den-

sity increased, more efficient heat was generated, and this tendency was remarkable at the higher frequency (418kHz) than the lower frequency (299kHz). The temperature of DM rose in proportion to the square of the magnetic field intensity. The temperature elevations were about 8deg/min and 14deg/min at frequencies of 299kHz and 418kHz respectively, using DM of 28mg Fe/ml and magnetic flux density of 5.2mT (detailed data is not shown in Fig. 4). These results equaled the heating characteristics in the old device.

In the DM-injected tumor of the treated group, the temperature could be maintained at about 43°C for 15-30 minutes without heating the normal tissue (Fig. 5 and Fig. 6). The time required for the increase to 43.0°C in the heated area was mostly within 15 minutes. Fig. 7 shows the VX-2 tumor growth curve after heating using this new device, and the tumor volume before heating was defined as a standard value of 100%. The growth inhibition of the VX-2 tumor was evident in the treated group ($P < 0.05$). All five control animals showed progressive disease, and two of them died from rapid tumor progression in the second and third weeks. In contrast, the survival of all eight treated animals was confirmed until the fourth week. As a result, the survival rate was higher in the treated group than in the control group, but a

Fig. 5. A) placing the animal onto the portable heating system

B) Temperature distribution obtained from thermography: DM-injected tumor was selectively heated to about 43.0°C without heating the normal tissue

Fig. 6. Typical temperature elevation curves during the heating treatment

significant difference was not statistically admitted during this observed period (Fig. 8: $P = 0.058$). From the autopsy findings, lung metastasis occurred in only two of eight treated animals (25%), and this ratio was significantly lower than that of control group (80.0%).

Discussion

The chemical structure of DM is similar to that of superparamagnetic iron oxide (SPIO) as magnetic resonance imaging (MRI) contrast media, but only DM generates heat under an AC magnetic field. This difference is primarily considered to be the result of differences in magnetism of the core substances¹²⁾. The LD50 of iron oxide is generally 300-600mg Fe/kg, whereas that of DM is 2,000-6,000mg Fe/kg

Fig. 7. Change of the tumor volume in each group after the treatment

Fig. 8. Survival curves in each group after the treatment

showing very low toxicity¹¹⁾. With a particle diameter at the nm like DM, the rotation of the magnetic vector of iron oxide particles and physical rotation of the particles were suggested to be closely involved in the mechanism of heat generation⁵⁾. It is considered that these mechanisms occur in the new portable system as well as the conventional heating system.

Our inductive heating system enables the target area to be selectively heated with the combination of the AC magnetic field and the DM. However, it is a problem that the device is extremely large and expensive for clinical application¹⁾⁷⁾⁸⁾. To overcome this problem, we developed a new portable device equipped with high-frequency inverters and a solenoid coil, and could reduce the inductive heating technology to about 0.4m×0.4m×0.4m, and 25kg. It seems that the performance of the new device equalled the existing device, and corresponded to the clinical use sufficiently. Consequently, the volume and weight of the new device are in 1/20 and 1/12, respectively compared with the old device. In the experiment on VX-2 tumor-bearing rabbits, the DM-injected target area was heated to about 43.0°C within 15 minutes without heating normal tissue. This result was a little inferior to past results when the DM-injected normal tongue at 56mg Fe/ml heated up to approximately 43.0°C within 2 minutes⁷⁾⁸⁾. However, the maintenance of temperature and the anti-tumor effect equaled the past experiment results for VX-2 or AH60C transplanted-tumor treatment using the old device¹⁾⁹⁾¹⁰⁾. As an effective DM administration method, Mitsumori *et al.* administered the DM-Lipiodol emulsion via the renal artery and observed an effective temperature rise of 12.7°C/10 minutes in the kidney¹³⁾. As this administration method is worth attempting in various organs of the body in clinical application, it is necessary to confirm the temperature rise in detail for this portable system.

In the future, we will try to increase the output of the generator for efficient heat generation and develop the coil shape to selectively concentrate the magnetic field on the tumor tissue. Moreover, it is necessary to decrease the production cost aimed at clinical application.

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デキストラン・マグネタイト複合体 (DM) を用いた携帯型誘導加温装置の開発 — in vitro における加温特性および家兎 VX-2 腫瘍における抗腫瘍効果 —

田澤賢次¹・和田重人²・八塚美樹¹・斎藤智宏³・田澤賢一³
長野 勇⁴・五十嵐功一⁵・古田 勲²・長江英夫⁶

¹ 富山医科薬科大学医学部成人看護外科

² 富山医科薬科大学医学部歯科口腔外科学講座

³ 富山医科薬科大学医学部第二外科学講座

⁴ 金沢大学大学院自然科学研究科電気情報システム科

⁵ 小松パワートロン株式会社

⁶ 名糖産業名古屋研究所

要 旨：デキストラン・マグネタイト複合体 (DM) は subdomain 粒子のコロイド状懸濁液であり、交流磁界中で効率良く発熱することが知られている。DM を用いた誘導加温は、従来の局所温熱療法の様々な問題点を克服しうる新しい方法として注目されている。臨床応用に際してより簡単に使用できることを目的とし、携帯型の誘導加温装置の開発を行ったので本稿において報告する。

新しい装置は 200V の交流電源 (最大出力 3kW) で使用が可能である。サイズは 0.4×0.4×0.4m ときわめて小型で、周波数は 100～500kHz の範囲で連続的に調節することが可能である。DM 懸濁液の濃度は過去の実験結果を参考に、in vitro の実験では 14mg Fe/ml (0.25M), 28mg Fe/ml (0.5M), 56mg Fe/ml (1.0M) を使用した。結果として 1) DM の温度は DM の濃度に比例して上昇、2) DM の温度は磁場強度の二乗に比例して上昇することが明らかになった。さらに DM 濃度 28mg Fe/ml, 磁束密度 5.2mT の実験条件下において、DM の温度上昇は周波数 299kHz で約 8°C/min, 418kHz で約 14°C/min であった。これらの結果は従来の装置における加温特性と同等であった。さらに家兎 VX-2 腫瘍において本装置による抗腫瘍効果を評価した。本装置を使用した温熱療法により、対照群 (n = 3) と比較して治療群 (n = 8) では有意な腫瘍成長阻害効果が確認され、肺転移の出現率が減少していた。以上本研究から、局所癌治療のための本携帯型加温装置の有用性が確認された。