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財団法人　日中医学協会　御中

貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：研究報告書

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1．助成金額：1,000,000 円

2．研究テーマ

－日中医学協会助成事業－　磁気温熱により誘導される抗腫瘍免疫に関する研究

3．成果の概要（100字程度）

移植乳癌の持つラットに、局所的に免疫アジュバントを投与した後、温熱療法を実施した。
移植巣を有意に縮小させただけではなく、消失させた症例も観察された。温熱により変性・壊死が誘導され、分解・吸収された腫瘍細胞の成分は抗原として免疫系を刺激し、腫瘍に対する免疫反応が引き起こされた。

※発表論文等

4．研究組織

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— 日中医学協会助成事業 —

磁気温熱により誘導される抗腫瘍免疫に関する研究

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Antitumor immunology induced by the magnetic induced hyperthermia

Abstract

The present study was carried to test effectiveness and mechanisms in treatment of mammary cancer using our magnetic induction hyperthermia system (MIHS). We found out that the thermotherapy at 50~55 ^{0}C by using MIHS is effective to inhibit the growth of mammary cancer. Furthermore, infiltration of the cancers by a large number CD4+ and CD8+ T cells indicates that there are various antitumor immunological responses. Such effects, however, were attenuated when immunoadjuvant was locally administered before thermotherapy. Such a combination of hyperthermia and immunoadjuvant resulted in not only the decrease in volume, but also the disappearance of cancers.

Key Words: **Hyperthermia, magnetism, cancer, immunology, mammary**

Introduction

Hyperthermia, as one of the procedures for treatment of malignant tumor, can directly kill the tumor cells, or at least inhibit their growth[1, 2]. Results *in vivo* in recent years have shown that the tumor-killing effects are related to the activation of various antitumor immunological responses[3]. It is considered that the components derived from the degeneration and necrosis of tumor cells induced by hyperthermia can be absorbed by the organisms[4]. These components in return can act as antigens that activate immunological system, which results in various antitumor effects. Those researches, however, are mostly focused on the conventional thermotherapy of which the temperature is about 43 ^{0}C,

combined with biotherapy, chemotherapy, or radiotherapy[5]. The present research was aimed to find out the ideal conditions between antitumor immunological responses and hyperthermia induced by magnetic irradiation.

Materials and Methods

The experiments were carried out in Wistar rats of 180~220 grams in body weight. The mammary carcinoma bearing rats were prepared by inoculation of 0.2 ml of Walker-256 cells ($2x10^6$/ml), a cell line derived from the same linage of Wistar rats in order to avoid immunological rejection. The cancer-bearing rats were divided into six groups (Table 1). In 7 days after the inoculation, two thermo-seeds that generate heat in magnetic field were implanted into the cancer on the right flank. To attenuate immunological response, immunoadjuvant was injected into the cancers of both flanks. The animals were irradiated in 3 days after the implantation. The cancer volumes were measured every 3 days. All tissue samples were collected in 14 days after the irradiation, observed morphologically and immunohistochemically.

Table 1 Groups and experiments of cancer-bearing rats

Days			1st	7th	8th	10th
Experiments			Inoculation	Thermo-seeds	Immunoadjuvant	Irradiation
G1#	Sides	R*	Yes	Yes	No	No
		L	Yes	No		
G2	Sides	R	Yes	Yes	No	42~46 °C, 30 min
		L	Yes	No		
G3	Sides	R	Yes	Yes	No	50~55 °C, 30 min
		L	Yes	No		
G4	Sides	R	Yes	Yes	Yes	42~46 °C, 30 min
		L	Yes	No		
G5	Sides	R	Yes	Yes	Yes	50~55 °C, 30 min
		L	Yes	No		
G6	Sides	R	Yes	No	Yes	No
		L	Yes	No		

*: R, right; L, left. #: 6 rats were divided into each group.

Results

Measurement of temperature within and around the cancers showed that the present methods can raise the temperature in the cancers as expected (Table 2). The

volumes of cancers treated with the higher temperature (group G1) were smaller than the controls (Table 3), although not significantly. The results indicate that magnetic induction hyperthermia system MIHS can generate hyperthermia enough for the present study. To attenuate the therapeutic effects while suppressing the damages to normal tissues around the cancers, immunoadjuvant was injected on both flanks before the irradiation in order to facilitate immunological response. It was found that the cancers were completely disappeared in 3 rats of group G5 (Table 3). In addition, the average volume of group G5 was significantly smaller than the other groups. The upper results indicate that the present system is suitable for treatment of the mammary cancer.

In group G5 rats treated with hyperthermia plus immunoadjuvant, the cancers on both flanks were infiltrated by a large amount of macrophages and lymphocytes. In addition, a relative clear margin could be observed between normal and malignant tissues. Results

Table 2 Increase of temperature to 50~56 °C in cancer during hyperthermia

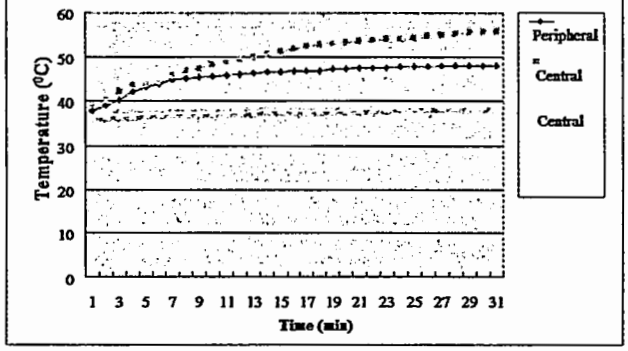


Table 3 Changes of cancer volumes on the right flank before and after hyperthermia

Groups	Number of rats	Averages of cancer volumes		Rate of final changes (%)
		Days after inoculation		
		10th Day	24th Day	
G1	6	2.75 ±1.23	3.07 ±0.76	11.6
G2	6	2.44 ±1.01	2.35 ±0.89	− 3.6
G3	6	2.83 ±0.66	2.34 ±0.49	− 17.3
G4	6	2.23 ±0.53	2.03 ±0.47	− 8.9
G5	6	2.86 ±0.34	1.87 ±0.33*	− 34.6
G6	6	2.62 ±0.76	3.03 ±0.75	25.6

Table 4 Changes of cancer volumes on the left flank before and after hyperthermia

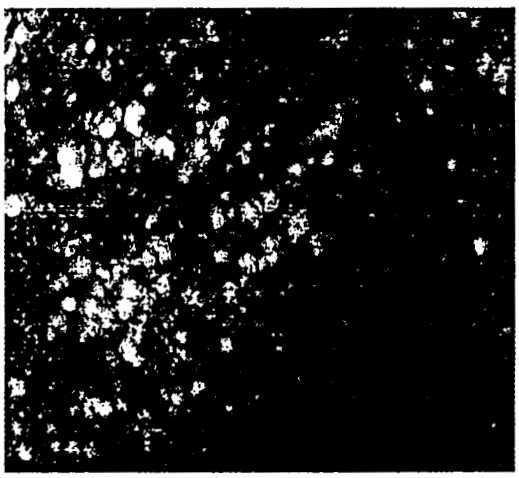
Groups	Number of rats	Averages of cancer volumes		Rate of final changes (%)
		Days after inoculation		
		10th Day	24th Day	
G1	6	1.60 ±0.43	1.81 ±0.39	13.0
G2	6	1.53 ±0.48	1.52 ±0.42	− 0.6
G3	6	1.79 ±0.61	1.56 ±0.55	− 12.8
G4	6	1.43 ±0.41	1.42 ±0.32	− 0.6
G5	6	1.67 ±0.33	0.67 ±0.76*	− 60.1
G6	6	1.76 ±0.3	2.09 ±0.63	18.0

Table 5 The number of $CD4^+$ T cell & $CD8^+$ T cell in each group (cells/HPF $\bar{x} \pm s$)

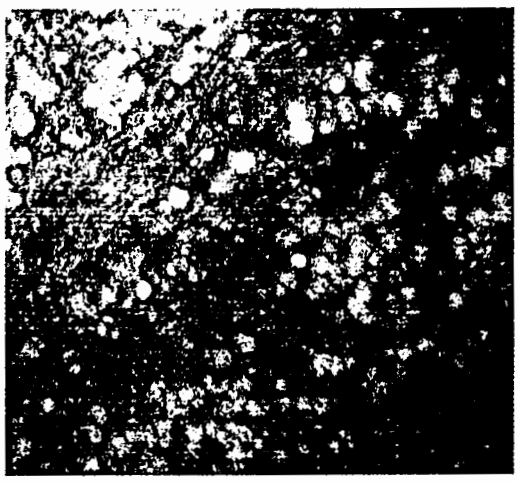
Groups	Number of rats	$CD4^+$ T cell	$CD8^+$ T cell
G1	6	1.43 ±0.65	1.57 ±0.88
G2	6	7.92 ±1.98	8.42 ±2.47
G3	6	8.51 ±2.06	10.31 ±2.23
G4	6	9.01 ±1.22	6.91 ±1.09
G5	6	16.52 ±1.26	19.18 ±2.31
G6	6	2.32 ±0.81	3.17 ±0.47

Control group G1 (x400)

Immunoadjuvant alone group G6 (x400)

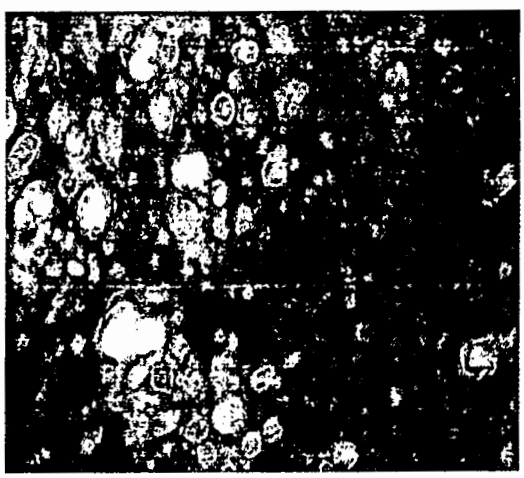


42~46 ^{0}C heating alone group G2 (x100)



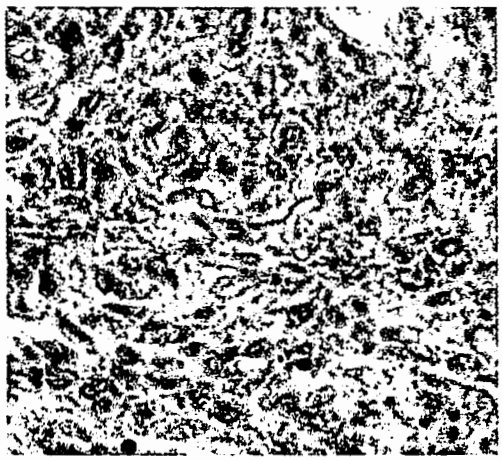
50~55 ^{0}C heating alone group G3 (x100)

42~46 ^{0}C plus immunoadjuvant group G4 (x400)

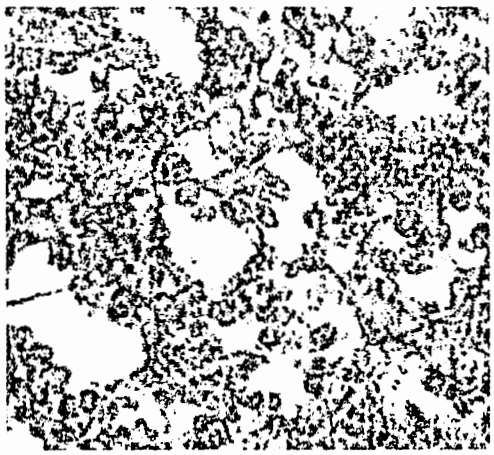


50~55^{0}C plus immunoadjuvant group G5 (x400)

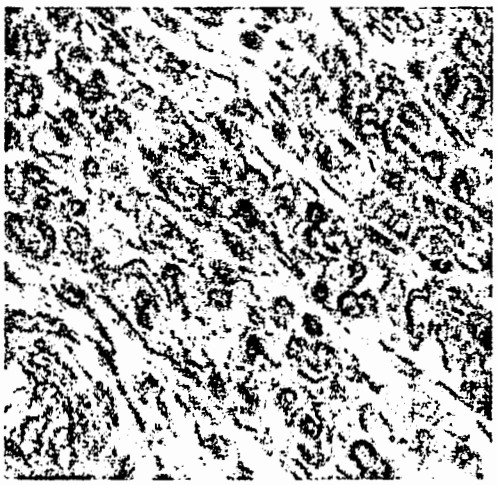
Fig. 1 Identification of CD4+ T cells following hyperthermia



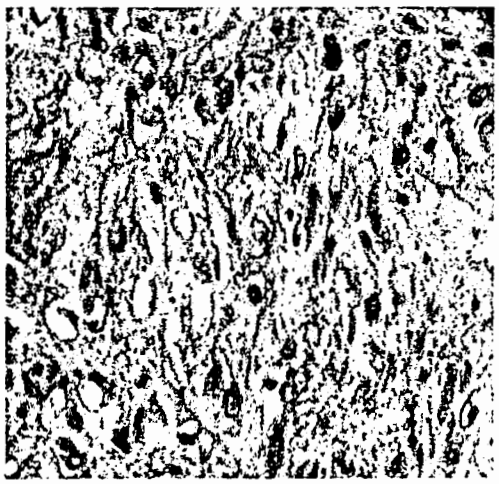
Control group G1



Immunoadjuvant alone group G6

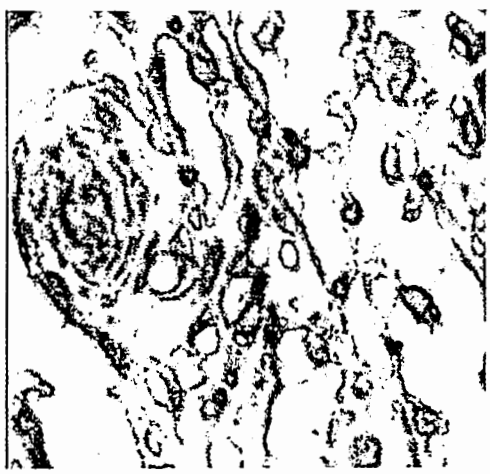


42~46 ^{0}C heating alone group G2



50～55 ^{0}C heating alone group G3

42-46 ^{0}C plus immunoadjuvant group G4



50～55^{0}C plus immunoadjuvant group G5

Fig. 2 Identification of CD8+ T cells following hyperthermia (x300)

from the immunological examinations showed that while there were only few CD4+ T cells in controls (Fig. 1), their numbers were significantly increased in the treated groups, especially groups G5 (Table 5). A similar tendency was also observed in the expression of CD8+ T cells CD8+ T cells (Fig. 2, Table 5). It is important to note that although the thermo-seeds were not implanted on the left flank, the average volume of cancers in group G5 was still significantly decreased. It is suggested that the immunological responses induced by hyperthermia can influence the cancers on the other part of the body.

Discussions

It is generally recognized that hyperthermia can kills the cancer cells directly, accompanied by induction of various immunological responses. The direct proofs are the inhibition of growth and disappearance of the tumor[3]. In our experiment, we found out that the thermotherapy at 50~55 ^{0}C by using the magnetic induction hyperthermia system (MIHS) is effective to inhibit the growth of mammary cancer. Furthermore, infiltration of the cancers by a large number CD4+ and CD8+ T cells indicates that there are various antitumor immunological responses. Such effects, however, were attenuated when immunoadjuvant was locally administered before thermotherapy. Such a combination of hyperthermia and immunoadjuvant resulted in not only the decrease in volume, but also the disappearance of cancers. Further studies are necessary to improve the efficiency of our system and clarify the immunological mechanisms involved.

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