Hyperthermia Enhances Immunotherapy in Cancer Patients: Clinical and Experimental Analyses

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Abstract: A symposium entitled "Immunological Science of Hyperthermia (basic and clinical)" was held during the 28\textsuperscript{th} annual meeting of the Japanese Society of Thermal Medicine in September, 2011. We presented the following clinical and experimental data in this symposium.

Clinical: We treated 1,386 patients with advanced or recurrent cancer by hyperthermia, immunotherapy or their combination, between July 2005 and August 2011. Hyperthermia was applied in 1,307 patients, activated lymphocytes therapy (CAT) in 995 patients, and dendritic cell therapy (DC) in 689 patients. The response to therapy was evaluated retrospectively in 1,343 patients, and 188 (14.0\%) responded to therapy, including 35 who showed a complete response. The response to immunotherapy increased from 8.1\% to 17.9\% when it was combined with hyperthermia, and the response was the highest in patients who received the combination of CAT+DC+hyperthermia.

Experimental: Mice inoculated with Lewis lung cancer (LLC) tumors were treated with hyperthermia or CAT. Each of hyperthermia and CAT reduced tumor growth and rate of lung metastasis, and the effects were more pronounced in animals treated by the two modalities. Each of hyperthermia and molecular target therapy reduced tumor growth and rate of lung metastasis, and increased the proportion of apoptotic cells in the tumor, and the effects were more pronounced in animals treated by the two modalities. The results suggest that hyperthermia enhances the effects of immunotherapy in cancer patients.

Key Words: dendritic cells, immunotherapy, hyperthermia, cancer

Introduction

Hyperthermia enhances immunotherapy in cancer patients. These clinical data and the mechanism of enhancement have attracted attention recently\textsuperscript{11}. A symposium entitled "Immunological Science of Hyperthermia (basic and clinical)" was organized during the 28\textsuperscript{th} annual meeting of the Japanese Society...
of Thermal Medicine in September 2011. That symposium was constituted by four researchers. Dr. Kokura showed the foundation of hyperthermic immunology and Dr. Terunuma and Dr. Tanigawa showed deployment clinical from the foundation. And I showed the accumulation result of clinical data and combined use with a new cure. Each doctor summarized review about the contents respectively.

In that symposium, we reported our results on the use of hyperthermic immunotherapy in more than 1,000 patients with advanced or recurrent cancer during the period of 2005-2011. In this paper, we report the clinical findings and the results of basic animal experiments.

1. Clinical
1.1. Patients

We treated 1,386 patients with advanced or recurrent cancer by hyperthermia or immunotherapy (between July 2005 and August 2011). Hyperthermia was applied using a Thermotron RF-8 heating device (Yamamoto Vinita, Osaka, Japan). Specifically, hyperthermia was used in 1,307 cancer patients, activated lymphocyte therapy (CAT) in 995 patients, and dendritic cell therapy (DC) in 689 patients. Activated lymphocytes (CAT) were prepared with anti-CD3 activated peripheral lymphocytes. Usually immature dendritic cells were injected to tumor or regional lymph node, but sometimes mature dendritic cells were used when there was no injectable tumor or lymph node.

Thus, 573 patients received the combination of CAT+DC+hyperthermia, 312 patients received CAT+hyperthermia, 92 patients received hyperthermia+DC, and 330 patients received hyperthermia only.

The percentage of chemotherapy which each therapy group of hyperthermia, CAT and DC received was 29.9%, 29.8% and 30.0%, respectively. The percentage of radiotherapy which each therapy group of hyperthermia, CAT and DC received was 2.5%, 3.1% and 2.8%, respectively.

1.2. Response to therapy

The effects of a treatment was judged according to the guidelines to evaluate the response to treatment in solid tumors (RECIST), such as a complete response (CR), a partial response (PR), stable disease (SD) and progressive disease (PD).

The response to therapy was evaluated in 1,343 patients. Clinical benefit case (CR, PR and long-term SD) was noted in 188 patients, including 35 who showed a CR. The beneficial effect of immunotherapy increased from 8.1% to 17.9% in patients who also received hyperthermia. The highest response rate (20.5%) was noted in patients who received the combination therapy of CAT+DC+hyperthermia. However, the response to hyperthermia alone was only 4.0% (Fig. 1).

Fig. 1. Number and percentages of clinical benefit cases that showed CR, PR and long SD. A, classification by the kind of treatment. B, classification by the combination therapy. A total of 188 patients (14.0%) among 1,343 evaluated cases showed such response. (between July 2005 and August 2011)
1.3. Case report

We present one case of 35 patients who showed a complete response to treatment. A 45-year-old female patient was diagnosed with colon cancer. Left hemicolectomy was performed in 2003, followed by chemotherapy. Two years after surgery, multiple lymph nodes were identified in the paraaortic and left cervical regions. Chemotherapy with various chemotherapeutic agents failed to induce a response and the tumors increased in size. She was referred to our clinic in October 2005. Treatment included weekly hyperthermia (from 2005/11/7 to 2006/1/5), injection of immature DC cells into the left cervical lymph nodes twice (2005/11/1, 12/12), followed by intravenous infusion of CAT cells seven times (from 2005/11/7 to 2006/1/5). PET/CT conducted three months later showed the disappearance of all lymph nodes, as well as the return of all tumor markers to the normal values (Figs. 2A and 2B). CT was enforced on 2005/10/28 and 2006/1/5. PET/CT was enforced on 2005/11/5 and 2006/4/12. This case was considered to have achieved a clinical CR\textsuperscript{31}.

![Graph A](image1.png)

**Fig. 2.** A, Cervical metastatic tumors disappeared and the level of CEA decreased to the normal range after the combination therapy of hyperthermia, intratumoral injection of DC cells and activated lymphocyte therapy. B, CT and PET taken before and after the combination therapy of hyperthermia, intratumoral injection of DC cells, and activated lymphocytes therapy. Note the disappearance of multiple metastases in the cervical and abdominal regions following the treatment.
2. Basic animal experiments

2.1. Hyperthermia enhances activated lymphocytes therapy

Mice (C57BL/6, male, 7 weeks) inoculated with Lewis lung cancer (LLC) tumors \((1 \times 10^6)\) were treated with hyperthermia (42°C, 20 min/session, 2 sessions/week, repeated for 5 weeks) or with activated lymphocytes (intravenous injection via the tail vein, \(2 \times 10^6\), 2 sessions/week, repeated for 5 weeks). Hyperthermia was applied using a Thermotron RF I.V. heating device (Yamamoto Vinita, Osaka, Japan). Frequency is 8 MHz. Output is 50~200 W. Heating method is condenser-type directric heating.

They were divided into the control group, CAT group, hyperthermia group, and hyperthermia + CAT group. The response to treatment was assessed by measuring tumor growth and lung metastasis. Both hyperthermia and CAT reduced not only tumor growth but also the frequency of lung metastasis. Furthermore, the combination of hyperthermia and CAT synergistically increased the reduction in tumor growth and the rate of lung metastasis\(^3\) (Figs. 3A and 3B). The significant difference was shown by Student’s t-test.

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![Graph A](image1.png)

**Fig. 3.** Animal experiments. Each of hyperthermia (HT) and CAT (A) reduced tumor growth and (B) the frequency of lung metastasis in experimental animals. Furthermore, their combination synergistically enhanced their effects on these parameters. Data are mean ± SD of n animals.

2.2. Hyperthermia enhances molecular target therapy

Mice inoculated with LLC tumors were treated with hyperthermia or molecular target drugs (erlotinib 25 mg/kg/day or sorafenib 10 mg/kg/day). The response to treatment was evaluated by
measuring tumor growth and the frequency of lung metastasis. The effect of treatment was also evaluated by immunohistochemical staining for human endothelial growth factor receptor (EGFR) -related 2 (HER2), vascular EGFR (VEGFR) and apoptosis. Each of hyperthermia and molecular target therapy reduced tumor growth and the rate of lung metastasis. Furthermore, the combination of hyperthermia and molecular target therapy further reduced tumor growth and the rate of lung metastasis. Hyperthermia and molecular target therapy reduced HER2 and VEGFR expression in the tumor. Furthermore, the two treatment modalities also synergistically increased the rate of apoptotic cells in the tumor\(^4\) (Figs. 4A-C).

**Fig. 4.** Animal experiments. Each of hyperthermia (HT) and molecular target therapy (A) reduced tumor growth, (B) reduced the frequency of lung metastasis, and (C) increased the proportion of apoptotic cells in the tumor. Furthermore, their combination synergistically enhanced their effects on these parameters. Data are mean ± SD of n animals.

**Conclusion**

Although the side effects of hyperthermia are minor, two problems related to the use of hyperthermia are the long period of time between its application and effect, and its limited effect. Hyperthermia is more effective when used in combination with chemotherapy or radiotherapy. Several studies have recently reported the beneficial therapeutic effects of the combination therapy of hyperthermia and immunotherapy\(^1,5\). Our study confirmed these findings both in humans and experimental animals.

It is often difficult to evaluate individual patients in a large study due to the different clinical background. However, we observed a good response to the combination of hyperthermia and immunotherapy in several cases, but no response to each component when used alone. The present study is an overview of the results of hyperthermic immunotherapy in more than 1,000 patients with advanced
or recurrent cancer over a period of six years.

How does hyperthermia enhance the effect of immunotherapy<ref> While this was not directly examined in the present study, other authors who participated in that symposium entitled “Immunological Science of Hyperthermia (basic and clinical)” held in the 28th annual meeting of the Japanese Society of Thermal Medicine in 2011, are discussing in another paper. But the following mechanisms have been proposed in previous studies with regard to cancer in general and its treatment by immunotherapy combined with hyperthermia: 1) Primarily the immune system has the capability to eliminate cancer, to some extent. 2) Advanced cancer suppresses the immune system and promotes invasion and metastasis. 3) Hyperthermia recovers and reinforces the limited immune system, thus eliminating cancer. Specifically, recovery and reinforcement of the antigen presentation system suppressed by cancer is one of the most important factors<ref>.

Previous studies focused mainly on the effect of hyperthermia on the local immune system. In our animal experiments, however, we demonstrated that hyperthermia is also effective by reinforcing immune-cell therapy. Furthermore, we showed that hyperthermia is effective in molecule target therapy.

Further studies are needed to examine the role of hyperthermia in the treatment of cancer.

References
1) Takeda H., Takeda T., Kobayashi S., Takeda T.: Hyperthermic immunotherapy was effective in 3 CR cases as the standard treatments had no effect or could not be enforced. Gan To Kagaku Ryoho (Jpn J Cancer Chemotherapy), 38 : 1939-1941, 2011. (Japanese).