Clinical Study of Micro-diameter Local Warming Device with Drug Local Injection Function for Deep Tumor Lesion

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Abstract

In order to provide effective treatment for relapsed and/or metastatic cancer in patients who completed a standard therapy, we investigated the simultaneous application of immunotherapy and percutaneous local thermal ablation, and its clinical potential for humans. Deep tumorous lesions were treated with puncture method using micro gauge thermal ablation tools which were equipped to locally inject immunosuppressive agents. The patients group consisted of 15 men (average age 66.7) and 31 women (average age 60.4) for a total of 46 participants (youngest age 37, oldest age 84, average age 62.4).

The primary disease categories were: ovarian cancer (1 case), renal caner (4 cases), malignant lymphoma (3 case), lung cancer (6 cases), breast cancer (10 cases), stomach cancer (2 cases), uterine cancer (5 cases), colorectal cancer (6 cases), pancreatic cancer (3 cases), Thymoma (1 case), esophageal cancer (1 case), bile duct cancer (2 case), head and neck cancer (1 case), pharynx cancer (1 case). The 182 ablation sites that were combined with immuno- therapy were evaluated using stratification that shows the categories of local injection of dendritic cells (DC), local injection of activated T cells (CTL), heat of 100℃, and heat of 60-70℃. Combination of this thermal method and immune cellular therapy in the cancer sites yielded results of 25％ complete response (CR), 30％ partial response (PR) and 18％ stable disease (SD), indicating that progression was suppressed in 73％ of all sites.

Out of 241 thermal therapy cases that were evaluated for safety, 16 cases had equipment failure, and 15 cases were recognized to have adverse events. Ten cases of equipment malfunction are thought to be initial failure. There were 3 comparatively serious adverse events due to equipment and/or percutaneous thermal ablation procedure, but the procedures were aborted immediately after the problem was identified. Patients were given pain medication and suffered no after-effects. Pain and/or physiological issues were recognized after 12 procedures, but these minor problems were resolved at the follow-up exam.

We verified that this equipment and percutaneous thermal ablation is safe for use in humans. A combination of percutaneous thermal ablation and immunotherapy shows potential to be effective against cancer sites and suppressing progression.

KEY WORDS Deep tumor lesion, Medicine drug local injection function, Micro-diameter local heating device, Micro heater, Trocar, Puncture warming procedure, Dendritic cells, Activated T cells, Cellular immunotherapy

Introduction

The authors have been working to find out if combining radiation with immunotherapy approach to treat advanced malignant tumor (cancer) patients is effective. The components of immunotherapy are patient-derived activated T cells and immature dendritic cells (iDCs) induced from Peripheral Blood Mononuclear Cells (PBMC). We have already reported the safety and feasibility of this protocol in a

small-scale clinical trial that included 26 patients with advanced cancer.[1] The result of the immuno-radiotherapy approach has also been reported for a larger cohort of 167 cases which were observed for one year after the last treatment cycle.[2] Autologous CD8+T cells isolated from the peripheral blood of 13 patients with relapse was proliferated in vitro with IL-2 and injected into 58 metastatic lesions, 59% Complete Response (CR) and 29% Partial Response (PR) was obtained among all cases. Our data indicated that combining immunotherapy with conventional radiotherapy may be effective to treat patients with severe cancer.

Radiation has been clarified to promote immunotherapy-induced anti-tumor immune response not only in clinical trials but also in the preclinical study. In addition to stimulating the anti-tumor activity of immune cells, radiation also increases the clarity of tumor cell antigenicity. Radiation induces apoptosis (self-death) and disappearance by necrosis of the malignant cells, and intratumoral administration of iDCs enables the immune system of the body to recognize Tumor Associated Antigens (TAA) as foreign bodies. Radiation therapy may also induce immunoenhancing effects by weakening the immunosuppressive microenvironment within the tumor.

There are few treatment options available for patients with recurrent and metastatic cancer who have completed the standard therapy, Admetech Corporation developed a local high temperature hyperthermia treatment with a puncture warming procedure in the veterinary field. Cutting edge technology was introduced in the veterinary field, and about 1,500 cases that mainly included dogs and cats for whom surgical excision was not indicated were treated only by warming at temperatures in the range between 60℃ to 80℃ for around 5 to 10 minutes. This treatment has demonstrated adequate anti-tumor effectiveness and there were no reports of serious adverse events.

Takagi et al. used the local high temperature hyperthermia treatment for the glioma rat model and showed that tumor growth was suppressed by heating at 60℃ to 70℃.[3] Takagi et al. observed the degeneration and disappearance of tumors in 3 dogs with superficial tumors (Rhabdomyosarcoma of the extremities, ceruminous adenocarcinoma, and perianal gland carcinoma) when warming treatment was implemented 1 to 3 times at 45℃ to 65℃ for 3 to 4 weeks.[4] Itoh et al. used a combination of warming treatment and immunotherapy on malignant melanoma in a dog's mouth.[5]

Since the puncture warming method was found to be safe and effective from these animal experiments and previous studies, we developed a device for deep progressive cancer in humans. As a result, the Micro-diameter Local Warming Device was developed as a living body heating instrument and controller.[6]

The authors examined the clinical potential to find out if local warming treatment can be combined with immunotherapy for effective treatment of patients with severe cancer and started this clinical study using the micro-diameter local warming device with a function to inject drugs locally for deep tumor lesions at the Tokyo Research Center, Hasumi International Research Foundation. We have prepared this report since the results of the investigator-initiated clinical study that was conducted with the combination of puncture warming treatment and immune cell therapy for patients with recurrent and metastatic cancer who had completed the standard therapy showed safety to humans, and suggested that the combination with immunotherapy was effective.

I Subject and Method

The ethics committee of the Tokyo Research Center, Hasumi International Research Foundation deliberated and gave the approval to conduct this study (January 27, 2014).

1. Patient

The patients considered for the study were patients referred to the Tokyo Research Center, Hasumi International Research Foundation with recurrent cancer after the completion of standard treatment or stage IV cancer patients who were not provided with the standard treatment during the period of this study. The patients were given detailed information on the treatment and protocols based on the Declaration of Helsinki and signed informed consent was obtained from them.

2. Overall protocol

The authors had proposed the method of collecting dendritic cells (iDCs) from patients and directly injecting the iDCs into the cancer tumors in the body of the same patient to identify cancer antigens (foreign bodies) in the body to facilitate healing and induce cytotoxic T cells (CTLs), and have reported the combination of this therapy with radiotherapy.[1,2] The protocol of the present study is a combination of the puncture warming treatment and immune cell therapy for patients with recurrent and metastatic cancer who have completed the standard therapy.

3. Preparation of immunotherapy drugs

The preparation details of the immunotherapy drug formulation from the patients are given in previous clinical papers, and the outline is given below.[1,2]

(1) Peripheral Blood Mononuclear Cells (PBMC) collected from the patients are separated using a leukocyte plasma separator (apheresis).

(2) Immature dendritic cells (iDCs): Adherent cells are separated from PBMC, iDCs are induced by using GM-CSF and IL-4, and then cryopreserved.

(3) Activated T cells (CTLs): CD8+T cells are separated from PBMC in the peripheral blood and cryopreserved.

4. Preparation of test device

1) Specification and performance of the test device

The device AMTC300 used in this study for conducting tests is a cylindrical microheater (hereinafter, referred to as "Microheater") with a fine diameter and sealed tip that is electrically heated. This instrument punctures and coagulates the living tissue (affected part) in the body of humans by heat or causes necrosis of tissue cells. The Microheater has a built-in heating element (hereinafter referred to as "Heater") near the tip that is insulated from the living body, and has an inbuilt thermocouple to measure and control the heater temperature. The device constantly measures the temperature with the thermocouple, and maintains the temperature at the set value by controlling the direct current applied to the Heater. The trocar needle, which also serves as a guide needle, percutaneously punctures the target tumor and affected part. The Microheater is then used by inserting into the trocar lumen.

Table 1 shows the specifications and performance of the test device AMTC300. **Fig. 1** shows (1) Photograph of the AMTC300 device, (2) Microheater with puncture and warming unit, and (3) Trocar needle.

2) Previous research information and puncture warming methodology

While percutaneous puncture-type ablation devices such as radiofrequency (RF) ablation have the same effect as surgical resection, the application to cancer such as hepatic cancer is limited because the principle involves applying a direct high frequency current to the living body presenting problems such as safety and temperature control. Also, a thick 15G puncture electrode needle (outer diameter 1.8 mm) is used.

We have developed a Microheater and control device retaining the advantages of percutaneous punctures that enables safe piercing and heating of the affected parts through constant automatic control using a sensor at the tip of a

fine diameter puncture needle, and without applying current to the living body.

The Microheater used in the puncture warming procedure has a 27G (outer diameter 0.42 mm) structure that is 150 mm long and a section of 10 mm at the tip generates heat. A 22G trocar needle with a length of 150 mm is used as the guide needle (outer diameter 0.72 mm). The trocar needle is pierced into the affected part under CT guidance and the Microheater is inserted into the lumen of the trocar needle. The Microheater can be used to heat any target near the single heater (radius 3 mm to 5 mm) by setting the temperature in the range between 50℃ to 100℃ for a duration ranging from 0.5 min to 10 min. Before and after the puncture and warming treatment, anesthetic, cell drugs, or a contrast medium can be locally administered through the trocar needle used for the puncture. Also, depending on the condition of the target tumor and affected part, 4 Microheaters can be simultaneously pierced to provide warming treatment with independent temperature control.

3) Safety test results

The electrical safety test results of the test device AMTC300 are shown in **Table 2**. The device was confirmed to meet the electrical safety standards of medical devices.

4) Performance test results

The heat generation performance test and timer operation checks were implemented for the Microheater of the device AMTC300. Heating simulation and heating experiments were carried out, and performance verification for clinical use of the puncture warming procedure was implemented.

Heating simulation equivalent to biological phantom tests is shown in **Fig. 2**, and results of the heating experiment performed on raw meat (chicken breast meat) are shown in **Fig. 3**.

The summary of warming tests using raw meat (chicken breast meat) with two heating needles is given below.

(1) Condition: Distance between needles 8 mm, heating element (heater) length 10 mm, heating element temperature (measured on the outer needle) approximately 80℃, heat genera- tion time 600 sec.

(2) Results: See **Fig. 3**.

(3) Discussion: The heated length of the needle was covered in white due to protein denaturation. The results were a match to the simulation results in the left panel of **Fig. 2** (Distance between needles 8 mm, length of heater element 10 mm, heating temperature 75℃, and heat generation time 600 sec).

5. Implementation of this study treatment: Patient characteristics, local injection and warming the lesions

1) Patient characteristics

A total of 46 patients (minimum age 37 years, maximum age 84 years, average age 62.4 years) participated in this study treatment, 15 were males (average age 66.7 years), and 31 were females (average age 60.4 years). When classifying cancer that is the primary disease, the cases included ovarian cancer (1 case), renal cancer (4 cases), malignant lymphoma (3 cases), lung cancer (6 cases), breast cancer (10 cases), stomach cancer (2 cases), uterine cancer (5 cases), colorectal cancer (6 cases), pancreatic cancer (3 cases), thymoma (1 case), esophageal cancer (1 case), bile duct cancer (2 cases), head and neck cancer (1 case), and pharynx cancer (1 case). Intensity Modulated Radiation Ther- apy (IMRT) and immunotherapy was provided as the disease stage was recurrent or stage III and above, but warming treatment was a prospective treatment for these patients due to exacerbation or metastasis.

2) Local injection of drug into the lesions (local injection)

Local anesthetic was injected after the puncture, and immature dendritic cells (iDCs) or cytotoxic T cells (CTLs) were administered after warming, a different protocol was followed for each subject.

3) Puncture to the lesions and limited warming

The Microheater with cord and trocar needle, which also serves as a guide needle, were wrapped in a sterilized bag. These were sterilized using ethylene oxide gas and used for the puncture warming treatment of the patients in the treatment room. For controlled warming of the target lesions, up to 4 guide trocar needles were pierced one by one depending on the condition of the target tumor and affected part under X-ray computed tomography (CT), and then Microheaters were inserted into the lumen of the trocar needles. Warming treatment was performed using separate protocols by setting the temperatures in the range between 60℃ to 100℃ for a duration ranging from 0.5 min to 10 min. Case list I is given in **Appendix 1.**

4) Procedure to determine the effect

The therapeutic effect on the tumors was determined by periodic observation of the target sites using Positron Emission Computed Tomography (PET-CT). Tumor markers or biochemical markers were used for the observation depending on the case.

II Results

1. Operability and malfunction of the warming device

Multiple doctors smoothly operated the main unit of the warming device, guide needle, and Microheater on 48 research subjects for warming treatment at 241 sites. The maximum time required for one treatment cycle per patient (puncture, increase in temperature, warming, lowering of temperature, and needle withdrawal) was 30 minutes, but treatment up to 150 minutes could be provided with 5 repetitions of the cycle.

Case list I (safety evaluation) is given in **Appendix 1**. In addition to 46 research subjects with 241 warming treatment sites, and 181 local injection sites, 2 other subjects with 4 events were evaluated (total 48 subjects). Device malfunctioned in 11 subjects with 16 events (**Table 3**), and adverse events were observed in 14 subjects with 15 events (**Table 4**). 10 malfunctions of the main unit were initial malfunctions and was addressed by improving the software for the display and control units, and setting up the hardware functions. A total of 6 malfunctions such as disconnection of the Microheater needle thermocouple and damaged warming needle base were also considered as initial malfunctions, and the manufacturing technology, inspection process, and hardware were improved to solve these problems.

2. Adverse events in this study treatment

Adverse events were observed in 14 subjects with 15 events (**Table 4**). There were 3 subjects with relatively serious adverse events due to the electric warming device and puncture treatment including sharp pain due to puncture and application of electric current, sharp pain, bleeding from the puncture site and vaginal bleeding. Puncture and warming treatment was stopped immediately after the onset of the symptoms and treated by prescribing analgesics, and sequelae was not observed in any of the subjects.

9 other subjects were observed to have pain or numbness after the treatment, but they were not severe, and remission was observed in all subjects with painkillers. Physiological reactions were induced in 3 subjects due to the puncture and warming treatment in the affected part and surrounding organs with 1 each of pneumothorax and degassing, vomiting of gastric juice, and post-treatment urinary urgency. There was remission in all the subjects during subsequent follow-up.

3. Determining the effectiveness evaluation for this study treatment

Case list II (effectiveness evaluation) that includes treatment conditions for effectiveness evaluation and the result of evaluation for 46 research subjects with 240 warming treatment sites is given in **Appendix 2**. Information on whether dendritic cells (DC) or cytotoxic T

lymphocytes (CTL) formulation was administered after warming treatment and the warming temperature range are given in the list. The classification of effectiveness judgment for the lesions targeted during the evaluation is given below.

CR (complete response)
PR (partial response)
SD (stable disease)
PD (progressive disease)

4. Effectiveness evaluation by total and various categories

The overall effectiveness evaluation for 182 sites where local warming was combined with immunotherapy is given in (**Table 5-1**), and the results based on various categories, DC local injection, CTL local injection, warming at 100℃ and warming range between 60℃ to 70℃ is given in (**Table 5-2**).

III Discussion

1. Findings from the previous studies and this study

After the fundamental phantom tests, experiments were conducted on animals with tumors in the high temperature hyperthermia region of 60℃ to 80℃, at the Ehime University Hospital[7]. Human renal cancer cell line Caki-1 was injected into the back of the nude mice and tumor growth was subjected to 3 levels of warming treatment between 45℃ to 65℃ and warming effect was observed based on tumor volume and tissue staining. A decrease in tumor growth rate was observed at the sites that were warmed compared to the sites that were not warmed. The expected heat generation characteristics and safety of the warming device were also verified.

In this study, the authors planned to examine if there was a clinical possibility of combining local warming treatment with immunotherapy for effective treatment of patients with severe cancer. The authors conducted this clinical study using the ultra-fine diameter local warming device with a function to inject immunological drugs locally for deep tumor lesions at the Tokyo Research Center, Hasumi International Research Foundation.

The results of the present study that combined the puncture warming treatment and immune cell therapy for patients with recurrent and metastatic cancer who had completed the standard therapy showed the safety of the combination therapy and puncture warming device to humans. The treatment when combined with immunotherapy was suggested to be effective and suppresses the progress of cancer in the target site.

2. Safety

For evaluation of safety, in addition to 56 research subjects with 241 puncture warming treatment sites and 181 local injection sites, 2 other subjects with 4 events were evaluated. Device malfunctioned in 11 subjects with 16 events (**Table 3**), and adverse events were observed in 14 subjects with 15 events (**Table 4**).

10 malfunctions of the main unit were initial malfunctions and could be addressed by improving the software for the display and control units, and setting up the hardware functions. A total of 6 malfunctions such as disconnection of the Microheater thermocouple and damaged warming needle base were also considered as initial malfunctions, and the manufacturing technology, inspection process, and hardware were improved to solve these problems. The device will be improved along with the manufacturing technology before use in future clinical applications.

There were 3 subjects with relatively serious adverse events due to the electric warming device and puncture warming treatment, sharp pain due to puncture and application of electric current including sharp pain, bleeding from the puncture site and vaginal bleeding. There were 9 subjects with pain or numbness after the treatment, and 3 other subjects with non-severe adverse events, 1 each of pneumothorax and

degassing, vomiting of gastric juice, and post-treatment urinary urgency. All these reactions could be considered as a physiological reaction induced due to the puncture and warming treatment in the affected part and surrounding organs. For the future clinical application of the device, warning on the adverse events will be provided to the practitioners in the form of "Precautions".

The target diseases included ovarian cancer, renal cancer, malignant lymphoma, lung cancer, breast cancer, stomach cancer, uterine cancer, colorectal cancer, pancreatic cancer, thymoma, esophageal cancer, bile duct cancer, head and neck cancer, and pharynx cancer. Puncture and warming sites included those with recurrence or metastasis of the corresponding cancer and sites involving other organs with metastasis, but if blood vessels and nerves were passing through or adjacent to the target lesion sites, then puncture and warming of such sites was avoided.

As pointed out by medical personnel, puncture warming procedure is contraindicated for vital organs that support life such as brain and heart, and blood vessels circulating blood, and nerves used for neurotransmission. Puncture and warming of lesion sites must also be avoided if blood vessels, and nerves are passing through or adjacent to the lesion targeted for treatment.

After taking into consideration the above safety findings of this clinical study and observing the contraindications and safety instructions, the clinical application of the device and puncture warming procedure is considered possible.

3. Effectiveness

The overall evaluation for 182 sites where local warming was combined with immunotherapy is given in (**Table 5-1**), and the results based on various categories, DC local injection, CTL local injection, warming at 100°C and warming range between 60℃ to 70℃ is given in (**Table 5-2**). This study was conducted for patients with recurrent and metastatic cancer who had completed standard treatments. The combined use of warming treatment and immune cell therapy indicated Complete Response (CR) in 25% of the sites, Partial Response (PR) in 30% of the sites, and Stable Disease (SD) in 18% of the sites and progression was suppressed in 73% of all the sites. This result is almost same as that of the trials conducted by combining with radiotherapy in small-scale clinical study[1)] with the suppression of progression in 78% of the sites including 48% Complete Response CR and excluding 29% Progressive Disease PD. This suggests the feasibility of a combination therapy that combines puncture and warming treatment with other therapies such as immune cell therapy that was used in this study.

4. Overview

An investigator-initiated clinical study was conducted by combining puncture warming treatment and immune cell therapy for patients with recurrent and metastatic cancer who had completed the standard therapy. From the results, the safety of the device and puncture warming treatment to humans and clinical application was confirmed. The effectiveness of combining this treatment with immunotherapy and feasibility of the combination therapy was also suggested.

Summary

To effectively treat patients with recurrent and metastatic cancer who had completed the standard therapy, local puncture and warming treatment was combined with immunotherapy, and the clinical feasibility in humans was examined. The authors conducted the procedure for deep tumor lesions using the micro-diameter local warming device with a function to inject immunological drugs locally. A total of 46 patients (minimum age 37 years, maximum age 84 years, average age 62.4 years) participated, 15 were males (average age 66.7 years), and 31

were females (average age 60.4 years).

When the primary disease was classified, the cases included ovarian cancer (1 case), renal cancer (4 cases), malignant lymphoma (3 cases), lung cancer (6 cases), breast cancer (10 cases), stomach cancer (2 cases), uterine cancer (5 cases), colorectal cancer (6 cases), pancreatic cancer (3 cases), thymoma (1 case), esophageal cancer (1 case), bile duct cancer (2 cases), head and neck cancer (1 case), and pharynx cancer (1 case). The results of overall evaluation and for various categories such as dendritic cells (DC) local injection, activated T cell (CTL) local injection, warming at 100°C, and warming range between 60℃ to 70℃ were shown for 182 sites where local warming was combined with immunotherapy. The combined use of warming method and immune cell therapy indicated Complete Response (CR) in 25% of the sites, Partial Response (PR) in 30% of the sites, and Stable Disease (SD) in 18% of the sites and progression was suppressed in 73% of all the sites.

In 241 warming treatment sites considered for safety evaluation, device malfunctioned in 16 subjects, and 15 subjects with adverse events were observed. Ten of the malfunctions were considered as initial malfunction. There were 3 subjects with relatively serious adverse events due to the device and puncture warming treatment. The treatment was stopped immediately after the onset of the symptoms and treated by prescribing analgesics, and sequelae was not observed in any of the cases. 12 other subjects were observed with pain and physiological reactions after the treatment, but all of them were non-severe and remission was observed in all subjects during follow-up.

This confirmed the safety of the device and puncture warming treatment to humans. This suggested that the puncture warming treatment when combined with immunotherapy is effective for cancer target sites, and suppresses the progress of cancer.

[**Conflict of Interest**] This is to disclose that there is no potential conflict of interest relationship between Tokyo Research Center, Hasumi International Research Foundation and Admetech Corporation (contribution of 1 million yen or more per year from the latter to the former).

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Table 1　Specification and performance of test device AMTC300

[Electrical Specifications]	
Power supply voltage	100 V AC ±10%
Power supply frequency	50/60 Hz
Power supply input	100 VA
DC output (4 channels) 1 channel	5 V 0.8 A
Medical devices regulations	Class 2 A
[Machine Specifications]	
Dimensions	Width 230 mm × Height 135 mm × Length 260 mm
Weight	7 kg
[Operating Environmental Conditions]	
Temperature	10 to 40℃
Humidity	30 to 75%RH
Pressure	700 to 1060 kPa
[Heating Performance]	
Temperature setting	40 to 100℃
Heating time	1 to 10 min (up to 60 min possible)

Fig. 1　Photo of test device

Left = Main unit, Upper right = Microheater, lower right = Trocar needle

Table 2 Electrical safety test results of test device AMTC300

	Item	Test method	Standard value	Judgment
Electrical safety	Leakage current	According to JIS T 0601-1: 1999. 19	Earth leakage current Exterior leakage current Patient leakage current Leakage current measured in patients	There is no problem within the specification range
	Dielectric strength	According to JIS T 0601-1:1999.20	Must meet the requirements of JIS T 0601-1:1999.20	Same as above
	Power supply	According to JIS T 0601-1:1999.10.2.2 a)	Single phase AC 100 V 1 A	Same as above
	Resistance of protective earth circuit	According to JIS T 0601-1:1999.18f)	Device with power socket: The impedance between the earthing blade of the power socket and any contactable metal part should not exceed 0.1 Ω	Same as above

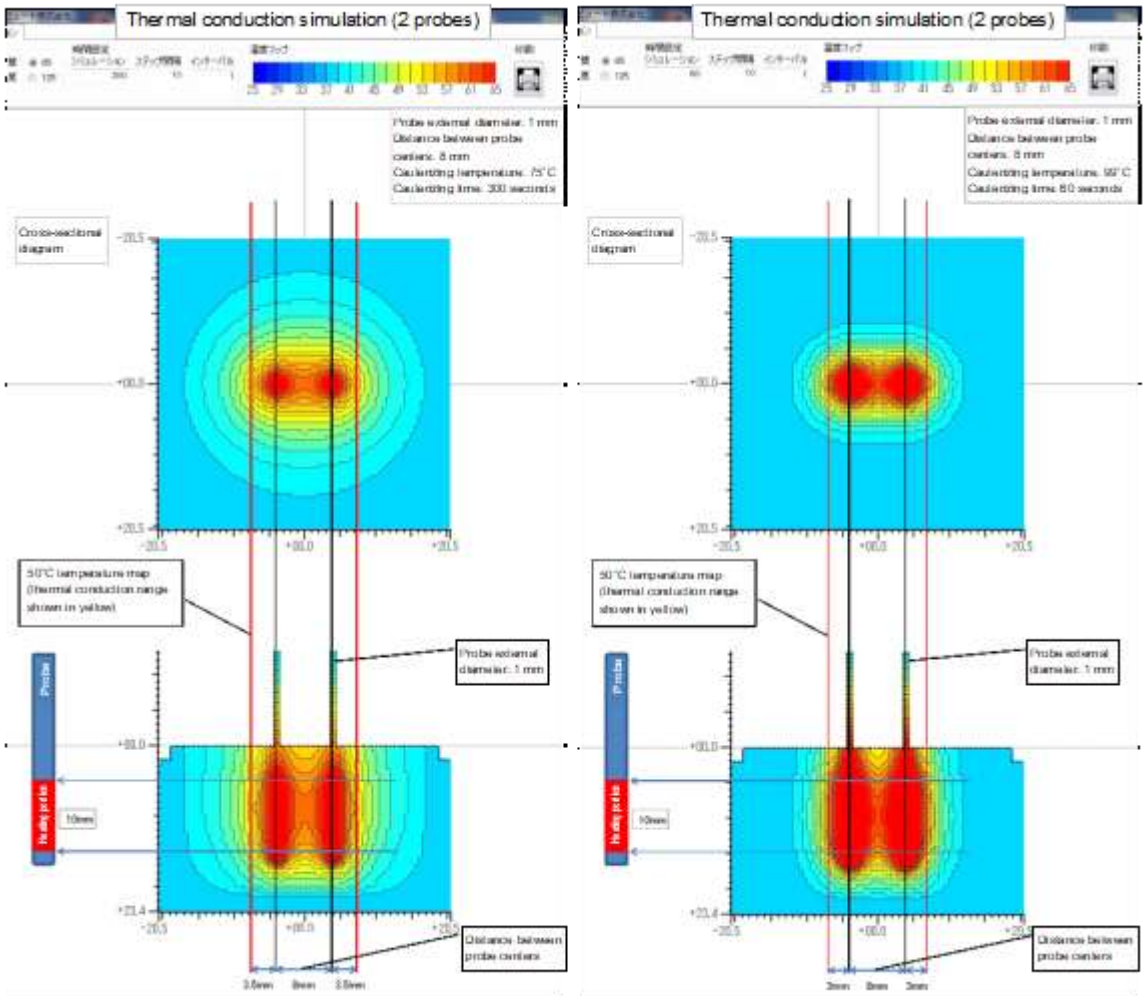

Fig. 2　Heating simulation equivalent to phantom test

The above figure is an example of computer-simulation for the state of heat transfer to a living body when two warming needles are pierced in parallel at intervals of 8 mm to generate heat. In the figure, cells within the red line (50°C) are presumed to undergo protein denaturation.

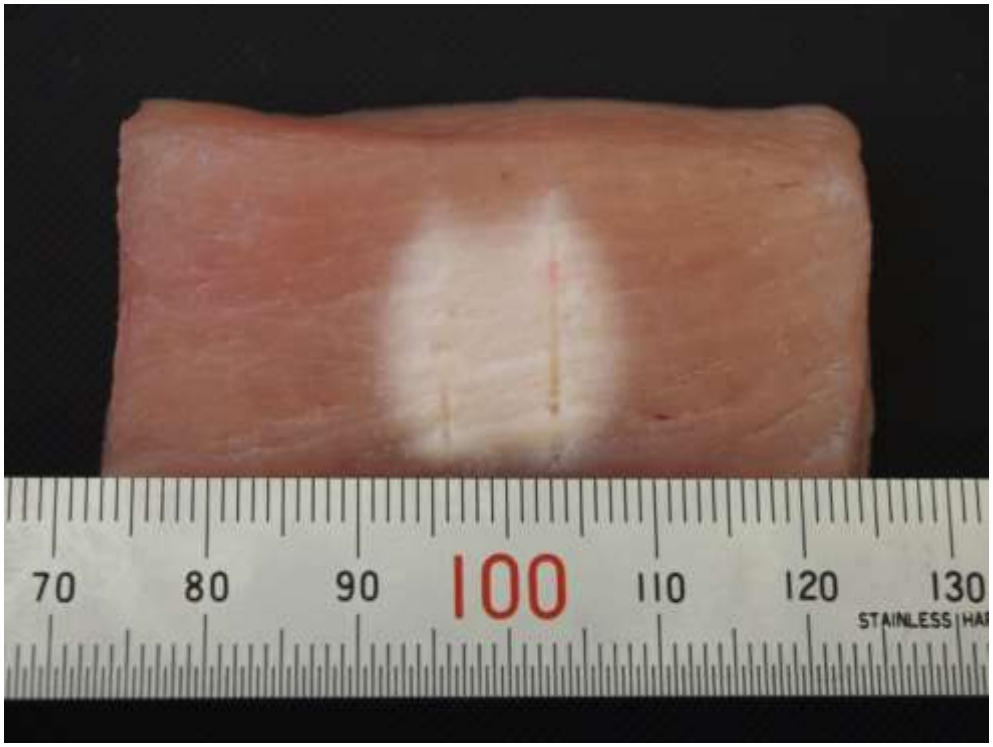

Fig. 3 Warming experiment using raw meat (chicken breast meat) with two heating needles

Table 3 Malfunction of warming device

Malfunction	Part of the device	No. of cases (Frequency%)	Note
Error display	Main display unit	1 (0.41)	Improvement of software for initial malfunction
LED display error	Main display unit	1 (0.41)	
Temperature display error	Main display unit	2 (0.83)	
Temperature setting error	Main display unit	1 (0.41)	
Temperature does not rise	Microheater and warming control	1 (0.41)	Improvement of software and hardware settings for initial malfunction
Temperature rise failure	Microheater and warming control	1 (0.41)	
Overshoot during warming	Warming control	2 (0.83)	
Error when temperature drops	Warming control	1 (0.41)	
Needle thermocouple disconnection	Microheater	5 (1.65)	Improvement of hardware for initial malfunction
Damage to the warming needle base	Microheater	1 (0.41)	
		Total 16 events / 11 subjects	
Remarks		Population parameter 241 sites	

Table 4 Adverse events in study treatment

Adverse events	Treatment site	No. of cases (Frequency%)	Note
Intense pain due to puncture and application of current	Lung cancer	1 (0.41)	Possibility of device malfunction
Intense pain	Colon cancer, passage of current through 4 locations	1 (0.41)	Warming discontinued
Pain and numbness of lower extremities	Breast cancer	1 (0.41)	
Chest pain	Breast cancer	1 (0.41)	
Pain the lower extremities	Colon cancer	1 (0.41)	
Pierced location, vaginal bleeding		1 (0.41)	Based on piercing treatment
Pain around the pierced location	Breast cancer	1 (0.41)	
Lower abdominal pain after treatment	Stomach cancer	1 (0.41)	
Post-treatment pain	Pancreatic cancer, puncture from diaphragmatic membrane	1 (0.41)	
Post-treatment pain	Malignant lymphoma, breast cancer, thyroid gland cancer	3 (1.24)	
Pneumothorax, degassing	Head and neck carcinoma	1 (0.41)	
Vomiting of gastric juice	Colon cancer	1 (0.41)	
Post-treatment urinary urgency	Uterine carcinoma	1 (0.41)	
		Total 15 events / 14 subjects	
Remarks		Population parameter 242 sites	

Table 5-1 Overall evaluation of the study treatment effectiveness

	CR	PR	SD	PD	Total
Number of sites (%)	46 (25.3)	54 (29.7)	32 (17.6)	50 (27.4)	182 (100)

CR＝complete response, PR＝partial response, SD＝stable disease, PD＝progressive disease

Table 5-2 Effectiveness evaluation of the study treatment for different categories

Number of sites	CR	PR	SD	PD	Total
DC local injection (%)	14 (26.4)	12 (22.6)	11 (20.8)	16 (30.2)	53 (100)
CTL local injection (%)	29 (25.2)	41 (32.8)	21 (16.8)	34 (27.2)	125 (100)
100℃ (%)	24 (30.8)	23 (29.4)	8 (10.3)	23 (29.4)	78 (100)
60~70℃ (%)	23 (22.1)	30 (28.8)	24 (23.1)	27 (26.0)	104 (100)

CR＝complete response, PR＝partial response, SD＝stable disease, PD＝progressive disease

Appendix 1　Case List I （Safety Evaluation）

Case number	Gender	Age	Classification	Disease stage	Number of treatments Number of times of warming (Count)	AMTC warming HB Temperature range	Local injection Number of sites (Evaluation instrument)	Device malfunction (11 subjects and 16 events)	Adverse events (14 subjects and 15 events)
1	F	53	Ovarian cancer	Recurrence	14	HB60℃ 10min HB70℃ 5min	14	Yes ① Error when temperature drops ② Needle thermocouple disconnection	None
2	M	68	Renal cancer	IV	12	HB60℃ 5min HB100℃ 0.5min HB70℃ 5min	12	None	None
3	F	61	Malignant lymphoma	Recurrence	9	HB100℃ 0.5min	6	None	None
4	M	84	Lung cancer	Recurrence	1	HB100℃ 0.5min	0	None	None
5	F	56	Breast cancer	IIIb	2	HB70℃ 5min	2	None	None
6	M	82	Stomach cancer	Recurrence	3	HB70℃ 10min	3	None	None
7	F	72	Lung cancer	IV	4	HB70℃ 10min HB100℃ 5min	4	None	None
8	F	49	Breast cancer	Recurrence	3	HB100℃ 0.5min	3	None	Yes ① Pain, lower extremities
9	M	76	Malignant lymphoma	Unidentified	1	HB100℃ 0.5min	1	None	None
10	M	66	Lung cancer	Recurrence	3	HB60℃ 5min	3	None	None
11	F	50	Breast cancer	Recurrence	10	HB100℃ 0.5min	6	None	None
12	M	73	Lung cancer	Recurrence	6	HB70℃ 10min	6	None	None
13	F	40	Breast cancer	IV	8	HB60℃ 10min	8	None	Yes ① Chest pain
14	F	68	Colorectal cancer	Recurrence	2	HB60℃ 5min	1	None	None
15	M	84	Renal cancer	Recurrence	1	HB100℃ 0.5min	1	None	None
16	F	63	Malignant lymphoma	IV	7	HB60℃ 5,10min HB100℃ 0.5min	6	Yes ① Error display ② Thermocouple disconnection	None
17	F	66	Uterine carcinoma	Recurrence	2	HB60℃ 5min	2	None	None
18	F	69	Lung cancer	IV	5	HB60℃ 10min	5	Yes ① Warming needle base tab damage	None
19	F	49	Colorectal cancer	Recurrence	7	HB60℃ 5min HB70℃ 10min	6	None	None
20	F	48	Breast cancer	Recurrence	3	HB70℃ 10min	3	None	Yes ① Pain around the pierced location
21	F	52	Stomach cancer	IV	3	HB70℃ 10min	3	Yes ① Warming overshoot ② Needle thermocouple disconnection	Yes ① Lower abdominal pain after treatment
22	M	39	Pancreatic cancer	Recurrence	3	HB70℃ 10min HB60℃ 10min	3	Yes ① Warming overshoot ② Needle thermocouple disconnection	None
23	F	83	Breast cancer	Recurrence	3	HB70℃ 5min		Yes ① Temperature display error	None
24	F	69	Pancreatic cancer	Recurrence	3	HB70℃ 5,10min		None	Yes ① Post-treatment pain
25	F	53	Breast cancer	Recurrence	3	HB100℃ 0.5min	3	None	Yes ① Post-treatment pain

26	F	65	Breast cancer	Recurrence	12	HB60℃ 10min HB70℃ 5min HB100℃ 0.5min	9	None	None
27	F	51	Colorectal cancer	Recurrence	19	HB70℃ 10min HB100℃ 0.5min	8	Yes ① Thermocouple disconnection	Yes ① Pain the lower extremities ② Pierced location, vaginal bleeding
28	M	50	Pancreatic cancer	IV	4	HB70℃ 5min	4	None	None
29	M	37	Pharyngeal cancer	Recurrence	2	HB100℃ 0.5min	2	None	None
30	M	64	Colorectal cancer	Recurrence	1	HB100℃ 0.5min	1	None	Yes ① Vomiting of gastric juice
31	F	59	Renal cancer	Recurrence	3	HB70℃ 10min HB100℃ 0.5min	2	Yes ① Temperature does not rise ② Temperature setting error	None
32	F	71	Uterine carcinoma	IV	1	HB70℃ 10min	1	None	None
33	F	63	Colorectal cancer	Recurrence	15	HB60℃ 10min HB100℃ 0.5min	14	None	None
34	F	62	Thymoma	IVb	2	HB60℃ 5min	2	None	None
35	M	80	Esophageal cancer	Recurrence	1	HB100℃ 0.5min	0	None	None
36	M	63	Renal cancer	Recurrence	2	HB100℃ 0.5min	2	None	None
37	F	66	Lung cancer	III	7	HB70℃ 5min HB100℃ 0.5min	6	None	None
38	F	56	Uterine carcinoma	Recurrence	2	HB100℃ 0.5min	2	None	None
39	F	56	Bile duct cancer	Recurrence	10	HB100℃ 0.5min	2	Yes ① LED display error	None
40	M	63	Bile duct cancer	Recurrence	16	HB100℃ 0.5min	6	Yes ① Temperature display error	None
41	M	71	Head and neck carcinoma	?IV	2	HB100℃ 0.5min	2	None	Yes ① Pneumothorax and degassing
42	F	73	Breast cancer	IV	2	HB100℃ 0.5min	2	None	None
43	F	52	Uterine carcinoma	Recurrence	1	HB100℃ 0.5min	1	None	Yes ① Post-treatment urinary urgency
44	F	75	Uterine carcinoma	IIIb	11	HB100℃ 0.5min	6	None	Yes ① Experienced pain after treatment
45	F	72	Breast cancer	III	4	HB100℃ 0.5min	4	None	None
46	F	49	Colorectal cancer	Recurrence	5	HB100℃ 0.5min	4	None	Yes ① Intense pain, warming discontinued
47	M	75	Lung cancer		3	HB70℃ 10min	3	Yes ① Temperature rise failure	Yes ① Intense pain due to puncture and application of current
48	F	47	Thyroid gland cancer		1	HB60℃ 10min	1	None	Yes ① Post-treatment pain

M = male, F = female

Appendix 2　Case List II（Effectiveness Evaluation）

Case number	Gender	Age	Classification	Pathological diagnosis	Disease stage	Number of treatments (DC/CTL)			Local injection Number of times of warming	AMTC warming HB	Local injection Number of sites	Evaluation (CR/PR/PD) distribution			
						DC	CTL	Others	(Count)	Temperature range	(Evaluation instrument)	CR	PR	SD	PD
1	F	53	Ovarian cancer	Peritoneal origin serous papillary carcinoma	Recurrence	5	9		14	HB60℃ 10min HB70℃ 5min	14	4	2	2	6
2	M	68	Renal cancer	Clear cell carcinoma	IV	12			12	HB60℃ 5min HB100℃ 0.5min HB70℃ 5min	12	6	3	3	0
3	F	61	Malignant lymphoma	Follicular type malignant lymphoma	Recurrence	3	6		9	HB100℃ 0.5min	6	2	3	1	0
4	M	84	Lung cancer	Large cell carcinoma	Recurrence	1			1	HB100℃ 0.5min	0				
5	F	56	Breast cancer	Invasive ductal carcinoma	IIIb		2		2	HB70℃ 5min	2		2		
6	M	82	Stomach cancer	Adenocarcinoma	Recurrence		3		3	HB70℃ 10min	3		1	1	1
7	F	72	Lung cancer	Adenocarcinoma	IV		4		4	HB70℃ 10min HB100℃ 5min	4	1	2		1
8	F	49	Breast cancer	Invasive ductal breast carcinoma	Recurrence		3		3	HB100℃ 0.5min	3		3		
9	M	76	Malignant lymphoma	Follicular lymphoma (G1)	Unidentified		1		1	HB100℃ 0.5min	1				1
10	M	66	Lung cancer	Adenocarcinoma	Recurrence		3		3	HB60℃ 5min	3				3
11	F	50	Breast cancer	Invasive ductal gland carcinoma	Recurrence	7		3	10	HB100℃ 0.5min	7	2	2		3
12	M	73	Lung cancer	Adenocarcinoma	Recurrence	6			6	HB70℃ 10min	6		3	1	2
13	F	40	Breast cancer	Papillotubular carcinoma	IV		8		8	HB60℃ 10min	8		3		5
14	F	68	Colorectal cancer	Adenocarcinoma	Recurrence	2			2	HB60℃ 5min	1			1	
15	M	84	Renal cancer	Renal cell carcinoma	Recurrence	1			1	HB100℃ 0.5min	1			1	
16	F	63	Malignant lymphoma	Malignant lymphoma	IV	2	5		7	HB60℃ 5,10min HB100℃ 0.5min	6	2	3	1	
17	F	66	Uterine carcinoma	Invasive squamous cell carcinoma	Recurrence		2		2	HB60℃ 5min	2				2
18	F	69	Lung cancer	Adenocarcinoma	IV	3	2		5	HB60℃ 10min	5			3	2
19	F	49	Colorectal cancer	Adenocarcinoma	Recurrence		7		7	HB60℃ 5min HB70℃ 10min	6			5	1
20	F	48	Breast cancer	Invasive ductal breast carcinoma	Recurrence		3		3	HB70℃ 10min	3		2		1
21	F	52	Stomach cancer	Signet ring cell ca.	IV		3		3	HB70℃ 10min	3		2	1	
22	M	39	Pancreatic cancer	Moderately differentiated adenocarcinoma	Recurrence		3		3	HB70℃ 10min HB60℃ 10min	3	1		1	1
23	F	83	Breast cancer		Recurrence		3		3	HB70℃ 5min					
24	F	69	Pancreatic cancer	Adenocarcinoma s/o	Recurrence		3		3	HB70℃ 5,10min					Necrosis
25	F	53	Breast cancer	Invasive ductal breast carcinoma	Recurrence		3		3	HB100℃ 0.5min	3	1	1		1
26	F	65	Breast cancer	Solid tubular carcinoma	Recurrence	4	8		12	HB60℃ 10min HB70℃ 5min HB100℃ 0.5min	9	1	4	2	2
27	F	51	Colorectal cancer	Adenocarcinoma	Recurrence	9	5	5	19	HB70℃ 10min HB100℃ 0.5min	8	4	1	3	
28	M	50	Pancreatic cancer	Adenocarcinoma s/o	IV		4		4	HB70℃ 5min	4	2	1	1	
29	M	37	Pharynx cancer		Recurrence		2		2	HB100℃ 0.5min	2		1	1	
30	M	64	Colon cancer	Adenocarcinoma	Recurrence		1		1	HB100℃ 0.5min	1				1
31	F	59	Renal cancer	Clear cell carcinoma	Recurrence	1	2		3	HB70℃ 10min HB100℃ 0.5min	2		2		
32	F	71	Uterine carcinoma	Squamous cell carcinoma	IV		1		1	HB70℃ 10min	1			1	
33	F	63	Colorectal cancer	Adenocarcinoma	Recurrence		15		15	HB60℃ 10min HB100℃ 0.5min	14	10	2	1	1
34	F	62	Thymoma	Malignant Thymoma	IVb		2		2	HB60℃ 5min	2	2			
35	M	80	Esophageal cancer	Neuroendocrine carcinoma	Recurrence		1		1	HB100℃ 0.5min	0				
36	M	63	Renal cancer	Clear cell carcinoma	Recurrence	2			2	HB100℃ 0.5min	2			2	

37	F	66	Lung cancer	Adenocarcinoma	III	5	1	1	7	HB70℃ 5min HB100℃ 0.5min	6	1	1		4
38	F	56	Uterine carcinoma	Clear cell carcinoma	Recurrence		2		2	HB100℃ 0.5min	2				2
39	F	56	Bile duct cancer	Adenocarcinoma	Recurrence	3		7	10	HB100℃ 0.5min	2	1			1
40	M	63	Bile duct cancer	Vater's papilla carcinoma	Recurrence	10	6		16	HB100℃ 0.5min	6		6		
41	M	71	Head and neck carcinoma	Unidentified	?IV	2			2	HB100℃ 0.5min	2	1			1
42	F	73	Breast cancer	Invasive ductal breast carcinoma	IV	2			2	HB100℃ 0.5min	2	1	1		
43	F	52	Uterine carcinoma	Squamous cell carcinoma	Recurrence		1		1	HB100℃ 0.5min	1				1
44	F	75	Uterine carcinoma	Squamous cell carcinoma	IIIb	7	4		11	HB100℃ 0.5min	6	3			3
45	F	72	Breast cancer	Invasive ductal gland carcinoma	III		4		4	HB100℃ 0.5min	4	1	3		
46	F	49	Colorectal cancer	Moderately differentiated adenocarcinoma	Recurrence		5		5	HB100℃ 0.5min	4				4
									240	Total	182	46	54	32	50

M = male, F = female