Development of a highly efficient implanted thermal ablation device: *in vivo* experiment in rat liver

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Objectives: To evaluate an implanted thermal ablation device that can be heated with high efficiency using a resonant circuit as the implant.

Methods: 16 rats were used. The implants, adjusted at a resonance frequency of 4 MHz, were fixed on the surface of the liver of rats under laparotomy. In 14 of 16 rats, an alternating magnetic field (AMF) was applied for 6 min with an output of 300 W from outside the body using a ferrite core applicator. The implant temperature during AMF exposure was measured. The 14 rats were divided into 5 groups, depending on time from AMF application until they were sacrificed (1 h, 1 day, 3 days, 7 days and 1 month after application). Two rats not exposed to AMF were used as controls. Livers were removed and evaluated; the cross-sectional area and width of the ablated region were measured.

Results: During AMF exposure, the implant temperature rose to $127.8 \pm 39.3^\circ\text{C}$ (mean $\pm$ standard deviation). The cross-sectional area of the ablated region was largest after 1 day and tended to decrease with time. The widths of the ablated region were 4.87 ± 0.22 mm, 4.15 ± 0.36 mm, 3.67 ± 0.58 mm and 3.24 ± 0.16 mm in the 1 day, 3 day, 7 day and 1 month groups, respectively. No significant differences ($p < 0.05$) were seen in either cross-sectional area or width of the ablated region.

Conclusion: Sufficient heat for ablation was obtained *in vivo* using a newly developed implanted thermal ablation device. This device may be a new option for thermal ablation therapy.

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Radiofrequency ablation (RFA) is a common treatment method for liver cancer and other malignant neoplasms [1]. With this method, an electrical current is generated in living tissue from an electric field produced by an electrode, causing thermal ablation of the tissue from frictional heat. Microwave ablation, in which water molecules in the body are made to vibrate and the frictional heat produced by this vibration causes an elevation in the temperature of living tissue, is similar [2]. Although the principles of RFA and microwave ablation differ, in both methods an electrode is inserted directly and heat is produced in the body tissue itself. Depending on the indication, these methods have a high level of local control, comparable with that of surgery, and have become widely used in recent years. However, RFA and microwave ablation devices have limitations in that the electrode must be inserted percutaneously and fixed, which can cause pain and distress in patients during long treatment, and a new puncture is required in cases of tumour recurrence.

On the other hand, a thermal ablation device was developed using the principle of inductive heating produced by application of an alternating magnetic field

(AMF) from outside the body, with placement of a small implant within the body [3, 4]. There have been reports of thermal ablation devices using materials science methods in which a ferromagnet is used as the implant. In *in vivo* experiments, however, the heat generated, approximately $50\text{--}60^\circ\text{C}$, is not sufficient for ablation. By contrast, a device with a design that increases heat efficiency using an electromagnetic engineering approach that adopts a resonant circuit as an implant, with which temperature elevation can be obtained with low output using a ferrite core in the heating device, has also been developed [5]. In *ex vivo* experiments, it has been reported that high heating efficiency is obtained with this device. The purpose of the present experiment was to investigate the heat generation capacity *in vivo* using this implanted thermal ablation device, and the histological changes in the region ablated with this device.

Methods and materials

Experimental design

All experiments were performed with approval from the experimental animal centre at our institution. The animals used were 16 male retired breeder rats (Jcl:SD; Clea Japan Inc., Tokyo, Japan), with body weights of 600–800 g.

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Implants and applicator system

The implant was a resonant circuit consisting of a coil and microcondenser (Figure 1). The coil was made of silver and had four windings, with a diameter of 5 mm and length of 5 mm. The microcondenser had a capacity of 22 000 pF and temperature characteristics of 60 parts per million °C⁻¹. The resonance frequency of the implant was adjusted to 4 MHz, and it was coated with silicon.

The AMF applicator was a copper solenoid in which a cylindrical ferrite core had been inserted to raise the magnetic flux density. The applicator was water-cooled to maintain a constant temperature and resistance. The distance between the applicators was set at 20 cm.

Heating procedure

The rats were anaesthetised using an intramuscular injection of a mixture of xylazine hydrochloride (Celactal, 3.3 mg kg⁻¹; Bayer Japan, Tokyo, Japan) and ketamine hydrochloride (Ketalar, 33 mg kg⁻¹; Sankyo Inc., Tokyo, Japan). A midline incision was made in the abdominal wall of the rats, and the implant was fixed to the liver surface with proline sutures. After closing the abdomen, the rats were placed at the median point between the applicators, and an AMF with frequency adjusted to 4 MHz with an oscillator and output of 300 W was applied for 6 min.

Temperature measurement

The temperature near the implant during AMF irradiation was measured with a fibre optic thermometer (HandyFlex; Neoptix Inc., Quebec, Canada). Immediately after the completion of AMF application, the body surface temperature of the rat was measured with infrared thermography (TVS-3000; Nippon Avionics Ltd, Tokyo, Japan).

Euthanasia and liver retrieval

12 of the 16 rats were divided into four groups by time from AMF application to sacrifice (1 hour, 1 day, 3 days, 7 days; three animals each). Two of the remaining four animals were sacrificed 30 days after the AMF application, and the other two were taken as controls in which an implant was placed but AMF was not applied. Controls were also sacrificed 30 days after implantation. The animals were sacrificed with intracardiac injection of pentobarbital sodium (Nembutal, 1 ml kg⁻¹; Dainihon Pharmaceutical Co., Osaka, Japan). The entire liver including the implant was removed soon after the animals were sacrificed, and it was fixed for 24 h in 10% neutral buffered formalin.

Microscopic observations

The implant was removed from the fixed specimen, and the ablated region was sliced with a thickness of 2 mm on an axial plane along the long axis of the implant. The slice with the largest area of discoloration when viewed grossly was selected. After the slice was dehydrated it was embedded in paraffin, and 3 µm slices were prepared. These slices were then deparaffinised and stained with haematoxylin–eosin (HE; Merck, Tokyo, Japan). Similar paraffin slices were stained using terminal transferase-mediated biotin d-UTP nick end labelling (TUNEL) stain and 70-kDa heat-shock protein (HSP70) stain.

70-kDa heat-shock protein stain

The prepared paraffin slices were deparaffinised, heat treated at 100 °C for 5 min in an autoclave, and washed in running water. They were then immersed in bovine albumin (Serological Research Institute, Richmond, CA) for 5 min at 37 °C, and incubated for 12 h at 4 °C with HSP70 antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA) diluted 200 times. After irrigating in

Figure 1. The implant is a simple circuit, 5 mm in diameter and 5 mm in length, consisting of a solenoid and microcondenser.

Tris-buffered saline (TBS; 500 mM sodium chloride and 20 mM TRIS, pH 7.5), the slides were incubated at 37°C for 30 min with biotin-labelled anti-mouse Ig goat antibodies (DAKO Japan, Tokyo, Japan) diluted 500 times. They were then washed further with TBS, incubated at 37°C for 30 min with peroxidase-labelled streptavidin (DAKO Japan) diluted 500 times, and washed again in TBS. The slides were stained in diaminobenzidine solution (3-3'-diaminobenzidine tetrahydrochloride; Wako Pure Chemical Industries, Osaka, Japan) and, after rinsing in running water, were stained with haematoxylin nuclear stain, dehydrated, cleared and mounted.

Transferase-mediated biotin d-UTP nick end labelling stain

After the prepared slides were deparaffinised, they were treated with protease K for 10 min and rinsed in running water. The slides were then immersed in 0.3% hydrogen peroxide methanol for 10 min at room temperature and washed. After washing four times in TBS, they were incubated at 37°C for 45 min in TUNEL reaction mixture (In Situ Cell Death Detection Kit; Roche Diagnostics, Tokyo, Japan). Then, after washing four times more in TBS, the slides were incubated at 37°C for 30 min in converter-peroxydase solution. Next, they were rinsed in running water and colour-reacted with diaminobenzidine solution. After again rinsing in running water, they were counterstained with methyl green (DAKO Japan), and dehydrated, cleared and mounted.

Measurement of ablated lesion

The specimen slices were scanned and digitised with a digital microscope (Olympus BX40; Olympus, Tokyo, Japan). The area and radius of the ablated area were measured using ImageJ v. 1.40g. The radius of the ablated area was taken to be the length of the perpendicular line from the centre of the implant contact area to the margin of the ablated area.

Statistics

All measured values are expressed as mean $\pm$ standard deviation. The measured values were examined for significant differences ($p < 0.05$) with the Kruskal-Wallis test using statistical software (Graphpad Prism 5.02; GraphPad Software Inc., San Diego, CA).

Results

No decreased appetite or abnormal behaviour was seen in any of the 16 rats during the course, and none had to be removed from the study.

Temperature measurement

The temperature of the implants rose quickly immediately after AMF application, and reached a plateau

in about 2 min. The mean maximum temperature was $127.8 \pm 39.3^\circ\text{C}$ (Figure 2).

In thermography, a temperature elevation of 3–4°C was seen in the skin directly above the area where the implant was fixed, but in other areas no large temperature increases were seen (Figure 3).

Macroscopic findings and histopathological assessment

The tissue near the implant turned white after AMF exposure, and haemostasis was seen on the periphery (Figure 4).

In HE-stained samples 1 h after AMF application, although some eosinophilic increase in the cytoplasm and dense staining of the nuclei were seen, regions with no major morphological changes were seen in a semi-circle around the implants (inner zone). There was a region on the periphery with concentric circular haemostasis and cytoplasmic reduction (outer zone; Figure 5). The margins of the inner and outer zones and normal liver parenchyma were indistinct. In the 1 day group, the margins of each of the zones were distinct, and eosinophilic cells with no nuclei and apoptotic bodies appeared in part of the outer zone (Figure 6). In the 3 and 7 day groups, there was a further increase in cells missing nuclei, and on the border edge of the outer zone and normal area infiltration of macrophages and granulocytes and fibrosis was seen. In the inner zone, cells with little morphological change were seen from 1 h to 7 days, but a tendency was seen for the range to decrease with time. In the 30 day group, the encapsulation of the ablation area margin and the shrinkage of the ablated area had become conspicuous.

In the control group, no gross or histological changes were seen in the tissue near the implant.

70-kDa heat-shock protein stain

In the 1 h group, the inner zone was negative for HSP70, and the outer zone had a small number of

Figure 2. Mean implant temperature during alternating magnetic field application. A plateau was reached in about 2 min, and the mean $\pm$ standard deviation maximum temperature was $127.8 \pm 39.3^\circ\text{C}$.

Figure 3. In thermography immediately after alternating magnetic field application, a temperature elevation of 3–4 °C was seen in the area of the implant, but no large increases in temperature were seen in any other regions (arrow indicates the anterior end of the rat).

positive cells, but the margin of each region was unclear. In the groups after 1 day, there was an increase in positive cells in the outer zone and a band with strongly positive cells appeared on the periphery. The border between the ablated area and normal area became distinct. In the 3 and 7 day groups, fibrosis appeared on the inner side of the strongly positive region.

Transferase-mediated biotin d-UTP nick end labelling stain

TUNEL-positive cells were seen in the inner zone in the 1 to 7 day groups. In the outer zone, TUNEL-positive cells were seen in some places in the 1 h group, and in the 1 day group many of the cells with remaining nuclei were positive. Many cells lacking nuclei were seen in the groups from 3 days and later.

Figure 4. On gross observation, a pale rim is seen on the periphery of the implant.

Figure 5. Schema of ablation area. The ablation area is divided into an inner and outer zone, and in the 3 day and later groups fibrosis appears on the periphery. The width of the ablation area is a perpendicular line from the implant to the margin of the ablation area.

Measurement of ablated area

In the 1, 3, 7 and 30 day groups, the region combining the inner and outer zones was taken to be the extent of the ablation area, and the cross-sectional area and distance from the implant to the ablation area margin were measured. In the 1 h group, the border between the outer zone and normal area was indistinct, and it was not measured.

The cross-sectional area of the ablated region in the 1 day, 3 day, 7 day and 30 day groups was $51.7 \pm 3.0 \text{ mm}^2$, $41.2 \pm 9.9 \text{ mm}^2$, $35.3 \pm 12.6 \text{ mm}^2$ and $23.9 \pm 0.3 \text{ mm}^2$, respectively (Figure 7), and the distance from the implant to

Figure 6. Tissue of the ablated region; the upper left side is the heating zone. (a) Haematoxylin–eosin (HE), 1 day. The border between the outer zone and normal area is distinct. (b) 70-kDa heat-shock protein (HSP70), 1 day. Area of strong HSP70 positivity (arrowheads) is seen on the periphery of the ablated region. (c) Transferase-mediated biotin d-UTP nick end labelling (TUNEL), 1 day. TUNEL-positive cells are seen in the inner zone. (d) HE, 3 days. Fibroblasts appear in the border area (arrows).

Figure 7. The ablated region cross-sectional area of the ablated region was largest after 1 day and tended to decrease with time.

Figure 8. The width of the ablated region is also largest in the 1 day group.

the ablation area margin was 4.87 ± 0.22 mm, 4.15 ± 0.36 mm, 3.67 ± 0.58 mm and 3.24 ± 0.16 mm, respectively (Figure 8). Statistical tests showed no significant differences ($p < 0.05$) in either cross-sectional area or distance, but both cross-sectional area and distance were maximal at 1 day and then tended to decrease with time.

Discussion

Inductive heating is a method of heating with metallic magnetic material present in a high-frequency magnetic field. There have been reports of rod or particle ferromagnets used as materials in implants as a thermal ablation device using inductive heating [6, 7]. In these devices, eddy currents produced within the metallic magnetic material are said to contribute to the generation of heat. The heat generation capacity in these reports is lower than with methods such as RFA or other capacitive heating methods in which an electrode is inserted directly into the body, and almost none report generation of heat equal to or above 60°C , which is said to be necessary for cell death in tissue [8, 9]. The thermal ablation device used in the present study increases the heating efficiency by making the implant an electrical circuit and using resonance characteristics. In this experiment, the mean temperature within the body was raised to above 120°C , confirming that the method has more than adequate heating capacity for coagulative necrosis of tissue.

Histologically, the border between the non-ablated and ablated areas was distinct from 1 day onwards, and the ablated area had an inner zone in which few morphological changes were seen, and an outer zone in which cell nuclei disappeared and cytoplasm was reduced. Cells of the inner zone were TUNEL-positive, and were thought to have died instantly from the high temperature and having fragmented DNA. Similar cell death occurs in tissue ablated with microwave ablation [10], but the tissue is reported to have few

morphological changes. In the outer zone, TUNEL-positive cells and apoptotic bodies were seen with a peak in the 1 day group, and with time the disappearance of nuclei and reduction of cytoplasm were seen. This so-called coagulative necrosis is thought to be a reduction over time with phagocytosis and absorption from cellular infiltration of macrophages and granulocytes. A region strongly positive for HSP70 was seen on the periphery of the ablated tissue. Heat shock protein is expressed at times of cell damage from heat or other causes, and HSP70 in particular is said to contribute strongly to the healing mechanism of damaged tissue [11]. It is reported that tissue that heals well has a high level of HSP70 expression [12, 13], and in the present experiment, the region of strong HSP70 positivity seen on the periphery of the ablation necrosis area is thought to be an area where the healing mechanism from the heat injury is actively working. The tissue image is similar following ablation with RFA and microwave ablation, which also exhibit these histological and immunohistological characteristics.

In this experiment, the area of the ablated region was found to decrease with time after the first day. In the ablated regions with RFA and microwave ablation, the ablation area in animal experiments is reported to reach a maximum size from 12 to 72 h after ablation and then decrease with time [10], and so the present results do not seem to contradict reports on conventional thermal ablation therapy. The width of the ablation range was 4.8 mm from the implant. Compared with RFA, in which an ablation range of about 3 cm in diameter is obtained with temperature elevation to about 70°C , a narrower ablation range is obtained despite the higher mean temperature of 120°C . The reason for this is thought to be that while RFA and microwave ablation heat the tissue itself with resistance heating, the implant used in the present experiment is heated and the tissue temperature is elevated by heat transfer, so the range of the temperature increase is small. Another reason is thought to be the short ablation time of 6 min. It may be possible to

enlarge the range by placing multiple implants or using intermittent AMF application.

The device implanted in the present experiment used the resonance features of electric circuits to improve heating efficiency. There was concern that heating efficiency like that *in vitro* could not be obtained because of changes in the magnetic field within the body, but results that may be even better than those with other inductive heating devices in past reports were obtained. In addition, the ability to raise the temperature with low power for an inductive heating device using a ferrite core applicator in the magnetic field generator will be crucial for the compactness and general purpose properties of the output device in clinical use. This is an implanted device with which treatment can be done multiple times once the implant is in place, which may make it useful in the treatment of large or recurrent tumours. The technique used in this thermal ablation device also has the advantage that it can be applied to implants of various shapes, including stent-like and plate implants [14]. In addition to ablation treatment, it may also be possible to use the device in heat therapy for hollow viscera or blood vessels. Future issues include expanding the ablation range by placing multiple implants and using intermittent AMF application, devising non-invasive methods for placement of implants, and confirming the safety within the body of the AMF application and placed implants.

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