Thermal Damage Threshold of Brain Tissue

—Histological Study of Heated Normal Monkey Brains—

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Abstract

The thermal damage threshold of normal brain tissue was evaluated from immediate and delayed histological changes caused by hyperthermia treatment of normal monkey (*Macaca fuscata*) brains. A 2450 MHz microwave antenna and an antenna cooling system devised by our group were used for interstitial hyperthermia treatment. The antenna within the cooling system was inserted through a small craniectomy under general anesthesia. The temperature at a reference point, 4 mm radially away from the surface of the cooling system, was maintained at 42, 43, 44, 45, or 46°C for 60 minutes. Eighteen animals were treated and sacrificed immediately after the treatment, while nine animals were treated and sacrificed 7 days after the treatment. The histological changes were studied microscopically on sections stained with HE or Kluver-Barrera's method. The non-survival experiment demonstrated that areas heated at 44°C or below showed no obvious irreversible changes. The survival experiment showed areas heated at 44°C or above developed coagulative necrosis. These histological findings indicate that thermal damage occurs in normal brain tissue after heating at 44°C or above for 60 minutes, suggesting that the safety limit for brain hyperthermia is 43°C for 60 minutes.

Key words: histological change, hyperthermia, interstitial microwave irradiation, normal brain tissue, thermal damage threshold

Introduction

Interstitial microwave irradiation is an effective method for inducing localized brain hyperthermia.^{14,15,18-20)} The optimum hyperthermia conditions for the treatment of human malignant brain tumors are determined by the thermal damage threshold of normal brain tissue. However, the effects of hyperthermia on normal brain tissue have not been studied widely.

Most previous information has been obtained from clinical cases with electroencephalographic or neurological changes after systemic fever.^{1,3-5,10,12,23)} Recently, non-survival animal studies have been reported,^{2,6,8,9,11,21,22)} but few have correlated the delayed histological changes caused by heating normal brain tissue.^{9,11)}

We previously developed an antenna cooling system to generate more uniform thermal fields without overheating.^{14,15)} In this study, the thermal damage threshold of normal brain tissue was estimated from the immediate and delayed histological changes caused by heating normal monkey brains with our antenna cooling system.

Materials and Methods

I. Hyperthermia apparatus

We used a microwave hyperthermia system, HMS-020 (Aloka Co., Ltd., Tokyo), consisting of a 2450 MHz microwave generator, thermometry equipment, and a microcomputer with color display. Silicone-coated interstitial microwave antennas 1.5 mm in diameter, with 40 mm radiation tip sections (HTA-2450-5-5; Aloka Co.), were used in all experiments. Intracerebral temperatures were measured by non-perturbing copper-constantan thermo-

couples. This system had 10 thermometry channels. Microwave power could be controlled automatically to maintain one arbitrary thermocouple at a constant temperature.

II. Antenna cooling system

We previously described the antenna cooling system consisting of a silicone tube 5.0 mm in outer diameter.¹⁴ These experiments used a more recently developed cooling system with a 4.0 mm outer diameter tube. The microwave antenna is inserted into the cooling tube and the coupled system is implanted as a unit. Overheating of tissue adjacent to the antenna can be avoided by a constant water flow through the cooling system. The water flow in the system is regulated by a drip infusion device connected to the inlet tube of the cooling system. The cooling system provides a more uniform thermal field, which allows easier comparison of histological changes in areas maintained at various temperatures.

III. Animal preparation

Our study was carried out on 20 adult Japanese monkeys (*Macaca fuscata*), weighing 5.2–9.8 kg. The animals were anesthetized with ketamine hydrochloride (10 mg/kg) and paralyzed with pan-

curonium bromide (0.2 mg/kg), orotracheally intubated, and mechanically ventilated. Catheters were placed in the femoral artery and vein to monitor arterial pressure, and provide arterial and venous blood samples for gas analysis. The body temperature was measured by an esophageal thermocouple. The respiratory volume and rate were adjusted to maintain the arterial pH and PO₂ within the physiological range. Care was taken to assure adequate anesthetic depth throughout the experiment.

The monkey's head was fixed to a stereotaxic frame (Universal Stereotaxic Instrument B-3000; Summit Medical Co., Ltd., Tokyo). A frontoparietal craniectomy 3 × 4 cm in size was performed, and the dura was incised. The basal plane was defined as passing through the external auditory meatus and the inferior orbital ridge based on Kusama's stereotaxic atlas of the Japanese monkey brain.⁷ The microwave antenna within the cooling system was inserted under stereotaxic guidance 12 mm lateral from the midline and 28 mm anterior to the external auditory meatus, to a depth of 23 mm from the brain surface. Thermocouples were placed in closed-tip catheters (Insyte; Desert Medical Inc., Sandy, Ut., U.S.A.), and inserted into the brain parallel to the antenna axis to a depth of 18 mm from

Fig. 1 Projection of the stereotaxic coordinates over the lateral (left) and dorsal views (right) of the monkey brain, showing the arrangement of the antenna cooling system and thermocouple catheters. The horizontal line indicates the plane in the brain section. The horizontal (H) 0 plane passes through the external auditory meatus and the inferior orbital ridge. The anterior (A) or posterior (P) 0 plane passes through both external auditory meatuses. The antenna cooling system was inserted under stereotaxic guidance 12 mm lateral from the midline and 28 mm anterior to the external auditory meatus, to a depth of 23 mm from the brain surface. The thermocouple catheters were inserted at distances of 0, 4, 9, 14, and 19 mm from the surface of the cooling system, to a depth of 18 mm. The reference point (RP) was located at a radial distance of 4 mm from the surface of the cooling system close to the putamen or caudate nucleus. Immediately or 7 days after the hyperthermia treatment, the brain was removed and cut parallel to the horizontal 0 plane into 4 mm thick horizontal sections.

the brain surface, at distances of 0, 4, 9, 14, and 19 mm from the cooling system along a line parallel to the midline (Fig. 1). The reference point was located at a radial distance of 4 mm from the surface of the cooling system, close to the putamen or caudate nucleus.

The microwave power was automatically controlled to maintain the reference temperature at 42, 43, 44, 45, or 46°C for 60 minutes. The maximum temperature adjacent to the cooling system was maintained to within 2°C of the reference temperature by regulating the water flow rate in the cooling system.

IV. Non-survival study

The non-survival study examined the bilateral hemispheres of 11 animals and the unilateral hemispheres of seven animals. The reference temperature was 42°C in five hemispheres, 43°C in five, 44°C in five, 45°C in four, and 46°C in four for 60 minutes. Six hemispheres were used as controls, by inserting the cooling system and thermocouple catheters into the brains without heating. The animals were sacrificed with an overdose of barbiturate, and the brains were fixed by perfusion with 10% buffered formalin immediately after the hyperthermia treatment, then removed and cut parallel to the horizontal 0 plane into 4 mm thick horizontal sections. The histological changes caused by heating were studied microscopically in sections stained with

HE or Kluver-Barrera's method.

V. Survival study

The survival study examined the bilateral hemispheres of two animals and unilateral hemispheres of seven animals. The reference temperature was 42°C in three hemispheres, 44°C in four, and 46°C in two for 60 minutes. Two hemispheres were used as controls as above. Seven days after the hyperthermia treatment, the animals were sacrificed with an overdose of barbiturate, and perfusion-fixation, sectioning, and staining of the brains performed. The histological changes were evaluated as in the non-survival study.

Results

The reference temperature reached the desired steady state about 6 minutes after starting the hyperthermia treatment and was maintained within $\pm 0.2^\circ\text{C}$ for 60 minutes (Fig. 2). The temperatures measured at the other points usually remained within approximately $\pm 0.3^\circ\text{C}$ of the initial steady temperature. The body temperature, measured at the esophagus, remained essentially constant in all animals.

I. Non-survival study

Macroscopic examination found a hematoma around the cooling system in one hemisphere, where

Fig. 2 Time-temperature plot during a brain hyperthermia experiment with the reference temperature maintained at 44°C. Temperatures were measured at radial distances of 0 (closed circles), 4 (open circles), 9 (closed squares), 14 (open squares), and 19 mm (closed triangles) from the surface of the cooling system. Body temperature is indicated by the open triangles. The reference temperature reached the desired steady state 6 minutes after starting hyperthermia and was maintained at 44°C $\pm 0.2^\circ\text{C}$.

Fig. 3 Horizontal sections of a monkey brain immediately after maintaining the reference temperature at 45°C for 60 minutes. *upper*: Photograph, showing no obvious morphological change except for the mechanical damage caused by insertion of the cooling system (*thick arrow*) or the thermocouple catheters (*arrowheads*). *middle*: The same section stained with Kluver-Barrera's method. The area around the cooling system, which was maintained at 46°C, appeared paler (*arrows*) than uninvolved areas. *lower*: Diagram of the same section. The dotted area (1) including the reference point was maintained at 45°C and the hatched area (2) around the cooling system was heated at 46°C.

Fig. 4 *left*: Photomicrograph of an area maintained at 44°C for 60 minutes, showing no cytological change immediately after the treatment. HE stain, $\times 400$. *right*: Photomicrograph of an area maintained at 45°C for 60 minutes immediately after the treatment, showing swollen cytoplasm in the putaminal neurons (*arrows*), and nuclei stained darker, suggesting slight shrinkage. The surrounding neuropil had a slightly coarse appearance. HE stain, $\times 400$.

the reference temperature was maintained at 46°C. No other obvious morphological changes were observed, except for the damage caused by insertion of the cooling system or the thermocouple catheters (Fig. 3 *upper*).

Microscopic examination revealed no obvious cytological changes in any hemisphere where the reference temperature was maintained at 42, 43, or 44°C (Fig. 4 *left*). All four hemispheres with the reference temperature at 45°C revealed swollen cytoplasm in the putaminal neurons, and nuclei stained more intensely, suggesting slight shrinkage. The surrounding neuropil appeared slightly coarse. The white matter showed edema (Figs. 3 *lower* and 4 *right*). Three of the four hemispheres where the reference temperature was maintained at 46°C had atrophied neurons surrounded by moderately coarse neuropil, with mild disruption of the myelin sheaths in the white matter. One hemisphere had some pyknotic nuclei and degenerated cells remaining in the necrotic area, suggesting necrobiosis, a precursor stage of necrosis. The lesions invariably extended chiefly in the white matter, not the cortex or the basal ganglia (Fig. 3 *middle, lower*).

All six control hemispheres contained minimal damage caused by insertion of the cooling system or the thermocouple catheters.

II. Survival study

Macroscopic examination of the three hemispheres in which the reference temperature was maintained at 42°C showed no obvious morphological changes

Fig. 5 Horizontal sections 7 days after the treatment with the reference temperature maintained at 42 (A), 44 (B), and 46°C (C); and without heating (D), showing damage due to the cooling system (*thick arrow*) and the thermocouple catheters (*arrowheads*). A: The area maintained at 42°C for 60 minutes showed no obvious morphological changes. B: The area maintained at 44°C or above showed coagulative necrosis, while the area maintained below 44°C showed no morphological change. C: The area maintained at 46°C was within the necrotic region, while the area maintained below 44°C showed no obvious morphological changes. D: The control showed only the mechanical damage caused by insertion of the cooling system and the thermocouple catheters.

in areas at 42°C or less. However, the area around the cooling system at 44–45°C showed coagulative necrosis (Fig. 5A). Three of the four hemispheres with the reference temperature maintained at 44°C showed coagulative necrosis in areas at 44°C or above, but no morphological changes in areas below 44°C (Fig. 5B). Both hemispheres with the reference temperature of 46°C showed coagulative necrosis in areas at 46°C or above. Areas heated at 43 or 43.5°C showed no morphological changes (Fig. 5C). All lesions extended chiefly in the white matter, not the cortex or the basal ganglia, as in the non-survival study. Both control hemispheres showed minimal damage caused by insertion of the cooling system or the thermocouple catheters (Fig. 5D).

Microscopic examination showed areas maintained at 42°C developed minimal edema of the white matter without obvious morphological changes of the neurons or neuropil in the caudate nucleus. Areas heated at 44°C or above showed coagulative necrosis surrounded by a sharply demarcated zone of proliferating blood vessels with active macrophages and scanty reactive astrocytes. Areas heated below 44°C showed no obvious morphological changes in the caudate nucleus (Fig. 6 *lower*), while widespread minimal edematous change was seen in the white matter around the necrotic region (Fig. 6 *upper*). Areas

heated at 46°C were within the region of coagulative necrosis. Areas heated at 43 or 43.5°C showed minimal edema of the white matter without obvious morphological changes in the caudate nucleus.

Discussion

Previous non-survival animal studies have demonstrated various effects of hyperthermia. Harris *et al.*⁶⁾ found that temperatures up to 42°C for 30 minutes were tolerated without gross pathological changes, using regional perfusion of the dog brain. Silberman *et al.*²²⁾ reported that the normal rabbit brain could tolerate radiofrequency heating at 42–43°C for 60 minutes, without apparent histological or clinical damage. In contrast, Salcman *et al.*²⁰⁾ reported that whole body heating of cats in precision-controlled water baths produced severe degeneration of the dentate nucleus, extending beyond the Purkinje cell layer. These changes were roughly proportional to both the temperature and the duration of heating and occurred after 1 hour at 43°C. Britt *et al.*²⁾ reported that ultrasound heating at 43°C for 50 minutes caused partial loss of neurons and edema in the white matter of cat brains, while no obvious damage was seen after heating at 42°C for 50 minutes. Lyons *et al.*⁸⁾ also reported that 915 MHz

Fig. 6 *upper*: Horizontal section of the specimen shown in Fig. 5B stained with Kluver-Barrera's method. Damage due to the cooling system (*thick arrow*) and the thermocouple catheters (*arrowheads*) is shown. The necrotic area maintained at 44°C or above showed reduced staining and disruption of myelin sheaths. Widespread edema was seen in the white matter around the necrotic region. CN: caudate nucleus. *lower*: Photomicrograph of the border zone in the caudate nucleus (boxed area in upper figure). The area heated at 44°C or above showed coagulative necrosis (1) surrounded by a sharply demarcated zone of proliferative blood vessels with active macrophages and scanty reactive astrocytes (2). The area maintained below 44°C showed no obvious morphological change (3). HE stain, $\times 100$.

interstitial microwave heating at 42.2°C for 60 minutes caused pyknotic cortical neurons and cerebral edema in dog brains. The results of these non-survival animal studies vary too much to allow any firm conclusions.

Our non-survival study showed that a temperature of 45°C or above was required to induce the degeneration of neurons. This thermal damage threshold was slightly higher than some previously

reported values (42.2–43°C, 50–60 minutes).^{2,8,21)} This divergence might be attributed to the uniform thermal field produced by our cooling system. Without the cooling system, the thermal damage may be greater due to a hot spot generated by a bare antenna. The greatest temperature gradient near a bare antenna is reported to be 1°C/mm.^{8,11,14)} We found comparison of the histological changes caused by heating the brain tissue difficult due to the rapid fall-off in the temperature generated by a bare antenna. The present study demonstrated that the cooling system provides a mild thermal gradient of 0.3–0.5°C/mm,¹⁴⁾ which makes it easier to compare the histological changes at various temperatures.

Matsumoto *et al.*¹¹⁾ studied heat damage in the canine brain by sequential quantitative computed tomography. Low-density regions corresponding to tissue heated above 44°C for 30 minutes were detected. Our survival study demonstrated that areas heated at 44°C or above for 60 minutes showed coagulative necrosis 7 days later, while no obvious irreversible changes were detected when the temperature was below 44°C (43 or 43.5°C) for 60 minutes, immediately or 7 days after the treatment. The histological findings indicated that thermal damage occurred in normal brain tissue after heating at 44°C for 60 minutes, suggesting that the safety limit for brain hyperthermia is 43°C for 60 minutes.

Both immediate and delayed histological examinations have detected no intrinsic morphological changes caused by heat. However, the distribution of degeneration or necrosis in the normal brain tissue was extremely characteristic with the lesion invariably extending in the white matter, avoiding the cortex or basal ganglia. Occlusive cerebrovascular disease causing an infarct confined to extensive areas of the white matter is rare, whereas multiple small necrotic zones are common.¹⁶⁾ This characteristic distribution of thermal lesions confined to white matter may be due to the following: Brain tissue perfusion is normally one-third to one-fourth lower in the white matter compared to the cortex or the basal ganglia,^{13,17)} so heat dissipation is greatest near the cortical surface of the exposed brain.^{3,8)} White matter has a lower water content than gray matter, a lower dielectric constant and higher conductivity, which result in a higher specific absorption rate of microwave radiation.²³⁾

Clinical application of local hyperthermia for malignant brain tumors requires accurate monitoring of the temperature in the heated brain, especially the white matter, in which hot spots tend to occur leading to necrosis.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Cancer Research (61015065) from the Ministry of Education, Science and Culture of Japan.

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