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. 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. Recently, non-survival animal studies have been reported, but few have correlated the delayed histological changes caused by heating normal brain tissue.

We previously developed an antenna cooling system to generate more uniform thermal fields without overheating. 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

1. 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. 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 pancuronium 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 stereotactic frame (Universal Stereotaxic Instrument B-3000; Summit Medical Co., Ltd., Tokyo). A frontoparietal craniectomy 3 x 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 stereotactic atlas of the Japanese monkey brain. The microwave antenna within the cooling system was inserted under stereotactic 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.
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 four, 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 perfusion-fixation, sectioning, and staining of the brains performed. The histological changes were evaluated as in the non-survival study.

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 +0.2°C for 60 minutes (Fig. 2). The temperatures measured at the other points usually remained within approximately +0.3°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 + 0.2°C.](image-url)
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.
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 minimal edema of the white matter without 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. 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. 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, Saleman et al. 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. 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. also reported that 915 MHz
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). 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. 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, which makes it easier to compare the histological changes at various temperatures.

Matsumoto et al. 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. 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, so heat dissipation is greatest near the cortical surface of the exposed brain. 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.

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.
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References


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