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# Ultrastructural Investigation of Neuronal Damage induced by Interstitial Radiofrequency Hyperthermia in Adult Dogs

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**Abstract** A study was conducted using electron microscopy to investigate hyperthermia-induced damage to neurons and myelinated nerve tracts in order to determine the thermal threshold. Twenty adult dogs were used. After parietal craniectomy, four electrode needles connected to a Thermotron-RF8 for interstitial 8-MHz radiofrequency hyperthermia were inserted in a 1 cm square pattern and placed at a depth of 1.5 cm from the brain surface. One thermocouple microprobe was set in the center of the square formed by the electrodes. One hour after heating for 15, 30, 45 and 60 minutes with set temperatures of 42°C and 43°C, the animal' s brain was removed and the ultrastructural investigation was performed.

The findings suggested that the normal brain adjacent to a tumor in vivo would be damaged irreversibly by a large thermal dose, and that neurons showed ultrastructural changes such as swollen or empty of mitochondria and central chromatolysis even after small and moderate thermal doses.

*Key words*:hyperthermia, radiofrequency, thermal threshold, ultrastructure

## Introduction

The effects of elevated temperature on the central nervous system have been studied by several investigators. Hyperthermia affects the electrical activity of the brain, its metabolic status, the blood-brain barrier permeability and morphology[1]. The reaction at the light microscopic level induced by hyperthermia in the brain has been studied by several groups of investigators[2,3,4,5,6,7,8]. However, the thermal threshold of the central nervous system remains to be confirmed, since biochemical studies have also shown that some of the damage undoubtedly occurs at the mitochondrial level[9,10]. Therefore, further studies, including ultrastructural investigation are necessary to determine the thermal threshold.

Since 1990 the authors have studying the feasibility of interstitial radiofrequency hyperthermia for the treatment of malignant brain tumors[5]. The aim of the present study was to investigate the thermal threshold of neurons and myelinated nerve tracts by electron microscopy.

## Materials and Methods

Twenty adult dogs, mean body weight 12 kg, were used. The animals were first sedated with ketamine (2 mg/kg, intramuscularly) and then anesthetized by intraperitoneal administration of pentobarbital sodium (20 mg/kg) and intubated. The animals breathed spontaneously throughout the experiment.

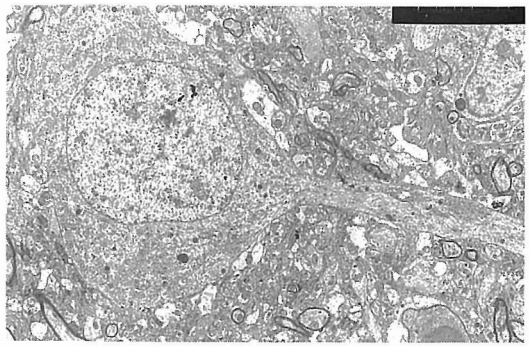

Fig. 1. Electron micrograph of brain tissue treated at 42°C for 15 minutes. No evidence of damage can be detected (×4,250).

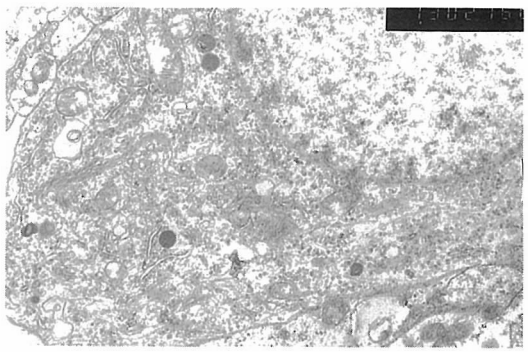

Fig. 2. Electron micrograph of brain tissue treated at 42°C for 30 minutes. Mitochondria in the neurons demonstrate empty swelling. The rough endoplasmic reticulum, Golgi apparatus and nucleus appear unchanged (×15,500).

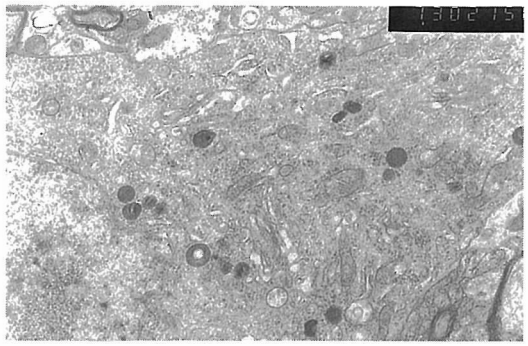

Fig. 3. Electron micrograph of brain tissue treated at 42°C for 60 minutes. Mitochondria in the neurons have a dense matrix. The rough endoplasmic reticulum is decreased in amount, and the polyribosomes show a tendency to be disaggregated, appearing mainly as monoribosomes. The Golgi apparatus enlarged with small coated vesicles, and a slight increae in the amount of lysosomes in evident. Several autolysosomes are also present. The nucleus contains clumped chromatin (×15,500)

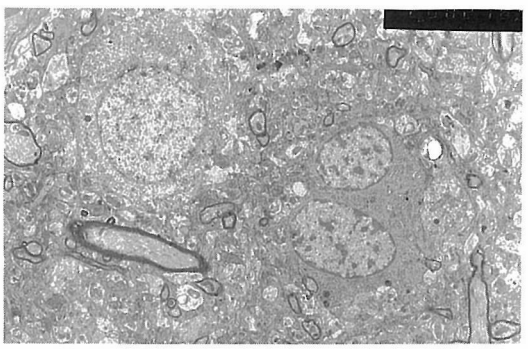

Fig. 4. Electron micrograph of brain tissue treated at 42°C for 60 minutes. Oligodendroglias, axons and myelin sheaths remain intact (×4,250).

A midline scalp incision was made and the temporalis muscle was reflected to expose the skull. After a parietal craniectomy measuring 5 cm×4 cm, the brain surface was exposed. Unilateral heating was performed in all animals. In four cases, the contralateral hemisphere served as a control for evaluating damage caused by insertion of electrode needles and a thermocouple microprobe into the brain without heating.

Four electrode needles, 1 cm in length and 1 mm in diameter, connected to a Thermotron-RF8 (YAMAMOTO VINYTER Co. Ltd.) for interstitial 8-MHz radiofrequency hyperthermia were used for heating, and one thermocouple microprobe (SENSOR-TEC Co. Ltd; Type IT-18) was used to measure the temperature of the tissue.

A boat-shaped acrylic template was placed over the brain, and five Teflon catheters (19 gauge) were inserted through the template.

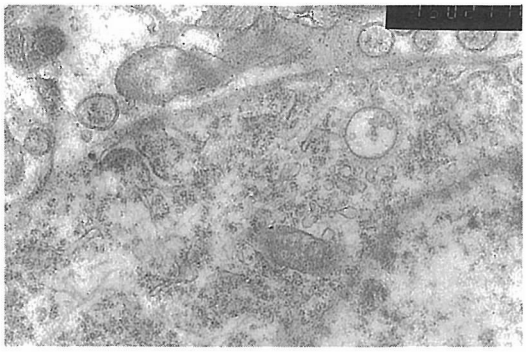

Fig. 5. Electron micrograph of brain tissue treated at 43°C for 15 minutes. The electron micrographical findings are almost the same as those after 60 minutes at 42°C (×31,800).

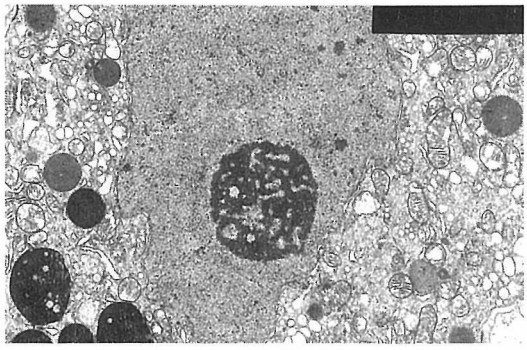

Fig. 6. Electron micrograph of brain tissue treated at 43°C for 60 minutes. In the perikaryon of the neurons, the rough endoplasmic reticulum disappears, and a number of destroyed mitochondria are present. The number of lysosomes is increased (×12,700).

The catheters for electrode needles were inserted at the corners of a 1 cm square, and were placed at a depth of 1.5 cm from the brain surface to bury the electrodes completely. The catheter for the thermocouple microprobe was set in the center of the square.

One hour after heating for 15, 30, 45 and 60 minutes with set temperatures of 42°C and 43°C, the brain of the animal was removed. Brain tissue within 5 mm from the tip of the thermocouple probe was cut into several small pieces and placed in 2.5% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.4) for 2 hours. After postfixation in 2% osmium tetroxide for 2 hours, the tissue was dehydrated and embedded in Epon. Thin sections were cut on an LKB Ultratome, placed on grids, and stained with uranyl acetate for 5 minutes, followed by lead citrate staining for 5 minutes. All specimens were examined in a JEM-200 CX electron microscope (NIHON DENSHI Co. Ltd.).

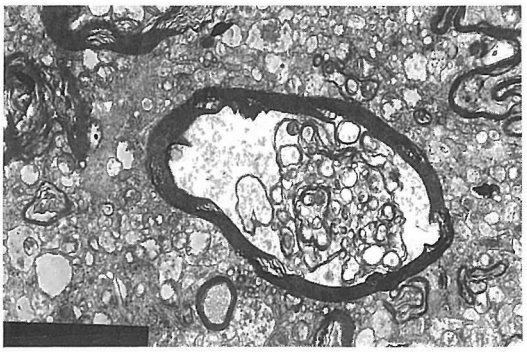

Fig. 7. Electron micrograph of brain tissue treated at 43°C for 60 minutes. A number of membranous dense bodies are present in the hypertrophic axons (×12,700).

## Results

In the control animals, thermocouple placement without heating left a small track easily identifiable microscopically, but did not cause damage to surrounding neurons or white matter.

After heating at 42°C for 15 minutes, no evidence of damage could be detected (Fig. 1). Histological findings of tissue damage however, were evident, after heating at 42°C for 30 minutes; mitochondria in neurons were swollen and empty, or showed more frequently a dense matrix. On the other hand, the rough endoplasmic reticulum, Golgi apparatus and nucleus seemed unchanged (Fig. 2). After heating at 42°C for 45 and 60 minutes, mitochondria were more severely destroyed. The rough endoplasmic reticulum was de-

creased in amount, and polyribosomes tended to be disaggregated, appearing mainly as monoribosomes. The Golgi apparatus enlarged with small coated vesicles, and lysosomes slightly increased. Several autolysosomes were also evident. The nucleus contained clumped chromatin (Fig. 3). Synapses showed empty swelling. Other cytological structures didn't change at this thermal dose (Fig. 4). After heating at 43°C for 15 and 30 minutes, the electron micrographical findings were almost the same as those after 60 minutes at 42°C (Fig. 5). After heating at 43°C for 45 and 60 minutes, some neurons showed shrinkage in the volume of both the cytoplasm and nucleus. The rough endoplasmic reticulum could not be seen in the cytoplasm. In the perikaryon of other neurons, the rough endoplasmic reticulum disappeared and many mitochondria were entirely destroyed. The number of lysosomes was increased (Fig. 6), and many membranous dense bodies were present in the hypertrophic axon (Fig. 7).

## Discussion

The ultrastructural changes in neurons induced by hyperthermia were classified into 3 groups. At a small thermal dose (42°C for 30 minutes), only mitochondria showed changes. At moderate thermal doses (42°C for 45 or 60 minutes, and 43°C for 15 or 30 minutes), central chromatolysis was observed in addition to the changes in mitochondria. Lysosomes were increased slightly in number. At large thermal doses (43°C for 45 or 60 minutes), the mitochondrial changes and central chromatolysis were more severe. In some neurons, the rough endoplasmic reticulum had completely disappeared and many mitochondria were destroyed. In the hypertrophic axon, many membranous dense bodies were seen. These findings appeared to be ascribable to the disconnection of the axon.

The effects of hyperthermia on brain tissue can be viewed in terms of changes in structure, function and metabolism. In each case, one must further consider whether the changes are reversible or irreversible. At large thermal doses, the changes produced are thought to be irreversible for two reasons. First, in some neurons, changes apparently ascribable to the rupture of the axon were seen[11]. Second, central chromatolysis is reversible if the injury is small. However, the central chromatolysis observed in this study was severe in all neurons.

At small and moderate thermal doses, there is a possibility that the changes are reversible. At these doses, central chromatolysis was less severe. Heine, studying Hela cells, observed a relationship between inhibition of RNA synthesis and corresponding morphological alterations in the form of degranulation of the nucleolus and monosome formation in the cytoplasm. However, the decreased RNA synthesis was found to be only transient, with complete recovery being evident within 1-2 days[12]. With regard to mitochondrial plasticity, it will be necessary to study several aspects further. Burger investigated changes in the rate of oxygen consumption ($QO_2$) using slices of rat cerebral cortex. The $QO_2$ of the cortex slices fell progressively with time at all temperatures (40-47.5°C). A decrease in $QO_2$ below that found at 38°C required about 40-50 minutes at 42 and 43°C[9]. Christiansen studied oxidative phosphorylation and respiratory control in mouse brain after preincubating the mitochondria in vitro at temperatures of up to 45°C. The results indicated that both inhibition of electron transport, loss of respiratory control and uncoupling of phosphorylation were likely to be the primary effects of hyperthermia. Leakage of endogenous cytochrome C into the surrounding medium was also demonstrated, indicating that membrane damage as an early effect of hyperthermia[10]. These biochemical studies showed that some of the damage undoubtedly occurred at the mitochondrial level. This is in good agreement with the observation that mitochondria of neurons were destroyed in the present study. However, this study was performed on animals sacrificed immediately after hyperthermia, so the late effects of thermal injury on normal brain tissue were not evaluated. The question therefore remains whether the structural changes in mitochondria seen immediately after heating is reversible or irreversible.

Our present findings suggest two things. First, the normal brain adjacent to a tumor in vivo may be damaged irreversibly by a large thermal dose. Second, neurons show ultrastructural changes such as destruction of mitochondria and central chromatolysis even at small and moderate thermal doses. In tissue culture studies of human glioblastoma cells, Gerweck indicated that hyperthermia at 42°C was only marginally lethal inasmuch as treatment for 300 minutes reduced survival to 70 to 80%[13]. At 43°C, however, cell killing was more pronounced, and survival dropped to approximately 10% after 300 minutes of treatment. As increased sensitivity to hyperthermia was seen under acid pH conditions, human glioblastoma cells would be more sensitive to heat in vivo. In fact, the changes in neurons in this study were different from those in tumor cells observed by Overgaard, who studied ultrastructural changes following local hyperthermia at 42.5°C for 30 minutes in a solid murine mammary carcinoma[14]. A few hours after treatment, pronounced lysosomal activity was observed in the cytoplasm of the tumor cells, together with mitochondrial destruction and disaggregation of polyribosomes. Overgaard concluded that the pronounced initial lysosomal activity in the tumor cells seemed to be of primary importance in the mechanism of cell injury, and selectively related to malignant cells. In the present study, normal neurons showed only minor alterations with a slightly increased amount of lysosomes. This may be explained by the difference in intra- and extracellular acidity[15]. However, there is a possibility that effective damage to malignant tissue may require a high temperature above the threshold of damage to normal brain[13].

It will be necessary to investigate in animals sacrificed after a long interval whether the structural changes in mitochondria observed after small and moderate thermal doses are reversible or irreversible, and whether these changes are accompanied by disturbance of energy metabolism and electrical dysfunction of neurons.

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