Bull Yamaguchi Med Sch 41(3-4): 89-97, 1994

The Effect of Hyperthermia on Cerebral Energy Metabolism Studied with in vivo ^{31}P Nuclear Magnetic Resonance Spectroscopy

Eiji Tsuchida

Department of Neurosurgery, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, *Japan*
(*Received June* 27, *revised December* 6 1994)

Abstract Energy metabolism in the Wistar rat brain was studied by means of in vivo nuclear magnetic resonance spectroscopy after heating. The ^{31}P-spectra were recorded at 10 minutes after heating the brain to 44 or 45°C (cortical temperature) for 30 minutes on 6 rats in each group. Thereafter, the ^{31}P-spectra were repeatedly recorded until 1 hour after heating. The ^{31}P-spectra showed no significant changes of pH and high-energy phosphate levels occurred for 1 hour after heating of the brain to 44°C for 30 minutes. However, those determined 10 minutes after heating the brain to 45°C for 30 minutes showed a statistically significant reduction of the PCr/Pi ratio without accompaning a pH shift and change in ATP concentration. No further changes occurred thereafter for 1 hour after heating of the brain. The heat-induced PCr/Pi ratio reduction observed in this study reflected mitochondrial dysfunction and not a decline in cerebral blood flow. The present study has demonstrated at least part of the heat-induced damage of the brain occurred at mitochondrial level in vivo, while cerebral energy metabolism was found to be preserved up to cortical brain temperature of 44°C.

Key words:Hyperthermia, Cerebral Energy Metabolism, ^{31}P-Nuclear Magnetic Resonance Spectroscopy, in vivo

Introduction

Hyperthermia holds promise as a new modality for the treatment of malignant brain tumors, either by itself or in combination with radiotherapy and chemotherapy[1]. Since 1990 Ikeda et al. studied the feasibility of interstitial radiofrequency hyperthermia for the treatment of malignant brain tumors[2]. However, there are also potential risks associated with the use of hyperthermia. Neurons do not replicate, and therefore it is imperative that heating takes place only in the tumor, and that the temperature in the normal surrounding brain tissue does not exceed the threshold for cell damage. Before a hyperthermia system can be designed for clinical use in the treatment of brain tumors, one must determine the thermal threshold at which either of structural or functional changes occur irreversibly in the cytological elements of the central nervous system[3]. Hyperthermia affects not only morphology, but also the electrical activity of the brain[4,5], its metabolic status, the blood-brain barrier permeability[6] and so on[1].

The reaction at the light microscopic level induced by hyperthermia in the brain has been studied by many investigators[2,3,7,8,9,10,11]. However, the thermal threshold of the central nervous system remains to be confirmed, since some damage of the neuron undoubted-

ly occurs at the mitochondrial level[12,13]. It is necessary to study further as to whether the cerebral energy metabolism is altered following hyperthermia in vivo or not.

In vivo nuclear magnetic resonance spectroscopy is a potentially useful tool for examining the physiological and biochemical natures of organs non-invasively and repeatedly. This technique enables changes in high-energy phosphate levels and intracellular pH (pHi) to be estimated and has been used to study the brain under a variety of experimental conditions, such as ischemia[14], hypoxia[15], hypoglycemia[16] and neurotrauma[17].

In this study, the relationship between heat and energy metabolism was investigated with magnetic resonance spectrometer after heating the rat brain to varying extents in order to estimate biochemical alterations by heat in vivo.

Materials and Methods

Eighteen adult male Wistar rats weighing 300-400 g were used in this study. Anesthesia was induced by intraperitoneal injection of pentobarbital (30 mg/kg) and maintained with 1% halothane in nitrous oxide (70%) and oxygen (30%). The rats were tracheostomized and mechanically ventilated. A polyethylene catheter (PE-50) was introduced into the right femoral artery, through which the arterial blood pressure (BP) was monitored continuously throughout the heating. A small amount of arterial blood was sampled at the beginning and at the end of brain heating and after the last ^{31}P spectral recording, and the arterial blood gases were analyzed.

Once fully anesthetized, each animal was placed in a stereotaxic frame and the scalp and temporal muscles were retracted to ensure that these tissues did not contribute to the ^{31}P spectrum. Two small burr holes were drilled in the skull over the bilateral parietal cortex (one was positioned 2 mm to the right of the midline and 2 mm posterior to bregma, the other was 2 mm to the left of the midline and 2 mm posterior to bregma). A thermocouple microprobe was inserted in the right burr hole at a depth of 2 mm from the brain surface to measure the cortical temperature and another was inserted in the other burr hole at a depth of 6 mm from the cranium to measure the deep cerebral temperature, where the ^{31}P-spectra signal intensities were maximal. A rectal temperature probe was also inserted in each rat. Brain heating was carried out using two incandescent photoflood lamps (National PRF-500WB) set 13-15 cm above the rat's head and the body was covered with insulating material to prevent overheating.

The animals were divided into three groups of 6 animals. The ^{31}P-spectra were recorded at 10 minutes after heating the brain to 44 or 45°C (cortical temperature) for 30 minutes on 6 rats in each groups and until 1 hour after heating. In the control group, the ^{31}P-spectra were measured at the same times after the surgical procedures without brain heating. In all the animals, the rectal temperature was maintained within normal range by bathing the whole body in warm air.

^{31}P-Spectral Measurements

The animals were placed in an in vivo nuclear magnetic resonance spectrometer (BEM-250/80, Otsuka Electronics Co., Tokyo, Japan; 4.7 Tesla equipped with a 20-mm diameter surface coil, operating at 81.079 MHz) in a prone position so as to center the cranial cavity on the volume of the homogeneous magnetic field of the instrument. Magnetic field homogeneity was optimized by shimming on the water proton signal. The spectral width was 5000 Hz and 1024 data points were collected in each scan. The spectra were collected as the free-induction decays using quadrature phase detection, digitized and processed with a computer and were obtained as 200 time-averaged free-induction decays at repetition times of 3.0 seconds. They were processed using a convolution difference technique to remove the broad baseline hump attributable to cranial bone phosphates and, to a lesser extent, phospholipids. The inorganic phosphate (Pi), phosphocreatine (PCr) and adenosine triphosphate (ATP) peak heights were measured to estimate the energy changes. The pHi was calculated from the extent of the Pi chemical shift relative to that of PCr[18]. All data were analyzed using unpaired t-test to determine the level of significance. A p value less than 0.05 was considered significant.

a. heating at 44°C for 30 minutes

b. heating at 45°C for 30 minutes

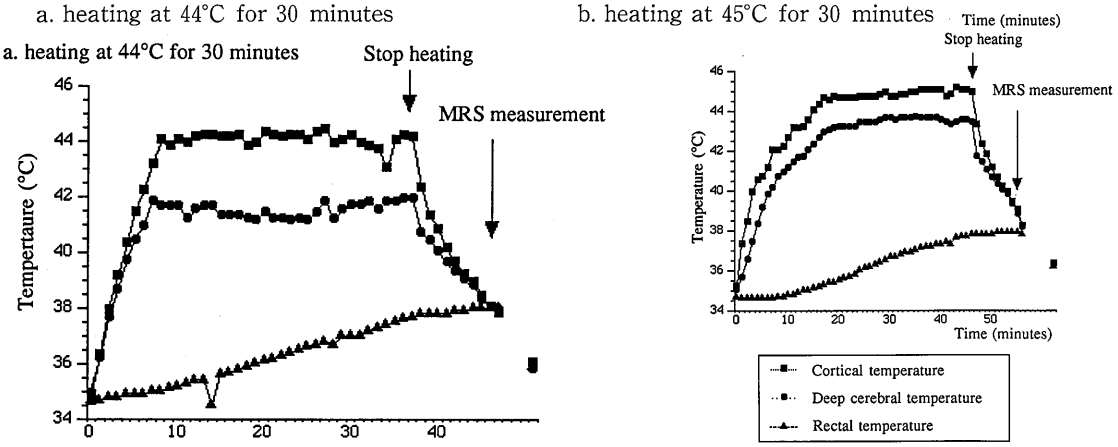

Fig. 1. Brain and rectal temperature gradients during brain heating
In each group all six cases showed similar changes in the temperature in the brain. Typical examples in each group are shown in this figure.

Table 1. Arterial blood gases and blood pressure in three groups

a. control (n=6)

Measurement	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	MABP (mmHg)
first	130.8± 8.5	38.0±2.6	7.35±0.05	110.5± 7.6
second	138.7± 9.8	34.2±4.9	7.40±0.55	102.8±15.3
third	102.7±13.5	33.3±3.9	7.46±0.38	94.3± 6.4

b. heating at 44°C for 30 minutes (n=6)

Measurement	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	MABP (mmHg)
first	136.8±16.1	36.7±2.3	7.36±0.48	104.5±6.5
second	136.2±19.4	37.3±2.2	7.35±0.03	96.7±6.1
third	97.3± 6.0	25.8±2.1	7.40±0.05	95.0±1.8

c. heating at 45°C for 30 minutes (n=6)

Measurement	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	MABP (mmHg)
first	136.3±6.0	34.8±2.0	7.36±0.03	104.0±8.2
second	136.0±7.2	35.8±2.9	7.34±0.04	92.0±4.6
third	108.7±7.4	38.5±3.7	7.33±0.05	90.3±1.6

* Values are expressed as means±standard deviations.
PaO_2; pressure of O_2 in arterial blood, $PaCO_2$; pressure of CO_2 in arterial blood. MABP; mean arterial blood pressure, first; at the beginning of the brain heating, second: at the end of its heating, third; after the last ^{31}P spectral recording.

Electroencephalogram(EEG)Recording

A carbon electrode was inserted into the right cerebral cortex through the burr hole and ECG recording was undertaken during brain heating in the experimental rats.

Results

Brain and Body Temperature Gradients

Using the technique described above, relatively normal rectal temperatures could be maintained while the cortical temperature was raised drastically (Fig. 1). Heating for 15-20 minutes was required to attain cortical temperatures of 44-45°C. The deep cerebral temperatures were usually about 2°C lower than the cortical temperatures.

Blood Gases and Blood Pressure

The mean arterial blood gas value and mean arterial blood pressure (MABP) determined in each group are summarized in Table 1. Neither hypotension nor hypoxia, which may have influenced the ^{31}P-spectra, occurred in any group.

^{31}P spectral changes

Fig. 2 shows typical ^{31}P-spectra recorded 10 minutes and 1 hour after sham operation in control rats. Those taken 10 minutes after sham operation exhibited strong PCr and ATP peaks and a small Pi peak. The PCr/Pi ratio was 7.57±1.11, the PCr/β-ATP ratio was 1.7±0.14 and the cerebral pHi was 7.11±0.10 (n=6; mean±SD). Thereafter no significant ^{31}P-spectral changes were observed (Table 2).

Table 2. The PCr/Pi ratio, pHi and PCr/β-ATP ratio in three groups

a. control (n=6)

Time after sham operation	PCr/Pi	pHi	PCr/β-ATP
10 minutes	7.57±1.11	7.11±0.10	1.70±0.14
1 hour	7.36±1.19	7.13±0.09	1.70±0.09

b. heating at 44°C for 30 minutes (n=6)

Time after brain heating	PCr/Pi	pHi	PCr/β-ATP
10 minutes	7.45±0.89	7.07±0.06	1.73±0.87
1 hour	7.39±0.87	7.07±0.08	1.77±0.10

c. heating at 45°C for 30 minutes (n=6)

Time after brain heating	PCr/Pi	pHi	PCr/β-ATP
10 minutes	1.47±0.12*	7.00±0.05	1.55±0.07
1 hour	1.48±0.11*	7.04±0.08	1.56±0.06

Values are expressed as means±standard deviations.
*Significant difference between heating and control group.

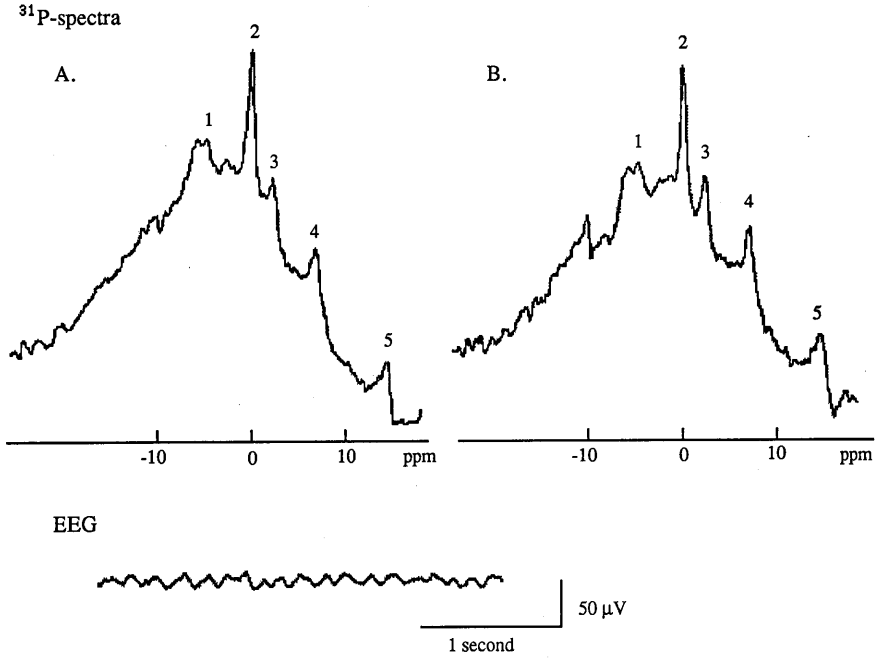

Fig. 2. ^{31}P-spectra and EEG recorded after sham operation.
Assignments of signal peaks are as follows:
1; Pi 2; PCr 3; γ-ATP 4; α-ATP 5; β-ATP.
Spectra obtained 10 minutes (A) and 1 hour (B) after sham operation showed strong PCr and ATP peaks and a small Pi peak.
No EEG changes were observed.

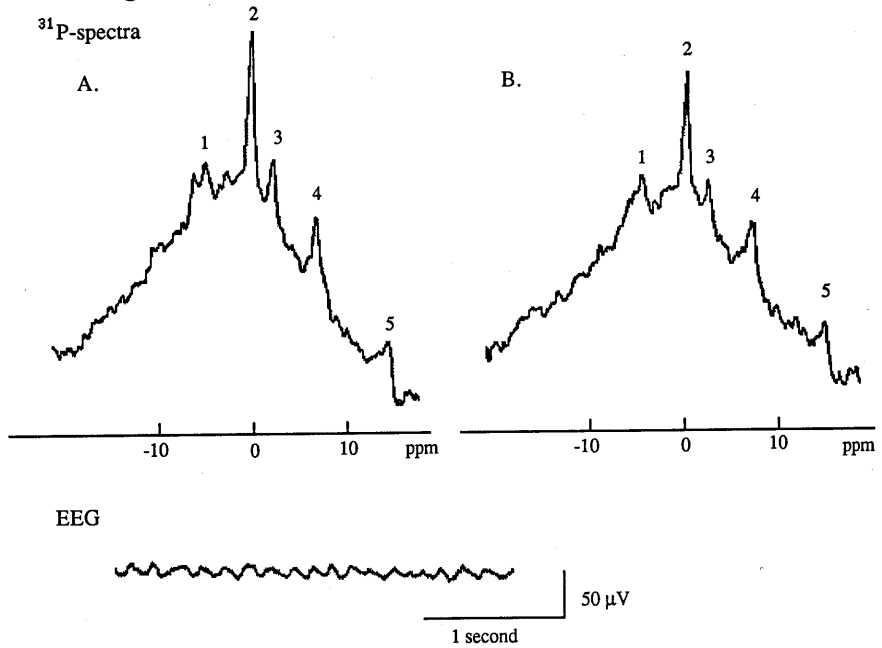

Fig. 3. ^{31}P-spectra and EEG recorded after heating at 44°C for 30 minutes
Assignments of signal peaks are as in Fig. 2.
No significant changes occurred in the Spectra at 10 minutes (A) and 1 hour (B) after heating.
No EEG changes were also observed.

Fig. 3 shows typical ^{31}P-spectra recorded 10 minutes and 1 hour after brain heating to 44°C for 30 minutes. Initially, the PCr/Pi ratio was 7.45±0.89, PCr/β-ATP was 1.73±0.87 and the cerebral pHi was 7.07±0.06 (n=6; mean±SD). Thereafter no further canges were observed (Table 2).

Fig. 4 shows typical ^{31}P-spectra taken 10 minutes and 1 hour after brain heating to 45°C for 30 minutes. The ^{31}P-spectra showed initially an increase in Pi and a decrease in PCr: the PCr/Pi ratio was 1.47±0.12 ($P<0.05$), the PCr/β-ATP ratio was 1.55±0.07 and the cerebral pHi was 7.00±0.05. Thereafter no recovery occurred and 1 hour after heating: the PCr/Pi ratio was 1.48±0.11, PCr/b-ATP was 1.56±0.06 and the cerebral pHi was 7.04±0.08 (Table 2).

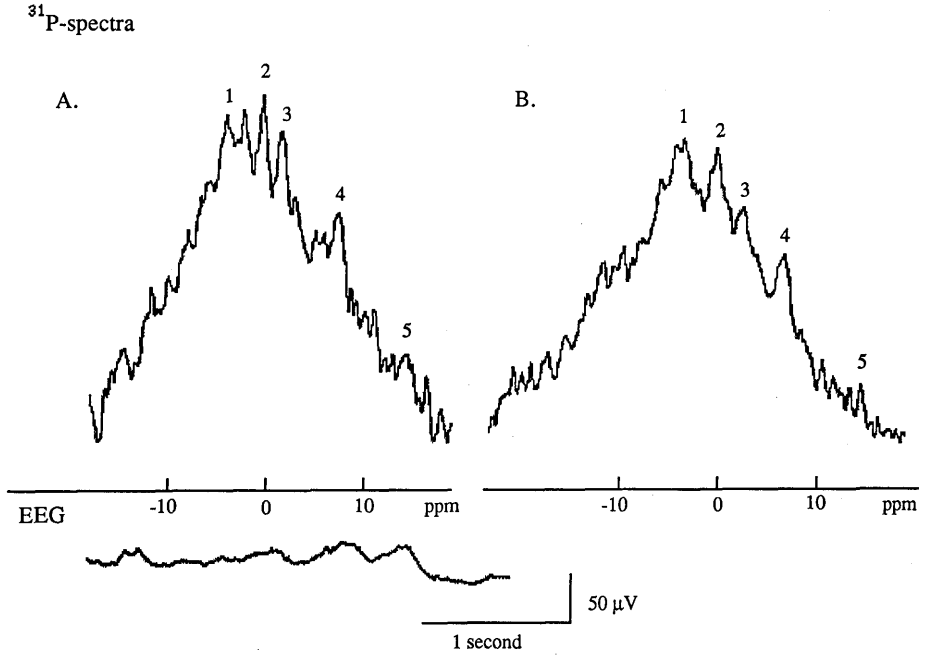

Fig. 4. ^{31}P-spectra and EEG recorded after heating at 45°C for 30 minutes
Assignments of signal peaks are as in Fig. 2.
^{31}P spectra obtained 10 minutes after heating showed an increase in Pi and a decrease in PCr without changes in ATP (A). Until 1 hour after heating no recovery occurred (B).
EEG obtained at the end of heating showed increase in the voltage and decrease in the frequency, but never became isoelectric.

EEG Changes

No EEG changes were observed in the rats subjected to brain heating to 44°C for 30 minutes, whereas in those heated to 45°C for 30 minutes, the EEG voltage increased and frequency decreased with time, but EEG never became isoelectric (Fig. 2, 3, 4).

Discussion

Several studies in vitro have been previously undertaken about the effect of hyperthermia on cerebral energy metabolism. Burger and Fuhrman investigated possible heat-injury criteria using rat cerebral cortex slices heated in vitro. They observed an initial increase in the oxygen consumption rate (QO_2) of the slices from 37°C to 45°C, and QO_2 declined progressively over 45°C. Moreover, they observed a decrease in QO_2 to below that found at 38°C required about 90-120 minutes at 40 and 41°C, and 40-50 minutes at 42 and 43°C[12]. Christiansen and Kvamme studied the oxidative phosphorylation and respiratory control of mouse brain mitochondria preincubated in vitro at temperatures up to 45°C. Their results indicated

the primary effects of heat tratment were likely to be inhibition of electron transport, loss of respiratory control and uncoupling of phosphorylation[13]. Although these results suggest that at least part of the heat-induced damage almost certainly occurs at the mitochondrial level, no reports have appeared documenting such biochemical alterations in vivo induced by heat. In our study, magnetic resonance spectroscopy techniques utilizing surface coils permitted non-invasive measurements of cerebral intracellular pH and high-energy phosphate levels to be carried out during the 1 hour post-heating period.

The ^{31}P-spectra recorded after heating the rat brain to 44°C for 30 minutes showed no pHi and high-energy phosphate level changes during the 1 hour post-heating period. However, those recorded 10 minutes after heating the brain to 45°C for 30 minutes showed a statistically significant reduction of the PCr/Pi ratio, which was not accompanied by a pH shift or change in the ATP concentration. These findings did not change thereafter during the 1 hour post-heating period.

A reduction in the PCr/Pi ratio is indicative of altered energy metabolism, which may signify inadequate tissue oxygenation or mitochondrial dysfunction [17,19]. It also may reflect intracellular buffering via the creatine kinase reaction:

$$PCr+ADP+H^{+}\rightarrow ATP+Cr.$$

As the reduction of the PCr/Pi ratio was not accompanied by a pH shift (acidosis), it was unlikely to be due to pH buffering[17].

The reduced PCr/Pi ratio may reflect a reduced cerebral blood flow and/or tissue oxygenation. In this study, hypotension and hypoxia, which may have influenced the ^{31}P-spectra were not observed[15]. Yamada studied changes in the blood pressure, cerebral blood flow, partial pressure of oxygen, pH in the cerebral cortex, electroencephalogram and somatosensory evoked potential during heating the heads of rabbits by bathing them in hot air, and observed the cerebral blood flow and partial pressure of oxygen increased as the cortical temperature increased to 45°C[4]. Moreover, reduction of PCr/Pi with pH shift has been shown to start at a cerebral blood flow level of 20 ml/ 100 g/min[14]. The blood flow which is lower than 20 ml/ 100 g/min would result in significant suppression of EEG activity (i. e. isoelectric EEG)[20,21]. In the present study, the author did not observe such significant EEG activity suppression, that is not different from the findings observed by Harris et al.[8]. Therefore, the PCr/Pi ratio reduction without pH shift observed in this study would appear to reflect mitochondrial dysfunction and not a decline in the cerebral blood flow.

In the present study the author could demonstrate that mitochondrial dysfunction was introduced by heat in vivo as well as in vitro study, while cerebral energy metabolism and electrical activity were found to be preserved up to a cortical brain temperature of 44°C. This finding is in good agreement with that observed in the studies, which showed that the cerebral functions could be preserved until the higher temperature in case of the head heating than in case of the whole body hyperthermia[4,5].

In is well known that hyperthermia (above 42°C) can produce selective and irreversible damage to the several experimental tumors both in vitro and in vivo with its inhibitory effect on the aerobic glycolysis[22,23,24,25]. Naruse and coworkers generated radiofrequency hyperthermia on rat glioma inoculated in the lumber region by applying the radiofrequency pulse using the surface coil in the nuclear magnetic resonance spectrometer with monitoring of the effects in the same device[26]. After heating the experimental tumor at 43.5°C for 60 minutes, they observed that the nucleoside triphosphate peaks decreased and Pi peak increased remarkably within 30 minutes. The Pi dominant pattern became even more prominent 3 hours later and lasted for 7 days. Because the ^{31}P-spectra recorded after heating the rat brain to 44°C for 30 minutes showed no changes in the cerebral energy metabolism during the 1 hour post-heating period in the present study, the therapeutic window of the hyperthermia for brain tumors is thought to exist at the temperature between 43 and 44°C so far as either the change of cerebral energy metabolism or electrical activity, which is induced by hyperthermia, is concerned.

Conclusion

The present study demonstrated that at least part of the heat-induced damage occurred at the mitochondrial level with in vivo nuclear magnetic resonance spectroscopy. Although the accurate thermal threshold for cerebral energy disturbance could not be determined because of heterogeneity of heat-induced tissue damage in this study, cerebral energy metabolism and electrical activity were found to be preserved up to a cortical brain temperature of 44°C, at which the antineoplastic effect of hyperthermia is apparent.

Reference

1) Salcman, M. and Samaras, G. M.: Hyperthermia for brain tumors: Biophysical rationale. *Newrosurgery* **9**:327-335. 1981.
2) Ikeda, N., Hayashida, O., Kameda, H., Ito, H. and Matsuda, T.: Interstitial radiofrequency (RF) hyperthermia for malignant brain tumors-experimental study about the heating effect (in Japanese). *Tumor and Infection:* **3**: 745-749, 1990.
3) Britt, R. H., Lyons, B. E., Pounds, D. W. and Prisonas, S. D.: Feasibility of ultrasound hyperthermia in the treatment of malignant brain tumors. *Med Instrum* **17**:172-177, 1983.
4) Yamada, N.: The effects of hyperthermia on cerebral blood flow, metabolism and electroencephalogram (in Japanese). *Brain and Nerve* (Tokyo) **41**:205-212, 1989.
5) Teschan, P. and Gllhorn, E.: Influence of increased temperature on activity of the cerebral cortex. *Am J Physiol* **159**:1-5, 1949.
6) Moriyama, E., Salcman, M. and Broadwell R. D.: Blood-brain barrier alteration after microwave-induced hyperthermia is purely a thermal effect: I. Temperature and power measurements. *Surg Neurol* **35**:177-182, 1991.
7) Britt, R. H., Pounds, D. W. and Lyons, B. E.: Feasibility of treating malignant brain tumors with focused ultrasound. *Prog Exp Tumor Res* **28**:232-245, 1984.
8) Harris, A. B., Erickson, L., Kendig, J. H., Mingrino, S. and Goldring, S.: Observation on selective brain heating in dogs. *J Neurosurg* **19**:514-521, 1962.
9) Lyons, B. E. Britt, R. H. and Stohbehn, J. W.: Localized hyperthermia in the treatment of malignant brain tumors using an interstitial microwave antenna array. *IEEE Trans Biomed Eng* **31**:53-62, 1984.
10) Lyons, B. E., Obana, W. G., Borcich, J. K., Kleinman, R., Singh, D., and Britt, R. H.: Chronic histological effects of ultrasonic hyperthermia on normal feline brain tissue. *Radiation Res* **106**:234-251, 1986.
11) Silberman, A. W., Morgan, D. F., Storm, F. K., Rand, R. W., Bubbers, J. E., Brown, W. J. and Morton, D. L.: Localized magnetic-loop induction hyperthermia of the rabbit brain. *J Surg Oncol* **20**: 174-178, 1982.
12) Burger, F. J. and Fuhrman, F. A.: Evidence of injury to tissues after hyperthermia. *Am J Physiol* **206**:1062-1064, 1964.
13) Christiansen, E. N. and Kvamme, E.: Effects of thermal treatment on mitochondria of brain, liver and ascites cells. *Acta Physiol Scand* **76**:472-484, 1969.
14) Crockard, H. A., Gadian, D. G., Frackowiak R. S. J., Proctor, E., Allen, K., Williams, S. R. and Russel, R. W. R.: Acute cerebral ischaemia: Concurrent changes in cerebral blood flow, energy metabolites, pH, and lactate measured with hydrogen clearance and ^{31}P and ^{1}H nuclear magnetic resonance spectroscopy. II. changes during ischaemia. *J Cereb Blood Flow Metab* **7**:394-402, 1987.
15) Litt, L., Gonzalez-Mendez, R. Weinstein, P. R., Severinghaus, J. W., Hamilton, W. K., Shuleshko, J., Murphy-Boesch, J. and James, T. L.: An in vivo ^{31}P NMR study of cerebral hypoxic hypoxia in rats. *Magn Reson Medi* **3**:619-625, 1986.
16) Cox, D. W. G., Morris, P. G., Feeney, J. and Bachelard, H. S.: ^{31}P-NMR studies on cerebral energy metabolism under conditions of hypoglycaemia and hypoxia in vitro. *Biochem J* **212**:365-370, 1983.
17) Vink, R., McIntosh, T. K., Weiner, M. W.

and Faden, A, I.: Effects of traumatic brain injury on cerebral high-energy phosphates and pH: A ^{31}P magnetic resonance spectroscopy study. *J Cereb Blood Flow Metab* **7**:563-571, 1987.

18) Petroff, O. A. C., Prichard, J. W. Behar, K. L., Alger, J. R., Hollander, J. A. and Shulman, R. G.: Cerebral intracellular pH by ^{31}P nuclear magnetic resonance spectroscopy. *Neurology* **35**:781-788, 1985.

19) Gyulai, L., Roth, Z., Leigh, J. S. and Chance, B.: Bioenergetic studies of mitochondrial oxidative phosphorylation using ^{31}Phosphorus NMR. *J Biol Chem* **260**:3947-3954, 1985.

20) Branston, N. M., Symon, L., Crockard, H. A. and Pasztor, E.: Relationship between cortical evoked potential and local cortical blood flow following acute middle cerebral artery occlusion in the baboon. *Exp Neurol* **45**:195-208, 1974.

21) Waltz, A. G.: Clinical relevance of models of cerebral ischemia. *Stroke* **10**: 211-213, 1979.

22) Dickson, J. A. and Shah, D. M.: The effects of hyperthermia (42°C) on the biochemistry and growth of a malignant cell line. *Eur J Cancer* **8**:561-571, 1972.

23) Dickson, J. A. and Muckle, D. S.: Total-body hyperthermia versus primary tumor hyperthermia in the treatment of the rabbit VX-2 carcinoma. *Cancer Res* **32**: 1916-1923, 1972.

24) Dickson, J. A. and Suzanger, M.: In vitro-in vivo studies on the susceptibility of the solid Yoshida sarcoma to drugs and hyperthermia (42°C). *Cancer Res* **34**: 1263-1274, 1974.

25) Muckle, D. S. and Dickson, J. A.: The selective inhibitory effect of hyperthermia on the metabolism and growth of malignant cells. *Brit J Cancer* **25**: 771-778, 1971.

26) Naruse, S., Hihuchi, T., Horikawa, Y., Tanaka, C. Nakamura, N. and Hirakawa, K.: Radiofrequency hyperthermia with successive monitoring of its effects on tumors using NMR spectroscopy. *Proc Natl Acad Sci* **83**:8343-8347, 1986.