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Radiofrequency hyperthermia with successive monitoring of its effects on tumors using NMR spectroscopy

(cancer/rf heating/^{31}P NMR/nucleoside triphosphate/tissue pH)

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ABSTRACT **Radiofrequency (rf) hyperthermia was generated on rat glioma inoculated s.c. in CD Fisher rats by applying the rf pulse using the surface coil in the NMR spectrometer, and the effect was monitored successively in the same spectrometer by measuring ^{31}P NMR spectra and ^{1}H NMR images. In the ^{31}P NMR spectrum at the preirradiation stage, nucleoside triphosphate peaks and a phosphomonoester peak were high and a P_i peak was low. After a rf pulse at a power of 5 W was applied continuously for 60 min, the nucleoside triphosphate peaks decreased and the P_i peak increased immediately, resulting finally in a dominant P_i peak pattern within 30 min in all 10 cases examined. These spectral changes occurred much earlier than the histological changes and lasted for at least 7 days. By the ^{1}H NMR imaging, the necrotic region was detected as a high-intensity lesion in spin echo and inversion recovery images 2 days after the irradiation. There were no changes either in the spectrum or in ^{1}H NMR images in any of 8 cases after irradiation with a rf pulse of <3 W. Thus, we could generate rf hyperthermia with the NMR spectrometer and the effects were monitored sensitively with the same spectrometer. It can be concluded that the NMR device can be used not only for diagnosis but also as a therapeutic tool.**

Radiofrequency (rf) hyperthermia is a matter of concern in cancer treatment (1). Though the mechanism and effects of this therapy have been studied extensively, many problems have been left unsolved—for example, the appropriate power of the rf pulse, proper duration of heating, proper design of the rf coil, effectiveness of combined therapy, and so on. This is partly due to the lack of an appropriate method for examining the mechanism and for evaluating the effect of the treatment *in vivo*. Meanwhile, there has been recent, rapid progress in the application of NMR to the medical field. In the course of looking for new applications of the NMR method to the medical field, we took notice of generating rf hyperthermia using a NMR spectrometer. As a NMR spectrometer transmits a rf pulse, it should be possible to produce hyperthermia using the rf pulse generated by the spectrometer in the magnet. It is also supposed that the effects so produced could be monitored sensitively with the same spectrometer by ^{31}P NMR spectrum measurements as in other therapies (2–5). Therefore, we intended to generate rf hyperthermia on experimental tumors using the NMR spectrometer with successive monitoring of the effects in the same device in an attempt to find a further application of NMR as well as to obtain informative data for hyperthermia as a cancer therapy.

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MATERIALS AND METHODS

Experimental Animals. A total of 54 CD Fisher rats was used for the experiments. A suspension of 10^6–10^7 cultured rat glioma (EA285) cells was inoculated s.c. in the lumbar region of the animal (2). Twenty-eight days after inoculating the tumor cells, when the tumors grew to >1.5 cm in diameter, the animals were divided into two groups—a control group and the rf hyperthermia group. The rf hyperthermia group was subdivided into three subgroups according to the power of the rf pulse applied. In each group, consisting of 8–10 rats, *in vivo* ^{31}P NMR spectra in the tumors were measured sequentially before and after the treatment using the surface coil method described below. In some of each group, ^{1}H NMR images (^{1}H MRI) were obtained at pre- and posttreatment stages with the same NMR device.

At an appropriate time, the tumor masses in each group were removed for histological examinations.

NMR Measurements. *In vivo* ^{31}P NMR spectrum measurements were done by the surface coil method with a SCM-200 spectrometer (JEOL, Japan; a 9-cm bore, 4.7-T magnet). After anesthetizing with an i.p. injection of sodium pentobarbital at a dose of 35 mg/kg, each rat was placed in a supine position in the magnet so that the inoculated tumor mass was located on a four-turn, surface coil (1.1 cm in diameter) and the resonant signal of ^{31}P at 80.75 MHz was obtained from the tumor. The homogeneity in the measured volume was about 0.15 ppm, the pulse width was 14 μsec, and scans were repeatedly made 400 times at 1.9-sec intervals. A 30-Hz noise filter was applied to the free induction decay signal prior to Fourier transformation. Tissue pH was calculated from the chemical shift (σ ppm) of P_i in each spectrum as reported (2).

The ^{1}H MRIs were obtained by using a 7-cm, saddle-type coil with the same NMR spectrometer. Spin echo (SE) and inversion recovery (IR) images were obtained by the ordinary two-dimensional Fourier transform method. The data matrix numbered 256 × 256.

rf Hyperthermia with the NMR Spectrometer. Following the measurements of the *in vivo* ^{31}P NMR spectrum in the tumor at the control stage, rf hyperthermia was generated by using the same surface coil in the same spectrometer. A rf pulse with the same resonant frequency as that of phosphorus nuclei was applied continuously to the tumor at various power levels for 60 min. The power of the rf pulse was measured with a power meter provided in the NMR spectrometer. The rats were divided into three subgroups according to the rf power level applied: (*i*) >5 W, (*ii*) 3–5 W, and (*iii*) <3 W. After this hyperthermia therapy, phosphorus spectra were measured sequentially again without changing the position of the rat over a period of 3 hr then were measured intermittently for 7 days. In this way, we generated rf

Abbreviations: ^{1}H MRI, ^{1}H NMR image; SE, spin echo; IR, inversion recovery.

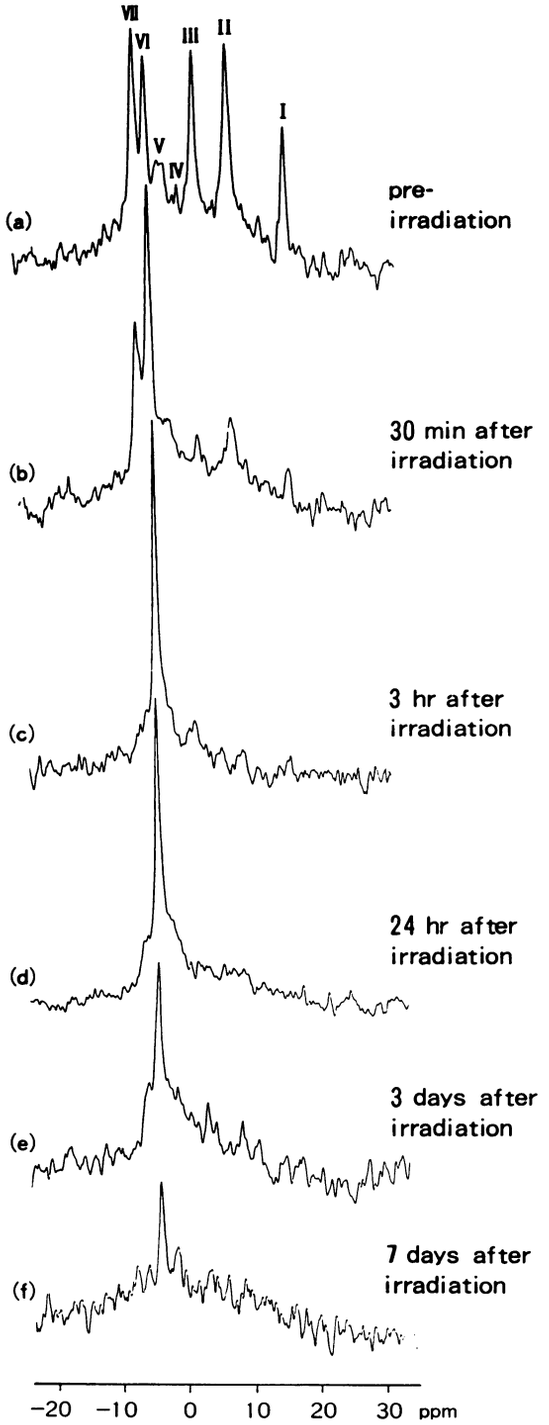

FIG. 1. ^{31}P NMR spectra of the rat glioma at various times before and after rf irradiation. Assignments of ^{31}P NMR signal peaks in the preirradiation stage are as follows: I, β-NTP; II, α-NTP and α-nucleoside diphosphate (NDP) with NAD^+/NADH; III, γ-NTP and β-NDP; IV, phosphocreatine; V, phosphodiesters; VI, P_i; VII, phosphomonoesters. The rf pulse was applied to the tumor continuously at a power of 5 W for 60 min with a surface coil in the magnetic field of the NMR spectrometer.

hyperthermia with the rf coil used for NMR spectrum measurements and were able to do successive monitoring of its effects with the same NMR spectrometer.

Measurements of Intratumoral Temperature During rf Hyperthermia. The temperature in the tumor tissue was measured in another series of rats that received the same rf hyperthermia therapy as described above. In each group, consisting of three or four rats, intratumoral temperatures at various depths under the skin were measured by using a 26-gauge needle thermister (model BAT-12, Sensortek, Clifton, NJ) during the course of the hyperthermia therapy.

RESULTS

***In Vivo* ^{31}P NMR Spectrum Changes After Generation of rf Hyperthermia.** The natural course of spectral changes during the tumor growth is the same as that reported (2). In the spectrum obtained 28 days after the inoculation of tumors, peaks of nucleoside triphosphate (NTP), phosphodiesters, P_i, and phosphomonoesters were clearly demonstrated (6). Tissue pH at this stage was 7.15 ± 0.07 (mean ± SD, n = 20). In this rat glioma model, spontaneous changes occurred slowly. Six to 8 weeks after the initial measurements, NTP peaks decreased and P_i peaks increased, and tissue pH decreased to 7.08 ± 0.10 (mean ± SD, n = 10).

In the group that had rf hyperthermia at a power of >5 W, drastic changes were seen in the ^{31}P NMR spectra after the rf irradiation. A typical change in the spectrum from this group is shown in Fig. 1. In the pretreatment period (Fig. 1, trace a), several peaks were clearly detected, notably NTP, phosphodiesters, P_i, and phosphomonoesters. After generation of rf hyperthermia, the NTP peaks decreased and the P_i peak increased remarkably within 30 min (Fig. 1, trace b). The P_i dominant pattern became even more prominent 3 hr later (Fig. 1, trace c) and lasted at least for 7 days, although peak heights decreased as the days passed during this period (Fig. 1, traces d–f). These spectral changes occurred in all 10 cases of this group. Tissue pH was 6.96 ± 0.10 (mean ± SD, n = 10) 3 hr after the irradiation when the spectrum showed a P_i dominant pattern. This value is significantly lower than that of the preirradiation period ($P < 0.1$).

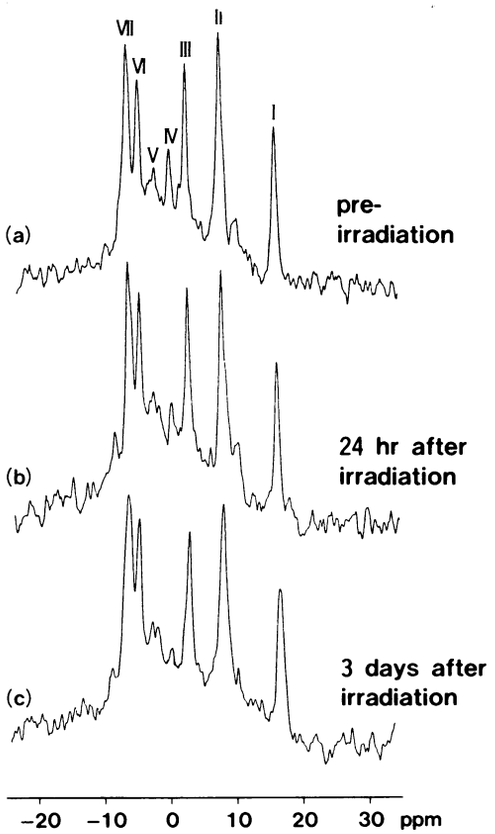

FIG. 2. ^{31}P NMR spectra in the rat glioma pre- and post-rf hyperthermia, with 60 min of irradiation at a power of 3 W. Assignments of ^{31}P NMR signal peaks are as in Fig. 1.

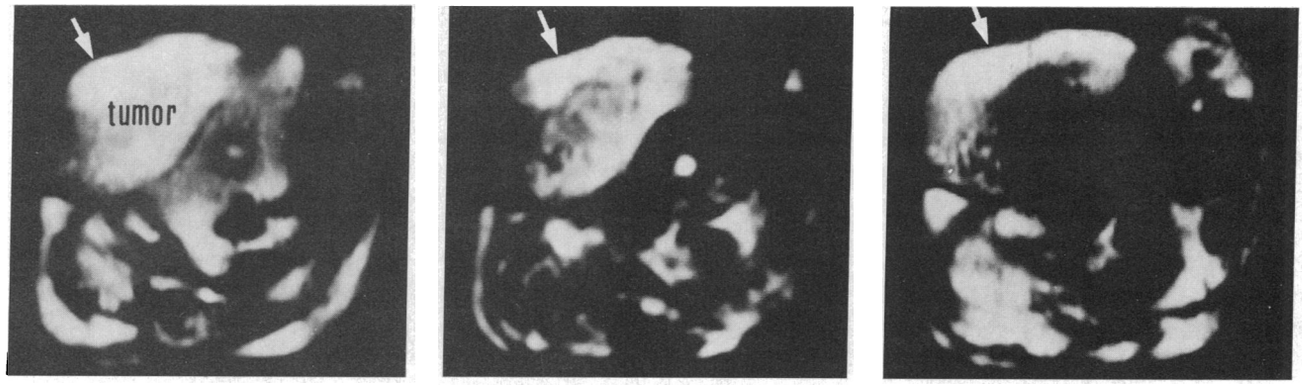

FIG. 3. ^{1}H MRI of the tumor in the rat 48 hr after rf irradiation at a power of 5 W. High-intensity regions (arrows) were detected in the area from the surface to the 5-mm depth in the SE image with long t_e and the IR image with 300-msec t_d.

In the group that had rf irradiation at a power of 3–5 W, 3 of 10 cases showed spectral changes similar to those of the group receiving irradiation >5 W. The remaining 7 cases showed no spectral changes after the irradiation.

In the group that had rf irradiation at a power of <3 W, no spectral changes were detected in any of the 8 cases examined. Typical spectra for pre- and post- rf irradiation in this group are shown in Fig. 2.

^{1}H MRI and Histological Examination After Generation of rf Hyperthermia. In the group that had the rf irradiation at a power of 5 W or more, no remarkable changes were demonstrated in the ^{1}H MRI of the tumor either directly after irradiation or up to 2 or 3 hr, when the ^{31}P NMR spectrum had already shown the P_i dominant pattern. Then, the irradiated regions, from the surface to 5 mm in depth, were detected as high-intensity areas in SE images with long echo times (t_e) and IR images with 300-msec interpulse intervals (t_d). These changes were clearly demonstrated 24 or 48 hr after the rf irradiation (Fig. 3).

Histological changes in the tumor tissue were not clearly recognized up to several hours after the rf irradiation at a power of 5 W. Two or 3 days after the irradiation, extensive necrosis was seen in the tumor tissue where the effective rf pulse was applied. This change was typically shown in a case extirpated 7 days after generation of the hyperthermia (Fig. 4). Necrosis was detected in the area from the surface to a depth of about 5 mm, to which rf power seemed to be applied effectively. This indicated that the changes in the ^{31}P NMR spectrum occurred earlier than the histological changes.

In the group that had rf irradiation at a power of <3 W, neither histological nor ^{1}H MRI changes were detected after the irradiation.

Intratumoral Temperature During rf Hyperthermia. In the group that had rf irradiation at a power of 5 W, all three cases showed similar changes in the temperature in the tumor tissue. A typical example is shown in Fig. 5. The temperature at a depth of 3 mm under the skin rose to 43.5°C within 5 min after inducing the rf irradiation and this level lasted during the rf irradiation. On the contrary, the temperature at a depth of 12 mm did not show any significant rise. In the area from a depth of 1 to 5 mm under the skin, the tumor temperature rose above 43.0°C. This region corresponded well to the necrotic portion demonstrated by MRI and histological examinations after generation of rf hyperthermia. Rectal temperature did not change during rf hyperthermia.

In the group that had rf irradiation at a power of 4 W, there was a variation of the changes in the tumor temperature among four cases examined. The first case showed the increase of the tumor temperature at a depth of 3 mm to 43.3°C during the steady stage. The second showed the increase of the temperature to 40.5°C. The remaining two cases showed a slight increase of the temperature to 39.0°C.

In the group that had rf irradiation at a power of <3 W, the tumor temperature at a depth of 3 mm did not exceed 39.0°C during the rf irradiation in all three cases examined.

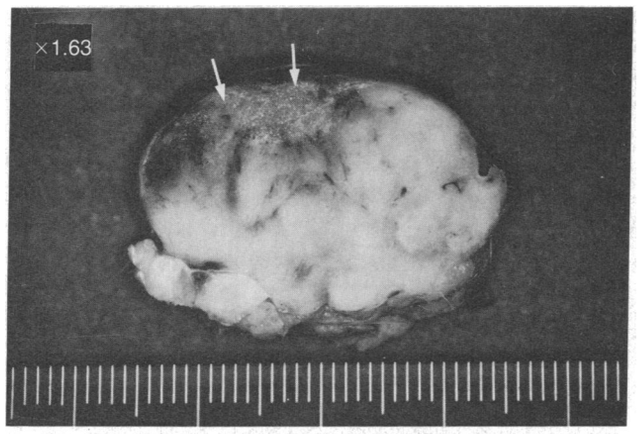

FIG. 4. Macroscopic finding of the tumor tissue 2 days after generation of the rf hyperthermia demonstrated in Fig. 1. A necrotic area is indicated in the region from the surface to a depth of 5 mm in the tumor (arrows), where the rf pulse was applied.

DISCUSSION

In this paper, we have demonstrated that the NMR spectrometer itself can be used as a therapeutic device by generating rf hyperthermia in tumors and can also be used to detect the results of the therapy by monitoring the effects in the same spectrometer. This is a further application of the NMR spectrometer. In general, the heating of the subject by rf irradiation causes serious problems in obtaining images using a MRI device, and many efforts have been made to minimize this detriment (7). By turning this disadvantage to an advantage for tumor treatment, the NMR spectrometer can be used as a rf hyperthermia device. However, there were still some limitations in the technique as used in this experiment. The rf coil used for hyperthermia was a flat-shaped surface coil, which was originally used for NMR spectrum measurements, and the heating method employed belongs to the inductive type (8, 9). Therefore, the region in which the rf pulse was effectively applied was only about 5–6 mm in depth. Accordingly, only a part of the whole tumor tissue was heated. This limitation of the effective heating area

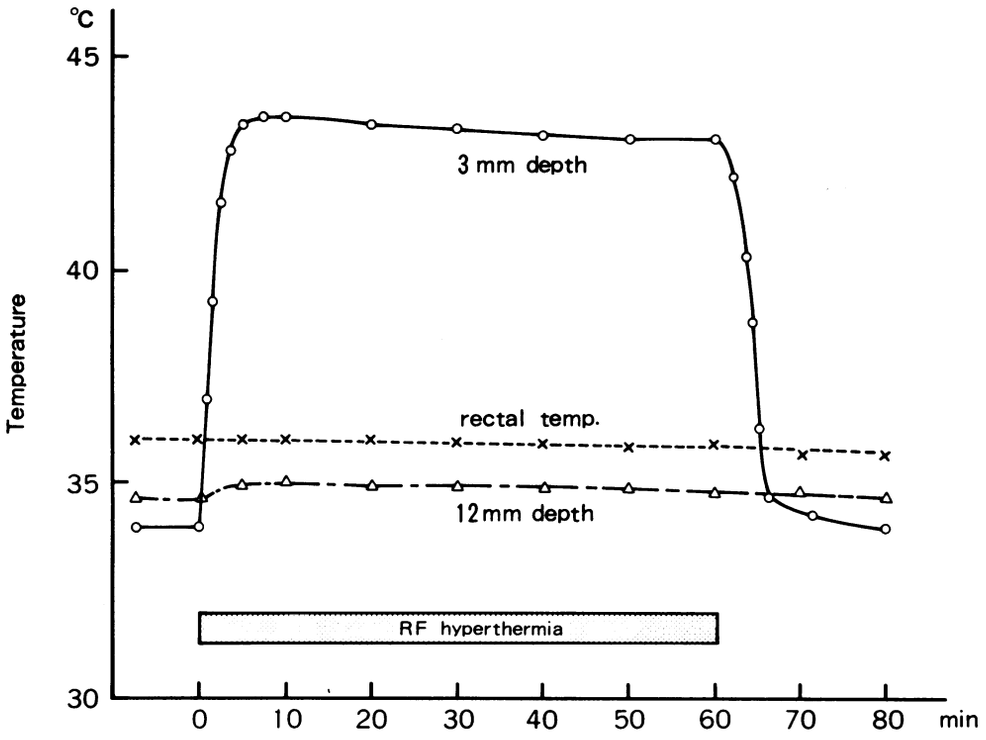

FIG. 5. Changes in the intratumoral temperature during rf hyperthermia. ○—○, Intratumoral temperature at a depth of 3 mm under the skin; △---△, intratumoral temperature at a depth of 12 mm; ×---×, rectal temperature.

was demonstrated by the histological examination and by the measurement of intratumoral temperature. This shortcoming could be overcome by an improvement in coil design (10). If the coil was appropriately made in a shape so as to wrap the tumor tissue completely, the whole tumor region would be affected. Moreover, another interesting application is expected in the hyperthermia therapy by the improvement of the rf coil. In this study, rf hyperthermia and the spectrum measurement were made by using the same surface coil, so that the spectrum could not be measured until the rf hyperthermia was completed. However, if the tumor was irradiated with a frequency of ^{1}H nucleus by one coil and the ^{31}P NMR spectrum measured simultaneously by another, or if the double-tuned coil with concurrent pulsing was used, the changes in the spectrum would be detected continuously even during the rf irradiation. In these cases, it might not be necessary to measure intratumoral temperatures, usually important in the hyperthermia therapy (11), because the effect would be directly detected by the spectral measurement.

In addition to these findings, several important results were obtained from the ^{31}P NMR spectral measurements. (*i*) The effects of the hyperthermia on the tumor tissue were monitored sensitively by the successive monitoring of the ^{31}P NMR spectrum. This was made possible by the fact that the spectrum changes occurred earlier than the histological and ^{1}H MRI changes. Consequently, ^{31}P NMR spectrum measurements can also be used as a sensitive monitor of hyperthermia therapy. (*ii*) The power of the rf pulse required for effective hyperthermia is determined by ^{31}P NMR spectral measurements. In this experiment, all of the tumor tissue that received rf irradiation at a power >5 W showed the spectral changes, but not at a power of <3 W. At a power of from 3 to 5 W, changes in the spectrum varied. This diversity probably refers to the susceptibility of tumor tissue to rf hyperthermia in relation to the stage of tumor growth (12) or the variety of vasculature in tumor tissue (13). (*iii*) The intratumoral pH could be measured *in vivo* by the ^{31}P NMR spectrum. It is very important to evaluate the intratumoral pH beforehand in hyperthermia therapy (14–16), because of the well-known fact that hyperthermia is more effective in tissue with a lower pH value (14, 15) and tumor tissue becomes acidotic after hyperthermia therapy (17). In this experiment, decrease in intratumoral pH could be easily demonstrated *in vivo* after generation of rf hyperthermia.

Thus, we demonstrated that rf hyperthermia therapy on the tumor could be generated using a NMR spectrometer and that the effects could be monitored successively with the same NMR device. In the future, these fundamental data could apply to a clinical MRI device with a high-powered magnetic field. With the advent of this method, the study of rf hyperthermia therapy will progress and consequently this therapy may become more available in the treatment of cancer.

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