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^{31}P-MR Spectroscopy of Experimental Tumors Following Irradiation

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Summary. **Phosphorus-31 magnetic resonance spectra were obtained by the JEOL JMN-GX270 spectrometer (6.3T) using the JEOL NM-G27TSPW surface coil probe from transplanted FM3A tumors grown subcutaneously in C3H mice at 3 h, 8 h, 1 day and 2 days after ^{60}Co irradiation with a single dose of 20 Gy or of 40 Gy. As there was no statistically significant difference between volumes of the tumors irradiated with 20 Gy and those with 40 Gy up to 3 days after irradiation, it is suggested that the observed changes in ^{31}P-MR spectra were due to a metabolic response to radiation,not due to the differences of tumor volumes. We found occasionally marked decreases in NTP levels at 3-8h after irradiation, which recovered 1-2 days later. Those changes,however,had no direct relation to later tumor growth. On the other hand, relative sequential changes observed from the 1st to the 2nd day, which included intracellular pHi of the tumor, Pi/β-NTP and PME/β-NTP ratios determined from ^{31}P-MR spectra, were related to later tumor growth. In responding tumors, those relative sequential changes showed increased pHi, decreased Pi/β-NTP and PME/β-NTP ratios, while reverse changes were found in control and non-responding tumors. It was concluded that relative sequential changes of those parameters could be used for predicting in early stages the tumor response to radiotherapy.**

INTRODUCTION

^{31}P-MR spectroscopy (^{31}P-MRS) can noninvasively provide information regarding the cellular phosphate compounds which participate in bioenergetics and membrane metabolism in tumor tissue, and may be useful for the diagnosing and staging of cancer and for the prediction and detection of the therapeutic response.[1-5] In particular,^{31}P-MRS has been used to monitor tumor response to radiotherapy,[6-11] hyperthermia[12,13] and chemotherapy[14,15] in several experimental animals and also in human patients.[19-22] However, ^{31}P-MR spectra of tumors vary from tumor to tumor and by stage and histology, and also exhibit various changes following therapy. Thus there are still considerable difficulties in interpreting the spectra, and further studies are required to determine if ^{31}P-MRS is of significance in predicting therapeutic effects.

^{31}P-MRS has been used to monitor tumor growth in experimental animals[16-18] and it has been shown that ^{31}P-MR spectra are considerably influenced by tumor volume. Therefore, it is of fundamental importarce to elucidate whether these spectral changes are due to biochemical responses to therapy or to differences in tumor volume. In this paper, ^{31}P-MR spectra of tumors grown subcutaneously in mice were measured at early stages (3 h, 8 h, 1 day and 2 days) after irradiation when there were no statistically significant differences in tumor volumes. We also examined whether ^{31}P-MRS could be a useful method for predicting response to radiotherapy by comparing those spectra with the later tumor growth.

MATERIALS AND METHODS

Tumors. FM3A cells (C3H/HeN mouse mammary adenocarcinoma) were transplanted subcutaneously in the thighs of male C3H mice. Tumors were studied when they reached volumes of approximately 400 mm^3 14-17 days after transplantation.

Irradiation. Localized irradiation of the tumor-bearing leg of mice was performed with photons from a ^{60}Co source with a dose of 20 Gy or 40 Gy (dose rate, 0.12 Gy/min). About 5 mm-thickness water bolus was

placed on the tumor. During irradiation the mouse was immobilized without anesthesia on a wooden plate with all limbs fastened to four fixed poles on a plate. Numbers of mice irradiated with 20 Gy and 40 Gy were 8 and 10, respectively. Tumor volumes, which were calculated by an ellipsoid approximation using the three orthogonal diameters ($V=(\pi/6)d_1 \times d_2 \times d_3$), were measured for 14 days after irradiation.

^{31}P-MRS. In vivo ^{31}P-MR spectra were taken using a JMN-GX270 spectrometer (JEOL, Japan; 6.3-T magnet) with a five-turn surface coil (10 mm in diameter).[16] Tumor spectra were obtained at each interval of 3 h, 8 h, 1 day and 2 days after irradiation while the mice were anesthetized with an interaperitoneal injection of sodium pentobarbital at a dose of 40 mg/kg. In the same way, spectra of control mice (n=6) were also measured at the same intervals. At measurement, a Faraday shield[16,23] was employed to reduce contamination of the signals from normal tissues outside the tumor. All spectra were acquired at 109 MHz, the pulse width was 13 μ sec, and scans were repeated 400-800 times at 2.0 sec intervals. A 30 Hz noise filter was applied to the free induction decay (FID) signal prior to Fourier transformation. To compare spectra,ratios of peak heights of interest were decided after a phase and baseline correction. Intracellular pH (pHi) of the tumor was estimated from the chemical shift of inorganic phosphate (Pi) relative to that of α-nucleotide triphosphate (NTP) as reported by Ng.[20]

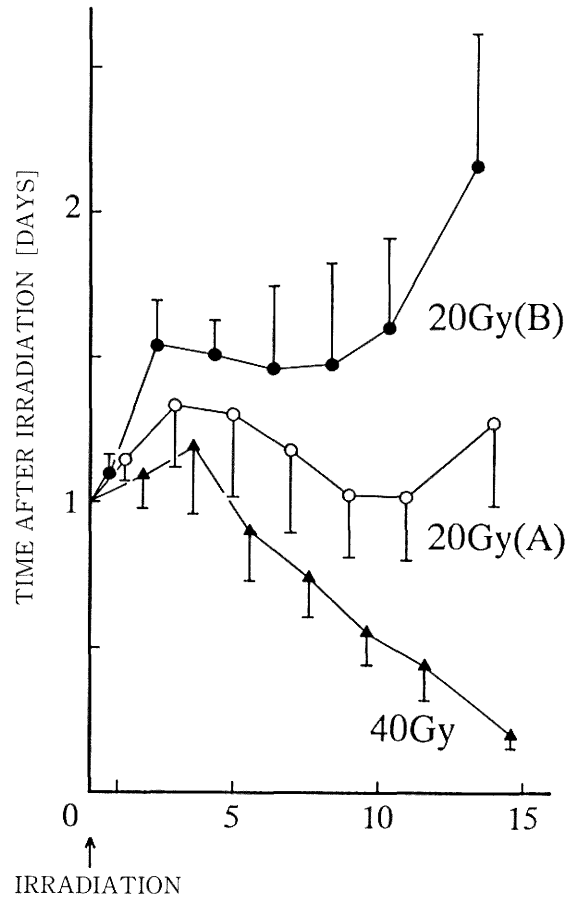

Fig. 1. Growth curves after irradiation. Tumors irradiated with 20 Gy were classified into two groups by volume on the 12th day after irradiation; less than (A group) and more than (B group) 1.2 times of that on day 0. Numbers of tumors in groups of 40Gy (▲), 20Gy (A —○), 20Gy (B—●) are 8, 6 and 4, respetively. Volume is expressed as a relative value of the absolute volume measured on day 0. Vertical bars indicate means ±SD.

RESULTS

Fig. 1 shows tumor growth curves after 20 Gy and 40 Gy irradiation. The tumors irradiated with 20 Gy were classified into two groups according to their volumes on the 12th day after irradiation; The A group being less than and the B group being more than 1.2 times of that on day 0. In every group, the tumor volume continued to increase until 2-3 days after irradiation, followed by a gradual decrease. Both groups of 20 Gy (A) and 20 Gy (B) showed regrowth about 10 days later. It is clear from the growth curves that tumors in the 40 Gy group showed the best response to radiotherapy, followed by the 20 Gy (A) group. The tumor volumes in the 20 Gy (B) group became significantly greater than those of the 20 Gy (A) at 9 days after irradiation. Up to 3 days after irradiation, there was no statistically significant difference in tumor volume among these three groups.

Fig. 2 shows typical ^{31}P-MR spectra at 3 h, 8 h, 1 day and 2 days after irradiation. In most studies, peaks of phosphocreatine (PCr) and phosphodiester (PDE) were either not detectable or extremely small, compared with phosphomonoester (PME), Pi and NTP. In every group some spectra at 3 h and 8 h after irradiation showed a temporarily marked reduction in NTP levels, which recovered 1-2 days later (Fig. 2-b, c, d). Some other spectra at 3-8 h, on the other hand, showed only slight changes in NTP levels (40 Gy; Fig. 2-a and 20 Gy (A), (B); not shown). Spectra at 3 h and 8 h after irradiation, therefore, showed extreme variation from tumor to tumor

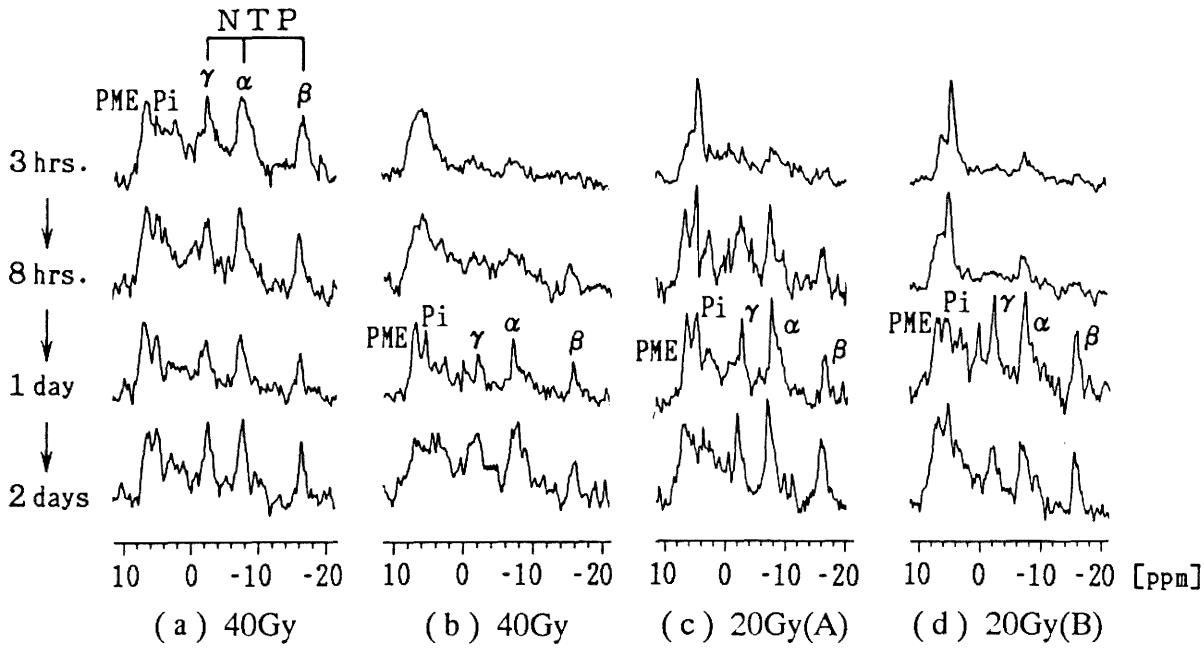

Fig. 2. ^{31}P-MR spectra after irradiation with 40Gy ((a), (b)) and 20Gy (A group; (c), B group; d)). Spectra are individually scaled for better demonstration.

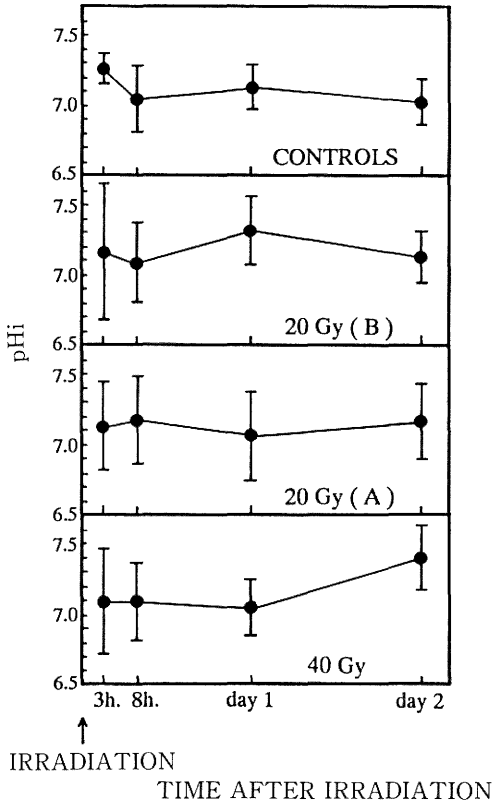

Fig. 3. Sequential change of pHi in controls and irradiated groups of 20Gy (B), 20Gy (A) and 40 Gy which are indicated in Fig. 1. Vertical bars indicate means±SD.

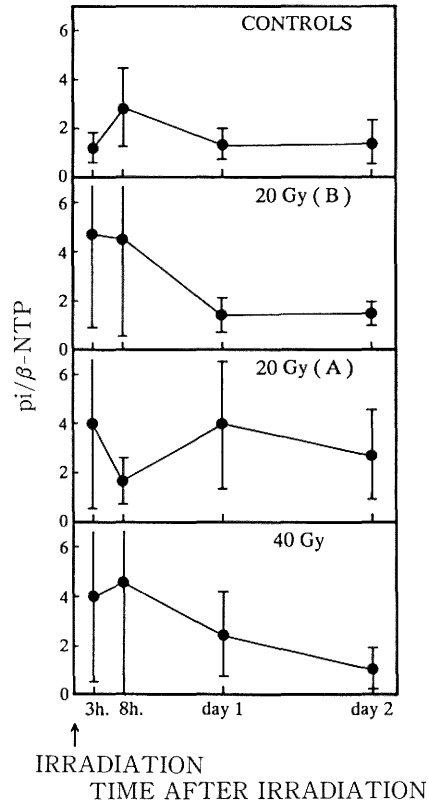

Fig. 4. Sequential change of Pi/β-NTP in controls and irradiated groups of 20Gy (B), 20Gy (A) and 40Gy. Vertical bars indicate means±SD.

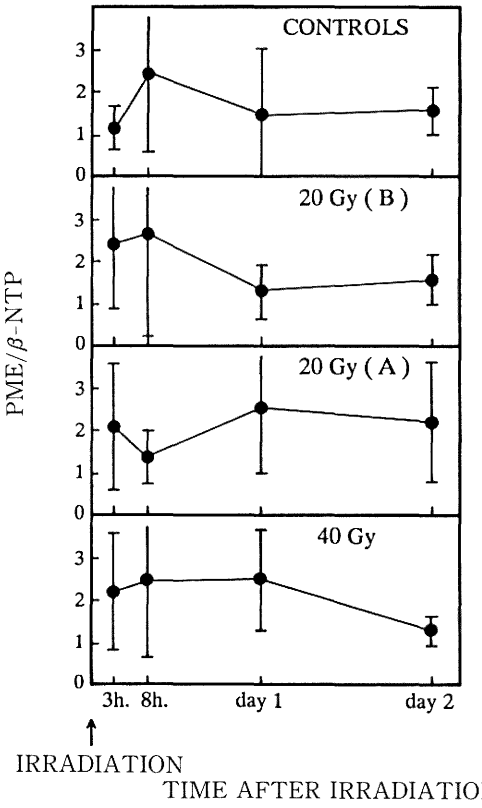

Fig. 5. Sequential change of PME/β-NTP in controls and irradiated groups of 20Gy (B), 20Gy (A) and 40Gy. Vertical bars indicate means±SD.

which was not related to radiation response.

Fig. 3, 4 and 5 show sequential changes in pHi, Pi/β-NTP and PME/β-NTP ratios in the control and the irradiated groups of 20 Gy (B), 20 Gy (A) and 40 Gy. In the control at 8 h, a considerable decrease of pHi and also a considerable increase in Pi/β-NTP and PME/β-NTP ratios were observed. Among the irradiation groups of 40 Gy, 20 Gy (A) and 20 Gy (B), there was no significant difference in pHi (Fig. 3-a), Pi/β-NTP (Fig. 3-b) and PME/β-NTP (Fig. 3-c) ratios at 3 h and 8 h. On the 1st day, however, the 20 Gy (B) group showed slightly higher pHi, slightly lower Pi/β-NTP and PME/β-NTP ratios than the other groups. In the 40 Gy group on the 2nd day, considerably higher pHi, lower Pi/β-NTP and PME/β-NTP ratios were observed, although these differences in each parameter were not statistically significant either on the 1st or the 2nd day.

From the 1st to the 2nd day, pHi decreased in the control and the 20 Gy (B) groups, contrarily to increase in the 20 Gy (A) and the 40 Gy groups (Fig. 3). Pi/β-NTP and PME/β-NTP ratios increased in the control and the 20 Gy (B) groups, on the other hand decreased in the 20 Gy (A) and the 40 Gy groups (Fig. 4 and 5). Fig. 6 shows relative sequential changes observed from the 1st to the 2nd day in pHi, Pi/β-NTP and PME/β-NTP ratios after irradiation. In the 40 Gy group, pHi increased while Pi/β-NTP and PME/β-NTP ratios decreased. In contrast, in the 20

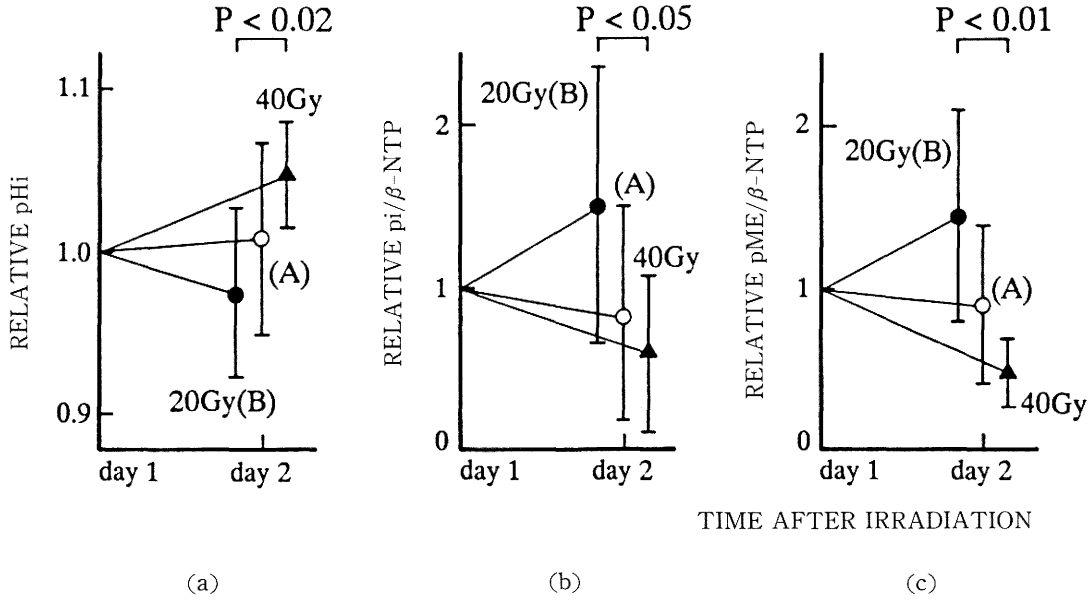

Fig. 6. Relative sequential changes in (a) pHi, (b) Pi/β-NTP and (c) PME/β-NTP ratios after irradiation. The symbols are indicated in Fig. 1. The value of each parameter on day 2 is expressed as a relative value of the absolute value measured on day 1. There is a statistically significant difference ($P<0.05$-0.01) between groups of 40Gy and 20Gy (B) for each parameter. Vertical bars indicate means±SD.

Gy (B) groups, pHi decreased while Pi/β-NTP and PME/β-NTP ratios increased. In the 20 Gy (A) group, every parameter showed only a slight change. Each parameter showed a statistically significant difference between the groups of 40 Gy and 20 Gy (B).

DISCUSSION

It has been shown that ^{31}P-MRS could potentially be an *in situ* predictor and/or sensitive monitor for therapeutic response of tumors. However, it has also been shown that ^{31}P-MR spectra have apparantly different patterns of response to therapy since spectra depend on tumor type, modality of therapy administrated dose and the tumor state which are determined by the blood and oxygen supply. In addition, the spectra may be influenced by anesthesia,[24] immobilization of the animal, measurement parameters, the types of coils and localization technique, signal processing and other factors. Therefore, these factors should be kept in mind even in an experimental system. In this study, we applied the Faraday shield which reduces contaminations from normal tissues outside the tumor. We also took care to maintain similar coditions in the tumor volumes, measurement parameters of spectra, signal processing and spectra analyzing. Nevertheless, measured spectra showed large variations, with the decrease in NTP levels observed at 3~8 h after irradiation being particularly variable. In some control tumors, spectral changes in the second measurement (at 8 h) also showed large variation. These spectral changes, therefore, may have been due to anesthesia and immobilization repeated at short (5h) intervals.

This study demonstrated that relative sequential changes observed from the 1st to the 2nd day in pHi, Pi/β-NTP and PME/β-NTP ratios were associated with later tumor growth. Those sequential changes in the 40 Gy group showed an increase of pHi and decrease of Pi/β-NTP and PME/β-NTP ratios. In contrast, changes in the 20Gy (B) group showed a decrease of pHi and increase of Pi/β-NTP and PME/β-NTP ratios. On the 2nd day after irradiation, there was no significant difference in tumor volume between the 40 Gy and the 20 Gy(B) group; in addition, no histological changes were recognized in any irradiation groups (histology not shown in this paper). It was, therefore, considered that those spectral changes were due to a metabolic response to radiation. Previous studies[4,6-8] indicated similar changes by comparing spectra between irradiation groups and non-irradiation groups, or by comparing between low-dose irradiation groups and high-dose groups, although the timing for the measurements of the spectra were different. The causes for these changes are considered to be due to the enhanced blood supply of the tumor tissue, activated production of ATP caused by reoxygenation and transition to the aerobic glycolysis of the metabolic pathway which leads to the alkalosis. On the other hand it has been shown that, under the condition of comparatively higher dose irradiation, an initial short term increase in Pi/β-NTP ratio and decrease in PCr level after irradiation are followed by recovery.[3,9-12] It is supposed that these changes are due to acute temporary damage to the tumor cells, especially mitochondria, and/or the vasculature of the tumor by irradiation. Therefore, after irradiation with a relatively higher dose, it is expected that spectral changes will show two phases; namely the initial transitory phase after irradiation is followed by the second long term phase in which spectral changes are the reverse of those in the initial phase. One previous study[11] indicated that, by revealing the correlation between spectral changes and the tumor tissue oxygen tension, changes in both the initial phase and the second phase could be indicators for the tumor response to radiotherapy in early stages. In our study, changes in the initial phase, which showed extremely decreased NTP levels at 3-8 h after irradiation, were observed in some tumors, but these changes had no direct relation to the later tumor growth. However, the relative sequential changes in pHi, Pi/β-NTP and PME/β-NTP ratios observed from the 1st to the 2nd day after irradiation were associated with later tumor growth. These later changes may imply a part of the biphasic process occurring after irradiation and could therefore be used for predicting the tumor response to radiotherapy at early stages.

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