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CEREBRAL METABOLISM IN BRAIN TUMOR OF MICE STUDIED BY IN VIVO ^{31}P-NMR SPECTROSCOPY

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SUMMARY: We now report a mouse model system of brain tumor for ^{31}P-NMR spectroscopic study of in vivo cerebral metabolism . In vivo ^{31}P-NMR (109 MHz) spectra were taken on the 9th day by the Faraday shield method of the brain of mice (3-week-old) transplanted intracerebrally with mKS·A tumor cells. In tumor-bearing mice, the amount of creatine phosphate decreased markedly and that of inorganic phosphate plus sugar phosphate increased accordingly. Furthermore, the broadening and splitting of individual signals were also noted with tumor-bearing mice; this is interpreted as indicating a variety of changes in chemical shift occurring in the brain of the animals due to heterogeneous distribution of pH. Binding or detaching of divalent cations to and from phosphometabolites may also be responsible for these changes.

High-resolution nuclear magnetic resonance (NMR) spectroscopy of ^{31}P-containing compounds is a powerful tool for investigation of in vivo cerebral metabolism (1). Several methods have been proposed to obtain ^{31}P-NMR spectra from a localized region in live animals. These include the surface coil method (2), topical magnetic resonance method (3), projection reconstruction method (4), sensitive point method (5), etc. Recently we have developed the Faraday shield method (6) which provides an additional method for obtaining NMR spectra from a localized region of live animals. Using this technique, we have succeeded in measuring ^{31}P-NMR spectra of the brain of mice transplanted intracerebrally with mKS·A cells.

The present paper describes the results of our experiments, demonstrating significant differences in phosphorus metabolism between tumored and normal brains.

MATERIALS AND METHODS

Transplantation of malignant and benign cells into mouse brain: mKS·A (SV40 virus-transformed BALB/c mouse kidney cells, transplantable) (7) or BK-

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BK cells (BK virus-transformed BALB/c mouse kidney cells, untransplantable) (7) were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. They were harvested from monolayer cultures by trypsinization and intracerebrally transplanted (1×10^6 cells/20 µl/mouse) to BALB/c mouse (3 to 5-week-old). On the 7th to 12th days after transplantation, the animals were subjected to in vivo ^{31}P-NMR spectroscopy and also histological examinations (8).

In vivo ^{31}P-NMR spectroscopy: The BALB/c mouse weighing about 20 g was anesthetized with sodium pentobarbital (60 mg/kg body weight). The animal body was covered, except for the head, with copper foil (Faraday shield) and placed in a 25-mmϕ tube as described previously (6). The spectra were obtained at 109.14 MHz with a JNM-GX 270 NMR spectrometer from 600 transients of 80 µsec (45°) delivered at 1.00-sec. intervals. In some experiments, in vivo ^{31}P-NMR spectra were measured also by the surface coil method (2) as modified by Yuasa et al. (9). The surface coil used (1 cm in diameter) consisted of three turns of gauze 16 copper wire insulated with vinyl coating. The following spectral parameters were used; 20 µsec (90°) pulse, 8192 data points, 1000 scans and 0.952 sec recycle time.

RESULTS AND DISCUSSION

In vivo ^{31}P-NMR spectroscopy has recently been used to determine the metabolic state of tumors (10-12); remarkable variations have been noted in the metabolic characteristics among tumors, even of the same kind at the same stage of growth (11). We now established a mouse model system of brain tumor with comparatively a small variation in the growth rate; this was shown by histological examination (8) and in vivo ^{31}P-NMR spectroscopy (see Figs. 2 and 3). Fig. 1 shows changes in body weight of mice after intracerebral transplantation of each of malignant tumor cells and benign cells. The mice transplanted with malignant tumor cells showed striking decreases in body weight, whereas those inoculated with benign cells did show increases in body weight, similar to control animals.

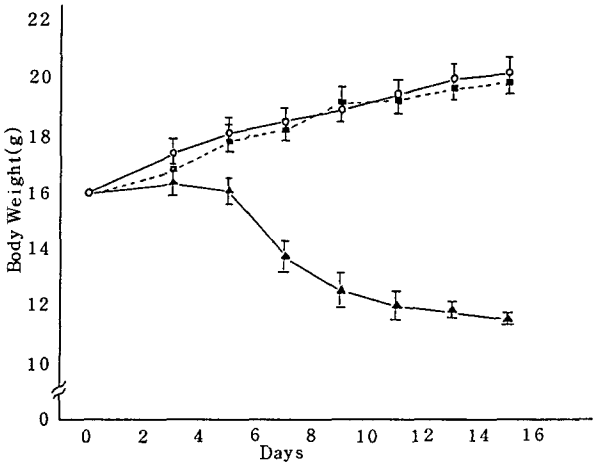

Fig. 1. Changes in body weight of mice after intracerebral transplantation with each of malignant tumor (mKS-A) cells and benign (BK-BK) cells.
○——○, control; ■---■, BK-BK; ▲——▲, mKS-A.

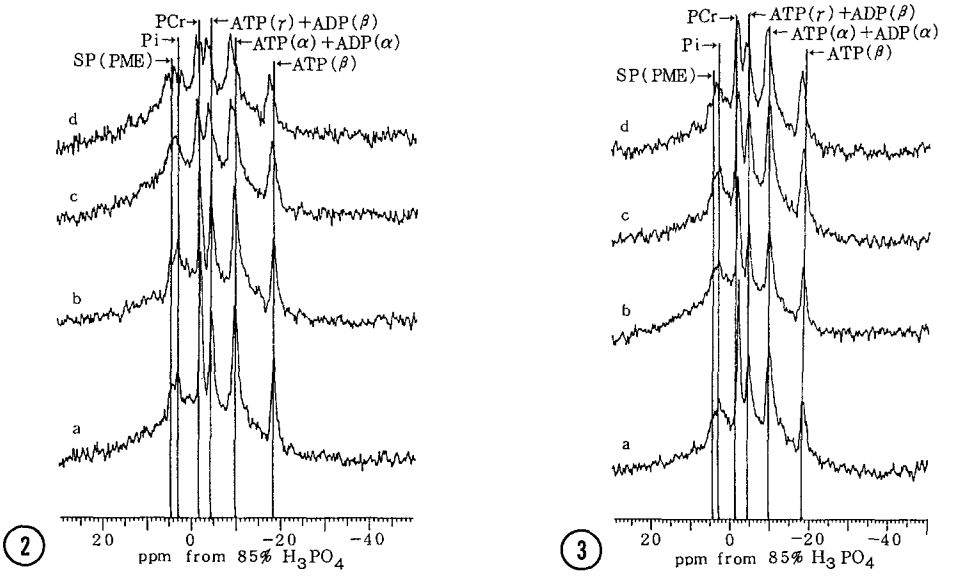

Fig. 2. In vivo ^{31}P-NMR (109 MHz) spectra on the 9th day of the brain of mice (3-week-old) transplanted intracerebrally with mKS-A tumor cells as measured by the Faraday shield method (25 mmϕ probe).
a,b, control mice: c,d, tumor-bearing mice; SP, sugar phosphate (phosphomonoesters); Pi, inorganic phosphate; PCr, creatine phosphate; ATP, adenosine triphosphate.

Fig. 3. In vivo ^{31}P-NMR (109 MHz) spectra on the 9th day of the brain of mice (4 to 5-week-old) transplanted intracerebrally with mKS-A tumor cells as measured by the Faraday shield method (25 mmϕ probe).
For symbols, see the legends to Fig. 2.

In vivo ^{31}P-NMR (109 MHz) spectra were taken by the Faraday shield method on the 9th day of the brain of mice (3-week-old) transplanted intracerebrally with mKS-A tumor cells, with the results shown in Fig. 2. The spectra were found to be highly reproducible among the same group of animals. In tumor-bearing mice (3-week-old), the amount of creatine phosphate (PCr) decreased markedly and that of inorganic phosphate plus sugar phosphate (PME, phosphomonoesters) increased accordingly. Furthermore, the broadening and splitting of individual signals were also noted with tumor-bearing mice. The cause of the changes in individual signals will be discussed later.

Fig. 3 shows the in vivo ^{31}P-NMR spectra, taken on the 9th day, of the brain of 4 to 5-week-old mice similarly transplanted with malignant tumor cells. The amount of creatine phosphate decreased to a lesser extent as compared with 3 week-old mice, and that of inorganic phosphate plus sugar phosphate (PME) also increased to a lesser extent. In other words, the effects of transplantation of the malignant tumor cells on cerebral metabolism were more pronounced in 3-week-old mice than in 4 to 5-week-old animals.

For the diagnosis of cancer, it is important to determine if the metabolism of malignant tumors differs sufficiently from that of benign tumors. In most tumors,

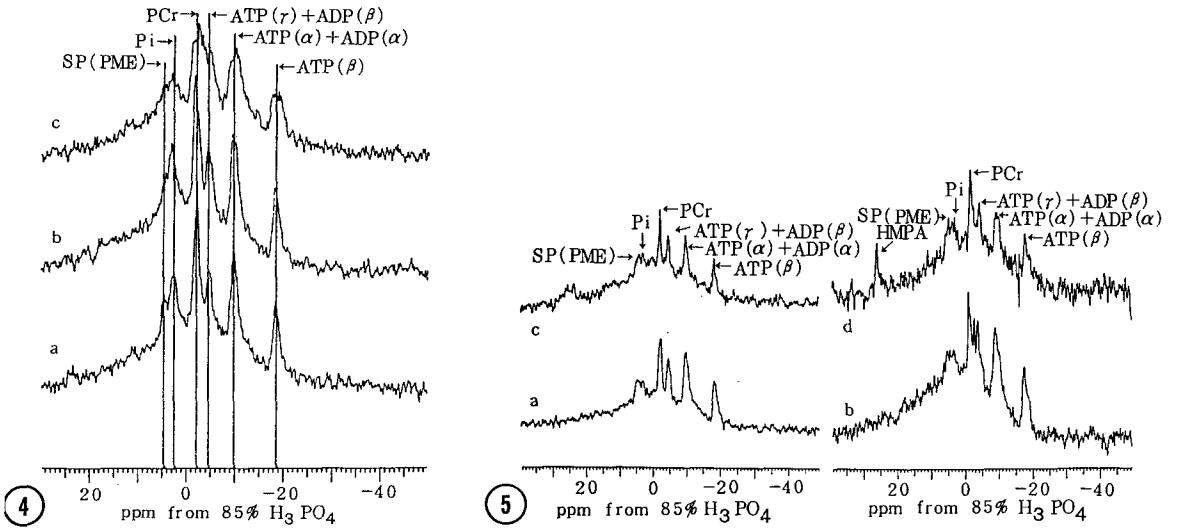

Fig. 4. In vivo ^{31}P-NMR (109 MHz) spectra of the brain of mice transplanted intracerebrally with malignant (mKS-A) and benign (BK-BK) cells as measured by the Faraday shield method (25 mmφ probe).
a, control; b, benign; c, malignant. For other symbols, see the legends to Fig. 2.

Fig. 5. In vivo ^{31}P-NMR (109 MHz) spectra of the brain of mice transplanted intracerebrally with malignant cells as measured by (a,b) the Faraday shield method (25 mmφ probe) and (c,d) the surface coil method.
a,c, control mice; b,d, tumor-bearing mice. HMPA (Hexamethyl phosphoro amide) used as an external reference.

it is well known that a low level of PCr peak and a relatively high level of the sugar phosphate (PME) peak are observed (10-12). In this respect, our ^{31}P-NMR spectral data shown in Fig. 2 are compatible with those of others (10-12). Fig 4. shows the in vivo ^{31}P-NMR spectra of the brain of the mice transplanted intracerebrally with each of malignant and benign cells as measured by the Faraday shield method (6). The spectra of the brain of the mice inoculated with the benign cells were greatly different from those of the animals inoculated with the malignant cells, but were very similar to those of control animals.

To evaluate the Faraday shield method (6), a comparison was made between the in vivo ^{31}P-NMR spectra obtained by this method and by the surface coil method reported previously (2). As shown in Fig. 5, no significant differences were observed between the in vivo ^{31}P-NMR spectra of the brain of tumor-bearing mice obtained by these two methods.

In our transplantation system, the tumor cells grew rather near the surface of the cerebrum under the skull, as demonstrated by histological examinations (8). This explains well for the consistency of the results obtained by these two methods. Needless to say, the surface coil method detects the NMR signals only from the part directly under the surface coil, whereas the Faraday shield method detects the signals from all parts of an unshielded localized region. In our recent study with

the primary brain tumor produced in syrian hamsters by simian adenovirus 7, the tumor cells were found to grow near the surface of the cerebrum and/or in its deep parts; in the latter case, the surface coil method often failed to detect signals from deep parts where main pathophysiological changes were developing, whereas the Faraday shield method succeeded in detecting such signals without failure. These results will be published elsewhere.

Since Warburg's classical work (13), it is well known that as tumor grows glycolysis becomes the predominant energy pathway; the pH of the tumor mass drops due to the enhanced production of lactic acid. In general, as the tumor grows progressively, its central part becomes necrotic due to insufficient blood supply; in the peripheral parts, in contrast, the tumor continues to grow actively. This may induce heterogenous pH distribution in the tumor mass (10). ^{31}P-NMR was most frequently used to determine the pH inside the tumored tissues (10).

As shown in Figs. 2-5, the broadening and splitting of ^{31}P-NMR signals of such compounds as ATP may be interpreted as indicating the diversity of the pH distribution in the tumor and its adjacent tissues in the brain of mice. The chemical shift of α-P of ATP, known to be independent of pH, was changed, suggesting that factor(s) other than pH should be responsible for this change of α-P in tumor-bearing mice. In this connection it is worth mentioning the findings of Roberts et al. (14) and others (15) that such divalent cations as Mg^{2+} and Ca^{2+} ions induced profound changes in chemical shift of ^{31}P-containing compounds. To examine the possibility if such divalent cations are involved, the effect of divalent cations on the chemical shift of PCr was determined. ^{31}P-NMR spectra were measured of PCr (10 mM) in 100 mM KCl and 2 mM Tris-HCl buffer (pH 7.4) in the presence of 5 mM $MgCl_2$ or 5 mM $CaCl_2$. The upfield change in chemical shift was found to be of levels of 0.22 and 0.07 ppm at a physiological concentration of Mg^{2+} and Ca^{2+} ions, respectively. The effect of Mg^{2+} ions on the chemical shift of PCr and ATP in the presence of 10 mM EDTA was also tested. As shown in Fig. 6, Mg^{2+} ions at concentrations higher than 10 mM added to ATP resulted in a striking downfield change in chemical shift of especially β-P signal of ATP. An excess amount of EDTA (10 mM) was added to the reaction mixture to eliminate any effect of divalent cations if present in it. On the contrary, a considerable upfield change in chemical shift of PCr was noted in Mg^{2+} ion concentration higher than 10 mM. The results demonstrated that the chemical shifts of PCr and ATP were greatly influenced by a physiological concentration of divalent cations, especially of Mg^{2+} ions.

In view of the foregoing results and discussions, the changes in chemical shift of phosphometabolites observed in the brain of tumor-bearing mice are not fully explained in terms of the changes in pH alone. Factors other than pH, for example, binding and detaching of divalent cations to and from phosphometabolites may also be responsible for these changes. At present the real cause of these changes is

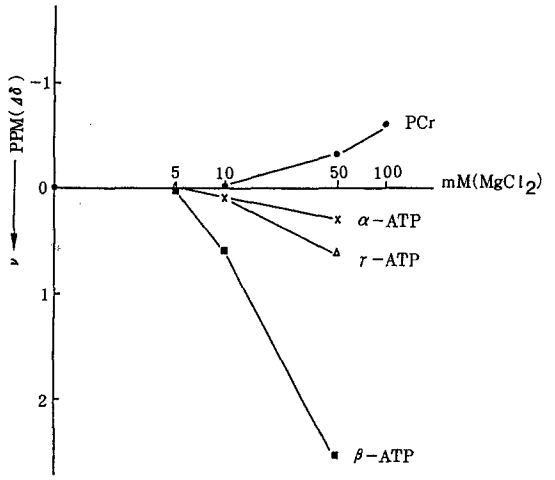

Fig. 6. Effects of Mg^{2+} ions on the chemical shift of PCr and ATP as measured by ^{31}P-NMR.
^{31}P-NMR spectra (109 MHz) were obtained of PCr (10 mM) and ATP (10 mM) in 100 mM KCl and 10 mM Tris-HCl containing 10 mM EDTA at pH 7.4 in the presence of $MgCl_2$ (10-100 mM).

not fully understood. Further study is needed, therefore, to clarify the factors affecting the chemical shift.

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