# Biological Malignancy and 18FdUrd Uptake in Glioma Patients - PET Study of Nucleic Acid Metabolism

# IV. 1. Biological Malignancy and $^{18}$FdUrd Uptake in Glioma Patients - PET Study of Nucleic Acid Metabolism

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## Introduction

Despite recent advances in neurosurgical treatment and sophisticated radio-chemotherapy, satisfactory results have not as yet been obtained in glioma patients.[32] To establish better treatment strategy, more must be known about the pathophysiology of the glioma. Positron emission tomography (PET) has allowed us to examine various physiological information and a number of studies of brain tumors have already been made.[2-5,7-10, 16, 19, 20, 23, 25, 28, 29, 31, 35, 36] No report on nucleic acid metabolism in brain tumor patients, however, has yet appeared in the literature. Since nucleic acid metabolism closely correlates with the proliferative potential of neoplasms[22], information concerning nucleic acid metabolism should be extremely useful in the diagnosis and treatment of glioma.

For these reasons, we undertook a clinical PET study of the nucleic acid metabolism of glioma. One of the fluorinated pyrimidines labeled with a positron emitter, $^{18}$F-fluoro-2'-deoxyuridine ($^{18}$FdUrd), was used in the present study as a tracer of nucleic acid metabolism.[1,17, 34]

## Materials and Method

### Patients

Twenty-two histologically defined glioma cases (17 males and 5 females, ranging in age from 27 to 73 years old, with a mean age of 44.0 years old) were studied with PET using $^{18}$FdUrd. Twelve patients were studied before radio-chemotherapy (Table 1). The

remaining ten patients were studied during or after radio-chemotherapy (Table 2). All of the patients underwent PET examination before radical surgery with the exception of case 13, who was scanned 3 months after resection of the tumor. Histological diagnoses were made from CT-guided stereotaxic biospies (10 cases) or operative specimens (12 cases). There were 5 cases of grade IV (4 glioblastoma and 1 pleomorphic xanthoastrocytoma). 11 cases of grade III (10 anaplastic astrocytoma and 1 anaplastic ganglioglioma), and 6 cases of grade II (5 fibrillary astrocytoma and 1 gemistocytic astrocytoma). The tumor tissue for histological diagnosis was obtained within 1 month from the PET study in 17 cases, 2 months in 1, 3 months in 3 and 4 months in 1. Informed consent was obtained from the patients and/or relatives. The present project was also approved by the Committee for Clinical PET Study of Tohoku University.

Scanner and procedure

The ECAT II (EG&G, Ortec)[30] and PT-931(CTI, Knoxville, Tennessee).[33]were employed. The spatial resolutions of the images were 17 and 8 mm, and the slice thicknesses were 18 and 7 mm in FWHM for the ECAT II and PT-931 respectively. $^{18}$FdUrd was synthesized using an automated synthesis system[18], which provides a radiochemical purity of over 98%. Following intravenous administration of 4 to 11 mCi of $^{18}$FdUrd, sequential scanning (each scan being of 5 minutes duration) was performed for 45 minutes, after which additional images in other positions were obtained. Usually three images were obtained with the ECAT II at 1 cm center to center spacing and 7 images were obtained simultaneously with the PT-931 at 7 mm spacing. The PET images were reconstructed suing a measured attenuation correction. The injected dose of $^{18}$FdUrd as 5-fluoro-2'-deoxyuridine was 2.0-5.8 mg, which was much lower than the doses of fluorinated pyrimidines used clinically as chemotherapeutic agents.[6]

Tissue uptake of $^{18}$FdUrd was also calculated and expressed as the differential absorption ratio(DAR)[21,24], which is related to % injected dose and is expressed as:

$$DAR=C_{pet}*W/D$$

where $C_{pet}$ is the tissue concentration of $^{18}$F expressed in mCi/g, W and D are body weight in g and the injected dose in mCi, respectively. Oval regions of interest were located on the brain tumor and the contralateral brain tissue in the sequential images using CT images as an anatomical guide, and the DAR was calculated. Statistical analysis was done by Welch's t test.

## Results

In all 16 cases of high grade glioma (III and IV) showed a positive image of the brain tumor clearly with high contrast (Fig. 1). On the other hand, a positive image could not be obtained in 6 cases of low grade glioma (II) (Fig. 2).

In the high grade glioma cases, the sequential value of the DAR in the tumor was much higher than that in the contralateral brain tissue throughout the scanning. The DAR of the low grade glioma cases, in contrast, was indistinguishable from that of the contralateral brain tissue (Fig. 3).

The results of DAR at 45-50 min after the administration of $^{18}$FdUrd are summarized in Tables 1 and 2. The mean DAR and standard deviation was 0.64 ±0.20 in the 5 grade IV tumors and 0.64±0.38 in the 11 grade III tumors. When these two groups were combined it was 0.64±0.34. The value for the 6 grade II tumors, on the other hand, was 0.21±0.042. A significant difference was observed between high grade glioma (III and IV) and low grade glioma ($p<0.01$), between grades IV and II ($p<0.005$) and between grades III and II ($p<0.05$). The large variation in rates for grade III glioma (59% SD) was mainly due to the high DAR value in case 9.

The mean DAR and standard deviation for contralateral brain tissue for the 23 patients was 0.18±0.053. There were no statistical difference in the values of the DAR with regard to histological grades, treatment or the different type of scanner.

The results of DAR ratio of tumor/contralateral brain tissue were more striking. A significant difference was observed between high grade glioma ($p<0.001$), between grades IV and II ($p<0.005$), and between grades III and II ($p<0.001$). The mean DAR value of grade II tumors, however, did not differ from that of homolateral brain tissue (Fig. 4). A clear differentiation between low grade and high grade glioma was observed at a DAR ratio of approximately 2.0.

## Discussion

PET provides a unique opportunity to obtain three-dimensional information on physiological processes in a noninvasive manner. Accordingly, there have been various metabolic studies of brain tumors.[2-5, 8-10, 16, 19, 23, 25, 28, 29, 31, 35, 36] Di Chiro et al. reported that FDG was useful in differentiating high and low grade glioma[8,9], and they also suggested that glucose metabolism significantly correlates with the prognosis of patients.[9,28] According to the recent report by Tyler et al.[35], however, the rate of glucose utilization did not correlate with tumor grade. Believing that information on nucleic acid

metabolism would provide us with the opportunity to investigate the proliferation potential of brain tumors, we undertook a PET study with $^{18}$FdUrd.

In the present study, $^{18}$FdUrd was used as a tracer of nucleic acid metabolism for the following reasons;1) fluorinated pyrimidines are known to show close correlation with the metabolism of nucleic acid[12], 2) in experiments using an implanted subdermal tumor, the intratumoral accumulation of $^{18}$FdUrd was high in comparison with other fluorinated pyrimidines such as 5-fluorouridine and 5-fluorouracil[1], 3) in a study using experimental rat brain tumor, the distribution pattern of 18FdUrd was suggested to closely correlate with the metabolism of nucleic acids.[34]

The results of our PET-$^{18}$FdUrd study showed a clear differentiation between high and low grade glioma. A region with a high accumulation of $^{18}$FdUrd was observed in all high grade glioma, whereas a positive image could not be obtained in low grade glioma cases. Not only was there a visual distinction in PET images, but also the DAR of the tumor disclosed a significant difference between the high and low grade glioma. In the present study, however, there was a large variation in the DAR in high grade glioma. There are several possible reasons for this; 1) A relatively small number of patients was examined in this study. 2) The heterogeneity of high grade gliomas, with a mixture of necrotic tissue and actively growing cells, and different tumor cell density, all of which can cause a wide variation in the DAR. In fact, the DAR of 0.26 in case 7 was relatively low incomparison with other grade III cases. Histological examination disclose, however, a mixture of oligodendroglioma (grade II) and anaplastic astrocytoma, which probably resulted in the low DAR value. 3) The limitations of the reliability of biopsies should not be overlooked.[26] Since only a small part of the tumor tissue can be obtained by this procedure, the existence of a grade IV component in the grade III group cannot be denied. 4) The effect of treatment may play another role. Since the metabolic state of brain tumors is known to change according to the treatment procedure[2,23], care must be taken in analyzing clinical data. 5) Finally, as reported by Hoshino et al.[14], the cell proliferative potential of high grade gliomas determined by S-phase fraction showed wide variation even in the same graded glioma. Other reports have stated that PET-FDG studies correlated better with the prognosis of brain tumor patients than did histologic subclassification.[9,10,28] These results may support the large deviation of DAR in high grade gliomas in the present study.

Di Chiro et al. emphasized the possible errors caused by a partial volume effect in PET-FDG study.[11] Although in the present series, false negative results were not observed in the high grade glioma patients, care should be taken to note possible errors

caused by this partial volume effect when studying very small tumors or tumors with a cavitation component surrounded by a thin rim of solid tumor.[8] In PET-FDG studies, however, even the glucose utilization rate of high grade glioma is sometimes lower than the contralateral gray matter.[35] Our PET-$^{18}$FdUrd study, on the contrary, clearly demonstrated a high grade glioma image, since the uptake of $^{18}$FdUrd in the brain is low. This may lead to fewer errors in diagnosing high grade glioma and also provide supplementary information suggesting the most appropriate target for biopsy[25,36].

The present results may be debated since the status of the blood-brain barrier was not investigated in this study. However, we believe that the $^{18}$FdUrd PET image mostly reflects the metabolism of nucleic acid for the following reasons: 1) In our experiments using rat brain tumors[34], double labeled autoradiography with $^{18}$FdUrd and $^{14}$C-thymidine revealed similar brain tumor images. In contrast, an autoradiographic comparison of $^{18}$FdUrd and $^{14}$C-aminoisobutyrie acid, which demonstrates impairment of the blood brain barrier, showed clearly different images. 2) Also, in an experimental study, the $^{18}$F radioactivity in tumor tissue remained at a constant level from 30 to 120 min, whereas a notable increase in $^{18}$F activity with time was observed in nucleotides and acid-insoluble fractions. 3) The sequential value of DAR stayed relatively constant following the administration of $^{18}$FdUrd. Since the clearance of $^{18}$FdUrd in blood is fast[17], if the $^{18}$FdURd accumulation in the tumor is dependent on breakdown of the blood-brain barrier, it should have been decreased with time due to the concentration gradient between the plasma and brain tumor tissue. 4) The analysis of Patlak's plot[27] was studied and the active uptake pattern of $^{18}$FdUrd was observed in high grade tumors (unpublished data).

Recent cell kinetic studies of brain tumors using the monoclonal antibody to bromodeoxyuridine[13-15] have attracted much attention since they have provided useful information for estimating biological malignancy of tumors in the human central nervous system in situ. PET studies using $^{18}$FdUrd will give us the opportunity to investigate the in vivo cell kinetics of brain tumor patients and should open a new filed for the management of these patients from the viewpoint of nucleic acid metabolism.

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Table 1. Patients scanned (PET) before treatment

| case | age | sex | histological grade | CT enhancement | DAR tumor | DAR contralateral | ratio |
|---|---|---|---|---|---|---|---|
| 1 | 59 | M | IV | + | 0.84 | 0.17 | 4.9 |
| 2 | 33 | M | IV | + | 0.73 | 0.23 | 3.2 |
| 3 | 63 | M | III | + | 0.85 | 0.24 | 3.5 |
| 4 | 27 | F | III | + | 0.41 | 0.15 | 2.7 |
| 5 | 49 | M | III | + | 0.69 | 0.23 | 3.0 |
| 6 | 42 | M | III | + | 0.46 | 0.15 | 3.1 |
| 7 | 54 | M | III | - | 0.26 | 0.08 | 3.3 |
| 8 | 35 | M | III | + | 0.40 | 0.15 | 2.7 |
| 9 | 73 | M | III | + | 1.73 | 0.23 | 7.5 |
| 10 | 37 | F | II | - | 0.23 | 0.26 | 0.9 |
| 11 | 43 | F | II | - | 0.24 | 0.24 | 1.0 |
| 12 | 28 | M | II | - | 0.17 | 0.12 | 1.4 |

DAR: differential absorption ratio, contralateral: contralateral brain tissue, ratio: DAR ratio of tumor/contralateral brain tissue

Table 2. Patients scanned (PET) during or after treatment

| case | age | sex | histological grade | CT enhancement | DAR tumor | DAR contralateral | ratio |
|---|---|---|---|---|---|---|---|
| 13 | 27 | F | IV | + | 0.42 | 0.12 | 3.5 |
| 14 | 45 | M | IV | + | 0.83 | 0.24 | 3.5 |
| 15 | 44 | M | III | + | 0.37 | 0.15 | 2.5 |
| 16 | 35 | M | III | ± | 0.51 | 0.12 | 4.3 |
| 17 | 44 | F | III | + | 0.73 | 0.12 | 6.1 |
| 18 | 36 | M | III | - | 0.54 | 0.20 | 2.7 |
| 19 | 50 | M | III | + | 0.44 | 0.09 | 4.9 |
| 20 | 45 | M | II | - | 0.29 | 0.24 | 1.2 |
| 21 | 38 | M | II | - | 0.17 | 0.19 | 0.9 |
| 22 | 49 | M | II | - | 0.18 | 0.18 | 1.0 |

DAR: differential absorption ratio, contralateral: contralateral brain tissue, ratio: DAR ratio of tumor/contralateral brain tissue

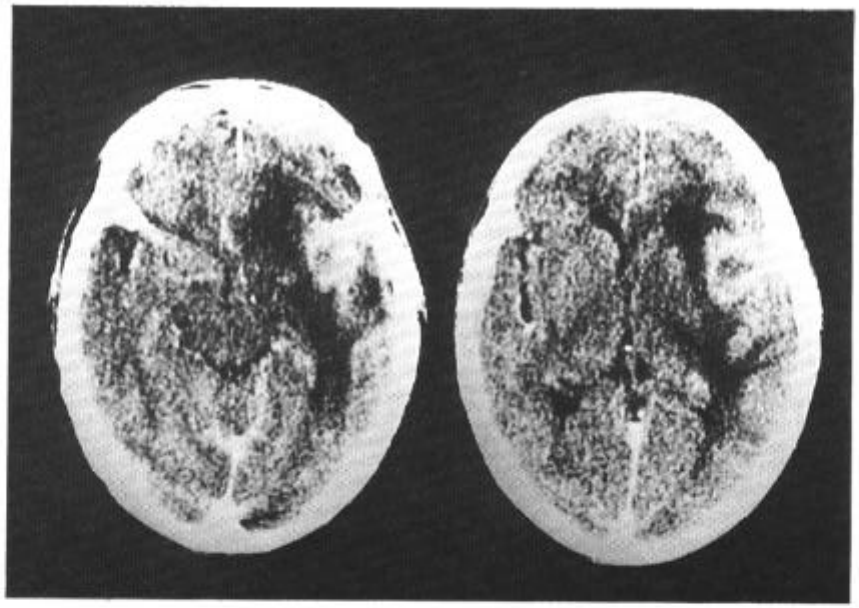

Fig.1A. A CT scan of case 3 revealed an enhanced tumor with a surrounding lowdensity area in the right fronto-temporal region .

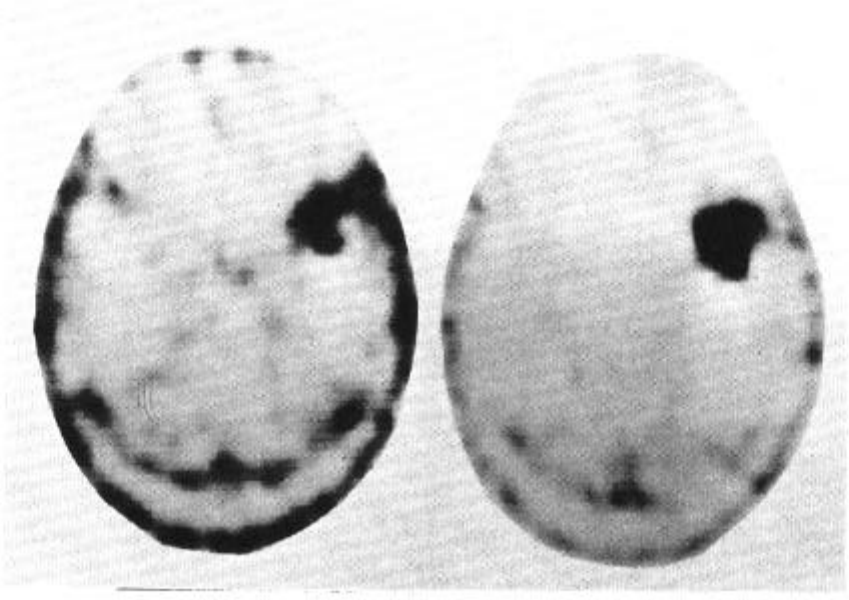

Fig. 1B. A PET scan with $^{18}$FdUrd clearly showed a lesion with high accumulation.

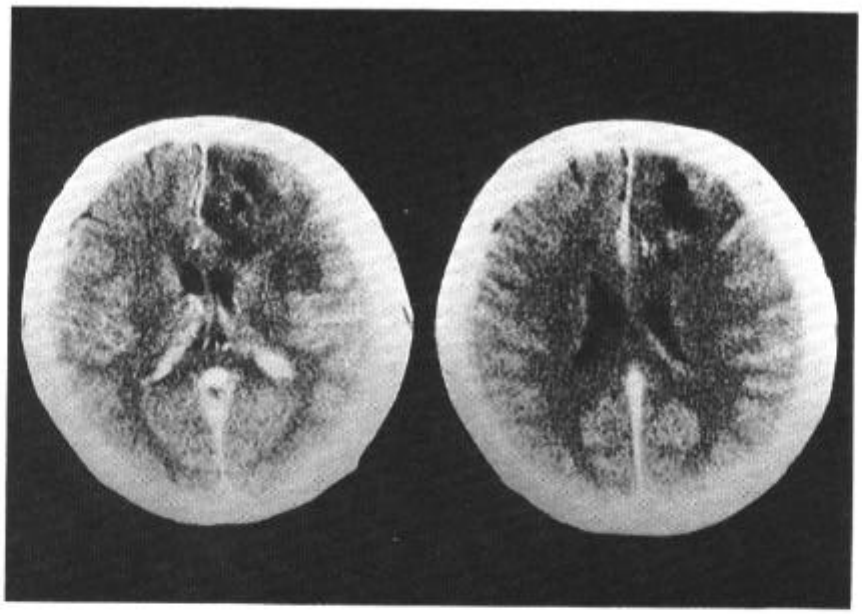

Fig. 2A. A CT scan of case 12 revealed a low density lesion in the right frontal region.

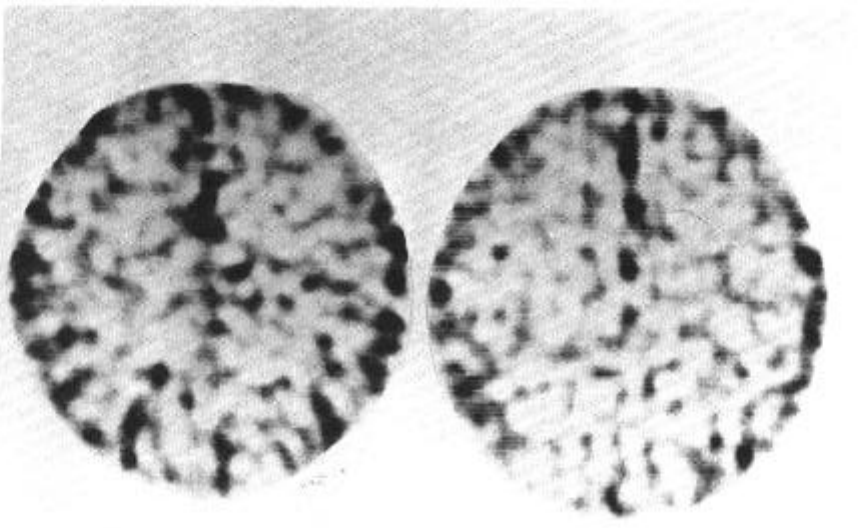

Fig. 2B. A PET scan with $^{18}$FdUrd showed no positive image.

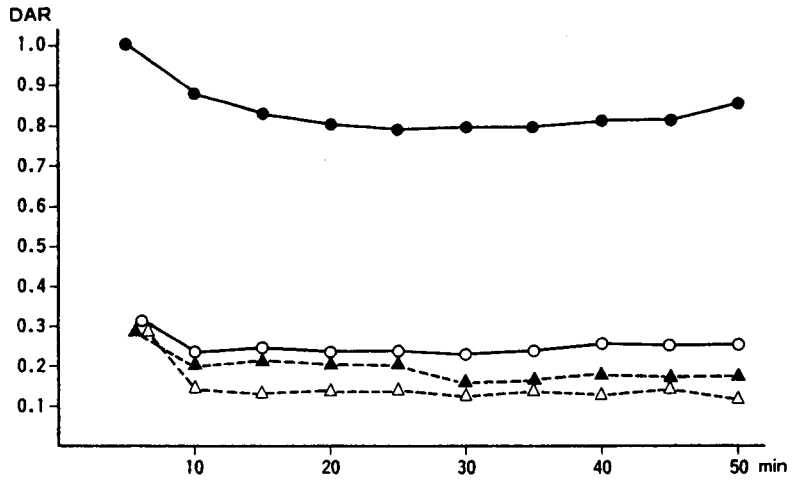


Fig. 3. Sequential changes in DAR in case 3 (closed circles: tumor, open circles: contralateral brain tissue), and case 12 (open triangle: tumor,closed triangle: contralateral brain tissue).

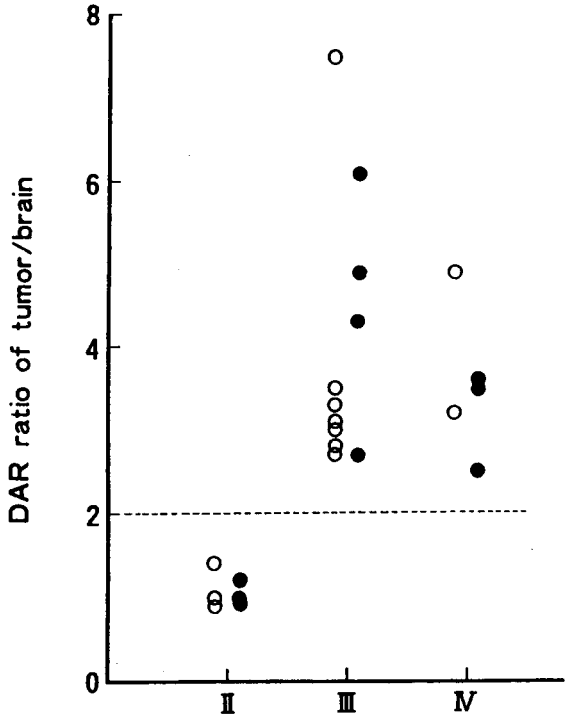


Fig. 4. DAR ratio of tumor/contralateral brain tissue. (open circle: before treatment, closed circle: during or after treatment)