Annals of Nuclear Medicine Vol. 18, No. 4, 291–296, 2004

Methionine positron emission tomography for differentiation of recurrent brain tumor and radiation necrosis after stereotactic radiosurgery —In malignant glioma—

Naohiro TSUYUGUCHI,*[1] Toshihiro TAKAMI,*[1] Ichiro SUNADA,*[2] Yoshiyasu IWAI,*[3] Kazuhiro YAMANAKA,*[3] Kiyoaki TANAKA,*[1] Misao NISHIKAWA,*[1] Kenji OHATA,*[1] Kenji TORII,*[4] Michiharu MORINO,*[1] Akimasa NISHIO*[1] and Mitsuhiro HARA*[1]

*[1]*Department of Neurosurgery, Osaka City University Graduate School of Medicine*
*[2]*Department of Neurosurgery, Saiseikai Ibaraki Hospital*
*[3]*Department of Neurosurgery, Osaka City General Hospital*
*[4]*Department of Nuclear Medicine, Osaka City University Graduate School of Medicine*

Object: Following stereotactic radiosurgery (SRS), we examined how to differentiate radiation necrosis from recurrent malignant glioma using positron emission tomography (PET) with ^{11}C-methionine (Met). ***Methods:*** Met-PET scans were obtained from 11 adult cases of recurrent malignant glioma or radiation injury, suspected on the basis of magnetic resonance images (MRI). Patients had previously been treated with SRS after primary treatment. PET images were obtained as a static scan of 10 minutes performed 20 minutes after injection of Met. We defined two visual grades (e.g., positive or negative Met accumulation). On Met-PET scans, the portion of the tumor with the highest accumulation was selected as the region of interest (ROI), tumor-versus-normal ratio (TN) was defined as the ratio of average radioisotope counts per pixel in the tumor (T), divided by average counts per pixel in normal gray matter (N). The standardized uptake value (SUV) was calculated over the same tumor ROI. Met-PET scan accuracy was evaluated by correlating findings with subsequent histological analysis (8 cases) or, in cases without surgery or biopsy, by the subsequent clinical course and MR findings (3 cases). ***Results:*** Histological examinations in 8 cases showed viable glioma cells with necrosis in 6 cases, and necrosis without viable tumor cells in 2 cases. Three other cases were considered to have radiation necrosis because they exhibited stable neurological symptoms with no sign of massive enlargement of the lesion on follow-up MR after 5 months. Mean TN was 1.31 in the radiation necrosis group (5 cases) and 1.87 in the tumor recurrence group (6 cases). Mean SUV was 1.81 in the necrosis group and 2.44 in the recurrence group. There were no statistically significant differences between the recurrence and necrosis groups in TN or SUV. Furthermore, we made a 2 × 2 factorial cross table (accumulation or no accumulation, recurrence or necrosis). From this result, the Met-PET sensitivity, specificity, and accuracy in detecting tumor recurrence were determined to be 100%, 60%, and 82% respectively. In a false positive-case, glial fibrillary acidic protein (GFAP) immunostaining showed a positive finding. ***Conclusion:*** There were no significant differences between recurrent malignant glioma and radiation necrosis following SRS in Met-PET. However, this study shows Met-PET has a sensitivity and accuracy for differentiating between recurrent glioma and necrosis, and presents important information for developing treatment strategies against post radiation reactions.

Key words: malignant glioma, recurrence, PET, methionine, stereotactic radiosurgery, radiation necrosis

Received November 14, 2003, revision accepted February 17, 2004.

For reprint contact: Naohiro Tsuyuguchi, M.D., Department of Neurosurgery, Osaka City University Graduate School of Medicine, Asahimachi 1–4–3, Abeno-ku, Osaka 545–8585, JAPAN.

E-mail: nao@med.osaka-cu.ac.jp

INTRODUCTION

After radiation treatment of brain tumor, it is difficult to differentiate between tumor recurrence and radiation necrosis using magnetic resonance imaging (MRI) and single photon emission computed tomography (SPECT).[4] However, some reports have shown that positron emission tomography (PET) using ^{11}C-methionine (Met) is effective for differentiating recurrent glioma from radiation induced changes, and can achieve early detection of recurrence.[10,11] Furthermore, we previously reported Met-PET as useful in detecting recurrent metastatic brain tumors after stereotactic radiosurgery (SRS).[13] The purpose of the present study was re-evaluate the clinical usefulness of Met-PET for differentiating recurrent malignant glioma from radiation necrosis after SRS.

Patients

This study forcused on 11 patients aged from 23 to 62 years (mean: 35.5 yrs), 8 males and 3 females, all with malignant glioma (grade III or IV). All subjects underwent operation, conventional radiotherapy (60 Gy irra-

Table 1 Case summaries of post SRS group (recurrence and necrosis)

Case	Age	Sex	Histological diagnosis (first operation)	Location	Radiation conventional + marginal dose (SRS) (Gy)	Size of lesion after SRS maximum diameter (mm)	Period (months) between SRS and PET	SUV	TN	Follow-up period (months)	Operation or biopsy	Visual inspection	Final diagnosis
1	35	M	GBM	Rt-fr	60 + 18	35	10	1.84	1.09	5	no	–	N
2	31	F	GBM	Lt-fr	60 + 15	29	3	1.06	0.69	12	no	–	N
3	25	M	AA	Lt-oc	60 + 17	32	3	1.92	1.19		yes	–	N
4	24	F	GBM	Lt-ba	60 + 14	30	19	1.68	1.52	5	no	+	N
5	33	M	GBM	Lt-te	60 + 18	45	6	2.57	2.06	5	yes	+	N
6	57	M	GBM	Lt-ce	60 + 16	29	7	2.83	1.88		yes	+	R
7	29	M	AA	Rt-fr	60 + 18	28	8	2.19	1.39		yes	+	R
8	23	M	GBM	Rt-fr, pa	60 + 17	54	5	3.46	3.03		yes	+	R
9	42	M	GBM	Rt-ba	60 + 14	38	4	1.78	1.61	death	no	+	R
10	62	M	GBM	Rt-te	60 + 17	29	5	1.95	1.84		yes	+	R
11	30	F	AA	Lt-fr	59 + 18	20	4	2.45	1.46	death	no	+	R

TN: tumor versus normal ratio, SUV: standardized uptake value, M: male, F: female, GBM: glioblastoma, AA: anaplastic astrocytoma, Rt: right, Lt: left, fr: frontal, pa: parietal, te: temporal, oc: occipital, ce: cerebellar, ba: basal ganglia, –: negative methionine uptake, +: positive methionine uptake, N: necrosis, R: recurrence

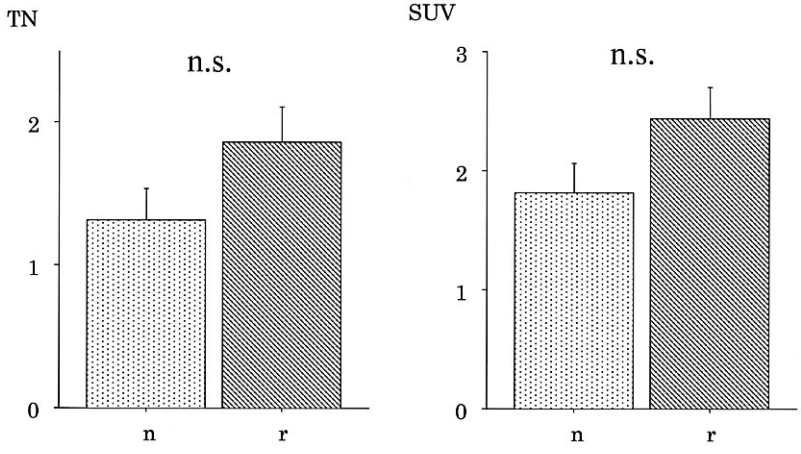

Fig. 1 Distribution of TN (*Left*) and SUV (*Right*) in the necrosis and recurrence groups. There are no significant differences in TN or SUV between the two groups. Error bar shows SEM (standard error of mean). The TN value is 1.31 ± 0.51, 0.23 (mean ± SD, SE) in radiation necrosis group (n = 5), and 1.87 ± 0.60, 0.25 (mean ± SD, SE) in tumor recurrence group (n = 6). The SUV is 1.81 ± 0.54, 0.24 (mean ± SD, SE) in radiation necrosis group (n = 5), and 2.44 ± 0.62, 0.25 (mean ± SD, SE) in tumor recurrence group (n = 6).
TN: tumor-versus-normal ratio, SUV: standardized uptake value, n.s.: not significant

diation) and chemotherapy (nimustine hydrochloride and interferon β) as initial treatment. On the basis of the MRI findings (Table 1), all 11 patients, who had undergone SRS (γ-knife or X-knife), were judged to be at high risk of tumor recurrence. The marginal dose was 14–18 Gy. All were examined using Met-PET. The mean period between SRS and PET was 6.7 months. Eight patients in the post-SRS group had a pathologic diagnosis based on biopsy or surgical resection within 3 weeks after PET. In three other patients who underwent no pathologic examination, the definitive diagnosis was based on the subsequent clinical course and MRI findings after long-term follow-up of more than 5 months. For histological studies, all sections were stained with hematoxylin-eosin (H-E). For necrosis case #5 in Table 1, sections were immunostained for glial fibrillary acidic protein (GFAP), leukocyte common antigen (LCA), and vessel endothelial growth factor (VEGF).

Eleven aged-matched, healthy volunteers (8 males, 3 females) underwent Met-PET study as controls. Their informed consent was a prerequisite for participation in the study. Females of childbearing age were not included.

Table 2 This is the 2 × 2 factorial cross table (accumulation versus diagnosis). There were two false positive cases and no false negative case. From this table, there was no significant difference between the recurrence and necrosis groups in Met accumulation. The sensitivity, specificity, and accuracy of Met-PET in detecting tumor recurrence were 100%, 60%, and 82%

Methionine	Recurrence	Necrosis
Accumulation	6	2
No Accumulation	0	3

(n = 11)

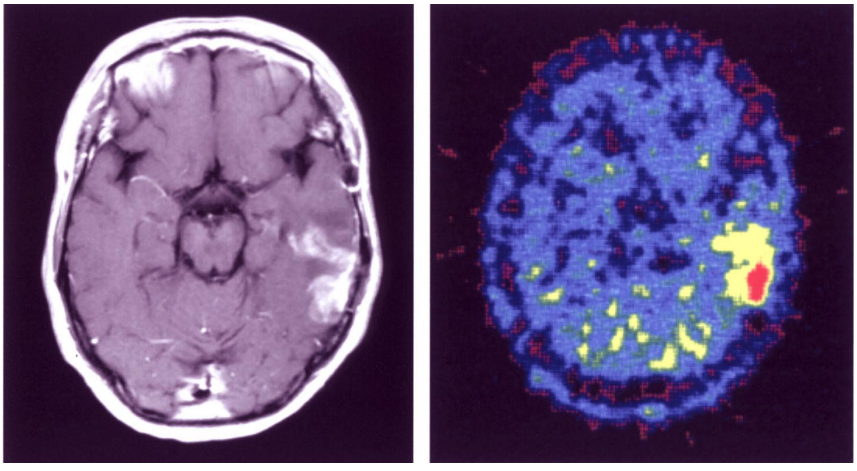

Fig. 2 Case #5: radiation necrosis. *Left:* MRI reveals an enhanced lesion in the left temporal lobe at six months after SRS. *Right:* Met-PET reveals increased uptake in the lesion and TN volume is high (2.06).

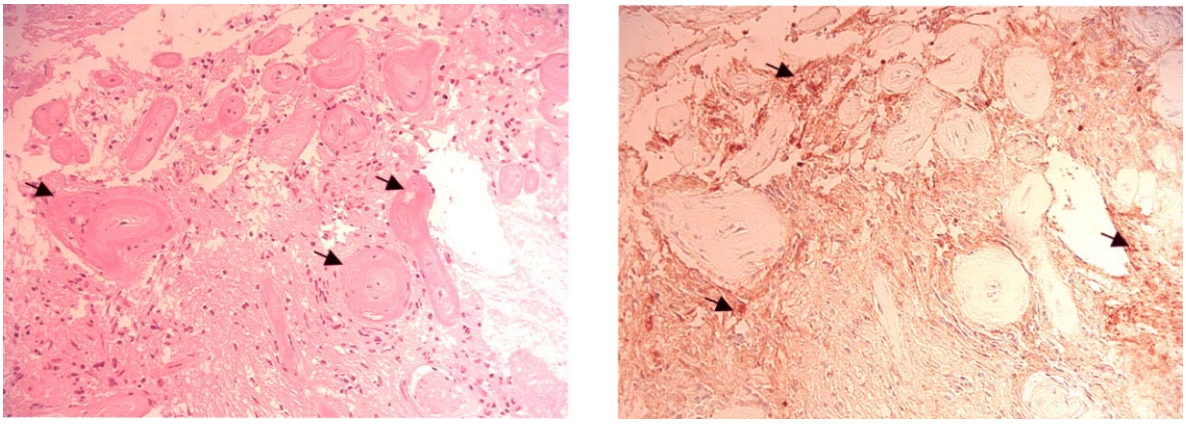

Fig. 3 Photomicrograph of a tissue section of Case #5. *Left:* Hematoxylin/Eosin (×100) The section of the tissue shows necrosis with vascular proliferation (*arrows*) and without viable tumor cells. *Right:* GFAP (glial fibrillary acidic protein) (×100) The section shows GFAP positive finding (*arrows*).

METHODS

We employed the same methods as in our previous study.[13] PET was carried out with HEADTOME IV PET scanners (Shimadzu, Kyoto, Japan) having a spatial resolution of 4.5 mm (full width at half maximum) and a slice thickness of 6.5 mm (14 slices). PET was performed parallel to the orbitomeatal line. After a transmission scan was obtained, a 10 minute static scan was performed at 20 minutes after injection of 370 MBq of Met within 30 seconds after a 4-hour fasting period. The PET images were reconstructed using measured attenuation correction. We divided two visual groups according to accumulation and no accumulation of Met in the lesion. The portion of the lesion with the highest Met accumulation was selected as the region of interest (ROI), and several circular ROIs with a diameter of 5 mm were located over the gray matter of the contralateral frontal lobe. If no abnormality could be detected, a circular ROI of the same size was located over the area corresponding to the MR abnormality. Lesion or tumor-versus-normal ratio (TN) was defined as the ratio of average radioisotope counts per pixel in the lesion or tumor (T) ROI, divided by average counts per pixel in several normal gray matter (N) ROI. The standardized uptake value (SUV: tissue activity of Met or FDG per body weight) was calculated over the same tumor ROI as follows: SUV = [(pixel count/pixel volume)/(injected radioisotope activity/body weight)] × calibration factor.

Scans were interpreted by two experienced nuclear medicine physicians.

Analysis

We compared TN (and SUV) in the tumor recurrence and necrosis groups and SUV of normal gray matter in control and patients, using the Mann-Whitney test.

We also made a 2 × 2 factorial cross table (accumulation or no accumulation, recurrence or necrosis) according to visual inspection. The p value was calculated using Fisher's exact test and Met-PET sensitivity and specificity in detecting tumor recurrence.

RESULTS

The clinical summary and PET result are shown in Table 1. Eight cases (#3, 5–11) underwent biopsy or surgical resection; 2 of these (#3 and 5) were histologically proven to have radiation necrosis without viable glioma cells. Three other cases (#1, 2, and 4) with no histological diagnosis were considered to have radiation necrosis; they had stable neurological symptoms and no massive enlargement of the lesion on MRI after more than 5 months.

Frontal lobe Met uptake in the 11 normal volunteers showed no laterality and the mean normal SUV was 1.41 ± 0.24 (mean ± SD). In the 11 tumor cases the SUV of the contralateral (non-tumor side) frontal lobe gray matter was 1.35 ± 0.30 (mean ± SD). There was no significant difference between the normal and tumor as regards frontal lobe SUV.

TN ranged from 0.69 to 2.06 (mean ± SE: 1.31 ± 0.23) in the radiation necrosis group (cases #1–5) and from 1.39 to 3.03 (mean ± SE: 1.87 ± 0.25) in the tumor recurrence group (cases #6–11). SUV ranged from 1.06 to 2.57 (mean ± SE: 1.81 ± 0.24) in the necrosis group and from 1.78 to 3.46 (mean ± SE: 2.44 ± 0.25) in the recurrence group. There was no significant difference between the recurrence and necrosis groups as regards TN or SUV (Fig. 1).

In terms of visual accumulation of Met, Met-PET did not show high accumulation in 3 cases (#1, 2 and 3) and showed visible accumulation in 8 cases (#4–11). In the 2 × 2 factorial cross table, there were two false-positive cases (#4 and 5) and no false-negative case (Table 2). This result shows that there were no significant differences between the recurrence and necrosis groups in terms of Met accumulation, with sensitivity, specificity, and accuracy of Met-PET in detecting tumor recurrence found to be 100%, 60%, and 82%, respectively.

REPRESENTATIVE CASES

Case #5 (Figures 2 and 3)

A 33-year-old male had been treated for glioblastoma of the left temporal lobe by surgery and conventional radiotherapy (total dose: 60 Gy) and chemotherapy (nimustine hydrochloride and interferon β). After these treatments, residual lesion was treated with γ-knife irradiation (marginal dose: 18 Gy). At six months after SRS, follow-up MRI revealed an enlarging enhanced lesion. Met-PET revealed increased uptake and a high TN volume (2.06). According to the visual findings, PET indicated that the lesion was recurrent tumor. The lesion was biopsied. Histological findings showed necrosis, with no viable tumor cells. LCA and VEGF showed negative findings, but GFAP showed positive finding. This suggested a false-positive case.

DISCUSSION

Met-PET provides good contrast with tumor uptake, because background uptake of amino acids in normal brain tissue is low. Met-PET has been reported as a useful technique for differentiating tumorous lesions from non-tumorous tissue,[7,9,10] for detecting recurrent or residual tumors, for distinguishing tumors from treatment-induced lesions, and for correlation with tumor's histological grade in glioma.[2] Met-PET images can identify the existence of tumor cells as a hot lesion, even in a small tumor lesion, the lesions being characterized by a marked uptake of Met. However, increased uptake of Met has occasionally been seen in brain hematoma and necrotic areas secondary to radiation therapy performed in treating brain

tumors.[3,8] In this way, Met-PET sometimes shows accumulation in non-tumorous lesions. Although the reason for this accumulation is not well known, there are several reports attributing such accumulation to enhanced metabolism of the amino acid itself, elevated permeability of amino acid due to blood-brain barrier damage, retention of amino acid by increased vascular beds, and activated membrane transport system, especially increased transport of amino acid through VEGF-induced angiogenesis.[2]

We previously evaluated the usefulness of Met-PET in terms of changes in metastatic brain tumors after SRS treatment.[13] The results showed different degrees of radiolabeled methionine accumulation between radiation necrosis and tumor recurrence; we reported Met-PET as useful in detecting recurrent tumors. In the present study, we investigated malignant glioma in the same manner as with metastatic brain tumors, but failed to find a statistically significant difference between radiation necrosis and glioma recurrence. This result may be attributable to pathological differences in post-treatment necrotic tissue between glioma and metastatic tumor. In radiation injury, the vascular changes are well characterized. There is proliferation of endothelial cells and fibroblasts and a perivascular inflammatory response characterized by the presence of lymphocytes, plasma cells, and macrophages. Furthermore, the radiation induced astrocytic and microglial responses that follow brain irradiation are indicative of reactive gliosis.[1,14] In the false-positive case (#5), we immunostained brain sections for GFAP as the marker of reactive gliosis, LCA as the marker of inflammation (reacting macrophages and histiocytes), and VEGF as a marker of vascular proliferation. We had positive finding of GFAP around the necrotic lesion, but no positive findings of LCA or VEGF in those lesions. The absence of immunostaining for LCA in our case excludes inflammatory change, and the absence of immunostaining for VEGF excludes any involvement of angiogenesis-related factor. On the other hand, the positive GFAP immunostaining demonstrated the presence of gliosis, indicating that proliferative change in glial cells is a possible mechanism of ^{11}C-Met accumulation.[6] The previous study revealed inflammatory cell infiltration in necrosis after metastatic brain tumors,[13] whereas the present study demonstrated that gliosis was more noticeable than lymphocyte infiltration. Additionally, there is a report suggesting gliosis as the mechanism of methionine accumulation in angioma[5]; gliosis is thought to involve a mechanism for inducing increased methionine metabolism and permeability.

The second item of note is the difference between the present study and the previous study of metastatic tumors, in that all subjects in the former underwent whole brain irradiation in the initial radiotherapy. Hence, methionine metabolism is possibly decreased due to radiation effect even in normal gray matter; it is thought that the TN ratio rises as the denominator decreases. In fact, comparison of the frontal lobe gray matter SUV in the normal subjects and the gray matter SUV on the intact side in the 11 glioma patients showed that the latter tended to be lower, though the difference was statistically insignificant. We used two parameters, TN and SUV, in comparing tumor recurrence and radiation necrosis in PET; however, similar results were obtained, with no statistically significant difference (Fig. 1). Although it is difficult to determine which is the appropriate parameter to evaluate accumulation rates in PET, it should be noted that SUV produces high SD even in normal gray matter, and that TN is based on the assumption of a constantly normal denominator, as described above.

Sonoda et al. reported that the ratio of tumor tissue to contralateral gray matter in Met-PET of recurrent glioma was significantly higher than that of radiation necrosis.[11] However, our results, unlike that report, showed that Met-PET was of low specificity to the extent of false-positive responses in some patients, with no significant difference between tumor recurrence and necrosis. The difference resides in the fact that Sonoda et al. reported on differentiation between recurrence and necrosis in conventional radiation for glioma, including benign cases, whereas we evaluated post-SRS changes exclusively in patients with malignant glioma of grade 3 or higher. Although it is difficult to accurately interpret the difference in the results of the two studies, one possible hypothesis is that the severity of post-treatment necrosis, inflammation, gliosis and other changes was greater severity because of high-dose irradiation administered within period in SRS than in conventional radiation.

We showed the high sensitivity and accuracy of Met-PET in detecting tumor recurrence, and no false-negative cases. Numerous reports have shown Met-PET to exhibit high sensitivity for brain tumors, which was also supported by the results of the present study. However, Szeifert et al. reported low Met accumulation of recurrent glioma after SRS.[13] In any case, if methionine accumulation is observed, subsequent treatment must be given full consideration.

CONCLUSION

Met-PET is a sensitive and accurate technique for differentiating between recurrent malignant glioma and radiation necrosis following SRS. This study noted a sensitivity, specificity, and accuracy of 100%, 60%, and 82% respectively. However, we could show there were no significant differences between the recurrence and necrosis groups in Met-PET, and so careful attention must be given to the possibility of false positivity. Furthermore, positive GFAP immunostaining demonstrated the presence of gliosis, indicating that proliferative change in glial cells is a possible mechanism of ^{11}C-Met accumulation.

This study presents information important for

developing treatment strategies against post radiation reactions. Immunostaining demonstrated the presence of gliosis, indicating that proliferative change in glial cells is a possible mechanism of ^{11}C-Met accumulation.

ACKNOWLEDGMENT

We are grateful to members of the Department of Neurosurgery and the Central Radiology Department of Osaka City University Medical School for technical support, especially the assistance of K. Hironaka, Y. Shimonishi, M. Yamazaki, and H. Tsushima in preparing our study.

REFERENCES

1. Chiang CS, McBride WH, Withers HR. Radiation-induced astrocytic and microglial responses in mouse brain. *Radiother Oncol* 1993; 29: 60–68.
2. De Witte O, Goldberg I, Wikler D, Rorive S, Damhaut P, Monclus M, et al. Positron emission tomography with injection of methionine as a prognostic factor in glioma. *J Neurosurgery* 2001; 95: 746–750.
3. Dethy S, Goldman S, Blecic S, Luxen A, Levivier M, Hildebrand J. Carbon-11-methionine and fluorine-18-FDG PET study in brain hematoma. *J Nucl Med* 1994; 35: 1162–1166.
4. Dooms GC, Hecht S, Brant-Zawadzki M, Berthiaume Y, Norman D, Newton TH. Brain radiation lesions: MR imaging. *Radiology* 1986; 58: 149–155.
5. Ichinose T, Tsuyuguchi N, Morino M, Sunada I, Ohata K, Takami T, et al. Discrepancy between [^{18}F]fluorodeoxyglucose and ^{11}C-methionine Positron Emission Tomography Findings in Sturge-Weber Syndrome—Case Report—. *Neurol Med Chir (Tokyo)* 2003; 43: 461–464.
6. Kracht LW, Friese M, Herholz K, Schroeder R, Bauer B, Jacobs A, et al. Methyl-[(^{11})C]-L-methionine uptake as measured by positron emission tomography correlates to microvessel density in patients with glioma. *Eur J Nucl Med Mol Imaging* 2003; 30: 868–873.
7. Lilja A, Lundqvist H, Olsson Y, Spannare B, Gullberg P, Langstrom B. Positron emission tomography and computed tomography in differential diagnosis between recurrent or residual glioma and treatment-induced brain lesions. *Acta Radiol* 1989; 30: 121–128.
8. Ogawa T, Hatazawa J, Inugami A, Murakami M, Fujita H, Shimosegawa E, et al. Carbon-11-methionine PET evaluation of intracerebral hematoma: distinguishing neoplastic from non-neoplastic hematoma. *J Nucl Med* 1995; 36: 2175–2179.
9. Ogawa T, Inugami A, Hatazawa J, Kanno I, Murakami M, Yasui N, et al. Clinical positron emission tomography for brain tumors: comparison of fludeoxyglucose F 18 and L-methyl-^{11}C-methionine. *Am J Neuroradiol* 1995; 17: 345–353.
10. Ogawa T, Kanno I, Shishido F, Inugami A, Higano S, Fujita H, et al. Clinical value of PET with ^{18}F-fluorodeoxyglucose and L-methyl-^{11}C-methionine for diagnosis of recurrent brain tumor and radiation injury. *Acta Radiol* 1991; 32: 197–202.
11. Sonoda Y, Kumabe T, Takahashi T, Shirane R, Yoshimoto T. Clinical usefulness of ^{11}C-MET PET and ^{201}Tl SPECT for differentiation of recurrent glioma from radiation necrosis. *Neurol Med Chir (Tokyo)* 1998; 38: 342–347.
12. Szeifert GT, Massager N, Brotchi J, Levivier M. Morphological redifferentiation in a malignant astrocytic tumor after gamma knife radiosurgery. *J Neurosurg* 2002; 97: 627–630.
13. Tsuyuguchi N, Sunada I, Iwai Y, Yamanaka K, Tanaka K, Takami T, et al. Methionine positron emission tomography of recurrent metastatic brain tumor and radiation necrosis after stereotactic radiosurgery: is a differential diagnosis possible? *J Neurosurg* 2003; 98: 1056–1064.
14. Yang T, Wu SL, Liang JC, Rao ZR, Ju G. Time-dependent astroglial changes after gamma knife radiosurgery in the rat forebrain. *Neurosurgery* 2000; 47: 407–415.