Effect of Expression of P-glycoprotein on Technetium-99m Methoxyisobutylisonitrile Single Photon Emission Computed Tomography of Brain Tumors

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Abstract

The expression of P-glycoprotein was investigated immunohistochemically in 26 brain tumor tissues and compared with the findings of technetium-99m methoxyisobutylisonitrile single photon emission computed tomography (99mTc-MIBI SPECT) to clarify the effect of P-glycoprotein on the diagnostic accuracy. P-glycoprotein labeling index of both tumor cells and vascular endothelial cells showed no clear relationship with the findings of 99mTc-MIBI SPECT imaging. Expression of P-glycoprotein has no effect on the diagnostic accuracy of 99mTc-MIBI SPECT.

Key words: brain tumor, P-glycoprotein, single photon emission computed tomography, technetium-99m methoxyisobutylisonitrile

Introduction

P-glycoprotein is a membrane protein that excretes some anti-cancer agents from tumor cells into the extracellular space. The multi-drug resistance 1 gene that encodes P-glycoprotein is overexpressed in many types of tumor cells, so an excess of P-glycoprotein is produced in these tumor cells.9,12) Technetium-99m methoxyisobutylisonitrile (99mTc-MIBI) may be excreted from tumor cells by P-glycoprotein.1,3,16) An in vitro study demonstrated that 99mTc-MIBI was accumulated less in strains of fibroblasts positive for P-glycoprotein than in strains negative for P-glycoprotein.3,16) Therefore, clinical cases of malignant glioma not demonstrated by 99mTc-MIBI single photon emission computed tomography (SPECT) were probably secreting P-glycoprotein.1) SPECT with 99mTc-MIBI is useful for assessing the malignancy of brain tumor.2,3,15) SPECT with 99mTc-MIBI provides clearer images and delineation of the tumor than SPECT with thallium-201 chloride. However, the influence of the expression of P-glycoprotein on 99mTc-MIBI SPECT has not been fully established.

The present study examined the expression of P-glycoprotein in 26 brain tumor specimens for comparison with the findings of 99mTc-MIBI SPECT to clarify the effect of P-glycoprotein on tumor imaging using 99mTc-MIBI SPECT.

Materials and Methods

I. Patient population

Thirty-four patients underwent 99mTc-MIBI SPECT before surgical removal of brain tumor. Immunohistochemical examination of paraffin-embedded materials was performed in 22 patients, nine males and 13 females aged 22 to 80 years (mean 48.4 years). Four patients also underwent 99mTc-MIBI SPECT at recurrence, so a total of 26 SPECT images and 26 surgical specimens were evaluated. The histological diagnosis was astrocytoma grade 2 in seven cases, mixed oligoastrocytoma in three, dysembryoplastic neuroepithelial tumor in one, astrocytoma grade 3 (anaplastic astrocytoma) in six, glioblastoma in six, metastasis from lung adenocarcinoma in one, and radiation necrosis in two. There were 14 initial cases and 12 recurrent cases. Nine patients were given chemotherapy before 99mTc-MIBI SPECT. Four patients were treated with in-
travenous or intraarterial infusion of nimustine hydrochloride (ACNU) at a dose of 2 mg/kg body weight. Another four patients were treated with our own PAV chemotherapy regimen (procarbazine hydrochloride, 100 mg/m² orally for 14 consecutive days; ACNU, 2 mg/kg intravenously on day 1; and vincristine, 1.4 mg/m² intravenously on days 1 and 8), which is a modification of the PCV chemotherapy regimen.7) Two patients were treated with the PE chemotherapy regimen (cisplatin and etoposide) based on the published protocol.6) II. 99mTc-MIBI SPECT

99mTc-MIBI SPECT was performed a few days before the surgical removal of the tumor. Early and delayed SPECT images were obtained immediately and 3 hours after the intravenous injection of 370 MBq of 99mTc-MIBI solution. SPECT data were acquired for 30 minutes using a Headtome set 050 (Shimadzu, Kyoto). SPECT images were reconstructed with Ramp and Butterworth filtering, and the absorption was corrected using reported methods.11) Regions of interest (ROIs) were selected in the tumor area and contralateral normal white matter on the SPECT images. MIBI indexes were calculated from the ratio of tumor ROI to contralateral normal ROI.

III. Histological examination

Surgical specimens were embedded and fixed in 10% formaldehyde solution. Histological diagnoses were made from the prepared specimens stained with hematoxylin and eosin. The paraffin-embedded tissues were dewaxed in xylene and rehydrated by passing through a 100–70% ethanol gradient for 3 minutes. After quenching, specimens were incubated with the monoclonal antibody to P-glycoprotein CLA51/1 (Medac Diagnostika, Hamburg, Germany) overnight at 40°C. This antibody is known to show stable staining with paraffin-embedded materials.19) The specimens were developed by immunoperoxidase sandwich staining using a LSAB kit (DAKO, Carpinteria, Calif., U.S.A.). The P-glycoprotein labeling index of tumor cells was calculated as the mean percentage of positive tumor cells in three view fields and more than 1000 cells were counted. The P-glycoprotein labeling index of the vascular endothelial cells was calculated in the same manner. Cutoff points were determined based on the median expression of P-glycoprotein among all specimens examined.

Results

I. P-glycoprotein labeling and MIBI indexes of tumor cells

The P-glycoprotein labeling index of tumor cells ranged from 0% to 10.2%, with a mean value of 3.48% and a median value of 2.1%. Tumors with a labeling index of more than 2% were defined as PG positive. There were 14 PG positive and 12 PG negative cases (Table 1). No differences between the PG positive and negative groups were found in age, sex, or diagnosis, except for the cases of radiation necrosis in the PG positive group.

Among the 14 initial cases, five were PG positive and nine were PG negative. Among the 12 recurrent cases, nine were PG positive and three were PG negative. PG positive cases were significantly more common among recurrent cases (Chi-square test, p < 0.001). The labeling index of recurrent cases (mean 5.37 ± 3.70) was significantly greater than that of initial cases (mean 1.86 ± 2.19) (Student’s t-test, p < 0.01).

All cases except for those of radiation necrosis

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Table 1  P-glycoprotein labeling index of tumor cells

<table>
<thead>
<tr>
<th></th>
<th>PG negative group (n = 12)</th>
<th>PG positive group (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-glycoprotein labeling index*</td>
<td>0–1.7 (0.62)</td>
<td>2.1–10.2 (5.93)</td>
</tr>
<tr>
<td>Age* (yrs)</td>
<td>22–80 (47.2)</td>
<td>22–75 (49.5)</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>4:8</td>
<td>5:9</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dysembryoplastic neuroepithelial tumor</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>astrocytoma</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>mixed oligoastrocytoma</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>anaplastic astrocytoma</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>glioblastoma</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>metastasis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>radiation necrosis</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*Range (mean). PG positive group: tumors with a P-glycoprotein labeling index of more than 2%.
were divided into low and high malignancy groups, based on the clinical and histological diagnoses. The low malignancy group included astrocytoma or mixed oligoastrocytoma, and the high malignancy group included anaplastic astrocytoma, glioblastoma, and metastasis. The early and delayed MIBI indexes of the low and high malignancy groups in the PG positive and negative groups are compared in Table 2. The high malignancy group showed significantly higher early and delayed MIBI indexes than the low malignancy group in the PG negative group (Student’s t-test, p < 0.05). The high malignancy group tended to show higher MIBI indexes than the low malignancy group in the PG positive group, but the difference was not statistically significant. The early and delayed MIBI indexes of PG positive group were higher than those of the PG negative group, but only the low malignancy group showed a statistically significant difference in delayed MIBI index (Student’s t-test, p < 0.05). The analyses were repeated with the subgroups of initial cases, and the same results were obtained. Statistical analysis of the MIBI index in the subgroups of recurrent cases was not possible due to the distribution of the cases.

No high malignancy tumor showed negative 99mTc-MIBI uptake but positive PG expression. Two cases which showed negative 99mTc-MIBI uptake and positive PG expression were low malignancy tumors.

99mTc-MIBI SPECT was performed twice, at initial diagnosis and at recurrence, in four cases. There was no histological transformation at recurrence except for one case of anaplastic astrocytoma at initial diagnosis that became radiation necrosis at recurrence. Two cases were treated with radiation and chemotherapy with ACNU or PAV regimen at initial MIBI examination. All four cases received radiation and chemotherapy with ACNU or PAV regimen at recurrence. All four cases showed an increase in P-glycoprotein labeling index and MIBI indexes at recurrence.

II. P-glycoprotein labeling and MIBI indexes of vascular endothelial cells

The P-glycoprotein labeling index of vascular endothelial cells ranged from 0% to 53.8%, with a mean value of 14.5% and a median value of 4.4%. Cases with a labeling index for vascular endothelial cells of more than 4% were defined as PG positive. The PG positive group included 13 cases and the PG negative group included 13 (Table 3). No differences were observed between the PG positive and negative groups with regard to age, sex, or diagnosis, except for the cases of radiation necrosis in the PG negative group.

Among the 14 initial cases, six were PG positive and eight were PG negative. Among the 12 recurrent cases, seven were PG positive and five were PG negative. The mean labeling index was 11.9 ± 17.0 for initial cases and 15.0 ± 19.7 for recurrent cases, with no significant difference.

The early and delayed MIBI indexes of the low and high malignancy groups in the PG positive and negative groups are compared in Table 4. No differences were statistically significant. The analyses were repeated with the subgroups of initial and recurrent cases, and the same results were obtained. 99mTc-MIBI SPECT was performed twice in four cases. Three cases showed an increase in P-glycoprotein labeling index at recurrence, and one case of radiation necrosis showed decrease.

III. Correlation of P-glycoprotein labeling and MIBI indexes

The correlations between P-glycoprotein labeling indexes and MIBI indexes were analyzed. Early
Table 3  P-glycoprotein labeling index of vascular endothelial cells

<table>
<thead>
<tr>
<th></th>
<th>PG negative group (n = 13)</th>
<th>PG positive group (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-glycoprotein labeling index*</td>
<td>0–3.5 (0.27)</td>
<td>5.2–53.8 (28.7)</td>
</tr>
<tr>
<td>Age* (yrs)</td>
<td>22–80 (50.8)</td>
<td>22–62 (46.0)</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>6:7</td>
<td>4:9</td>
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<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dysembryoplastic neuroepithelial tumor</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>astrocytoma</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>mixed oligoastrocytoma</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>anaplastic astrocytoma</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>glioblastoma</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>metastasis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>radiation necrosis</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Range (mean). PG positive group: cases with a P-glycoprotein labeling index for vascular endothelial cells of more than 4%.

Table 4 Comparison of 99mTc-MIBI indexes of vascular endothelial cells in the PG positive and negative groups

<table>
<thead>
<tr>
<th></th>
<th>99mTc-MIBI index</th>
<th>P-glycoprotein labeling index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Delayed</td>
</tr>
<tr>
<td>Low malignancy group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG positive group</td>
<td>2.62 ± 1.94</td>
<td>4.69 ± 4.28</td>
</tr>
<tr>
<td>PG negative group</td>
<td>1.26 ± 0.12</td>
<td>0.98 ± 0.20</td>
</tr>
<tr>
<td>High malignancy group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG positive group</td>
<td>6.09 ± 6.41</td>
<td>6.13 ± 4.87</td>
</tr>
<tr>
<td>PG negative group</td>
<td>6.35 ± 4.80</td>
<td>16.82 ± 22.76</td>
</tr>
<tr>
<td>Radiation necrosis</td>
<td>6.38</td>
<td>3.60</td>
</tr>
</tbody>
</table>

MIBI indexes showed a weak positive correlation (r = 0.45), but delayed MIBI indexes showed almost no correlation with P-glycoprotein labeling index of tumor cells (r = 0.20). Early and delayed MIBI indexes showed no correlation with P-glycoprotein labeling index of vascular endothelial cells (r = -0.29 and -0.21). No clear negative correlation between MIBI indexes and P-glycoprotein labeling index was recognized in our study. No correlation was found between the P-glycoprotein labeling indexes for tumor cells and for vascular endothelial cells (r = 0.28).

Discussion

The present study found a higher P-glycoprotein labeling index in recurrent tumors. The high malignancy group showed higher early and delayed MIBI indexes than the low malignancy group, but no such tendency was found with the P-glycoprotein labeling index. In our series, no case of false negative 99mTc-MIBI SPECT finding due to high expression of P-glycoprotein was observed. Our results indicate that the expression of P-glycoprotein has no obvious effect on the diagnostic accuracy of 99mTc-MIBI SPECT.

Functional imaging of P-glycoprotein is possible using 99mTc-MIBI SPECT both in vitro and in vivo. Under controlled conditions such as the same tumor viability or blood supply, the total amount of P-glycoprotein may be directly related to the findings of 99mTc-MIBI SPECT imaging, but many factors influence the findings in a clinical setting. The amount of P-glycoprotein is only one of these factors, whereas the most important may be tumor viability or the malignant potential of the tumor cells. The amount of 99mTc-MIBI taken up into tumor cells is proportional to the function of calci-
um-dependent sodium potassium adenosine triphosphatase on the cell membrane of the tumor cells. However, we could not detect any influence of P-glycoprotein on 99mTc-MIBI SPECT.

The CLA51/1 monoclonal antibody of P-glycoprotein is known to show stable staining of paraffin-embedded materials. There are several commercially available monoclonal antibodies for P-glycoprotein. Some monoclonal antibodies do not cause stable staining of paraffin-embedded materials. Many investigations of P-glycoprotein expression in brain tumors were conducted using such unstable monoclonal antibodies and fresh frozen tumor samples. P-glycoprotein labeling indexes using fresh frozen tumor samples varied from 0–20%, and agree with the P-glycoprotein labeling indexes in our study. There may be subtypes of P-glycoproteins recognized by different monoclonal antibodies. Details of the structure and the function of P-glycoprotein detected by various monoclonal antibodies should be further investigated.

Blood vessels in normal specimens or benign glioma specimens are generally reported to stain positively for P-glycoprotein. However, P-glycoprotein expression in the vascular endothelial cells of anaplastic primary brain tumors is heterogeneous or absent. In our study, the vascular endothelial cells in some cases in the low malignancy group did not express P-glycoprotein, whereas some cases in the high malignancy group showed high labeling indexes for P-glycoprotein. Our results indicate that P-glycoprotein labeling index of endothelial cells is not directly related to the malignancy of the tumor. Also, the 99mTc-MIBI index had no relationship with the P-glycoprotein labeling index of endothelial cells.

Only some tumor cells are positive for P-glycoprotein. Examination of histological specimens cannot clarify the relationship between P-glycoprotein expression and the clinical functions of P-glycoprotein on blood vessels or tumor cells. The capillary endothelium acts as the blood-brain barrier in normal brain, but does not in malignant glioma, although both express P-glycoprotein. The relationship between expression of P-glycoprotein by the capillary endothelium or tumor cells and the function of P-glycoprotein remains unclear. Consequently, the clinical significance of P-glycoprotein expression is also unknown. However, expression of P-glycoprotein in tumor cells is directly related to the uptake of 99mTc-MIBI in vitro and in vivo. Therefore, the P-glycoproteins in blood vessels have little effect on the uptake of 99mTc-MIBI.

The P-glycoprotein labeling indexes of both tumor cells and vascular endothelial cells showed no clear relationship with 99mTc-MIBI uptake. The presence of P-glycoprotein in tumor tissue had little influence on 99mTc-MIBI SPECT imaging. Therefore, the diagnostic accuracy of 99mTc-MIBI SPECT is not affected by expression of P-glycoprotein.

Acknowledgments

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11) Hirose Y, Ikeda Y, Higashi Y, Koga K, Hattori H,

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Commentary

The authors examined the effect of P-glycoprotein on tumor imaging using ⁹⁹mTc-MIBI SPECT. They evaluated 26 ⁹⁹mTc-MIBI SPECT images and surgical specimens. The heterogeneous group of tumors consisted of astrocytoma grade 2, mixed oligoastrocytoma, dysembryoplastic neuroepithelial tumor, astrocytoma grade 3, glioblastoma, metastasis from lung adenocarcinoma and radiation necrosis. Fourteen cases were treated initially, 12 recurrently. Nine of the 26 patients had chemotherapy before ⁹⁹mTc-MIBI SPECT. P-glycoprotein was detected histologically using the monoclonal antibody CLA51/1. ⁹⁹mTc-MIBI is a substrate that accumulates passively in malignant brain tumors, P-glycoprotein is a 170 kDa transmembrane glycoprotein that acts as an efflux pump of the blood brain barrier and is also expressed in different levels in human brain tumors. ¹) P-glycoprotein is associated with multi-drug resistance and therefore of interest in determining the efficacy of chemotherapy in brain tumors. ²) The efficacy of chemotherapy has a great variability in individuals, sometimes with severe side effects, and is expensive. Therefore, methods that determine the efficacy of the therapy for the individual will be of great interest. The authors found that the presence of P-glycoprotein in tumor tissue had little influence on ⁹⁹mTc-MIBI imaging. Therefore, they concluded that the diagnostic accuracy of ⁹⁹mTc-MIBI SPECT in detecting malignant brain tumors was not affected by expression of P-glycoprotein. This publication is a worthwhile contribution in the still ongoing debate for the relevance of ⁹⁹mTc-MIBI SPECT in predicting chemoresistance of malignant brain tumors associated with P-glycoprotein. ³)

References


Rainer RITZ, M.D., Dirk FREUDENSTEIN, M.D., and Ernst H. GROTE, M.D.

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The authors have studied the expression of P-glycoprotein in 26 brain tumor samples and compared the findings of $^{99m}$Tc-MIBI SPECT to the expression of P-glycoprotein. They found that there was no clear relationship between P-glycoprotein expression and $^{99m}$Tc-MIBI SPECT imaging. This is an important study because it relates a carefully studied patient population to an immunohistochemical marker. It has previously been suggested that overexpression of P-glycoprotein in malignant gliomas is associated with poor imaging of the tumor by $^{99m}$Tc-MIBI SPECT. Numerous in vitro studies would support this statement. However, this study clearly illustrates the difficulty we have going from in vitro to in vivo studies. In the in vivo situation, many factors can influence the imaging of a tumor by SPECT including the vascular endothelium, the degree of necrosis within the tumor, and various membrane channel dependent mechanisms. I congratulate the authors on bringing this important observation forward.

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Since MIBI labeling index was found to be a strong indicator of tumor proliferation and malignancy, efforts have been made to utilize MIBI in predicting tumor malignancy before treatment. SPECT with $^{99m}$Tc-MIBI is one of such technologies developed and is considered promising. However, there are several questions remaining. One of them is delivery and uptake of intravenously administered $^{99m}$Tc-MIBI in the tumor cells. If adequate $^{99m}$Tc-MIBI is not accumulated in the tumor cells, SPECT does not assess the proliferation of tumors. One of the important factors known to control the intracellular uptake of drugs is the P-glycoprotein level in tumor cells, which is subject to change depending on tumor malignancy. In this study, Shibata et al. investigated if P-glycoprotein levels in the tumor cells have any influence on $^{99m}$Tc-MIBI SPECT. They measured P-glycoprotein levels in glioma cells from 26 patients and did not find any effect of the levels on the findings of SPECT with $^{99m}$Tc-MIBI. This is an important study that answers one of the questions in the clinical setting of $^{99m}$Tc-MIBI SPECT and supports its usefulness in predicting the malignancy of gliomas preoperatively. If the actual MIBI labeling indices of tumor cells had been measured and taken in this analysis, more precise data may have been obtained.

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