Pharmacokinetics and Tumor Localization of \( {^{131}}\text{I}-\text{Labeled Anti-Tenascin Monoclonal Antibody 81C6} \) in Patients with Gliomas and Other Intracranial Malignancies

Michael R. Zalutsky, Robin P. Moseley, Hugh B. Coakham, R. Edward Coleman, and Darell D. Bigner

Departments of Radiology [M. R. Z., R. E. C.], Pathology [M. R. Z., D. D. B.], and the Preuss Laboratory for Brain Tumor Research [D. D. B.], Duke University Medical Center, Durham, North Carolina 27710; Brain Tumor Research Laboratory, Department of Neurosurgery, Frenchay Hospital, Bristol, England [R. P. M., H. B. C.]

ABSTRACT

We previously have reported that radiodinated anti-tenascin monoclonal antibody 81C6 exhibits therapeutic potential against both s.c. and intracranial human glioma xenografts in athymic mice and rats. Herein we report the selective tumor localization of \( {^{131}}\text{I}-\text{labeled 81C6} \) in patients with gliomas and other intracranial malignancies. Nine patients were simultaneously administered 5–50 mg of \( {^{131}}\text{I}-\text{labeled 81C6} \) and 1–2 mg of \( {^{131}}\text{I}-\text{labeled} \) 45.6, an isotype-matched control monoclonal antibody. The blood clearance half-time for 81C6, normalized to that of 45.6 in the same patient, appeared to decrease with 81C6 protein dose. Gamma camera images obtained at 1 to 3 days exhibited increased uptake of 81C6 in regions corresponding to tumor with varying degrees of contrast to surrounding normal brain. Biopsy specimens of tumor and normal brain were obtained and analyzed histologically for tumor content. The average uptake of 81C6 in tumor ranged from 0.6 to 4.3 \( \times 10^{-3}\% \) of the injected activity corresponding to regions of glioma as determined by computed tomography (7–11).

One MAb of interest for radioimmunodiagnosis and therapy is 81C6, an \( \text{IgG}_{2b} \) immunoglobulin which reacts with an epitope of the extracellular matrix antigen tenasin, found in human glioma cell lines, glioma xenografts in athymic rodents, primary human gliomas, but not in normal adult or fetal brain (12). The potential utility of radioiodinated 81C6 MAb for the diagnosis and treatment of gliomas has been documented in several preclinical studies. Paired-label studies in intracranial and s.c. human glioma xenograft models have shown that tumor uptake of radioiodinated 81C6 is specific and permits the gamma camera imaging of intracranial xenografts as small as 20 mg (13, 14). In the intracranial model, paired-injection protocols were used to demonstrate that intracarotid administration increased tumor delivery of radioiodinated 81C6 by 20% over i.v.-injected MAb (15). With regard to its therapeutic potential, treatment with \( {^{131}}\text{I}-\text{labeled} \) 81C6 has resulted in significant tumor growth delay and regression (16) in athymic mice bearing s.c. D-54 MG human glioma xenografts and in prolongation of median survival for athymic rats bearing intracranial tumors (17). Lack of response after treatment with \( {^{131}}\text{I}-\text{labeled} \) control MAb demonstrated the specificity of these therapeutic effects.

The pharmacokinetics of \( {^{131}}\text{I}-\text{labeled} \) MAb antibody 81C6 have been evaluated in patients with gliomas and other intracranial malignancies. Using paired-label protocols (18), we have demonstrated that uptake of radioiodinated 81C6 in intracranial tumors is significantly higher than coadministered isotype-matched control MAb. In addition, although the magnitude of tumor uptake of radioactivity is low, it does occur at levels comparable to those reported for other MAbs in extracranial lesions.

INTRODUCTION

The management of patients with gliomas and other intracranial malignancies is an extremely frustrating problem for the clinician. Current approaches to the treatment of these tumors are largely ineffective. Even multimodality regimens including surgery, external beam radiotherapy, and chemotherapy have extended the median survival of patients with malignant glioma by only a few weeks (1). While a multiplicity of factors contribute to the lack of effective therapeutic measures for primary and metastatic brain tumors, a central limiting factor is the nonspecific nature of these therapies which results in dose-limiting toxicity to normal brain tissue.

The potential for exploiting antibodies to deliver diagnostic and therapeutic radionuclides selectively to brain tumors was first explored by Day and coworkers (2) using polyclonal rabbit antisera. More recent studies have demonstrated that radiolabeled MAbs directed against human glioma-associated antigens can localize specifically in s.c. and intracranial human glioma xenografts in animals (3–6). The feasibility of utilizing radioiodinated MAbs for selectively delineating and treating gliomas has also been investigated in patients. Brain scans obtained after administration of \( {^{125}}\text{I}-\text{labeled} \) F(ab); fragments and \( {^{131}}\text{I}-\text{labeled} \) intact antibodies have shown increased levels of radioactivity corresponding to regions of glioma as determined by computed tomography (7–11).

One MAb of interest for radioimmunodiagnosis and therapy is 81C6, an \( \text{IgG}_{2b} \) immunoglobulin which reacts with an epitope of the extracellular matrix antigen tenasin, found in human glioma cell lines, glioma xenografts in athymic rodents, primary human gliomas, but not in normal adult or fetal brain (12). The potential utility of radioiodinated 81C6 MAb for the diagnosis and treatment of gliomas has been documented in several preclinical studies. Paired-label studies in intracranial and s.c. human glioma xenograft models have shown that tumor uptake of radioiodinated 81C6 is specific and permits the gamma camera imaging of intracranial xenografts as small as 20 mg (13, 14). In the intracranial model, paired-injection protocols were used to demonstrate that intracarotid administration increased tumor delivery of radioiodinated 81C6 by 20% over i.v.-injected MAb (15). With regard to its therapeutic potential, treatment with \( {^{131}}\text{I}-\text{labeled} \) 81C6 has resulted in significant tumor growth delay and regression (16) in athymic mice bearing s.c. D-54 MG human glioma xenografts and in prolongation of median survival for athymic rats bearing intracranial tumors (17). Lack of response after treatment with \( {^{131}}\text{I}-\text{labeled} \) control MAb demonstrated the specificity of these therapeutic effects.

The pharmacokinetics of \( {^{131}}\text{I}-\text{labeled} \) MAb antibody 81C6 have been evaluated in patients with gliomas and other intracerebral malignancies. Using paired-label protocols (18), we have demonstrated that uptake of radioiodinated 81C6 in intracerebral tumors is significantly higher than coadministered isotype-matched control MAb. In addition, although the magnitude of tumor uptake of radioactivity is low, it does occur at levels comparable to those reported for other MAbs in extracranial lesions.

MATERIALS AND METHODS

Monoclonal Antibodies. Monoclonal antibody 81C6 and a control antibody of the same \( \text{IgG}_{2b} \) isotype, 45.6 (produced by the myeloma cell line 45.6TG1.7), were harvested from the ascitic fluid of athymic mice carrying intraperitoneal 81C6 or 45.6 hybridoma. MAbs were purified from hybridoma culture supernatant using Protein A-Sepharose 4B. Immunohistological analyses have shown that MAb 45.6 does not display specific antigen-mediated binding to normal human tissues (12). Purified MAbs were dialyzed against 0.05 M phosphate buffered saline (pH 7.4), passed through a 0.22-µm filter (Millipore) and stored at 4°C until used. The purity of each batch of MAb was examined by high-pressure liquid chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The MAbs were proven free of bacterial and viral contamination by culture in routine medium and murine antibody production testing in accordance with the guidelines suggested by the Food and Drug Administration.

Radioiodination of MAbs. MAbs 81C6 and 45.6 were labeled with \( {^{125}}\text{I} \) (Amersham) and \( {^{131}}\text{I} \) (Amersham), respectively, using the stationary-phase chloramide, Iodogen (Pierce Chemical Co.) (19). Radioiodinated MAbs were
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separated from free 131I and 125I by passage through a gas-sterilized, 10-
ml Sephadex G-25 column. The binding characteristics of radiiodi-
nated 81C6 and 45.6 were analyzed in an immunoreactivity assay
performed at antigen excess. Between 50 and 100 ng of each labeled
MAb was incubated overnight in triplicate with 300 mg of both D-54
MG human glioma tumor homogenate and normal rat liver homoge-
nate in 1 ml 0.15 M phosphate buffer containing 1% bovine serum
albumin. The amount of 81C6 bound to rat liver after washing was
considered to represent nonspecific binding and was subtracted from
the percentage 81C6 bound to tumor in order to calculate the specific
binding of 131I-labeled 81C6. Specific binding also could be calculated
by subtracting the percentage 45.6 bound to tumor from the percentage
81C6 bound to the same homogenate. For all the preparations of 131I-
labeled 81C6 and 125I-labeled 45.6 used in these studies, greater than
97% of the radioligand activity was precipitable in trichloroacetic acid.
The absence of aggregates in the labeled MAb was demonstrated by
chromatography over a 1.6 x 60 cm Sephacryl S300 gel (Pharmacia),
which showed a single peak with a retention time corresponding to that
of intact IgG.

Patients. Permission for this study was obtained from the Ethical
Committee of Frenchay Hospital. Each patient, along with a close
relative, gave informed written consent. Nine patients with clinically
presumed, malignant glioma were entered into this study. As shown in
Table 1, the histological diagnoses, based on the evaluation of tumor
samples obtained at craniotomy, revealed that six patients had glo-
blasta multiforme, and one each had an oligodendroglioma, an
anaplastic astrocytoma, and a metastatic adenocarcinoma (but sus-
pected initially of having an anaplastic astrocytoma). All patients were
scheduled for treatment by surgical resection of tumor. Clinical evalu-


tion included computerized tomography (CT) scan, performed with
a single peak with a retention time corresponding to that of intact IgG.

Analysis of Tissue Samples. As part of their normal course of therapy,
all patients underwent surgical resection of their tumors 29 to 77 ha-
fter injection of 131I-labeled 81C6 and 125I-labeled 45.6 MAbs (Table
3). Between four and 10 samples considered to be tumor at opera-
tion were analyzed from each patient. Separate tissue samples were sent for
pathological evaluation for use in patient management. In addition, samples of temporalis muscle and normal brain were obtained whenever
permitted by the operative approach (muscle, Patients 1, 2, 4–8; normal
brain, Patients 1, 2, 4–9). All tissue samples were stored, placed in
sterile, phosphate buffered saline prior to

RESULTS

Radiolabeled MAbs. The specific activities of the 131I-labeled
81C6 preparations ranged between 0.06 and 0.4 µCi/µg. For
125I-labeled 45.6, the specific activities were between 0.2 and
1.8 µCi/µg. In all cases, the trichloroacetic acid precipitability
of the administered 131I and 125I activity was greater than 97%.
No evidence for aggregate formation was found in the Sephra-
cryl S300 chromatographic analyses of the labeled MAb used
in these studies. The immunoreactivity of the 131I-labeled 81C6
preparations was determined in vitro by measuring differential
binding to antigen-positive D-54 MG human glioma tumor and
antigen-negative normal rat liver homogenates. From the data
presented in Table 1, one can calculate that the specific binding
values ranged between 54 and 71%. When specific binding was
calculated as the difference between the percentage 81C6 and

Table 1 Patient studies with 131I-Labeled 81C6

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Histological diagnosis</th>
<th>Dose 81C6 (µg)</th>
<th>D-54 MG tumor (%)</th>
<th>Rat liver (%)</th>
<th>Specific (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>GBM*</td>
<td>5</td>
<td>67</td>
<td>13</td>
<td>54</td>
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<td>GBM</td>
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<td>54</td>
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<td>14</td>
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<td>4</td>
<td>GBM</td>
<td>20</td>
<td>76</td>
<td>5</td>
<td>71</td>
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<tr>
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<td>GBM</td>
<td>20</td>
<td>76</td>
<td>5</td>
<td>71</td>
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<tr>
<td>6</td>
<td>MA</td>
<td>20</td>
<td>75</td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>7</td>
<td>GBM</td>
<td>50</td>
<td>75</td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>8</td>
<td>Oligo</td>
<td>50</td>
<td>72</td>
<td>2</td>
<td>70</td>
</tr>
<tr>
<td>9</td>
<td>AA</td>
<td>50</td>
<td>72</td>
<td>2</td>
<td>70</td>
</tr>
</tbody>
</table>

* GBM, glioblastoma multiforme; MA, metastatic adenocarcinoma; Oligo, oligodendroglioma; AA, anaplastic astrocytoma.
nonspecific control MAb 45.6 binding to D-54 MG tumor, nearly identical specific binding percentages were obtained (data not shown).

Pharmacokinetics of 131I-labeled 81C6 and 125I-labeled 45.6. Nine patients received from 5 to 50 mg (1.8–3.5 mCi 131I) of 81C6 and 1.0–1.7 mg (0.2–1.3 mCi 125I) of isotype-matched control MAb 45.6 (Tables 1 and 2). Blood and urine samples were obtained serially over the next 7 days. Half-times for the disappearance of 131I and 125I activity from the blood pool are summarized in Table 2. Since Patient 6 died at 27 h after MAb administration, insufficient data were available for determination of blood clearance half-times. The clearance of 81C6 was better fit by least squares analysis to a biexponential equation; in contrast, the clearance of 45.6 was better fit to a monoexponential equation.

For 81C6, the mean first component half-time ($t_1$) ranged from 2.4 h at 5 mg to as short as 0.1 ± 0.05 h at the 50-mg dose. Although there appeared to be an inverse relationship between 81C6 protein dose and first component half-time, this correlation was significant ($r = -0.89$) only when Patient 7 was not included. The second component half-time ranged from about 35 h at 5 mg to as low as 22.4 ± 0.6 h at 50 mg. An inverse relationship between 81C6 protein dose and second component half-time again was apparent ($r = -0.81$) only when Patient 7 was excluded.

With the exception of Patient 7, the half-time for clearance of 45.6 control MAb ranged between 33.4 ± 0.8 and 46.0 ± 1.0 h with no correlation with either 45.6 or 81C6 protein dose observed. The clearance half-time for 45.6 in Patient 7 was 62.8 ± 1.9 h. If the second component half-time for 81C6 is normalized to the 45.6 blood clearance half-time, there is an excellent inverse correlation ($r = -0.93$) between this ratio and 81C6 protein dose administered (Fig. 1), even if the data from Patient 7 are excluded.

The cumulative urinary excretion of radioiodine following the injection of 131I-labeled 81C6 and 125I-labeled 45.6 is illustrated in Fig. 2. No significant differences between 81C6- and 45.6-associated radioiodine levels in the urine were observed at any time. When the data from all nine patients were combined, 18.3 ± 6.0% and 19.3 ± 3.6%, respectively, of the 131I and 125I were found in the urine 24 h after injection. Similarly, 37.2 ± 8.4% of the 131I and 38.7 ± 4.7% of the 125I administered were found in the urine at 48 h. The standard deviation for the variable protein dose 81C6 also was higher than that for the constant protein dose 45.6. On Days 3 through 7, suggesting the possibility that the rate of urinary excretion may be dose dependent. However, the correlation coefficients for the relationship between 81C6 dose and cumulative urinary excretion were less than 0.5 at all time points.

In addition, the protein-associated fraction of radioiodine activity in the blood and the urine was determined for both nuclides by TCA precipitability. In the blood, the TCA precipitable activity for both nuclides decreased from initial values of 97–99% to 93–96% at 4 to 7 days. No significant differences between 81C6- and 45.6-associated radioiodine TCA values were observed at any time point, nor were any dose-related effects seen. The fraction of both 131I and 125I in the urine that was TCA precipitable was less than 5% at all time points.

Table 2 Blood clearance of 131I and 137I activity in patients injected with 131I-labeled 81C6 and 137I-labeled 45.6

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Dose (mg)</th>
<th>131I-labeled 81C6</th>
<th>137I-labeled 45.6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First component</td>
<td>Second component</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T_1$ (h)</td>
<td>$T_2$ (h)</td>
<td>$T_1$ (h)</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>2.3 ± 0.3</td>
<td>34.7 ± 1.0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>2.5 ± 0.3</td>
<td>36.0 ± 0.9</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>1.4 ± 0.3</td>
<td>34.9 ± 2.1</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0.7 ± 0.2</td>
<td>28.6 ± 1.6</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>1.1 ± 0.2</td>
<td>24.8 ± 1.4</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>2.5 ± 0.8</td>
<td>36.1 ± 2.6</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>0.5 ± 0.2</td>
<td>22.4 ± 0.6</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>0.1 ± 0.05</td>
<td>27.6 ± 0.9</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>0.1 ± 0.05</td>
<td>27.6 ± 0.9</td>
</tr>
</tbody>
</table>

Fig. 1. Relationship between 81C6 protein dose and the ratio of the second component blood clearance half-time of 81C6 to the blood clearance half-time for 45.6 in each patient.

Fig. 2. Cumulative urinary excretion of 131I and 125I during the week following injection of 131I-labeled 81C6 and 125I-labeled 45.6 MAbs.

Phosphoryl S300 gel permeation chromatography was performed on at least one sample from each patient obtained between 12 and 36 h after MAb administration. In all cases, greater than 90% of both the 131I and the 125I activity eluted with the anticipated retention time for unmodified IgG immunoglobulin. The remainder of the activity eluted as a low molecular weight species and was presumed to reflect free iodide.

Immunoreactivity in Serum. These studies were performed on
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Monoclonal antibody 81C6 was selected for pharmacokinetic evaluation in humans because of the wealth of preclinical information which suggests that this MAb may be useful for delivering radionuclides to gliomas and other neoplasms that express tenascin (3, 14, 16, 17). In these studies, both $^{131}I$-labeled 81C6 and $^{125}I$-labeled 45.6 control MAb were administered intracarotidally because a delivery advantage of approximately 20%, relative to i.v. injection, was observed in paired-injection studies in the D-54 MG glioma xenograft model (15). However, after studying the first seven patients, it was determined by paired-injection analysis, that intracarotid administration did not offer a delivery advantage for 81C6 in the human (20). Since there is a small, but finite risk associated with carotid angiography, the last two patients received MAbs via i.v. injection.

In this report, we have demonstrated that $^{131}I$-labeled 81C6 was accumulated in brain tumors in concentrations sufficient to permit external imaging. Thus, disruption of the blood-brain barrier was confirmed beginning 10 min after MAb administration with the last set of images obtained about 2–4 h prior to surgery. At 1 h, prominent blood pool activity and hepatic accumulation were seen. Activity in the liver, and in seven patients the spleen, was also observed in the later images. Uptake of $^{131}I$ in the liver and the spleen was more pronounced, relative to blood pool, in patients injected with lower protein doses (5–10 mg) of 81C6. Some evidence of bone marrow accumulation was observed on scans obtained after 24 h.

[99mTc]Glucoheptonate scanning performed prior to MAb administration revealed blood-brain barrier impairment in all nine patients. Known sites of tumor could not be identified on scans obtained earlier than 24 h after $^{131}I$-labeled 81C6 MAb injection. In Patients 3, 4, 7, and 8, tumor could be identified on later images. Selective localization of $^{131}I$-labeled 81C6 in tumor was more apparent in Patients 1, 2, 5, 6, and 9. Anterior and lateral planar images of Patient 5, which were obtained 24 h after injection of a 20-mg dose of $^{131}I$-labeled 81C6, are shown in Fig. 4. In these scans, accumulation of $^{131}I$ in the region corresponding to tumor is clearly demonstrated.

Imaging. The nine patients were scanned at various intervals beginning 10 min after MAb administration with the last set of images obtained about 2–4 h prior to surgery. At 1 h, prominent blood pool activity and hepatic accumulation were seen. Activity in the liver, and in seven patients the spleen, was also observed in the later images. Uptake of $^{131}I$ in the liver and the spleen was more pronounced, relative to blood pool, in patients injected with lower protein doses (5–10 mg) of 81C6. Some evidence of bone marrow accumulation was observed on scans obtained after 24 h.

Fig. 3. Immunoreactivity of $^{131}I$ activity in sera of patients numbered 4 (○), 5 (□), and 6 (△) injected with 20 mg of $^{131}I$-labeled 81C6. Immunoreactivity defined as the percentage binding to D-54 MG human glioma homogenates in vitro of $^{131}I$, derived from 81C6, minus the percentage binding of $^{125}I$, derived from 45.6 control MAb.
of the brain obtained 24 h after injection of a 20 mg (3.4 mCi) dose of $^{131}$I-labeled 81C6. Patient 5, glioblastoma multiforme.

Table 3 Tumor uptake and tumor to normal brain ratios of $^{131}$I-labeled 81C6 determined from patient biopsy samples

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Dose to surgery (mg)</th>
<th>Tumor uptake (% ID/g)</th>
<th>Tumor:Normal brain ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>$8.1 \times 10^{-4}$</td>
<td>3.5 1.6-6.0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>$1.3 \times 10^{-3}$</td>
<td>2.6 1.1-8.6</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>$7.5 \times 10^{-4}$</td>
<td>4.3 2.4-6.6</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>$6.8 \times 10^{-4}$</td>
<td>4.3 2.4-6.6</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>$4.1 \times 10^{-4}$</td>
<td>76 26-93</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>$4.3 \times 10^{-3}$</td>
<td>56 10-205</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>$1.2 \times 10^{-3}$</td>
<td>14 11-17</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>$1.0 \times 10^{-3}$</td>
<td>15 6-26</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>$6.4 \times 10^{-4}$</td>
<td>2.3 1.0-3.6</td>
</tr>
</tbody>
</table>

* Composition of biopsy samples not determined by pathology.

Fig. 4. Lateral and anterior planar images of the brain obtained 24 h after injection of a 20 mg (3.4 mCi) dose of $^{131}$I-labeled 81C6. Patient 5, glioblastoma multiforme.

Table 4 Localization indices in tumor biopsy samples from patients injected with $^{131}$I-labeled 81C6 and $^{45}$I-labeled 45.6

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Dose 81C6 (mg)</th>
<th>Muscle</th>
<th>Normal brain</th>
<th>Tumor range (average)</th>
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<tr>
<td>1#</td>
<td>5</td>
<td>0.99</td>
<td>1.3-1.9 (1.5)</td>
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<tr>
<td>2#</td>
<td>5</td>
<td>1.25</td>
<td>1.1-2.4 (1.8)</td>
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</tr>
<tr>
<td>3#</td>
<td>10</td>
<td>-</td>
<td>1.2-1.4 (1.3)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>1.09</td>
<td>1.7-3.4 (2.3)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.95</td>
<td>3.4-5.0 (3.7)</td>
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</tr>
<tr>
<td>6</td>
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<td>1.23</td>
<td>1.3-5.0 (3.2)</td>
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<td>7</td>
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<td>9</td>
<td>50</td>
<td>-</td>
<td>1.3-4.0 (2.3)</td>
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# Localization index =

$81C6$ in tissue/$81C6$ in blood

45.6 in tissue/45.6 in blood

* Composition of biopsy samples not determined by pathology.

Tissue samples not available.

Table 5 Effect of correcting for histologically determined biopsy composition on tumor uptake and tumor to normal brain ratios

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tumor uptake</th>
<th>Tumor:Normal brain ratio</th>
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<tbody>
<tr>
<td></td>
<td>% ID/g</td>
<td>Raw</td>
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<tr>
<td>Normal brain</td>
<td>3.0 x 10^{-4}</td>
<td>7.6 x 10^{-3}</td>
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<tr>
<td>Normal brain</td>
<td>2.7 x 10^{-4}</td>
<td>5.5 x 10^{-3}</td>
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<tr>
<td>Tumor</td>
<td>3.0 x 10^{-4}</td>
<td>7.6 x 10^{-3}</td>
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<tr>
<td>Tumor</td>
<td>3.0 x 10^{-4}</td>
<td>7.6 x 10^{-3}</td>
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<td>3.0 x 10^{-4}</td>
<td>7.6 x 10^{-3}</td>
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</table>

barrier was sufficient to allow access of labeled immunoglobulin to intracerebral tumors. The contrast between tumor and surrounding normal brain seen on the scans increased with time, becoming optimal 24–48 h after MAb injection. In general, better tumor images were obtained in those patients with higher $^{131}$I uptake levels as determined by gamma counting of their tumor biopsy specimens.

The amount of $^{131}$I localizing in brain tumors after $^{131}$I-labeled 81C6 injection was quite low, with average values ranging between 1 and $4 \times 10^{-3}$ % per gram. The magnitude of 81C6 uptake in cerebral tumors was similar to that observed by Richardson et al. (10) following the injection of MAb UJ13A labeled with $^{131}$I. While it is possible that an incompletely compromised blood-brain barrier might contribute to the low level of MAb uptake in brain tumors, it is important to point out that these values are comparable to those observed in tumor biopsies following i.v. MAb injection in patients with melanoma, breast, ovarian, and colorectal carcinoma (22–28).

One of the principal limitations of external beam radiotherapy for the treatment of primary brain tumors is radiation-induced toxicity to adjacent normal brain. When the biopsy data from the eight patients from which a specimen of adjacent normal brain are pooled, the average tumor:brain ratio for $^{131}$I-labeled 81C6 was greater than 5:1. If only those specimens for which fractional tumor content was determined histologically are considered (Patients 4–9), then the average tumor:normal brain ratio for 81C6 increases to 25:1. MAb protein dose may be a pertinent factor since the average tumor:brain ratio for biopsies at 20 mg 81C6 was 38:1, in comparison to 9:1 for samples from patients receiving 50 mg of 81C6 MAb.

The tumor:normal brain uptake ratios seen in biopsies for 81C6 IgG are quite favorable when compared to those found in the literature for other MAb and fragments. After injection of radioiodinated UJ13A IgG into six patients with primary brain tumors, tumor:normal brain ratios at 3 to 16 days ranged from 3 to 13:1 in surgically resected tissues (10). Using the lower molecular weight F(ab')2 fragment of Mel-14, Behnke et al. (7) observed tumor:brain ratios of 2 to 18:1 in four patients with gliomas.

Since nonspecific uptake of proteins in brain tumors is well known (29), it is essential to demonstrate that radiolabeled MAb accumulation in cerebral malignancies is related to specific processes. To address this concern, all patients receiving $^{131}$I-labeled 81C6 also were injected with an $^{125}$I-labeled, isotype-matched control MAb (45.6) so that specific and nonspecific immunoglobulin uptake in brain tumors could be differentiated. Unlike the results of a previous study in glioma patients in
which no difference between specific UJ13A and nonspecific HMFG2 uptake in tumor was seen (11), we have observed that the localization indices for 81C6 uptake in cerebral malignancies were as high as 5. For the patients whose biopsy composition was determined histopathologically, the average localization index for tumor was 2.83, compared to 1.07 and 1.11 for normal muscle and brain, respectively. These results indicate that the uptake of radioiodinated 81C6 in brain tumors is specific.

The uptake of 81C6 MAb in tumor specimens from individual patients was quite variable, ranging from a 15-fold difference for Patient 6 to a 50% difference for Patient 7. Patient 6, who had the greatest tumor uptake, was not known to have a metastatic adenocarcinoma until after histological evaluation of the biopsy. 81C6 reacts with tenascin, which is expressed in many tumor types, including fibrosarcoma, carcinoma of the breast and Wilms tumor (12); however, quantitation of differences in tenascin expression among tumor types or among different tumors of the same type has not yet been performed. Imaging of extracranial lesions could not be evaluated in Patient 6 since no evidence of primary or other sites of metastases was seen at autopsy. Studies to evaluate imaging of other tumor types with radiolabeled 81C6 are under consideration.

Heterogeneities in vascular permeability and antigenic expression may contribute to differences in 81C6 accumulation in a particular tumor. Since uptake of 45.6 MAb reflects nonspecific processes, regional variations in brain tumor uptake are related to heterogeneities in tumor blood flow and vascular permeability. In each patient’s biopsy specimens, the variation in 45.6 uptake ranged from 25 to 500%; and, except for Patient 4, about a threefold lower variation in tumor accumulation of 45.6 compared to 81C6 was seen. These differences, in concert with the large variation in localization indices observed for 81C6 in most patients, suggest that regional variation in antigenic expression is an important factor influencing the heterogeneity of 81C6 accumulation in brain tumors. The importance of regional variations in antigen distribution in affecting 81C6 uptake has also been demonstrated in D-54 MG human glioma xenograft models using quantitative autoradiography (30). These studies suggest that it may be necessary to use mixtures of MAbs reactive with different glioma-associated antigens in order to obtain maximal and uniform uptake of radiolabeled MAbs in gliomas.

The influence of protein dose on MAb pharmacokinetics is not clear, partly because of intrinsic differences in the labeled MAb systems that have been investigated. In xenograft animal model studies, there is disagreement as to the effect of MAb dose on the magnitude of tumor uptake and tumor-to-normal-tissue ratios (31–37). Most clinical studies investigating the effect of MAb protein dose have been performed with 111In-labeled MAbs (38–42). Lesion detectability appears to increase with increasing protein dose of ZME-018, 9.2.27, and 96.5 anti-melanoma MAbs (38–40, 42). However, higher protein doses of MAb also result in prolonged retention of 111In activity in the blood pool with some MAbs (38, 39, 42), but not for others (39, 41). Since the catabolism of 111In- and 131I-labeled MAbs is different, particularly with regard to the prolonged retention of 111In-labeled catabolites (43), the relevance of these studies to the current investigation is questionable. In at least one study with an 131I-labeled MAb (B72.3), immunoglobulin dose did not influence MAb pharmacokinetics and lesion detectability (23).

Although it is not possible to make a definitive statement concerning the effect of 81C6 dose on tumor uptake, it appears that expedited blood clearance is associated with higher protein doses of 81C6. This behavior is in marked contrast to that reported in the literature, particularly for 111In-labeled MAbs. One possible explanation is that, at higher doses of 81C6, the rate of catabolism is increased. In contrast to 111In-labeled MAb, radiiodinated MAb degradation products are excreted relatively rapidly via the urine and, thus, increased catabolism could lead to more rapid clearance. However, no dose-dependent effects were observed for the urinary excretion of 81C6 and, at all time points, the concentration of both 81C6 and 45.6 derived radioiodine in the urine was quite similar. It is worth noting that the cumulative urinary excretion observed for both 81C6 and 45.6 was about twice as high as that observed previously for 131I-labeled B6.2 MAb (44). Since the tenasin antigen, which 81C6 recognizes, also is present in normal liver and spleen red pulp sinusoids (12), antigen-mediated binding to these tissues could also influence the pharmacokinetics of 81C6 associated radiolabel.

In summary, we have demonstrated that gliomas and an intracerebral metastatic adenocarcinoma can be detected in patients by gamma camera imaging after injection of 131I-labeled 81C6. The magnitude of brain tumor uptake was small but comparable to that reported in the literature for other types of tumors. Analysis of biopsy specimens from patients injected with 20–50 mg of 81C6 revealed an average tumor-to-normal-brain ratio of about 25:1. By coadministering an 125I-labeled control MAb, we were able to determine that the localization indices in tumor were as high as 5. In contrast, the localization indices in normal brain and muscle were approximately one, indicating that uptake of 81C6 in these cerebral malignancies was specific.

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GLIOMA LOCALIZATION OF MONOCLONAL ANTIBODY IN PATIENTS

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