Specific Localization of Thallium 201 in Human High-Grade Astrocytoma by Microautoradiography

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ABSTRACT

The ability to accurately distinguish remaining or recurrent high-grade astrocytoma from necrosis or edema following treatment is essential to optimal patient management. Thallium 201 planar γ-camera imaging has been shown to be helpful in detecting recurrent high-grade astrocytoma; however, due to tissue heterogeneity adjacent to and within tumor, the cellular specificity and quantitation of 201TI uptake are largely unknown. In order to determine which tissues are responsible for the radioisotope uptake, microautoradiographic techniques were used to examine multiple tissue sections from five patients with high-grade astrocytoma. Each patient received 5 mCi of 201TI i.v. 1 h prior to tumor removal. Additionally, all patients received computerized tomographic and 201TI planar γ-camera scans prior to surgery. Following surgery, the excised tissue specimens were tentatively classified by gross pathological examination and then immediately processed for dry mount autoradiography; grain density was determined over regions containing tumor, adjacent and uninvolved brain tissue, necrotic tissue, and background. Highly significant differences were found in grain densities (201TI uptake) between tumor and uninvolved brain tissue, as well as between uninvolved brain tissue and necrotic tissue; there was no significant difference between background grain density and that in necrotic tissue. Mean grain densities (grains/cm² ± 1 SD) across patients were: tumor, 102 ± 23; adjacent, uninvolved brain tissue, 29 ± 11; necrotic tissue, 6.2 ± 1.1; and background, 7.0 ± 4.1. We conclude that the ability of 201TI to selectively image high-grade astrocytoma is due to its preferential uptake into tumor cells.

INTRODUCTION

High-grade astrocytoma (WHO Grade III anaplastic astrocytoma and WHO Grade IV glioblastoma multiforme) is a highly malignant tumor for which palliative treatment includes surgical debulking, chemotherapy, or radiation (1-5). Because complete eradication of this tumor is not usually possible, posttherapy imaging which is sufficiently sensitive and specific to detect the presence of remaining, or recurrent, tumor is essential to optimal patient management (1-5).

CT1 scanning and standard 99mTc radionuclide imaging following treatment of high-grade astrocytoma usually demonstrate pathological contrast enhancement or increased 99mTc uptake, respectively, regardless of the absence or presence of viable recurrent tumor (6, 7). These findings are due to necrosis and associated disruption of the blood-brain barrier at the site of the lesion; therefore, use of these methods to detect residual or recurrent viable tumor may yield ambiguous results (6, 7). Recent work using positron emission tomography has shown promise in differentiating tumor from necrosis (8-10), but this imaging modality is not widely available.

Recent investigations using the radioisotope 201TI, however, have shown promise in imaging these tumors because uptake of the radionuclide has demonstrated excellent correlation with the location of viable tumor (11). Furthermore, 201TI is routinely available in regional nuclear medicine facilities. Planar γ-camera imaging of the distribution of 201TI is useful in quantifying residual or recurrent tumor and has been shown to have greater sensitivity for detecting tumor recurrence compared with magnetic resonance imaging or CT (12, 13). The uptake of 201TI into high-grade astrocytoma is generally ascribed to its action as a potassium analogue, and the increased cellular uptake into tumor is thought to reflect increased potassium transport due to enhanced Na+/K+-ATPase activity characteristic of tumor cell membranes (11, 14, 15). Necrosis and breakdown of the blood-brain barrier adjacent to the tumor, however, make determination of the specific cellular uptake of 201TI ambiguous at the gross tissue level (11, 13). Therefore, we undertook a study using microautoradiographic techniques that provide resolution at the cellular level in order to determine which tissues—tumor; adjacent, uninvolved brain; or necrotic brain—were responsible for the uptake of 201TI.

MATERIALS AND METHODS

Five patients with residual or recurrent high-grade astrocytoma were inducted into this study. Planar γ-camera scintigraphy with 201TI was performed on each patient within 1 wk prior to surgical debulking of the tumor; each patient received 5 mCi of 201TI i.v. 1 h before image acquisition. The imaging protocol has been previously described in detail (13). In addition, 1 h prior to tumor removal, 5 mCi of 201TI were injected i.v. to assure that the relative tissue uptakes would correspond to those of the previously obtained scintigraphic results (13).

The excised tissue specimens were tentatively classified by gross pathological examination and then immediately placed on cryostat slides frozen in dry ice to preserve cellular integrity. The pedicles were subsequently mounted in a cryostat at −25°C, and the specimens were sectioned at 10 μm. Alternate sections were dried-mounted onto slides precoated with Kodak NTB-3 emulsion or mounted on gelatin-coated slides and fixed in paraformaldehyde (4% in 0.1 M phosphate buffer, pH 7.4). The fixed sections were stained with hematoxylin-eosin for positive identification of cellular histology. The autoradiographic sections were exposed in the dark for 3 to 12 days at −90°C; the low temperature reduced the effects of negative chemography and preserved tissue cellular integrity (16, 17). After development of the autoradiographs in Kodak D-19 developer and fixation in paraformaldehyde, grain density on tissue sections from each patient which best contained tumor, normal, and necrotic cellularity was determined by counting individual silver grains using dark-field microscopy on Days 7 to 9 of emulsion exposure. Grain densities were determined separately over regions containing tumor; adjacent, uninvolved brain tissue of normal cellularity by histological examination; necrotic tissue; and background. Results are expressed as grains/cm². Histology was determined by a board-certified neuropathologist (P. E. M.).

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2 To whom requests for reprints should be addressed, at Division of Nuclear Medicine, University of Michigan Medical Center, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0028.
3 The abbreviation used is: CT, computed tomography.

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RESULTS

Table 1 is a summary of the history of the 5 patients examined in this study. A comparison between preoperative planar $^{201}$Tl and CT images in a 77-yr-old female (E. C.) with high-grade astrocytoma is illustrated in Fig. 1, A and B. These images show concordance between tumor visualized by CT (A) and the enhanced uptake of $^{201}$Tl seen on planar $\gamma$-camera imaging (B). Fig. 2, A and B shows the results of repeat CT and $^{201}$Tl scans, respectively, performed 5 days postoperatively in the same patient; whereas there has been a significant reduction of $^{201}$Tl uptake after surgical removal of essentially all viable tumor, the persistence of pathological contrast enhancement and abnormality on the CT scan demonstrates the relative inability of CT to differentiate between the presence or absence of viable tumor. Photomicrographs of two serial tissue sections from this patient viewed in bright- and dark-field optics (Fig. 3, A and B, respectively) show tumor invading normal tissue. The dark-field image demonstrates increased grain density over the tumor compared with that of the adjacent brain tissue.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Tumor location</th>
<th>Clinical history</th>
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<tbody>
<tr>
<td>E. H.</td>
<td>70/M</td>
<td>Left frontal</td>
<td>Radiation therapy 2 wk prior to debulking and tissue sampling</td>
</tr>
<tr>
<td>L. V.</td>
<td>22/F</td>
<td>Left parietal</td>
<td>Partial tumor resection 6 mo prior to debulking and tissue sampling</td>
</tr>
<tr>
<td>M. K.</td>
<td>54/F</td>
<td>Right frontal</td>
<td>No prior resections. Radiation therapy 1 yr prior to debulking and tissue sampling</td>
</tr>
<tr>
<td>E. C.</td>
<td>77/F</td>
<td>Right frontal</td>
<td>Tumor biopsy 9 days prior to subtotal tumor debulking</td>
</tr>
<tr>
<td>P. C.</td>
<td>37/M</td>
<td>Left frontal</td>
<td>Prior biopsy 2½ yr ago, followed by radiation therapy. Recurrent symptoms 1 mo prior to debulking and tissue sampling</td>
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Values for grain densities over tumor; adjacent, uninvolved brain tissue; necrotic tissue; and background for each of the five patients are provided in Table 2. The mean values (grains/cm$^2$) ± 1 SD for each are: tumor, 102 ± 23; adjacent, near-normal brain tissue, 29 ± 11; necrotic tissue, 6.2 ± 1.1; and background, 7.0 ± 4.1. Paired $t$ tests revealed highly significant differences in count densities between tumor and uninvolved brain tissue [$t = 9.7$, $df = 3$, $P$ (one-sided) = 0.001] and between uninvolved and necrotic brain tissue [$t = 4.3$, $df = 3$, $P$ (one-sided) = 0.01]. There was no significant difference between the count densities of necrotic brain tissue and the background [$t = -0.55$, $df = 4$, $P$ (one-sided) = 0.31].

DISCUSSION

The results of this study show that the ability of $^{201}$Tl to selectively image high-grade astrocytoma is due to its preferential uptake into tumor cells: microautoradiographic analysis following in vivo cellular uptake of $^{201}$Tl revealed a 3.5-fold greater concentration of this radioisotope in the tumor cells compared with that in uninvolved brain tissue and no significant difference in uptake between necrotic tissue and background. This preferential tracer-uptake may reflect the high level of Na$^+$/K$^+$-ATPase activity in the cell membranes of this neoplasm.

The relative inability of CT or magnetic resonance imaging to differentiate between the presence or absence of viable tumor severely limits their utility as diagnostic tools in the management of high-grade astrocytoma. Previous studies have shown that planar $\gamma$-camera imaging of patients with high-grade astrocytoma can accurately detect tumor recurrence (11, 13) and that the uptake of $^{201}$Tl is probably tumor specific since its distribution does not characteristically include regions of blood-brain barrier breakdown (11). The results of our microautoradiography experiment lend further support to the sensitivity and specificity of $^{201}$Tl for the cells of high-grade astrocytoma; the preferential uptake of this radiotracer by these cells makes
Fig. 2. This pair of photographs compares the CT image (A) and the planar, vertex $^{201}$TI (B) in the same patient shown in Fig. 1, but following surgical debulking of the tumor. Note the lack of concordance between the CT and $^{201}$TI images. The CT image continues to show pathological contrast enhancement in the region of surgical excision due to its relative inability to differentiate viable tumor from necrosis; the $^{201}$TI image, however, shows no abnormal tracer activity, reflecting the relative absence of residual tumor mass following surgery.

Fig. 3. A, bright-field microscopic section demonstrating tumor (T, lower left) infiltrating normal brain tissue (N, upper right). B, dark-field microscopic section of the same region shown in A, demonstrating increased grain density over tumor (T, lower left) compared with that over normal brain tissue (N, upper right).

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<th>Table 2 Autoradiographic results</th>
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<td>Patient</td>
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* Average ± SD.

this agent a useful in vivo marker for the presence of residual or recurrent tumor cells.

REFERENCES

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