Prognostic implications of the proliferative potential of low-grade astrocytomas


Department of Neurological Surgery, Brain Tumor Research Center, and Department of Pathology, School of Medicine, University of California, San Francisco, California

The proliferative potential of low-grade astrocytomas was estimated in 47 patients. Each patient received an intravenous infusion of bromodeoxyuridine (BUDR), 150 to 200 mg/sq m, at the time of craniotomy to label cells in deoxyribonucleic acid (DNA) synthesis; the percentage of S-phase cells, or BUDR labeling index (LI), of each tumor was determined immunohistochemically. In 29 patients (60%), the tumors had BUDR LI's of less than 1%, indicating a slow growth rate; only three (10%) of these patients died of recurrent tumor during a follow-up period of up to 38 years. In contrast, of the 18 patients (40%) whose tumors had BUDR LI's of 1% or more, 12 (67%) had a recurrence and nine died during the same follow-up period. These results show that the proliferative potential, as reflected by the BUDR LI, is an important prognostic factor that separates low-grade astrocytomas into two groups and provides a more scientific rationale for selecting treatment for individual patients.

KEY WORDS: brain neoplasm • astrocytoma • bromodeoxyuridine • prognosis

Regardless of the degree of histopathological differentiation or anaplasia, all intracranial gliomas are clinically malignant because without appropriate treatment they are usually fatal. The histologically diverse tumors known as "low-grade astrocytomas," however, are separated by an indistinct diagnostic borderline from more anaplastic gliomas because they behave quite differently. Low-grade, or well-differentiated, astrocytomas are often considered benign because they usually grow very slowly and the afflicted patients survive longer than those with anaplastic astrocytoma or glioblastoma multiforme. Although low-grade astrocytomas have some malignant features, such as the lack of a clear border between tumor and surrounding tissue, they carry a far better prognosis than malignant astrocytomas. Nevertheless, some low-grade astrocytomas, histologically indistinguishable from the rest, progress rapidly and carry a poor prognosis.

The biological and clinical manifestations of intracranial malignancy depend largely on the rate of tumor growth, as the bone enclosure of the skull provides limited space for a brain tumor to grow. Thus, the prognosis should correlate with the size and proliferative potential of the tumor. It has been assumed, based on the histopathological findings and the results of earlier cell kinetics studies, that low-grade astrocytomas have a low proliferative potential. Recent studies, however, have shown considerable variation in the proliferative potential of these tumors as reflected by the S-phase fraction, or percentage of cells engaged in deoxyribonucleic acid (DNA) synthesis.

Measurement of the S-phase fraction became feasible with the development of monoclonal antibodies against bromodeoxyuridine (BUDR), a thymidine analogue. S-phase cells incorporate BUDR into their nuclei and can be readily identified immunohistochemically. Since 1984, we have used BUDR to investigate the proliferative potential of central nervous system tumors in situ. Our previous studies have suggested that a significant proportion of low-grade astrocytomas have growth potentials similar to those of malignant astrocytomas. In this report of BUDR labeling studies in 47 patients with low-grade astrocytomas, tumors with high S-phase fractions are contrasted with those having low S-phase fractions.

Clinical Material and Methods

Permission to administer BUDR was received from the Committee on Human Research at the University of California, San Francisco (UCSF), and from the
National Cancer Institute. Informed consent was obtained from each patient or a responsible relative. Forty-seven patients with moderately anaplastic astrocytomas who underwent surgery between May, 1986, and July, 1987, entered the study. There were 27 males and 20 females aged 2 to 68 years; 17 patients were under 15 years of age. The histological diagnosis was established using criteria developed at UCSF. Moderately anaplastic astrocytoma was defined as an astrocytoma with mildly to moderately increased cellularity, enlarged astrocytic nuclei, and relatively uniform cytoplasm but without mitotic activity or vascular endothelial proliferation. These tumors are histologically similar to grade II astrocytomas in the Kernohan classification. Juvenile pilocytic astrocytomas were excluded from this analysis.

Each patient received a 30- to 60-minute infusion of BUdR, 150 to 200 mg/sq m, at the start of surgery but before biopsy of the tumor. Excised tumor specimens were fixed in 70% ethanol, embedded in paraffin, and cut into sections 6 μm thick. The slides were deparaffinized, immersed for 30 minutes in methyl alcohol containing 0.3% H₂O₂ to block endogenous peroxidase activity, rinsed with distilled water, and analyzed immunohistochemically as described below.

Immunohistochemical Studies

The immunohistochemical staining method used to detect BUdR-labeled cells has been described in detail elsewhere. Briefly, the tissue sections were denatured with 2 N HCl and immersed in purified anti-BUdR monoclonal antibodies, either B-44 or IU-4, at a concentration of 1:50 and 1:5000, respectively. The slides were reacted with a 1:50 dilution of peroxidase-conjugated anti-mouse rabbit immunoglobulin G antibodies, developed with diaminobenzidine tetrahydrochloride and H₂O₂ in Tris buffer, and counterstained with hematoxylin.

Bromodeoxyuridine Labeling Index

The BUdR LI was calculated from each slide as the percentage of BUdR-labeled nuclei among total nuclei scored, excluding those of vascular components and hematogenous cells. Only areas that had an even distribution of labeled cells were analyzed; marginal zones where the tumor approached involved brain were avoided because we could not always differentiate normal from neoplastic astrocytes. In each area, 200 to 600 cells were counted, and at least 1000 cells (average 5000) were evaluated to determine the average LI of each specimen.

Statistical Analysis

Patients were arbitrarily divided into two groups: those whose tumors had LI's of less than 1% and those whose tumors had LI's of 1% or more. The duration of survival in these groups was estimated nonparametrically from incomplete observations by the method of Kaplan and Meier.

Results

Twenty-nine (62%) of the 47 low-grade astrocytomas had average BUdR LI's of less than 1%, and 18 (38%) had LI's of 1% or more (Fig. 1). Five tumors with LI's under 1% and six with LI's of 1% or over were recurrent at the time of study. Twenty-four patients whose tumors had low LI's and 16 whose tumors had higher LI's received radiation therapy; 10 patients in each group received chemotherapy.

The patients were followed for 4 months to 32 years. The outcome is summarized according to the BUdR LI for all patients and for those under the age of 15 years in Table I. Except for three patients who died of recurrent tumor, patients whose tumors had LI's of less than 1% were doing well without obvious recurrence. In contrast, 12 of 18 patients whose tumors had LI's of 1% or more had a recurrence, and nine died (Fig. 2).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. OF CASES</th>
<th>SEX</th>
<th>ALIVE</th>
<th>RECURRENCE</th>
<th>DEAD</th>
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</thead>
<tbody>
<tr>
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<td>47</td>
<td>27/20</td>
<td>35</td>
<td>15</td>
<td>12</td>
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<tr>
<td>BUdR LI &lt; 1%</td>
<td>29</td>
<td>18/11</td>
<td>26</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>BUdR LI ≥ 1%</td>
<td>18</td>
<td>9/9</td>
<td>9</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>patients &lt; 15 years</td>
<td>17</td>
<td>11/6</td>
<td>15</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
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<td>9/2</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BUdR LI ≥ 1%</td>
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<td>2/4</td>
<td>5</td>
<td>3</td>
<td>1</td>
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</table>

* BUdR LI = bromodeoxyuridine labeling index.
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With one exception, all patients whose tumors had LI's over 3% either suffered a recurrence or died.

Nonparametric analysis demonstrated a significantly higher 3-year survival rate in patients whose tumors had a BUdR LI of less than 1% compared with patients whose tumors had higher LI's (85% vs. 10%, \( p < 0.01 \); Gehan modification of Wilcoxon rank-sum analysis, \( z = 2.84668 \)) (Fig. 3). The median duration of survival among the latter patients was approximately 80 weeks.

**Discussion**

The results of this study demonstrate that approximately 40% of low-grade astrocytomas have BUdR LI's of 1% or more. These tumors have a higher proliferative potential and carry a far worse prognosis than histologically similar tumors with BUdR LI's under 1%. It is difficult to correlate the BUdR LI with the actual growth rate of low-grade astrocytomas because they are not well demarcated from surrounding tissue and therefore their size cannot be measured accurately from contrast-enhanced computerized tomography scans. Moreover, in this study, the two groups were not matched exactly for those receiving chemotherapy or radiation therapy, or for recurrent versus primary tumor. Similarly, it is difficult to correlate the BUdR LI with the duration of survival because many factors, including patient age, tumor location, extent of resection, and the type and amount of adjuvant therapy, determine the fate of patients with low-grade astrocytomas. \(^{2,17,19,26}\) Nevertheless, survival time is an indicator of how fast a brain tumor grows, as the brain provides limited space for a tumor to grow before it kills the patient.

Although the BUdR LI could not be precisely correlated with survival of individual patients, low-grade astrocytomas with a BUdR LI of 1% or more carried a worse prognosis than those with lower BUdR LI's, similar to that of highly anaplastic astrocytomas or malignant astrocytomas. \(^{3,4}\) We are not certain why low-grade gliomas with low LI's grow so slowly, but it is probable that spontaneous cell loss closely balances proliferation of neoplastic astrocytes. Among the patients whose tumors had BUdR LI's of 3% or more, only one survived more than 3 years without recurrence. This patient underwent gross total removal of a right frontal tumor at 4 years of age and received no adjuvant therapy. The tumor had an LI of 8.3%. It is possible that the neoplastic astrocytes were eradicated by the surgical intervention or that the neoplastic cells left behind ceased to proliferate, as is seen in some pediatric tumors such as hamartomas, juvenile pilocytic astrocytomas, and neurofibromatosis which proliferate for a certain period and then appear to cease growing spontaneously.

Therapeutic attitudes regarding low-grade gliomas differ, \(^{2,5,6,17,19,21,22,25,26}\) some authors favor radiation therapy or chemotherapy after surgery, while others report that adjuvant treatment has no benefit. The results of this study, although preliminary, have important implications for the treatment of these patients. Low-grade astrocytomas are not biologically uniform tumors: some follow a benign course, while others behave like malignant astrocytomas. The latter tumors may, like malignant gliomas or glioblastomas multiforme, respond better to radiation therapy, even though the treatment may not be curative. \(^{24}\) Conversely, low-grade gliomas with low LI's may not respond to adjuvant therapies but nevertheless have a far better prognosis, as reflected by
longer survival times. Surgical removal is the mainstay of treatment for these tumors; radiation or chemotherapy might be reserved until malignant changes or signs of faster growth are observed. This approach should be evaluated as quickly as possible because aggressive radiotherapy or chemotherapy may be an inefficient treatment for tumors with low LI's, and a conservative postoperative approach (observation) may deny potentially beneficial adjuvant treatment for patients whose tumors have high LI's.

Quantitation of the proliferative potential appears to be crucial in selecting optimal treatment for patients with low-grade astrocytomas. In order to understand the biological and clinical characteristics of these tumors, differences in the proliferative potential must be considered in evaluating the effects of various treatments on survival. It is also important to determine why such differences occur in tumors with similar morphologies. Although various assumptions have been presented to explain some of these features, further efforts are required to elucidate the basic biology of low-grade astrocytomas. This knowledge may provide a more scientific rationale for therapeutic intervention and improve the prognosis of patients with these tumors.

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Address reprint requests to: Takao Hoshino, M.D., c/o Department of Neurological Surgery, The Editorial Office, 1360 Ninth Avenue, Suite 210, San Francisco, California 94122.