Prediction of tumor doubling time in recurrent meningiomas

Cell kinetics studies with bromodeoxyuridine labeling

KYUNG G. CHO, M.D., D.M.Sc., TAKAO HOSHINO, M.D., D.M.Sc., TADASHI NAGASHIMA, M.D., JUDITH A. MUROVIC, M.D., AND CHARLES B. WILSON, M.D.

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

Eight patients with recurrent meningiomas (four malignant, two hemangiopericytic, and two nonmalignant) were given intravenous bromodeoxyuridine (B UdR), 200 mg/sq m, at the time of surgery to label cells in the deoxyribonucleic acid (DNA) synthesis phase; labeled cells were detected in excised tumor specimens by immunoperoxidase staining using anti-B UdR monoclonal antibody. These tumors showed a wide range of B UdR labeling indices (LI's), calculated as the percentage of B UdR-labeled cells divided by the total number of cells scored, from 0.3% to 5.4%. The tumor doubling times (Td's), estimated from serial computerized tomography scans, ranged from 8 to 440 days and showed a close inverse correlation with the B UdR LI's. A semilogarithmic linear regression analysis of these values yielded a correlation coefficient of 0.99. Tumor doubling time (Td) can be estimated using the formula: Td = 500 × Exp (−0.73 × LI), where Exp signifies the natural log base. By predicting the growth rate of meningiomas, the B UdR LI may supplement histopathological diagnosis and improve both the determination of prognosis and the design of treatment modalities in individual patients.

Key Words • meningioma • cell kinetics • labeling index • bromodeoxyuridine • immunohistochemistry • brain neoplasm

Most meningiomas are well-encapsulated slow-growing tumors that are amenable to total surgical removal and are therefore considered benign. Some meningiomas, however, grow very fast, invade surrounding normal brain tissue or skull, and recur more frequently than "classic" meningiomas. The biological behavior of meningiomas often cannot be predicted from the histopathological findings alone, and definition of malignant meningiomas is still subject to ambiguities. Ultrastructural and tissue culture studies have not been conclusive in elucidating the biological behavior of these tumors. Nevertheless, invasiveness and potential for rapid growth are important factors in predicting the malignancy of meningiomas. Although current histopathological techniques can demonstrate invasiveness, the actual proliferative potential of a meningioma cannot be determined unequivocally from its histology.

The development of monoclonal antibody against bromodeoxyuridine (B UdR) has enabled us to study the cytokinetics of human tumors in situ more rapidly and with fewer restrictions than was possible with autoradiographic studies using tritiated (3H) thymidine. Recent studies have shown that the B UdR labeling index (LI), which is calculated as the number of B UdR-positive cells divided by the total number of neoplastic cells after exposure to B UdR, provides a reliable estimate of the proliferative potential of individual tumors. We have measured the B UdR LI, or S-phase fraction, of several recurrent meningiomas in the past 2 years. In this study, these values were correlated with the tumor doubling times (Td's) estimated from serial computerized tomography (CT) scans of patients with meningiomas to determine whether the rate of tumor growth could be predicted from the B UdR LI.
Tumor doubling time in recurrent meningiomas

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Major Histology</th>
<th>Tumor Location</th>
<th>Recurrences Time Period (yrs)</th>
<th>BUdR LI (%) Td (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46, F</td>
<td>meningiotheliomatous meningioma</td>
<td>clival</td>
<td>5.4</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>2</td>
<td>34, M</td>
<td>hemangiopericytoma</td>
<td>rt temporal</td>
<td>8.5</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>39, F</td>
<td>meningiotheliomatous meningioma</td>
<td>tentorial</td>
<td>13.0</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>69, M</td>
<td>malignant meningioma</td>
<td>lt parietal</td>
<td>2.0</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>49, M</td>
<td>malignant meningioma</td>
<td>rt parasagittal</td>
<td>1.0</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>6</td>
<td>25, F</td>
<td>hemangiopericytoma</td>
<td>rt ventricular</td>
<td>3.8</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>7</td>
<td>8, F</td>
<td>malignant meningioma</td>
<td>falcial</td>
<td>3.8</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>8</td>
<td>49, M</td>
<td>malignant meningioma</td>
<td>lt parietal</td>
<td>1.8</td>
<td>5.4 ± 0.2</td>
</tr>
</tbody>
</table>

* BUdR LI = bromodeoxyuridine labeling index; Td = tumor doubling time.
† Measured from preoperative growth.
‡ Measured from postoperative growth.

Materials and Methods

Source of Tumor Tissue

Between June, 1984, and November, 1985, eight patients with meningiomas underwent surgery for removal of recurrent tumor at the University of California, San Francisco. Four of the tumors were malignant meningiomas, two were meningiotheliomatous, and two were hemangiopericytic meningiomas. Permission to administer BUdR was obtained from the Human Experimentation Committee at the University of California, San Francisco, and from the National Cancer Institute. All eight patients consented to receive BUdR at the time of craniotomy. Shortly after the induction of anesthesia but before biopsy of the tumor, each patient was given a 1-hour intravenous infusion of BUdR, 200 mg/sq m. Excised tumor tissues were fixed in chilled 70% ethanol for a minimum of 12 hours.

Peroxidase Immunohistochemistry

The staining procedure used to identify BUdR-labeled nuclei has been described in detail elsewhere. Briefly, the ethanol-fixed excised tumor tissue was embedded in paraaffin, cut into 6-μm sections, and deparaffinized. To avoid endogenous peroxidase activity, the slides were incubated for 30 minutes in methanol with 0.3% H2O2, denatured for 60 minutes in 2N HCl, and reacted with a 1:30 dilution of purified anti-BUdR monoclonal antibody* in phosphate-buffered saline solution (PBS) containing 2% bovine serum albumin and 0.5% Tween 20 for 45 minutes at room temperature. The slides were then covered with a 1:50 dilution of peroxidase-conjugated anti-mouse immunoglobulin G antibody† in PBS for 45 minutes and reacted with 15 mg of diaminobenzidine tetrahydrochloride and 6 μl of 30% H2O2 in 60 ml of Tris buffer for 10 to 15 minutes. Ten percent Gills hematoxylin was used to counterstain the tissue sections. Cells labeled with BUdR were counted in six to 15 microscopic fields to determine the average BUdR LI, which was expressed as a percentage of the total number of cell scored.

Tumor Doubling Time

The volume of each tumor was estimated from serial CT scans. The area of tumor in each scan was determined by planimetry; tumor volume was calculated as the sum of the products of the area of tumor multiplied by the slice thickness. Tumor doubling time (Td) was calculated as t x log 2/(log VB - log Va), where Va is the initial volume of the tumor and VB the volume of the tumor after t days. When postoperative CT scans did not demonstrate residual tumor, it was postulated that 0.1 cu cm of tumor remained even after gross total removal at the most recent operation.

Results

The clinical data on the eight patients in this study, the BUdR LI's of their tumors, and the Td's estimated from serial CT scans are summarized in Table 1. The BUdR LI's, or S-phase fractions, ranged from 0.3% to 5.4% (mean 3.0% ± 2.0% standard error). Cases 1 and 2, with long intervals to recurrence (more than 5 years), had low BUdR LI's (0.3% and 0.5%) and long Td's (440 days and 281 days, respectively). In the other patients, who had shorter intervals to their first recurrence (less than 1 year), the BUdR LI's were more than 1% and the Td's were less than 100 days, except in Case 3. In Cases 1 to 4, Td was calculated from preoperative tumor growth because no postoperative growth was detected; in Cases 5 to 8, Td was calculated from postoperative tumor growth. The mean Td's were 65.8 ± 56.9 days and 17.4 ± 7.5 days, respectively, in these two groups.

The Td's of these eight meningiomas were plotted...
FIG. 1. Tumor doubling time estimated from serial computerized tomography scans is plotted against bromodeoxyuridine (BUDR) labeling index (LI) for each of the eight tumors. Error bars indicate 1 standard deviation, showing the amount of LI variability from area to area within the same tumor.

Discussion

Since Craig proposed that meningiomas be classified as benign or malignant on the basis of their histology and clinical behavior, many histopathological analyses have been undertaken to predict the biological behavior of these tumors. Several histological variants, including syncytial, hemangiopericytic, and papillary meningiomas, have been considered malignant. However, Jellinger and Slowik found no definite relationship between other subtypes of meningiomas and their biological tendency to recur. Most authors agree that, in meningiomas, microscopic features such as a high mitotic rate, focal necrosis, increased cellularity, and cortical invasion correlate with a faster growth rate and more frequent recurrence. On the other hand, Henschen, Simpson, and Adegbite, found little correlation between the histological characteristics and the growth rate of these tumors. Their findings are supported by the occasional observation of mitosis, necrosis, and even cortical invasion in meningiomas that do not recur after surgical removal. Jääskeläinen, concluded from a study of 43 patients with meningiomas that the mitotic index correlated grossly with the growth rate, but its value in predicting Td was only suggestive. Moreover, the mitotic rate of meningiomas is too low to be properly measured, and the distribution of mitotic figures is too inconsistent to provide the basis for either a reliable estimate of proliferative potential or prediction of the growth rate of individual tumors. Cytogenetic studies may be useful in the future, but at present one must still rely on clinical and histological criteria to predict the biological behavior of meningiomas. In order to improve our ability to predict the growth rate and the probability of recurrence, and also to design more effective treatment for individual patients, a more quantitative measurement of proliferative capacity of these tumors is needed.

Like 3H-thymidine, BUDR is incorporated into nuclear deoxyribonucleic acid (DNA) during DNA synthesis, but is neither radioactive nor myelotoxic at the dose used for in vivo labeling studies. Although cytotoxic or teratogenic effects may occur with prolonged administration at high doses, BUDR has been used without serious side effects as a radiosensitizing agent for brain tumors and cancers of the head and neck. The immunocytochemical method of detecting BUDR-labeled cells requires only 2 to 3 hours to complete, and the results are available within a few days after biopsy of the tumor.

Hoshino, et al., have administered intravenous BUDR, 150 to 200 mg/sq m, to patients with various brain tumors at the time of surgery and determined the LI immunocytochemically using anti-BUDR monoclonal antibodies. These studies have shown that the BUDR LI's of individual tumors correlate reasonably well with their clinical behavior and therefore afford a more quantitative indication of their proliferative capacity.

The growth rate of a neoplasm can be calculated by serial volume measurement. Yamashita and Kuwabara demonstrated that the ratio of the volume of tumors estimated from CT scans obtained at two different times under the same conditions was consistently similar to the ratio of tumor volumes determined at surgery. This method is especially suitable for calculating the Td’s of meningiomas. First, meningiomas are so well demarcated and homogeneous on the enhanced CT scans that tumor volume can be estimated more accurately than is possible with gliomas. Second, unlike other neuroectodermal tumors, meningiomas (even some malignant forms) do not contain prominent or

### Table 2

<table>
<thead>
<tr>
<th>BUDR LI (%)</th>
<th>Doubling Time (days)</th>
<th>Time to Recurrence†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>347</td>
<td>8.5 yrs</td>
</tr>
<tr>
<td>1.0</td>
<td>241</td>
<td>6 yrs</td>
</tr>
<tr>
<td>2.0</td>
<td>116</td>
<td>2.8 yrs</td>
</tr>
<tr>
<td>3.0</td>
<td>56</td>
<td>1.4 yrs</td>
</tr>
<tr>
<td>4.0</td>
<td>27</td>
<td>8 mos</td>
</tr>
<tr>
<td>5.0</td>
<td>13</td>
<td>4.5 mos</td>
</tr>
</tbody>
</table>

* BUDR LI = bromodeoxyuridine labeling index.
† Time required for a tumor to grow from 0.1 cu cm to 50 cu cm.
massive areas of necrosis; thus, cell loss appears to be more uniform and consistent. Because tumor recurrence is caused by a minute volume of residual tumor, we assumed that 0.1 cu cm of tumor remained after apparent gross total removal, even though no tumor was seen on the postoperative CT scan. This is a reasonable assumption that has been made by others.19

Six of the eight recurrent meningiomas had BUdR LI's greater than 1%. These values are in accordance with shorter Td's and faster recurrence than are typical of classic meningiomas. A BUdR LI greater than 1% therefore implies faster tumor growth and more frequent recurrence, and may be regarded as a characteristic of "malignant" meningioma. Because meningiomas with BUdR LI's greater than 3% had Td's of less than 30 days and recurred after a mean interval of only 9 months, such tumors should be carefully monitored and perhaps should be treated as aggressively as malignant gliomas in order to prevent recurrence.

Hemangiopericytomas are generally considered to be more aggressive biologically than other classic meningiomas.20,21,22,23,24,25 The two recurrent hemangiopericytic meningiomas in this study displayed markedly different behavior. In Case 6, the tumor had an LI of 4.1% and a Td of 27 days and recurred four times in 3.8 years. This tumor can therefore be diagnosed as a malignant meningioma. In Case 2, however, the tumor had an LI of 0.5% and a Td of 281 days; it did not recur until 8.5 years after the initial surgical removal.

The median BUdR LI of the five recurrent malignant meningiomas in our study, including one hemangiopericytoma, was 4.1%. This BUdR LI is higher than those of classic meningiomas,11 pituitary adenomas,25 and low-grade gliomas,14,15,16 but lower than those of malignant gliomas, including glioblastoma multiforme.14,15,16 The Td's of the malignant meningiomas were close to those of the malignant gliomas in Yamashita and Kuwabara's study,37 in which the Td's calculated from CT scans ranged from 15.0 to 21.1 days.

Our study of eight meningiomas has shown that the BUdR LI's of these tumors correlated closely with the actual Td's estimated from serial CT scans. Linear regression analysis has demonstrated that Td can be calculated from the BUdR LI using the equation: Td = 500 × Exp ((−0.73 × LI)). Table 2 shows the Td's calculated from hypothetical meningiomas with BUdR LI's of 0.5% to 5% and indicates the time required for such tumors to grow from 0.1 to 50 cu cm. Because growth potential and rate of recurrence are of major prognostic importance in treating patients with meningiomas, determination of the BUdR LI is an extremely helpful diagnostic procedure.

Acknowledgments

The authors thank Frances James for manuscript preparation and Stephen Ordway for editorial assistance.

References

22. Ludwin SK, Rubinstein LJ, Russell DS: Papillary menin-

Manuscript received January 31, 1986.
This study was supported by Grant PDT-159 from the American Cancer Society and Grants CA-13525 and CA-09291 from the National Cancer Institute.
Address reprint requests to: Takao Hoshino, M.D., c/o The Editorial Office, Department of Neurological Surgery, 1360 Ninth Avenue, Suite 210, San Francisco, California 94122.