Thymidine kinase in brain-tumor cysts

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A recently developed method for deoxythymidine kinase (TK) determination was applied to brain-tumor
cyst fluid and fluid from a non-neoplastic intracerebral cyst. The fluid from all tumors tested positive for TK
whereas the non-neoplastic cyst lacked TK. Cyst fluid was also analyzed for TK before and after intracystic
instillation of BCNU. It is suggested that TK activity in the fluid in cystic brain lesions could prove useful in
deciding whether an intracerebral lesion is neoplastic. Also, TK activity can be used to evaluate the effect of
topical therapy.

KEY WORDS • thymidine kinase • brain tumor • tumor marker •
cystic brain tumor • intratumoral drug delivery

DEOXYTHYMIDINE kinase (TK) belongs to the
pyrimidine metabolism cycle, and acts as a
scavenger enzyme in the synthesis of deoxy-
thymidine monophosphate. Different isoenzymes of
TK appear in different kinds of cells. The cytosolic iso-
enzyme, TK-F, is present only in proliferating cells,
including tumor cells, where it represents the greater
part of the TK activity. In resting differentiated cells,
the mitochondrial isoenzyme, TK-A, predominates. Be-
cause TK-F is present only in proliferating cells, it can
be used as a tumor marker, since proliferation is a
major feature of neoplastic cells.

The development of a new improved TK enzyme
assay utilizing iodine-125 (125I)-iododeoxyuridine as a
substrate has made it possible to measure TK-F activity
in human serum. Large concentrations of TK were
found in sera of patients with leukemia, lymphoma,
and small-cell carcinoma of the lung.7,9 In an extensive
study of patients with non-Hodgkin's lymphoma, it was
concluded that TK activity in serum was a reliable
prognostic marker.7

We have recently demonstrated that TK activity can
also be measured in the cerebrospinal fluid (CSF) of
patients with several kinds of primary and secondary
brain tumors.10 The results are promising, and further
studies may show TK to be of practical value as a CSF
marker of brain tumors.

There are no exact figures for the frequency of cystic
brain tumors, but it is estimated that about 10% of all
brain tumors are cystic. Little attention has been paid
to the fluid that accumulates in cystic tumors, although
chemical analysis has yielded important information
about the tumor. The use of the cyst fluid for malign-
cy grading has been suggested by Szliwowski and
Cumings16 and Buckell, et al.,5,6 who studied the lac-
todehydrogenase content of cyst fluid. Chemical grad-
ing could prove especially valuable when diagnosis is
attempted from a small biopsy specimen, in which
classification is often difficult.4 In the present study we
looked for TK (EC 2.7.21) in the fluid from cystic brain
tumors.

In gliomas, the fluid that accumulates in cysts may
be in direct contact with the extracellular fluid of
the tumor cells. Therefore, such a cyst could be used
as a reservoir for topically applied chemotherapeutic
agents.14 A study is currently being conducted on dif-
ferent forms of nonoperative therapy for centrally lo-
cated cystic malignant gliomas. It involves the instilla-
tion of chemotherapeutic agents into tumor cysts with
the aid of an Ommaya reservoir.14 Using this device,
cyst fluid can also be removed for chemical analysis. In
three patients in our series, we examined the fluid for
TK activity before and after instillation of BCNU (1,3-
bis(2-chloroethyl)-1-nitrosourea), to establish whether
BCNU administered in this way influenced the amount
of TK in the fluid.
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TABLE 1

<table>
<thead>
<tr>
<th>Diagnosis of Lesion</th>
<th>No. of Lesions</th>
<th>TK Activity (units/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.08</td>
<td>0.08–2.4</td>
</tr>
<tr>
<td>astrocytoma IV</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>astrocytoma III</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>hypothalamic glioma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>oligodendroglioma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>primitive neuroectodermal tumor</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>colloid cyst</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>subarachnoid cyst</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Clinical Material and Methods

Preparation of Specimens

Specimens of fluid from 18 cystic brain lesions were obtained for TK analysis. The lesions were identified by contrast-enhanced computerized tomography (CT), and routine histological classification was performed. Most histological diagnoses were made from a small specimen obtained by a stereotaxic biopsy procedure or on a larger specimen removed at craniotomy. Cyst fluid was aspirated during these procedures and centrifuged at 900 G, and the supernatant was separated and stored at -20°C until required. In some patients, lumbar CSF and serum were also collected for TK analysis. The albumin content of cyst fluid, CSF, and serum was measured in some cases.

BCNU Treatment

Three patients with inoperable cystic gliomas had an Ommaya reservoir implanted into the tumor cyst at the time of the stereotaxic biopsy procedure. About 1 week later, when the wound had healed and the diagnosis was histologically confirmed, some fluid was aspirated for TK analysis and 100 mg of BCNU, dissolved in ethanol and saline, was instilled into the tumor cyst. Specimens of fluid were subsequently removed after various intervals for TK analysis. In two of the patients, serum and CSF were also collected and examined for TK activity.

TK Enzyme Assay

Cyst fluid, CSF, and serum were analyzed as described elsewhere. The TK activity found was linear with time and the amount of cyst fluid, CSF, or serum studied. All enzymatic activities were recalculated to units/µl of analyzed specimen. One unit of TK activity is defined as 1.2 × 10^-14 katal. In healthy people, the mean serum TK level is 2.4 ± 1.25 units (± standard deviation) and the mean CSF TK concentration is less than 0.08 units.

Results

The specimens from all tumors showed TK activity. The activity varied between tumors of different types as well as within the same tumor group (Table 1). Astrocytoma grades III and IV yielded activities ranging from 0.6 to 7.5 units/µl. A hypothalamic glioma showed low TK activity (0.84 units/µl). In the oligodendroglioma fluid the TK activity was 2.4 units/µl. The primitive neuroectodermal tumor fluid contained 4.6 units/µl; this tumor was highly malignant and recurred within 6 months. A surprisingly high TK activity, 6.4 units/µl, was found in the fluid-like matter in a benign colloid cyst of the third ventricle. The highest TK activities, 9.0 and 18.2 units, were noted in two metastases, one secondary to a mammary carcinoma and the other to a lung carcinoma of unknown type.

The fluid of an expansive subarachnoid cyst in the mesencephalon was also analyzed. Biopsy performed at the same time showed that the lesion was not neoplastic. No TK activity was found. A shunt was placed in the cyst, and subsequent follow-up examinations have further convinced us that the lesion was not a tumor.

Illustrative Case Reports

Case 1

This patient, who had a recurrent astrocytoma grade III, had previously been treated by surgery, irradiation, systemic chemotherapy, and interferon administration. The recurrent tumor contained a large solitary cyst in which a catheter connected to an Ommaya reservoir was placed. Fluid aspirated immediately before instillation of BCNU showed TK activity of 5.7 units/µl. Cerebrospinal fluid sampled at the same time contained 1.8 TK units/µl (this value is characteristic of malignant gliomas). The serum TK level was 3.6 units/µl. The albumin content was 33 gm/liter in the cyst fluid and 36 gm/liter in the serum. After instillation of BCNU as described above, there was a considerable increase in cyst fluid TK activity: 3 days after instillation the TK level was 9.5 units/µl, and 3 days later it was 5.4 units/µl. The albumin content of the cyst fluid did not change significantly.

Case 2

A CT scan (Fig. 1) and biopsy revealed that this patient had an inoperable astrocytoma grade IV deep in the left parietotemporal region. The changes in TK and albumin content in the cyst fluid and CSF after BCNU instillation are given in Table 2. It is noteworthy that in this highly malignant tumor instillation of BCNU was followed by a more than fourfold increase in cyst TK activity. The CSF TK level was 0.124 units/µl initially; the highest value, 0.286 units/µl, was seen after 9 days.

Case 3

A CT scan in this patient disclosed a large cystic...
tumor with areas of calcification (Fig. 2), and histological examination of a biopsy specimen showed an oligodendroglioma with signs of anaplasia. The cyst fluid TK level was 2.4 units/µl. The changes in TK and albumin levels in the cyst fluid, CSF, and serum during the 4 days after BCNU instillation are listed in Table 3. The increase in the cyst fluid TK level was less striking than in Cases 1 and 2: at 24 hours after BCNU administration the cyst fluid TK activity was 3.7 units/µl, and 3 days later a maximum of 5.0 units/µl was noted. No TK was found in the CSF until 4 days after the instillation of BCNU, when a level of 0.17 units/µl was recorded.

Discussion

The study of brain-tumor cyst fluid is important for several reasons. Chemical analysis will sometimes show whether or not a lesion is neoplastic, it can indicate the degree of malignancy, and in some cases it may even determine the nature of a cystic tumor. Chemical analysis grading can be particularly valuable when the diagnosis is made from small biopsy specimens in which histological classification can be difficult. The cystic cavity of gliomas appears to be in direct contact with the extracellular space of the tumor cells, which makes the use of intracystic application of chemotherapeutic agents attractive. Cystic craniopharyngiomas have been successfully treated by intracystic deposition of a radio-nuclide. Immunosuppressive factors have been found in tumor cyst fluid, suggesting that they are produced locally by the tumor.

In this paper, we describe a new tumor marker, deoxythymidine kinase (TK). This enzyme is present in proliferating cells (that is, during the later G₁ and S phases). Traditionally, the rate of cell division has

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**TABLE 2**

Case 2: Changes in TK activity and albumin content after intracystic instillation of BCNU*

<table>
<thead>
<tr>
<th>Time Post-BCNU (hrs)</th>
<th>TK (units/µl)</th>
<th>Albumin (gm/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyst</td>
<td>CSF</td>
</tr>
<tr>
<td>0</td>
<td>7.5</td>
<td>0.124</td>
</tr>
<tr>
<td>24</td>
<td>8.0</td>
<td>0.229</td>
</tr>
<tr>
<td>48</td>
<td>14.8</td>
<td>0.280</td>
</tr>
<tr>
<td>72</td>
<td>11.5</td>
<td>0.100</td>
</tr>
<tr>
<td>96</td>
<td>13.6</td>
<td>0.125</td>
</tr>
<tr>
<td>6 days</td>
<td>31.6</td>
<td>—</td>
</tr>
<tr>
<td>8 days</td>
<td>17.4</td>
<td>—</td>
</tr>
<tr>
<td>9 days</td>
<td>11.0</td>
<td>0.286</td>
</tr>
<tr>
<td>13 days</td>
<td>5.4</td>
<td>—</td>
</tr>
</tbody>
</table>

*TK = deoxythymidine kinase; CSF = cerebrospinal fluid; — = not sampled.

**TABLE 3**

Case 3: Changes in TK activity and albumin content after intracystic instillation of BCNU*

<table>
<thead>
<tr>
<th>Time Post-BCNU (hrs)</th>
<th>TK (units/µl)</th>
<th>Albumin (gm/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyst</td>
<td>CSF</td>
</tr>
<tr>
<td>0</td>
<td>2.4</td>
<td>&lt; 0.08</td>
</tr>
<tr>
<td>12</td>
<td>2.3</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>3.7</td>
<td>&lt; 0.08</td>
</tr>
<tr>
<td>36</td>
<td>4.7</td>
<td>—</td>
</tr>
<tr>
<td>48</td>
<td>4.8</td>
<td>&lt; 0.08</td>
</tr>
<tr>
<td>72</td>
<td>5.0</td>
<td>&lt; 0.08</td>
</tr>
<tr>
<td>96</td>
<td>3.2</td>
<td>0.170</td>
</tr>
</tbody>
</table>

*TK = deoxythymidine kinase; CSF = cerebrospinal fluid; — = not sampled.
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been measured by autoradiographic study of the incorporation of radioactive thymidine into deoxyribonucleic acid (DNA). The catalyst for such incorporation is TK. Direct assay of cell division-specific proteins such as TK (in contrast to cell-incorporation procedures) is possible for measuring the accumulation of the enzyme in body fluids such as serum, CSF, and tumor cyst fluid.

We found TK activity in the cyst fluid of all tumors, whereas a non-neoplastic cyst that we tested lacked TK. The highest TK activity was noted in the fluid of the metastases, which agrees with the findings of Buckell, et al., who reported the highest lactodehydrogenase activity in the fluid of cystic metastases. The amounts of TK in astrocytomas grades III and IV were lower than those in the metastases. An oligodendroglial tumor with an unusual degree of anaplasia showed a TK activity within the range of the malignant astrocytomas. A cystic primitive neuroectodermal tumor in a child was clearly malignant and recurrent; the TK of this tumor was also within the range of the malignant astrocytomas. We studied one case of low-grade glioma. This was a hypothalamic glioma with a TK level of 0.84 units/µl.

The TK activity in the fluid of the cystic astrocytomas grades III and IV varied considerably. The reason is unclear. In most cases, the histological classification was based on small biopsy specimens. No attempts were made to correlate TK activity with tumor cyst volume, tumor location, or clinical course, so it remains uncertain whether or not the variation in TK in this tumor group reflects differences in proliferation characteristics. Further studies are in progress. Nor is it known whether certain tumors have an amplified expression of TK in their cells. Surprisingly, the fluid-like content of a benign colloid cyst of the third ventricle showed high TK activity. However, TK itself is not a tumor marker, but may also be present in non-neoplastic proliferative tissues. In a colloid cyst, proliferating cells could be shed into the cyst, where the TK activity is retained.

In most cases, the cyst-fluid TK level was higher than in serum, indicating that the TK in cyst fluid was derived from the tumor cells and had not leaked into the cyst from the blood through a defective blood-brain barrier. It was found that cyst fluid contained about 15% to 20% less albumin than did serum, suggesting a blood-brain barrier defect in cystic gliomas. The protein TK is similar in molecular size to albumin and may enter a tumor cyst from the circulation in roughly the same way as does albumin. In certain tumors, the cyst-fluid TK content was less than 15% to 20% of the serum concentration, indicating that the tumor cells did not synthesize TK or that the release of TK into the tumor cysts was in some way impaired.

Serum TK may be elevated in systemic malignancies. However, the cyst-fluid TK activity found by us in two cerebral metastases was not due to high serum TK concentrations, because these patients' sera, sampled the same day as the cyst fluids, contained 3.9 and 2.9 units/µl respectively; in other words, the cyst-fluid TK values were roughly 2.5 and 6 times greater than the serum concentrations.

The protein profiles of the cystic glial fluids were similar to those in serum in the two patients studied (data not shown). A similar relationship between serum and cyst fluid was reported by Kikuchi and Neuwelt. It is tempting to assume that the fluid in cystic gliomas represents a collection of liquid vasogenic edema. Should this be the case, such edema could be studied directly.

Instillation of BCNU into tumor cysts caused a dramatic local increase in TK activity. It seems probable that BCNU, dissolved in ethanol and saline, had a cytotoxic effect on the tumor cells, and that the release of TK into the cyst indicated tumor cell injury and/or death. There was a time lag of about 24 to 48 hours between the instillation of BCNU and the increase in TK activity in the cyst fluid, and a maximum TK activity was recorded 3 to 6 days after the procedure. We have no explanation for these changes, but further studies may show whether they can throw light on how BCNU acts.

Instillation of BCNU did not significantly influence the clinical course of the disease. However, the results suggest that TK activity could prove useful in monitoring the effect of a specific treatment in an individual patient. This may be of particular interest because modern concepts of glioma therapy embrace a variety of treatments, so a method for evaluating their effects on tumor cells would be welcome.

Conclusions

It is suggested that measurement of cyst-fluid TK could prove useful in deciding whether an intracerebral cystic lesion is a tumor. The TK activity can also be used to evaluate the effect of topical therapy. Future studies may show whether TK activity can be used in studies on the kinetics of tumor cell proliferation.

References

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