Correlation of tumor plasminogen activator with peritumoral cerebral edema

A CT and biochemical study

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Extracts from 15 human cerebral tumors were tested by a fibrin-plate plasminogen-dependent assay for levels of tumor plasminogen activator (TPA) activity. The TPA activity was correlated with the amount of perineoplastic edema as quantified on computerized tomography (CT) brain scanning. Analysis of the results showed a correlation coefficient of 0.72 when all tumors were included. Analysis of the nine tumors with the highest TPA levels showed a correlation coefficient of 0.96. One metastatic tumor had the highest level of TPA activity, equivalent to a pure 100-μg/ml solution of urokinase, and the greatest amount of cerebral edema on CT. Meningiomas generally had the next highest levels of TPA activity and edema, followed by astrocytomas of varying grades, which generally had the lowest level of TPA activity. However, three astrocytomas that had low TPA activity also had significant edema surrounding the tumor, indicating that more than one mechanism may be producing peritumoral edema. There was no correlation between tumor size and the amount of perineoplastic edema. These preliminary results suggest that TPA's may be involved in the production of peritumoral cerebral edema and that treatment of patients with currently available plasminogen activator inhibitors may be successful in reducing peritumoral edema.

KEY WORDS • plasminogen • plasminogen activator • cerebral edema • cerebral neoplasm

Tumor plasminogen activators (TPA's) have been implicated in several processes of tumor growth, including tumor invasion, metastasis, and vascular permeability. In vitro studies have shown that TPA's are produced by many tumors, including central nervous system (CNS) tumors. Despite the wide interest and numerous biochemical and in vitro studies on TPA's, no clinical correlation study has been performed that demonstrates the clinical effects of TPA's. In this study the TPA levels in human cerebral tumors removed at operation have been correlated with the amount of preoperative cerebral edema shown on computerized tomography (CT) scans and with the histological type of the tumor. Human CNS tumors are an ideal model to study this association since, unlike tumors elsewhere in the body, it is usually possible to image the entire extent of the tumor and of the surrounding edema.

Peritumoral edema is a significant cause of mortality and neurological morbidity in patients with primary or metastatic cerebral tumors. In addition, cerebral edema can complicate and delay various treatment options such as surgery, radiation therapy, and chemotherapy. In clinical practice, corticosteroids are used to control and ameliorate the perineoplastic cerebral edema. In vitro studies have shown that corticosteroids are potent inhibitors of TPA production by malignant cultured cells. This fact has further stimulated the present investigation of the potential relationship between TPA and cerebral edema in the hope that additional therapeutic agents may be proposed for peritumoral cerebral edema.

Materials and Methods

Clinical and Pathological Material

This study was confined to operable intra-axial tumors located in the cerebral or cerebellar hemispheres since these areas can be imaged by CT scanning with minimal artifact and accurate determinations of peritumoral edema can be obtained. The surgically removed tumor specimen was placed on ice and delivered.
to the Department of Pathology. The neuropathologist divided the specimen so that adequate tissue was placed in fixative for appropriate tissue processing. Any excess tumor specimen was immediately frozen and stored at -70°C. After the final pathological slides had been processed and the diagnosis of the tumor confirmed, the neuropathologist released the frozen tissue for plasminogen activator studies.

Preparation of TPA Extracts

One gram of tumor tissue was homogenized in a Wheaton glass tissue homogenizer with 5 volumes of a 0.1-M Tris-glycine buffer (pH 8.0). The homogenate was clarified by centrifugation at 10,000 G for 30 minutes and adjusted with buffer to obtain a protein concentration of 2.5 mg/ml by absorbance at 280 nm using $E_{280} = 1.5$, then separated into aliquots and stored at -70°C. Repeated assays of aliquots from the same tumor showed no detectable change in TPA activity over a period of up to 4 months at 4°C and 12 months at -70°C with one freeze-thaw cycle. In addition, there appeared to be no significant difference in TPA activity with standard centrifugation of the homogenate as described above or with ultracentrifugation at 100,000 G for 15 minutes in a Beckman air ultracentrifuge.*

Fibrin-Plate Radial Diffusion Enzyme Assay for TPA Activity

The fibrin-plate assay for TPA activity was based on the technique of Schumacher and Schill,14 using fibrinogen containing agar gel. Plasminogen-free bovine fibrinogen† was dissolved in 0.1 M of warm (40°C) Tris-glycine buffer (pH 8.0) to a concentration of 10 mg/ml and mixed with an equal volume of 1.5% Sea-Plaque agarose‡ at 40°C. Human plasminogen§ was added to a final concentration of 7 μg/ml along with 10 NIH units/ml of bovine thrombin. The entire solution was gently stirred and poured onto plastic plates of Gelbond film II to produce a gel 1 mm thick and was allowed to cool. A 3-mm punch was used to make circular test wells in the plate. Then 10 ml of test solution and standards was added to the wells, and the plate was incubated at 37°C for 18 hours. The resultant lytic zones were measured with a millimeter rule and re-

Results

Fifteen human cerebral tumors removed at craniotomy met the above-mentioned selection criteria. These included seven primary gliomas, five meningiomas, one metastatic tumor, one medulloblastoma, and one tissue sample of reactive gliosis surrounding an arteriovenous malformation (Table 2). A straight-line plot of TPA activity was obtained in every experiment when the log$_{10}$ of the concentration of urokinase in the standard dilutions was plotted against the diameter of the corresponding lytic zones. This technique was quite reproducible provided that incubation time and temperature were carefully controlled. Tumor-soluble extracts were tested on fibrin plates which included human urokinase (5000 Plough units)* which were added to every plate. Human urokinase, 1 mg/ml, when diluted to concentrations of 1/10, 1/20, 1/100, and 1/400, gave a straight-line curve on the plates when the diameter of the lytic zone was plotted against log$_{10}$ of the concentrate after 18 hours of incubation at 37°C (Table 1). Control plates were prepared exactly as above except that plasminogen was omitted. Test samples were added to these plates to detect protease activity which was not plasminogen-dependent.

Evaluation of CT Scans

The preoperative contrast-enhanced CT scans of the selected patients were retrospectively analyzed by tracing on paper the area of tumor mass and the peritumoral edema area on each slice of the scan and by cutting out the paper tracings and weighing them. On each scan there was a scale in centimeters, and a known area on the scan was similarly measured and weighed. Since each CT slice was 1 cm thick, the tracing weights were added and converted to volume in cubic centimeters based on the weight/standard area ratio. Some tumors were excluded from the study because of lack of enhancement of the tumor and the inability to distinguish between the low density of infiltrating tumor and that of peritumoral edema. Such tumors were usually low-grade astrocytomas.

<table>
<thead>
<tr>
<th>Urokinase Dilution</th>
<th>Diameter of Lytic Zone (mm)</th>
<th>Log Conc of TPA</th>
<th>TPA Value (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/10</td>
<td>16.00</td>
<td>2.00</td>
<td>100.0</td>
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</tr>
<tr>
<td>1/400</td>
<td>7.25</td>
<td>0.40</td>
<td>2.5</td>
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* Log Conc of TPA = log concentration of tumor plasminogen activators.
the urokinase standards and their lytic zone diameters plotted on the standard curve. The concentration of TPA could then be expressed in terms of micrograms/milliliter of urokinase. The results of such an experiment are shown in Table 2.

The single metastatic tumor produced the largest lytic zone, equivalent to that of a solution of purified urokinase of 100 μg/ml. Three of the meningiomas had TPA’s in the range of 50 to 10 μg/ml urokinase along with two moderate-grade astrocytomas. An assortment of tumors were in the low TPA range (< 10 μg/ml): low-grade astrocytomas, reactive gliosis, high-grade astrocytomas, and a medulloblastoma. The single case of reactive gliosis (Sample No. 1Bl0) consisted of white matter surrounding an arteriovenous malformation. This sample was included in the series to provide some indication of the baseline level of endogenous tissue plasminogen activator which might be present in abnormal white matter that does not contain tumor tissue.

Figure 1 graphically demonstrates the level of TPA found in the various tumor types. All of the meningiomas had TPA levels greater than that of the reactive gliosis. Meningiomas generally had the highest level of TPA and edema, followed by astrocytomas of various grades and types. However, three astrocytomas which had low TPA activity also had significant peritumoral edema. This could indicate that more than one mechanism may be responsible for the edema seen on CT scans. Alternatively, the CT scans could be overestimating the amount of edema seen in these types of tumors since it is often difficult with infiltrating tumors to distinguish between low density representing tumor infiltrating the parenchyma and low density representing peritumoral edema.

The volume of peritumoral cerebral edema on CT scans was plotted against the log of TPA concentration for samples in which the log concentration of TPA was greater than 0.5 (Fig. 2), since those values fall within the range of the urokinase standards and within the confidence sensitivity and accuracy of the assay. Figure 2 also includes the best-fit line for the data as well as the confidence limits for ±2 standard deviations. The correlation coefficient was 0.97. When all the samples were included in the linear regression analysis, a correlation coefficient of 0.72 was obtained. There was no correlation between tumor volume and peritumoral edema, as demonstrated in the scattergram shown in Fig. 3.
Discussion

Tumor plasminogen activators have been consistently found in relatively elevated amounts in various tumor cell types and are frequently associated with cell transformation in tissue culture. One study has shown that TPA's from several different types of human tumor cells have molecular weights different from those of normally occurring human plasminogen activators, including urokinase. These TPA's were 60,000 daltons in size as opposed to the usual 48,000-dalton size found for urokinase. Tissue culture cells which have been transformed with deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) tumor viruses have been shown to produce TPA's, whereas normal cell-line control samples did not. This observation has stimulated others to use this characteristic to develop techniques to detect transformed cells and colonies.

When human primary cerebral tumor cell lines, including gliomas and meningiomas, were tested for plasminogen activator activity, all of the tumors were found to have elevated levels when compared to normal human brain. Anti-urokinase antibody did not inhibit the TPA activity from the tumors, indicating that the TPA activity was immunologically distinct from normal human urokinase-like activity. This study concluded that further investigations were needed to determine if the TPA activity observed in primary cerebral tumors could be correlated with tumor invasiveness or perineoplastic edema. Other studies have shown that glioma cell TPA activity coincides with the mitotic activity of the cells as they increase when a mitotic wave is seen in the cell culture. A clinicopathological study of human meningiomas and surrounding cerebral edema correlating CT findings with pathological findings has shown changes in the surrounding cortex which could be best explained by a humoral factor released by the tumor. The often-observed clinical finding of a small cerebral tumor with a disproportionately large amount of surrounding edema has led many clinicians to suspect that an excretory tumor factor may be responsible for the edema. The tumors frequently responsible for this type of presentation are often metastases or meningiomas.

In vitro studies using embryonic lung cell cultures and tumor cell cultures of several different cell types have shown that physiological concentrations of the glucocorticoid dexamethasone inhibited the plasminogen activator activity of all of the cells tested except those derived from melanoma. In addition, another study has shown that macrophage plasminogen activator can be inhibited by dexamethasone. Because of the well-known, clinically observed effect of dexamethasone in reducing peritumoral cerebral edema, it is tempting to hypothesize that tumor plasminogen activators may play a role in producing such edema.

Although this study tested only a few tumors, the results do suggest a strong correlation between TPA and peritumoral edema, especially for meningiomas and metastatic tumors. It is the first study to confirm, based on fresh tumor tissue from cerebral tumors, the large amounts of TPA found in tissue culture studies of similar tumors. It is likely that other tumor humoral factors released by cerebral tumors may affect adjacent normal brain and produce peritumoral edema. A correlation has been found between peritumoral cerebral edema and the level of tumor leukotrienes and vasoactive peptidolipids measured in fresh tumor specimens obtained at craniotomy (KL Black, et al., unpublished data). Metastatic tumors produced the highest amounts of leukotrienes and cerebral edema, whereas gliomas had less of both. Meningiomas were not included in the study.

The results of this limited study are interesting considering clinical experience. The fact that meningiomas and a metastatic tumor showed the highest levels of TPA and peritumoral edema correlates with clinical observations in the CT scan era. The fact that there is
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no correlation between tumor size and peritumoral edema also corresponds to the clinical impression. In this study, astrocytomas had less TPA activity and less peritumoral edema. They may represent those tumors where another factor is responsible for surrounding edema. In addition to its possible direct effects on normal tissue through the production of plasmin, plasminogen activator is known to activate the kinogen-bradykinin pathway. Althuous measuring the amount of vasoactive peptide bradykinin has been implicated as one cause of vasogenic edema. Although measuring the amount of TPA in the tumor itself is interesting, it would seem more interesting to measure the amount of TPA in the edematous normal brain surrounding the tumor. In this small series, there was no case where removal of surrounding normal brain was justified. Furthermore, because of the presence of normal tissue and endothelial plasminogen activators, a specific marker for TPA would be required before such a study could be carried out. The problem is complex when one considers a study in which a polypeptide secreted by mouse melanoma PG19 cells induced plasminogen activator production by normal human fibroblasts. Nevertheless, the results of this study have stimulated further experiments to answer these questions.

Despite the small number of tumors tested, the data suggest that there is a relationship between the TPA level and the amount of peritumoral edema. This would indicate that a more extensive study is warranted to determine if this relationship is causal. Such a study would for the first time give new knowledge about the cause and treatment of peritumoral cerebral edema.

References

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