Vascular permeability induced by protein product of malignant brain tumors: inhibition by dexamethasone

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Serum-free conditioned medium derived from confluent monolayer cultures of malignant human astroglial tumors contains a substance that rapidly increases capillary vascular permeability after intradermal injection into guinea pigs. Accumulation of vascular permeability factor (VPF) activity occurs with increasing duration of tumor incubation in vitro. Expression of this activity is inhibited by incubation of cell cultures with cycloheximide or dexamethasone. This VPF is an acid-stable heat-labile macromolecule that is inactivated by trypsin and pepsin and binds immobilized heparin. Activity is retained by ultrafiltration with 30,000-dalton cut-off microconcentrators. Pretreatment of test animals with systemic dexamethasone prior to intradermal injection of VPF diminishes microvascular permeability. Furthermore, VPF activity is not inhibited by antihistamines. Secretion of VPF may cause the vasogenic brain edema that is frequently associated with malignant primary and metastatic intracerebral tumors. Inhibition by dexamethasone of both VPF expression in tissue culture, and VPF activity at the microvascular level in test animals, is in keeping with the known efficacy of this agent in treating the vasogenic edema associated with brain tumors.

KEY WORDS • brain neoplasm • vascular permeability factor • astrocytoma • glioblastoma

Cerebral edema is a significant cause of the neurological deficits and elevated intracranial pressure associated with malignant brain tumors. This form of cerebral edema is produced by disruption of the blood-brain barrier with increased vascular permeability and excessive interstitial fluid accumulation. Prior investigations into the origin of tumor-induced increased cerebrovascular permeability implicate biochemical mediators, structural alterations in tumor-induced blood vessels, destruction of the cerebral capillary endothelium, increased capillary hydrostatic pressure, and nonspecific tumor secretory products. Glucocorticoids such as dexamethasone effectively reduce neurological deficits and intracranial hypertension associated with peritumoral brain edema. The pharmacological basis for their efficacy is, however, largely empirical.

The purpose of this study was to investigate the possibility that brain tumors produce and release a specific substance that evokes cerebral edema by increasing vascular permeability. To accomplish this, conditioned medium of confluent monolayer cultures derived from a variety of human brain tumors was harvested and the Miles assay was used to measure capillary permeability in normal skin following intradermal injection of test substances.

Materials and Methods

Tissue Culture Techniques

Cells derived from tissue explants of human brain tumors were cultured at 37°C in 95% room air/5% CO₂ and 100% humidity in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% L-glutamine, 1% penicillin (10,000 U/ml), 1% streptomycin (10,000 µg/ml), and 10% fetal calf serum. Confluent cultures grown in Falcon tissue-culture flasks (175-sq cm growth area) were washed three times with Hank's balanced salt solution without the addition of calcium, magnesium, or phenol red, and incubated with 25 ml of serum-free DMEM for 24 hours. Flasks typically contained 2 × 10⁷ to 4 × 10⁷ cells. The washing process was then repeated, and conditioned serum-free DMEM was collected 5 to 7 days later and either 1) assayed for vascular permeability factor (VPF) activity, 2) concentrated fivefold by vacuum dialysis using collodion mem-
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branes with a 25,000-molecular weight (MW) cut-off, or 3) concentrated 20- to 40-fold by wet-tubing dialysis (MW cut-off 25,000) against 0.05 molar ammonium bicarbonate titrated to pH 7.4 with glacial acetic acid. The medium was then subjected to shell freezing in super-cooled acetone and lyophilization for 48 hours. The lyophilized product was then reconstituted using Dulbecco’s phosphate-buffered saline (PBS) without calcium or magnesium and was filtered through a 0.45-µ membrane; it was then subjected to assay for VPF activity or biochemical characterization steps. Flasks of confluent cells were subcultured every 5 to 20 days as necessary using standard procedures.

Miles Assay

The Miles assay is a biological assay of induction of capillary permeability in normal skin following the intradermal injection of test substances. Modifications of the original assay were used to more precisely quantify VPF activity. After induction of anesthesia with diethyl-ether, previously shaved male Hartley strain guinea pigs, each weighing 450 to 500 gm, were given an intracardiac injection of 1% Evans blue dye and 10 µCi (2.22 × 10⁶ disintegrations/min) of iodine-125-labeled bovine serum albumin (125I-BSA) in a total volume of 1.0 ml. Test samples (0.1 ml) of conditioned medium from benign and malignant human brain tumors, fibroblasts, and control substances were then injected intradermally using No. 30 needles. All samples were tested in triplicate. Histamine standards (1.0 µg) were included when indicated.

Test samples that induce cutaneous vascular permeability cause a visible blue stain at the injection site due to extravasation of the circulating Evans blue dye. After 20 minutes the animals were exsanguinated, and the injection sites were excised and counted on a Beckman Gamma 4000 counter to measure 125I-BSA extravasation. The tissue tags were then allowed to dry for 5 days, after which they were weighed. Activity of VPF was expressed as counts per minute per milligram dry-tissue weight (cpm/mg). Activity of un.injected (blank) skin tags was subtracted as background to provide values for the increase in vascular permeability induced by the sample.

Brain Tumor-Derived Vascular Permeability Factor Studies

Cycloheximide Study. Tissue culture medium derived from malignant glial tumors was assayed after 12 hours of incubation with cycloheximide (20 µg/ml). Control studies were performed on plain DMEM, conditioned medium from 12-hour cultures not exposed to cycloheximide, and plain DMEM to which cycloheximide was added just before intradermal injection of the test substances.

Dexamethasone Studies. Tissue culture medium derived from malignant glial tumors was assayed after incubation for 40 and 72 hours with graded concentrations of dexamethasone (10⁻⁵, 10⁻⁷, 10⁻⁹, and 10⁻¹¹ M). Controls included 40- and 72-hour preparations of medium not exposed to dexamethasone and conditioned medium to which similar concentrations of steroid were added just before intradermal injection of the test substances. To determine the effect of pretreatment of the animals with systemic glucocorticoid on VPF activity, dexamethasone (2 mg/kg) was administered intraperitoneally 6 hours and 1 hour before intradermal injection of the test substances. Control animals received an intraperitoneal injection of an equivalent volume of Dulbecco’s PBS.

Pyrilamine/Cimetidine Study. To determine if the VPF acted via a pathway in common with histamine, guinea pigs were pretreated with pyrilamine (5 µmole/kg, subcutaneously) and cimetidine (500 µmole/kg, intraperitoneally) prior to intradermal injection of the test substances.

Experiments to Determine Physical Characteristics of VPF

Separate solutions containing lyophilized conditioned medium in Dulbecco’s PBS were treated with heat (95°C for 20 minutes), acidification (pH 7.4 to 3.0 for 10 minutes followed by titration to pH 7.4), and trypsinization (incubation at 37°C with 1.0 mg trypsin/ml VPF solution) followed by neutralization of trypsin with soybean trypsin inhibitor. Peptidic degradation was achieved by acidifying the 1.0 mg/ml pepsin-VPF mixture to pH 3.4 and incubating at 37°C for 30 minutes followed by retitration to pH 7.4 to inactivate the pepsin’s proteolytic capability. Soybean trypsin inhibitor (1000 µg/ml) was also coinjected with conditioned medium to determine whether VPF activity was related to Hageman factor activation (clotting Factor XIIa).

Results

Conditioned medium from low-passage confluent monolayer cell cultures of human malignant astrocytoma (glioblastoma multiforme) lines evoked increased microvascular permeability, whereas medium from benign astrocytoma, meningioma, and fibroblasts had little or no activity (Table 1). Fluid aspirated from a cystic glioblastoma had very high VPF activity, whereas no activity was evident in samples of cerebrospinal fluid from a normal volunteer, a patient with a sacral chordoma, or a patient with a malignant cerebral glioma (Table 1).

Induction of capillary permeability by glioblastoma...
TABLE 1

Expression of vascular permeability factor (VPF) activity*

<table>
<thead>
<tr>
<th>Source of Test Sample</th>
<th>VPF Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment A</td>
<td></td>
</tr>
<tr>
<td>control (DME)</td>
<td>2.4 ± 4.0</td>
</tr>
<tr>
<td>fibroblast</td>
<td>7.4 ± 4.3</td>
</tr>
<tr>
<td>meningioma 1</td>
<td>5.9 ± 3.2</td>
</tr>
<tr>
<td>meningioma 2</td>
<td>7.7 ± 4.0</td>
</tr>
<tr>
<td>astrocytoma</td>
<td>15.2 ± 5.8</td>
</tr>
<tr>
<td>glioblastoma 1</td>
<td>25.7 ± 3.9</td>
</tr>
<tr>
<td>glioblastoma 2</td>
<td>50.3 ± 12.9</td>
</tr>
<tr>
<td>Experiment B</td>
<td></td>
</tr>
<tr>
<td>control (DPBS)</td>
<td>13.3 ± 7.9</td>
</tr>
<tr>
<td>CSF (normal volunteer)</td>
<td>7.2 ± 5.5</td>
</tr>
<tr>
<td>CSF (sacral chordoma)</td>
<td>8.6 ± 5.1</td>
</tr>
<tr>
<td>CSF (malignant glioma)</td>
<td>5.1 ± 3.9</td>
</tr>
<tr>
<td>cyst fluid (glioblastoma) 1</td>
<td>365.2 ± 80.6</td>
</tr>
<tr>
<td>cyst fluid (glioblastoma) 2</td>
<td>643.3 ± 16.6</td>
</tr>
</tbody>
</table>

*The VPF activity for all Miles assays is expressed as mean counts per minute/mg tissue ± standard error of the mean. Experiment A: VPF activity in conditioned medium from human tissue lines; Experiment B: VPF activity in cerebrospinal fluid (CSF) and cystic glioblastoma fluid. DME = Dulbecco's modified Eagle's medium; DPBS = Dulbecco's phosphate-buffered saline.

Discussion

Several substances which increase the permeability of normal blood vessels have been derived from normal and tumorous tissues. Previously described VPF's include histamine (MW 111), serotonin (MW 222), leukotriene (MW 336 to 522), prostaglandin (MW 350 to 355), prostacyclin (MW 352), thromboxane (MW 352 to 370), bradykinin (MW 1060), kallidin (MW 1188), leukokin (MW 2500), lymphocyte permeability factors (MW 12,000 and 39,000), and kallikrein (MW 108,000). We have demonstrated that human malignant gliomas produce and release a substance in vitro that rapidly induces microvascular permeability in the Miles assay with onset of activity by 1 to 2 minutes, no significant effect upon VPF activity, whereas heating attenuated activity by 46% to 67%. Exposure of brain tumor-derived VPF to polyclonal immunoglobulin G raised against the VPF derived from guinea pig line 10 tumor eliminated induction of vascular extravasation. Furthermore, both factors have been shown to bind heparin-Sepharose (data not shown).

Fig. 1. Histogram showing induction of vascular extravasation of 125I-bovine serum albumin as a function of cell culture incubation time for glioblastoma line 1 samples. Vascular permeability factor activity is expressed as mean counts per minute (CPM)/mg + 1 standard error of the mean (SEM).
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Physical and chemical characterization of VPF*

<table>
<thead>
<tr>
<th>Sample Identification</th>
<th>VPF Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
</tr>
<tr>
<td>control (DPBS)</td>
<td>0</td>
</tr>
<tr>
<td>conditioned medium</td>
<td>56.9 ± 8.4</td>
</tr>
<tr>
<td>conditioned medium (heat)</td>
<td>26.3 ± 3.5</td>
</tr>
<tr>
<td>conditioned medium (acid)</td>
<td>48.9 ± 0.9</td>
</tr>
<tr>
<td>conditioned medium (SBTI)</td>
<td>73.7 ± 8.6</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
</tr>
<tr>
<td>control (DPBS)</td>
<td>0</td>
</tr>
<tr>
<td>conditioned medium (trypsin)</td>
<td>195 ± 10.0</td>
</tr>
<tr>
<td>conditioned medium (pepsin)</td>
<td>3 ± 0.4</td>
</tr>
</tbody>
</table>

* Vascular permeability factor (VPF) activity for all Miles assays is expressed as mean counts per minute/mg tissue ± standard error of the mean. Experiment 1: exposure of conditioned medium from glioblastoma line 1 to heat, acid, and soybean trypsin inhibitor (SBTI); Experiment 2: proteolytic digestion of VPF. DPBS = Dulbecco's phosphate-buffered saline.

Cerebral edema is associated with many primary and metastatic malignant brain tumors as well as with certain benign tumors. The mechanism of this "vasogenic" brain edema derives from a physiological and ultrastructural alteration in the blood-brain barrier. This results in increased microvascular permeability and subsequent accumulation of a plasma ultrafiltrate in the cerebral interstitial space. Our findings indicate that malignant glial tumors produce and release a substance that causes increased permeability in normal cutaneous capillaries. Furthermore, the production or secretion of VPF in vitro, as well as its action in vivo, is inhibited substantially by dexamethasone. The elaboration of the VPF may be responsible for the peritumoral cerebral edema frequently associated with malignant brain tumors. Suppression of this factor's production and its microvascular effects by dexamethasone suggests an explanation for the efficacy of glucocorticoids in symptomatic brain edema associated with cerebral neoplasms. This is consistent with the occurrence of steroid receptors in cerebral tumors and the lack of steroid efficacy with ischemic, toxic, hemorrhagic, and traumatic cerebral edema.

Acknowledgments

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