Tumor-associated neurological dysfunction prevented by lazarois in rats

WESLEY A. KING, M.D., KEITH L. BLACK, M.D., KIVONOBU IKEZAKI, M.D., SCOTT CONKLIN, B.S., AND DONALD P. BECKER, M.D.

Brain Research Institute, Jonsson Cancer Center, and Division of Neurosurgery, University of California School of Medicine, Los Angeles, California

The efficacy of U-74006F and U-78517F in the treatment of blood-tumor barrier permeability and tumor-associated neurological dysfunction was evaluated in a brain-tumor model in rats. U-74006F is a 21-aminosteroid and U-78517F is a 2-methylamino chroman. Rats with stereotactically implanted Walker 256 tumors were treated with methylprednisolone, U-74006F, U-78517F, or vehicle (0.05 N HCl) on Days 6 through 10 following implantation. Neurological function and vascular permeability were assessed on Day 10. Methylprednisolone and U-74006F were equally effective at preventing neurological dysfunction compared to the control group (p < 0.01); U-78517F was slightly less effective than U-74006F and methylprednisolone but was significantly better than vehicle in preventing neurological dysfunction. Delivery of methylprednisolone resulted in a significant decrease in tumor vascular permeability (p < 0.006) while U-74006F and U-78517F had no effect on permeability. This suggests that U-74006F and U-78517F prevented tumor-associated neurological dysfunction by a mechanism other than decreasing permeability in tumor capillaries, and that U-74006F or U-78517F could prove useful in the treatment of brain tumors.

KEY WORDS • blood-brain barrier • brain neoplasm • glucocorticoid • lazarois • lipid peroxidation • methylprednisolone • rat

VASOGENIC edema is a major cause of neurological decline in patients with brain tumors. The mechanism of edema production is unknown, although a number of physical and biochemical factors have been implicated.2,24 Glucocorticoids are routinely used in treating peritumoral edema but, as yet, the mechanism for their beneficial effects is not fully understood.

The synthetic glucocorticoid, methylprednisolone, has been studied in models of central nervous system (CNS) trauma, ischemia, and cerebral edema. It has been shown to have numerous biochemical actions, including the ability to alter metabolic states, decrease inflammatory response, and inhibit lipid hydrolysis and peroxidation. In high doses, this agent has demonstrated a protective role in experimental spinal injury, cerebral trauma, and following aneurysmal subarachnoid hemorrhage (SAH).8,10-12,24 Methylprednisolone will also effectively attenuate tumor-associated vasoergic edema. Unfortunately, treatment with methylprednisolone is complicated by the well-known glucocorticoid and hormonal side effects.

Recently, two novel series of potent inhibitors of iron-catalyzed lipid peroxidation, collectively known as "lazarois," were developed.6,9 The first series are the 21-aminosteroids which lack glucocorticoid, mineralocorticoid, or other hormonal activities. One of these compounds, U-74006F, has been found effective in treating experimental spinal cord injury, spinal trauma, concussive head injury, and cerebral ischemia.6,7,13,16-20 It has also been found efficacious in primate and rabbit models of vasospasm following SAH.22,23,25 The second series are the 2-methylamino chromans (U-78517F, for example) which are even more potent antioxidants than the 21-aminosteroids and appear to possess equivalent cerebroprotective activity.15 In the present study, a rat tumor model was used to study the effect of iron-dependent lipid peroxidation inhibition with U-74006F and U-78517F on vascular permeability and tumor-associated neurological deterioration.

Materials and Methods

Laboratory Preparation

Walker 256 carcinomas were used as an experimental metastatic tumor model. Tumor cells were
TABLE 1

<table>
<thead>
<tr>
<th>Grade</th>
<th>Criteria</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>asymptomatic</td>
</tr>
<tr>
<td>2</td>
<td>minimally symptomatic, no hemiparesis</td>
</tr>
<tr>
<td>3</td>
<td>moderately symptomatic, mild hemiparesis</td>
</tr>
<tr>
<td>4</td>
<td>markedly symptomatic, moderate or marked hemiparesis</td>
</tr>
<tr>
<td>5</td>
<td>moribund</td>
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</table>

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maintained as a suspension in tissue culture using Ham’s F-12 nutrient medium with 10% fetal calf serum at 37°C and 5% CO2 in a humidified incubator. Twenty-five female Wistar rats, weighing 80 to 100 gm each, received 1 × 10⁷ viable Walker 256 cells stereotactically implanted to a depth of 4 mm into the left hemisphere. On the 6th day following implantation, rats were randomly assigned to four treatment groups. The rats received vehicle, methylprednisolone, U-74006F, or U-78517F. The compounds were dissolved in 0.05 N HCl in phosphate-buffered saline (pH 3.0). Solutions were prepared fresh prior to administration and the injection volume was held constant at 0.2 ml per rat. Each rat received glucocorticoid, aminosteroid, or methylamino chroman in a dose of 10 mg/kg given as an intraperitoneal injection in a twice-daily dosage. The vehicle control group received 0.2 ml of 0.05 N HCl. Treatment was continued until Day 10 when the morning dose was given prior to permeability testing and histological examination.

Neurological Evaluation

Neurological function was rated according to the criteria in Table 1. Asymptomatic rats were given a rating of Grade 1. Those minimally symptomatic (Grade 2) displayed no hemiparesis, but had diminished spontaneous activity or decreased grooming. Grade 3 rats had at least a mild hemiparesis, but were able to right themselves rapidly when placed on their sides. Rats rated as Grade 4 had moderate or marked hemiparesis and righted slowly. A rating of Grade 5 was given to those rats that appeared frankly moribund.

Vascular Permeability

Permeability studies were performed on rats with a neurological rating of Grade 4 or better. On Day 10 following tumor implantation, animals were anesthetized with ketamine (50 mg/kg) and xylozine (0.8 mg/kg) given intramuscularly. Femoral vessels were cannulated and physiological parameters monitored. Body temperature was maintained at approximately 37°C with a heating pad. A bolus of ¹⁴C-labeled α-aminoisobutyric acid (¹⁴C-AIB) (100 μCi/kg) was injected into a femoral vein. Arterial blood was continuously withdrawn for 10 minutes for plasma radioactivity determination.

Rats were killed by decapitation 10 minutes after the ¹⁴C-AIB bolus; the brain was rapidly removed and frozen in isopentane (−40°C). Sections of the frozen brains 20 μ thick were cut in a cryomicrotome, placed on coverslips, dried rapidly on a warming plate at 65°C, mounted on paper, and exposed to x-ray film for 14 days. Tissue radioactivity in regions of interest was determined using computerized densitometry on the autoradiographs, and a vascular permeability constant (Kv) was calculated by the method of Blasberg, et al., and expressed in ml/gm/min. Maximum tumor diameter was measured by staining slides with thionin following autoradiographic exposure.

Data were expressed as means ± standard error of the means. Statistical analysis was performed using Student’s t-test, with a probability of 0.05 or less being considered significant.

Results

Regardless of the experimental group, there was no significant difference between levels of blood pO2, pCO2, pH, temperature, or hematocrit among the rats.

Neurological Function

The neurological function in the rat groups 10 days after tumor implantation is presented in Table 2. Of the seven rats in the control group treated with vehicle only, two rats remained asymptomatic while the rest were moderately symptomatic or worse. Six rats were tested in each of the other treated groups. Methylprednisolone, U-74006F, and U-78517F significantly prevented neurological dysfunction compared to controls (p < 0.01 for methylprednisolone and U-74006F and p < 0.03 for U-78517F). All rats in the methylprednisolone group and those treated with U-74006F were without neurological deficits. Four rats in the U-78517F group were asymptomatic while two had minimal symptoms.

Vascular Permeability

Six rats in each group were tested for tumor vascular permeability. The results are presented in Table 3. The methylprednisolone group had a Kc value of 0.24 ± 0.026 ml/gm/min, which was significantly less than that

TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Rats</th>
<th>Grade</th>
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</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>7</td>
<td>28.5%</td>
</tr>
<tr>
<td>vehicle</td>
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<td>100%</td>
</tr>
<tr>
<td>vehicle</td>
<td>6</td>
<td>66.7%</td>
</tr>
</tbody>
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* Mean values are expressed ± standard error of the means. For definition of grades see Table 1. Significance of difference by Student’s t-test vs. vehicle-treated rats: * = p < 0.01; † = p < 0.03.

§ MP = methylprednisolone.
of the vehicle-treated rats (0.351 ± 0.024 ml/gm/min) (p < 0.006). Rats treated with U-74006F and U-78517F had K values of 0.300 ± 0.033 and 0.362 ± 0.022 ml/gm/min, respectively. These values were not significantly different from those of the control animals.

Mean tumor size is listed in Table 4. The average maximum tumor diameter in the vehicle-treated group was 6.25 ± 0.214 mm. This was significantly larger (p < 0.001) than the mean tumor size in the groups treated with methylprednisolone, U-74006F, and U-78517F, which were 2.67 ± 0.167, 3.75 ± 0.403, and 4.17 ± 0.380 mm, respectively.

Discussion

Peritumoral Edema

The pathophysiology of brain edema associated with tumors is unknown, although there is likely an interplay of both structural and biochemical factors. A number of potential mediators of edema formation have been identified.2,3,24 These include various amino acids, biogenic amines, members of the kallikrein-kinogen-kinin system, arachidonic acid, leukotrienes, and free radicals. Release of free fatty acids by phospholipid hydrolysis has been shown in tissue surrounding cerebral neoplasm.2 Arachidonic acid, which is the most abundant free fatty acid in brain, is then oxidized to a group of biologically active compounds collectively known as eicosanoids. These include the prostaglandins, thromboxanes, leukotrienes, and prosta cyclin. A highly reactive free radical superoxide anion can also be produced following membrane release of arachidonic acid.

Free radicals have long been suggested to participate in the membrane changes responsible for cerebral edema. Free radical formation is a chemical reaction that occurs following many types of tissue injury, especially that due to irradiation. Molecular oxygen is particularly susceptible to becoming a radical, which can then interact with lipids forming reactive liperoxides. This latter reaction is catalyzed by iron or copper complexes. Peroxidative products of arachidonic acid may interfere with membrane functional integrity. This in theory could result in both vasogenic and cytotoxic edema. The exact role of free radicals in brain edema formation is not thoroughly understood.

Peritumoral edema is a major cause of acute neurological decline in patients with intraparenchymal brain neoplasms. Routine use of synthetic glucocorticoids has been the mainstay of effective treatment of vasogenic edema. Steroids have many biochemical actions and they likely decrease brain edema by a variety of mechanisms. Possible mechanisms include the ability of glucocorticoids to decrease arachidonic acid release by inhibiting the enzyme phospholipase A2 and their ability to inhibit lipid peroxidation.4

Lazaroids

Recently, a group of nonglucocorticoid steroids, termed “lazaroids,” have been developed which are known to be potent inhibitors of iron-dependent lipid peroxidation. The nature of the anti-lipid peroxidation action is considered to include an α-tocopherol-like membrane antioxidant action, scavenging of superoxide anions, and possibly a membrane-localized iron-chelating effect. Earlier work has demonstrated the ability of these compounds to attenuate the effects of posttraumatic spinal cord ischemia in rats, experimental head injury in mice, and spinal cord injury. Chronic cerebral vasospasm following SAH may also be reduced with U-74007F.

This study demonstrates the ability of the 21-amino steroid U-74006F and the 2-methylamino chroman U-78517F to prevent neurological dysfunction in rats with experimental brain tumors. Results showed that U-74006F was as effective as methylprednisolone, and that U-78517F was slightly less effective than U-74006F. In our experiments, methylprednisolone significantly decreased vascular permeability within the tumors, which is in agreement with the findings of others. The lazaroids, on the other hand, showed no effect on the vascular permeability constants. Hence, the mechanism of action of these compounds must be at least in part separate from reducing vascular permeability within tumors. Perhaps they affect intracellular water content.

Tumor size was also decreased following glucocorticoid and lazaroid administration. This too could explain the improved neurological improvement observed. Steroids have previously been implicated as playing a role in inhibiting cerebral neoplasm metabolism. This has been investigated most extensively in
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meningiomas, although glioma growth (at least in culture) may also be inhibited by steroid administration. Megyesi and coworkers\(^1\) found no significant decrease in tumor growth or vascular permeability in an experimental astrocytoma model following U-74006F treatment. Whether the decrease we found in the tumor size is secondary to decreased water content within tumors or to altered tumor growth should be further examined.

The beneficial effects of lazaroids could be attained by other mechanisms in addition to their iron-dependent lipid peroxidation inhibition capabilities. Like conventional steroids, these compounds may affect multiple biochemical pathways. For example, U-74006F inhibits iron- or iodoacetate-induced membrane release of arachidonic acid in cultured cells.\(^6\) Other theoretical mechanisms could include a reduction in interstitial edema by reducing cellular secretory activity or increasing interstitial resistance to bulk flow of edema. Although the mechanism of reduced neurological dysfunction with lazaroids remains unclear, current findings suggest that further study of these compounds in the management of CNS tumors is warranted.

References


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Address reprint requests to: Wesley A. King, M.D., Division of Neurosurgery, 74-140 CHS, UCLA School of Medicine, 10833 Le Conte Avenue, Los Angeles, California 90024.