The effect of 5-lipoxygenase inhibition on blood-brain barrier permeability in experimental brain tumors

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To determine if leukotrienes are important mediators of vascular permeability in brain tumors, the effect of 5-lipoxygenase inhibitors on blood-tumor barrier permeability in rats harboring HK Walker 256 brain tumors was examined using quantitative autoradiography with \( \alpha^{-14} \)C-aminoisobutyric acid. The 5-lipoxygenase enzyme converts arachidonic acid to leukotrienes. Three 5-lipoxygenase inhibitors were utilized: BW755C, nordihydroguaiaretic acid, and AA-861. All three 5-lipoxygenase inhibitors significantly decreased vascular permeability both within the tumors and in brain adjacent to tumor. This suggests that capillary permeability in and adjacent to tumors is influenced by endogenous leukotrienes and that leukotrienes play an important role in brain tumor edema.

KEY WORDS - blood-brain barrier - brain neoplasm - leukotriene - lipoxygenase - rat

We have demonstrated previously that leukotrienes \( \text{C}_4 \) (LTC\(_4\)) infused into the carotid artery ipsilateral to an experimental gial tumor will increase the unidirectional transfer constant for permeability (K\(_{\text{u}}\)) within the tumor twofold, while no effect on permeability is seen in normal brain. Intracarotid infusion of LTC\(_4\) also selectively increases blood-brain barrier (BBB) permeability within ischemic brain tissue in rats. A significant correlation between vasogenic brain edema surrounding brain tumors and LTC\(_4\) levels in tumor tissue has been shown, as well as high-affinity binding sites for LTC\(_4\) on isolated brain capillaries. These observations have led us and others to speculate that leukotrienes could be important mediators of capillary permeability in pathological conditions in which the ability of brain capillaries to inactivate LTC\(_4\) is impaired.

Since leukotrienes are oxidized products of arachidonic acid through the 5-lipoxygenase pathway, we sought in this study to determine whether inhibition of the 5-lipoxygenase enzyme would decrease BBB permeability in brain tumors. The inhibitory agent BW755C primarily acts on 5-lipoxygenase but also has some effects on cyclooxygenase, which metabolizes arachidonic acid to prostaglandins. Nordihydroguaiaretic acid (NDGA) and AA-861 selectively inhibit 5-lipoxygenase but not cyclooxygenase. Here we report on the effects of these three 5-lipoxygenase inhibitors on the blood-tumor barrier permeability in rats harboring HK Walker 256 brain tumors and discuss the biological implications.

Materials and Methods

Twenty-eight female Wistar rats, each weighing 110 to 150 gm, were used for this study. Prior to intraperitoneal administration in the rats, BW755C, NDGA, and AA-861 were dissolved in cottonseed oil and prepared as an emulsion (10% oil) in 0.8% polyoxyethylene sorbitan mono-oleate (TWEEN 80) phosphate-buffered saline (PBS). Alpha-[\( \text{I}^{-14} \)C]-aminoisobutyric acid (AIB) (50.0 mCi/mmol) was used for quantitative autoradiographic studies.

Tumor Inoculation

The HK Walker 256 tumor cells were maintained in F-12 medium with 10% calf serum. The rats were anesthetized with intraperitoneal pentobarbital sodium (Nembutal, 40 mg/kg). Tumor cells (5 \( \times \) 10\(^3\)) in a 5-\( \mu \)l solution were stereotactically injected by means of a Hamilton syringe into the right cerebral hemisphere through a burr hole 5 mm lateral to the bregma and 5 mm vertically deep to the dural surface.

5-Lipoxygenase Experiments

The rats were divided into four groups and given

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* BW755C was a gift from Wellcome Reagents Ltd., Beckenham, England; NDGA was supplied by Sigma Chemical Co., St. Louis, Missouri; AA-861 was a gift from Takeda Medical Co., Osaka, Japan.
+ Alpha-[\( \text{I}^{-14} \)C]-aminoisobutyric acid supplied by New England Nuclear, Boston, Massachusetts.
† F-12 medium and 10% calf serum obtained from Gibco, Grand Island, New York.
intrapertoneal injections of either BW755C (10 mg/kg), NDGA (1.2 mg/kg), AA-861 (20 mg/kg), or cottonseed oil (10%) emulsion in 0.8% Tween 80 PBS (vehicle control). The injections were administered every 8 hours beginning 3 days after tumor implantation. Six days after tumor implantation and 2 to 4 hours after the last intrapertoneal drug administration, the rats were again anesthetized and both femoral arteries and one femoral vein were cannulated. Body temperature was maintained at 37°C, and arterial blood gas levels, blood pressure, and hematocrit were monitored. Animals with abnormal physiological parameters were eliminated from the study. An intravenous bolus of 14C-AIB (100 μCi/kg) was injected, and a peristaltic withdrawal pump was used to obtain femoral arterial blood at a constant rate of 0.083 ml/min immediately after injection of 14C-AIB for determination of serum radioactivity. Ten minutes after the injection of 14C-AIB, the animals were killed by decapitation and the brains were rapidly removed and frozen.

**Autoradiography**

The frozen rat brains were mounted onto pedestals with M-1 embedding matrix, and 20-μm coronal sections were cut using a cryotome. The sections were thaw-mounted onto coverslips and autoradiograms were generated by coexposing the sections on Kodak XAR-5 film with tissue-calibrated 14C standards* for 2 weeks. After exposure, the sections were stained with hematoxylin and eosin to correlate areas of histologically verified tumor with autoradiograms. Quantitative analysis was performed using a computer-assisted digital image analyzer.18 A unidirectional blood-to-brain transfer constant (K) was calculated using the method of Blasberg, et al., and was expressed in μl/gm/min.

**Definition of Regions**

For K, measurements, the tumor periphery was defined as the outer half of the tumor radius, the tumor center as the inner half of the tumor radius, and brain adjacent to tumor as an area of the adjacent basal ganglia in the vascular territory of the lenticular striate vessels.

**Data Analysis**

The K, values for the experiments were calculated by measuring the three regions of interest described above in three consecutive sections. Analysis of variance and unpaired Student’s t-test were applied to the mean values from separate experiments. Mean values are expressed ± standard deviation.

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* Pump manufactured by Gibson Medical Electronics, Inc., Middleton, Wisconsin.  
† M-1 embedding matrix supplied by Lipshaw Manufacturing, Detroit, Michigan.  
* 14C standards obtained from Amersham Corp., Arlington Heights, Illinois.

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T. Baba, C. C. Chio, and K. L. Black

**Results**

**Physiological Status**

Arterial blood gas levels were measured in all rats used in the experiments. The mean pH, PaCO2, and PaO2 were 7.432 ± 0.028, 38.4 ± 3.6 mm Hg, and 108.2 ± 19.1 mm Hg, respectively. The mean arterial blood pressure was 109.4 ± 9.2 mm Hg and hematocrit was 32.6% ± 3.9%. There was no statistically significant difference in these physiological parameters among the four rat groups.

**Brain Tumor Size**

The size of the rat brain tumors was measured in the section with the largest tumor area. The average size was 7.9 ± 2.7 sq mm, and there was no statistically significant difference among the four rat groups.

**BBB Permeability**

The mean BBB permeability (K) in the seven rats in the control group was 300.9 ± 87.7 μl/gm/min in the tumor center, 212.9 ± 60.1 μl/gm/min at the tumor periphery, and 45.1 ± 12.1 μl/gm/min in brain adjacent to tumor. Administration of BW755C decreased the mean K, value to 174.9 ± 79.2, 98.7 ± 35.2, and 22.3 ± 8.0 μl/gm/min in the tumor center, tumor periphery, and brain adjacent to tumor, respectively. These changes were all significant compared to the control group (p < 0.005, p < 0.001, and p < 0.001, respectively). A mean significant decrease in the K, value after NDGA administration was also noted compared to the control group (173.3 ± 39.3 (p < 0.005), 113.0 ± 39.4 (p < 0.001), and 19.9 ± 6.5 μl/gm/min (p < 0.001) in the tumor center, tumor periphery, and brain adjacent to tumor, respectively). Delivery of AA-861 also decreased the mean K, value to 214.4 ± 55.3, 129.8 ± 33.0, and 26.2 ± 13.0 μl/gm/min in the tumor center, tumor periphery, and brain adjacent to tumor, respectively. These differences were also statistically significant compared to the control group (p < 0.05, p < 0.005, and p < 0.005, respectively). The mean K, value in the extratumoral normal tissue of the control group was 3.8 ± 4.0 μl/gm/min in the ipsilateral frontal cortex, 3.8 ± 4.0 μl/gm/min in the contralateral frontal cortex, 3.8 ± 4.0 μl/gm/min in the ipsilateral basal ganglia, and 4.2 ± 3.7 μl/gm/min in the contralateral basal ganglia. These mean values were 2.2 ± 2.9, 3.1 ± 3.0, 4.6 ± 5.4, and 3.1 ± 3.0 μl/gm/min, respectively, in the BW755C-treated group; 1.1 ± 2.9, 1.1 ± 2.9, 2.5 ± 3.3, and 1.1 ± 2.9 μl/gm/min, respectively, in the NDGA-treated group; and 2.2 ± 3.3, 2.2 ± 3.3, 2.9 ± 3.6, and 2.6 ± 3.3 μl/gm/min, respectively, in the AA-861-treated group. There were no significant differences among these groups. These data are summarized in Fig. 1.

**Discussion**

Leukotrienes are powerful metabolites of arachidonic acid which have been proposed to promote vascular permeability.5,6,13,17,19 A correlation between vasogenic
Lipoxygenase inhibition in brain tumors

Fig. 1. Graph showing the effect of the 5-lipoxygenase inhibitors BW755C (10 mg/kg), nordihydroguaiaretic acid (NDGA, 1.2 mg/kg), and AA-861 (20 mg/kg) on permeability (K_i) values in HK Walker 256 tumor-implanted rat brains. Values are expressed as the mean ± standard deviation. Abbreviations: tumor peri = tumor periphery; BAT = brain adjacent to tumor; n = number of rats in each group. Statistical significance: * = p < 0.05; ** = p < 0.005; *** = p < 0.001, compared to the control group.

edema surrounding brain tumors and the level of LTC_4 in tumor tissue has been shown, as well as high-affinity binding sites for LTC_4 on isolated brain capillaries. We also demonstrated that LTC_4 infused into the carotid artery ipsilateral to experimental glia tumors in rats could selectively increase permeability within the tumor twofold, without increasing permeability in normal brain. However, LTC_4 markedly increases vascular permeability in a variety of systemic capillary beds. Normal brain capillaries, unlike systemic capillaries, appear to resist the vasoactive effects of leukotrienes.

Gamma Glutamyl Transpeptidase

Gamma glutamyl transpeptidase (γ-GTP) is an enzyme that inactivates LTC_4, LTD_4, and LTE_4 to LTF_4. Both γ-GTP and aminopeptidase (an enzyme that inactivates LTD_4 to LTE_4) are enzymes unique to brain capillaries and are not present in systemic capillaries. Unlike normal brain capillaries, which are rich in γ-GTP, tumor capillaries appear to lack γ-GTP. This led us to speculate that normal brain capillaries might use γ-GTP as an enzymatic barrier to inactivate LTC_4, which is a potent mediator of vascular permeability in systemic capillaries, capillaries that lack γ-GTP. Interestingly, LTC_4 slightly increased BBB permeability in brain adjacent to tumors where γ-GTP was also moderately decreased.

Recently, we have demonstrated that the BBB could be selectively opened by intracarotid infusions of LTC_4 in ischemic tissue 48 to 72 hours after middle cerebral artery occlusion, but it could not be opened in ischemic tissue 24 hours after middle cerebral artery occlusion. We suggested this was due to loss of γ-GTP in ischemic capillaries over time. Almost all γ-GTP activity in brain capillaries is lost in ischemic tissue by 72 hours after occlusion; however, moderate amounts of γ-GTP activity still remain in capillaries of ischemic tissue 24 hours after occlusion.

Leukotrienes and Edema

In the present study, we demonstrated that inhibitors of 5-lipoxygenase will decrease permeability within brain tumors and in brain tissue adjacent to tumors. This suggests that capillary permeability within and adjacent to brain tumors is, at least in part, related to levels of endogenous leukotrienes. After cerebral injury, the leukotriene content is increased.

Increased levels of leukotrienes coupled with increased susceptibility to their vasoactive effects suggest that leukotrienes might play an important role in vasogenic edema in pathological lesions.

Unterberg et al. reported that arachidonic acid itself and not its degradation products are responsible for the induction of barrier opening, and concluded that leukotrienes do not promote brain edema or act as mediators of cerebral edema. Their conclusion is based mainly on their examination of normal brain tissue, where the enzymatic barrier is intact and protecting brain tissue from the vasoactive effects of leukotrienes. Recently, however, they reported that BW755C did not affect brain edema in the cold-induced injury of rabbit brains. Cold-induced injury is a severe form of capillary injury which results in mechanical destruction of brain capillaries. Cold injury is a poor model, therefore, to study how biochemical mediators might influence capillary permeability in pathological conditions such as tumors or ischemia. Their evidence against leukotrienes as mediators of brain edema is true primarily in normal brain tissue where enzymatic barrier is intact. In the pathological conditions that occur clinically, if the enzymatic barrier is damaged, we speculate that leukotrienes are one of perhaps many mediators that influence brain edema.

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References


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