Selective blood-tumor barrier disruption by leukotrienes

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The authors have previously reported that intracarotid infusion of 5 µg leukotriene C₄ (LTC₄) selectively increases blood-tumor barrier permeability in rat RG-2 tumors. In this study, rats harboring RG-2 tumors were given 15-minute intracarotid infusions of LTC₄ at concentrations ranging from 0.5 µg to 50.0 µg (seven rats in each dose group). Blood-tumor and blood-brain barrier permeability were determined by quantitative autoradiography using ³²C aminoisobutyric acid. The transfer constant for permeability (Kₜ) within the tumors was increased twofold by LTC₄ doses of 2.5, 5.0, and 50.0 µg compared to vehicle alone (90.00 ± 21.14, 92.68 ± 15.04, and 80.17 ± 16.15 vs. 39.37 ± 6.45 µl/gm/min, respectively; mean ± standard deviation; p < 0.01). No significant change in Kₜ within the tumors was observed at the 0.5-µg LTC₄ dose. Blood-brain barrier permeability was selectively increased within the tumors. At no dose in this study did leukotrienes increase permeability within normal brain.

To determine the duration of increased opening of the blood-tumor barrier by LTC₄ administration, Kₜ was measured at 15, 30, and 60 minutes after termination of a 15-minute LTC₄ infusion (seven rats at each time point. The mean Kₜ value was still high at 15 minutes (92.68 ± 15.04 µl/gm/min), but declined at 30 minutes (56.58 ± 12.50 µl/gm/min) and 60 minutes (55.40 ± 8.10 µl/gm/min) after the end of LTC₄ infusion.

Sulfidopeptide leukotrienes LTC₄, LTD₄, LTE₄ and LT₄ were infused to compare their potency in opening the blood-tumor barrier. The mean leukotriene E₄ was the most potent, increasing the permeability value 3.5-fold compared with vehicle alone (139.86 ± 23.95 vs. 39.37 ± 6.45 µl/gm/min).

Key Words • leukotriene • brain neoplasm • blood-brain barrier • rat

The blood-tumor barrier within the capillaries of primary brain tumors is considered to be an obstacle to delivering water-soluble antineoplastic agents to brain-tumor tissue.¹,¹⁰,¹²,¹⁷ One approach to brain tumor therapy has therefore focused on methods that open or modify the brain-tumor barrier and increase drug delivery. In a previous study, we demonstrated that leukotriene C₄ (LTC₄) infused into the carotid artery ipsilateral to experimental RG-2 gliomas in rats increased the unidirectional transfer constant for permeability (Kₜ) twofold within the tumors, while no effect on permeability was seen in normal brain.⁶ Based on this observation, we suggested that LTC₄ may be useful clinically in selectively increasing the delivery of antineoplastic agents to tumors. The purpose of this study was to determine the optimal dose and the duration of opening of the blood-tumor barrier by LTC₄ administration. The potencies of various sulfidopeptide leukotrienes (LTC₄, LTD₄, LTE₄, and LT₄) were also compared.

Materials and Methods

Eighty-four female Wistar rats, each weighing 130 to 170 gm, were used for this study. The sulfidopeptide leukotrienes* were obtained at a concentration of 0.1 mg/ml in a 1:1 phosphate-buffered saline (PBS)-ethanol solution. Aliquots of LTC₄, LTD₄, LTE₄, and LT₄ were diluted to experimental concentrations in PBS and the final ethanol concentration was adjusted to 2.5%. Vials containing the leukotrienes were sealed under nitrogen and stored at −80°C. Alpha-[¹⁴C]-aminoisobutyric acid (AIB) (57.6 mCi/mmoll)† was used for quantitative autoradiographic study.

Tumor Inoculation

The RG-2 glioma cells were maintained in a monolayer culture in F-12 medium with 10% calf serum.‡ The rats were anesthetized with intraperitoneal pentobarbital sodium (Nembutal, 40 mg/kg). Tumor cells (5 × 10⁹ in a 5-µl solution) were stereotactically implanted by means of a Hamilton syringe into the right cerebral

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* Leukotrienes supplied by Cayman Chemicals, Ann Arbor, Michigan.
† Alpha-[¹⁴C]-aminoisobutyric acid supplied by New England Nuclear, Boston, Massachusetts.
‡ F-12 medium and 10% calf serum supplied by Gibco, Grand Island, New York.
hemisphere 5 mm lateral to the bregma and 5 mm deep to the dural surface.

**Dose Response Study**

Ten to 11 days after tumor implantation, the rats were again anesthetized and a polyethylene (PE-50) catheter was inserted retrograde through the external carotid artery to the common carotid artery bifurcation ipsilateral to the tumor. The external carotid artery was then ligated, and both femoral arteries and one femoral vein were cannulated. Body temperature was maintained at 37°C. Arterial blood gas levels, blood pressure, and hematocrit were monitored. Animals with abnormal physiological parameters were eliminated from the study. Infusions of LTC₄ at concentrations of 0.5, 2.5, 5.0, and 50.0 μg/0.8 ml or 0.8 ml of vehicle (2.5% ethanol in PBS, pH 7.1) were administered into the right carotid artery of seven rats in each dose group using a constant-infusion pump at a rate of 53.3 μl/min for 15 minutes. Five minutes after the start of the intracarotid infusion, an intravenous bolus of 100 μCi/kg of ¹⁴C-AIB was delivered. A peristaltic withdrawal pump was used to withdraw femoral arterial blood at a constant rate of 0.083 ml/min immediately after injection of ¹⁴C-AIB for determination of serum radioactivity. Fifteen minutes after the start of intracarotid infusions, the animals were killed by decapitation and the brains were rapidly removed and frozen.

**Time Course Study**

Ten to 11 days after tumor implantation, the rats were cannulated and physiological parameters were measured as described above. An intracarotid infusion of 0.8 ml LTC₄ at a dose of 5 μg was delivered for 15 minutes, and Kᵢ was measured at 15, 30, and 60 minutes after the termination of LTC₄ infusion.

**Potency of Leukotrienes**

Various sulfidopeptide leukotrienes (LTC₄, LTD₄, LTE₄, and LTF₄), at a dose of 5 μg, were infused for 15 minutes into seven rats in each leukotriene group and the Kᵢ value was measured as described above.

**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

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§ Catheter manufactured by Clay Adams, Parsippany, New York.

¶ Infusion pump manufactured by Harvard Apparatus, Inc., Milis, Massachusetts.

∗ Withdrawal 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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**Definition of Regions**

For Kᵢ 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 Kᵢ 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.

**Results**

**Physiological Status**

Arterial blood gas levels were measured in all rats used in the experiments. The mean pH, PaCO₂, and PaO₂ were 7.394 ± 0.056, 37.6 ± 5.5 mm Hg, and 96.8 ± 12.2 mm Hg, respectively (± standard deviation). The mean arterial pressure was 132 ± 16.8 mm Hg and the hematocrit was 36.2% ± 6.2%. The physiological parameters were not altered after leukotriene infusion.

**Brain Tumor Size**

The size of the rat brain tumors was measured in the histological section with the largest tumor area. The average size was 9.2 ± 3.1 sq mm, and there was no statistically significant difference among the subgroups.

**Dose Response Study**

An infusion of LTC₄ at a dose of 0.5 μg failed to demonstrate any significant change in mean Kᵢ (± standard error of the mean) within the tumors compared to vehicle (36.99 ± 11.26 vs. 39.37 ± 6.45 μl/gm/min). The mean Kᵢ values in the 2.5-, 5.0-, and 50.0-μg LTC₄ dose groups all increased approximately twofold compared to vehicle alone (90.00 ± 21.14, 92.68 ± 15.04, and 80.17 ± 15.15 vs. 39.37 ± 6.45 μl/gm/min, respectively; p < 0.01). There was no significant mean change in blood-brain barrier (BBB) permeability of the ipsilateral frontal cortex (4.03 ± 4.46, 5.33 ± 3.27, and 3.36 ± 2.44 vs. 3.22 ± 1.97 μl/gm/min), contralateral frontal cortex (3.47 ± 4.72, 3.79 ± 3.65, and 2.61 ± 2.44 vs. 2.84 ± 1.86 μl/gm/min), ipsilateral basal ganglion (4.95 ± 4.15, 4.65 ± 3.73, and 3.82 ± 3.02 vs. 3.11 ± 1.82 μl/gm/min), and contralateral basal ganglion (3.47 ± 4.72, 3.79 ± 3.65, 2.81 ± 2.73 vs. 2.74 ± 2.02 μl/gm/min) (Fig. 1). No acute side effects were seen in the rats, even in those receiving the
Leukotrienes and blood-tumor barrier permeability

FIG. 1. Graph showing permeability ($K_I$) values of various doses of leukotriene C$_4$ intracarotid infusion. The values are expressed as the mean ± standard error of the mean (vertical bars). Abbreviations: peri = periphery; BAT = brain adjacent to tumor; ipsi = ipsilateral; contra = contralateral; Fr Cx = frontal cortex; BGG = basal ganglion; $n$ = number of rats in each dose group; * = statistically significant ($p < 0.01$) compared with vehicle.

The highest dose. An infusion of 50 $\mu$g LTC$_4$ did not, however, elevate the $K_I$ value more than recorded with an infusion of 5 $\mu$g.

Time Course Study

Fifteen minutes after the termination of LTC$_4$ infusion, the mean $K_I$ value remained elevated by almost twofold compared with vehicle alone ($92.68 \pm 15.04$ vs. $39.37 \pm 6.45 \mu$g/min). The mean $K_I$ value significantly declined after 30 and 60 minutes ($56.58 \pm 12.50$ and $55.40 \pm 8.10 \mu$g/min, respectively) compared with that after 15 minutes ($p < 0.01$) (Fig. 2).

Potency of Leukotrienes

All sulfidopeptide leukotrienes (LTC$_4$, LTD$_4$, LTE$_4$, and LTF$_4$) increased the mean $K_I$ value within the tumors significantly compared to vehicle alone ($92.68 \pm 21.14$, $84.19 \pm 22.16$, $139.86 \pm 23.95$, and $100.35 \pm 11.00$ vs. $39.37 \pm 6.45 \mu$g/min, respectively; $p < 0.01$). The most potent was LTE$_4$, which increased the $K_I$ value within the tumors about 3.5-fold (Fig. 3).

Discussion

Leukotrienes are metabolites of arachidonic acid generated via the 5-lipoxygenase pathway. Levels of leukotrienes in brain tissue are increased during postischemic reperfusion and in brain tumors, subarachnoid hemorrhage, and concussive brain injury. We previously showed a correlation between LTC$_4$ levels and brain edema surrounding tumors in man. Gaetani, et al., also demonstrated that perilesional brain tissue has a significant capacity for an ex vivo synthesis of eicosanoids, and the capacity to synthesize LTC$_4$ is significantly correlated with the extent of edema, particularly in cases of neuroepithelial and metastatic tumors. Leukotrienes have also been suggested to be biochemical mediators of ischemic brain edema.

In a prior study, we incorrectly suggested that the intracerebral injection of high doses of leukotrienes in rats increased BBB permeability in normal brain. Intracerebral injections of leukotrienes in these studies did not in fact increase permeability in normal brain; however, permeability did increase under the area where drilling for the burr hole had occurred, suggesting that injury to brain is prerequisite for leukotrienes to increase permeability.

Subsequently, we have demonstrated that intracarotid-
id infusion of LTC4 selectively increases capillary permeability in tumors and ischemic brain but not in normal brain.\(^2,6\) This may be due in part to the fact that normal brain capillaries, unlike systemic capillaries, contain high concentrations of the enzyme gamma glutamyl transpeptidase. This enzyme inactivates LTC4 and LTE\(_4\) and is lost in brain tumor capillaries and in brain capillaries after ischemia.\(^2,6\) In effect, normal brain capillaries, unlike capillaries within brain-tumor tissue or ischemic brain, appear to have an "enzymatic barrier" to protect them against the vasoactive effects of leukotrienes.

Neuwelt and coworkers\(^7-20\) have shown that osmotic barrier disruption with hypertonic mannitol will non-selectively increase the delivery of chemotherapeutic agents and monoclonal antibodies to brain tissue in humans. However, osmotic disruption of the BBB results in a large increase in drug delivery to normal brain.\(^6\) One advantage of leukotrienes selectively opening the blood-tumor barrier is that it leaves the barrier in normal brain intact,\(^6\) protecting normal brain from the adverse effect of antitumor compounds.

This study extends our prior report, which demonstrated the ability of LTC4 to selectively open the blood-tumor barrier. The optimal dose range of LTC4 infusion for increasing permeability in rat RG-2 tumors is 2.5 \(\mu\)g or 5.0 \(\mu\)g. Opening of the blood-tumor barrier is reversible and decreases significantly 30 minutes after termination of LTC4 infusions. Leukotriene E\(_4\) appears to be the most potent sulfidopeptide leukotriene to open the blood-tumor barrier.

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References


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