an immune-mediated mechanism in delayed radiation injury to the brain, principally because of inflammatory infiltrates that are so frequently seen on microscopical examination [8, 10, 11]. Recently, Hart and associates [3] demonstrated that cerebral vasculitis can be produced by transfer of lymphocytes activated in vivo or in vitro after exposure to cerebral capillary endothelial cells. Endothelial cells injured or altered by radiation may also be capable of serving as a source of antigen to initiate a cerebral vasculitis.

Several approaches can be envisioned to define whether immune-mediated mechanisms contribute to the pathogenesis of delayed radiation necrosis. First, human biopsy or autopsy material can be studied immunohistochemically to search for antibody, complement, or immune complex localization. Second, the possibility of an immune contribution should be studied in a more controlled setting in experimental animals, in which models of radiation vasculitis have been developed [7, 11]. If an immune mechanism contributes in any way to the pathogenesis of delayed radiation necrosis, then immunosuppressive therapy may be beneficial in radiation brain injury.

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# Increased Leukotriene C4 and Vasogenic Edema Surrounding Brain Tumors in Humans

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Leukotrienes are pharmacologically active compounds that promote vascular permeability. In this study we sought to determine whether tissue leukotriene–like immunoreactivity was increased in intracranial tumors associated with peritumoral edema. In 20 patients undergoing craniotomy tissue specimens were immediately frozen after removal and tissue leukotriene $C_4$ levels were determined by radioimmunoassay. An index of peritumoral edema was estimated from preoperative contrast-enhanced computed tomographic scans. There was a significant correlation between brain edema and tissue leukotriene levels ($p < 0.003$). Metastatic tumors (n = 8) had the highest leukotriene $C_4$ level at 13.8 ± 8.5 pg/mg tissue (mean ± SE) and the highest index of edema 5.7 ± 1.8. The mean leukotriene $C_4$ level in the gliomas (n = 5) was 6.2 ± 2.3 pg/mg tissue and the edema index was 2.1 ± 0.6. There was no edema and no neoplasm in the temporal lobes removed for seizure (n = 2), and their level of leukotriene $C_4$ was 0.4 ± 0.1 pg/mg tissue. The formation of leukotriene $C_4$ is stimulated by intracranial tumors. Leukotrienes increase blood–brain barrier permeability and may be important in the formation of vasogenic edema surrounding tumors.

Leukotrienes (LTs) are biologically active hydroxy lipids ($LTB_4$) and peptidolipids ($LTC_4$, $D_4$, and $E_4$)

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formed from the unsaturated fatty acid arachidonic acid [14]. Leukotrienes have been shown to increase vascular permeability in postcapillary venules [1, 18], elicit erythema and wheal formation in skin [16], and produce coronary [10] and cerebral [17] vasoconstriction. We recently demonstrated that leukotriene $B_4$, $C_4$, or $E_4$ injected directly into brain parenchyma will significantly increase blood–brain barrier permeability, and we speculated that leukotrienes may be important in the generation of vasogenic brain edema [2, 3]. Since intracerebral neoplasms produce vasogenic brain edema, we sought to determine if leukotrienes could contribute to the development of peritumoral vasogenic edema.

## Materials and Methods

Brain or tumor tissue was obtained from 20 patients undergoing craniotomy. Tissue was obtained from 8 patients with metastatic tumors, 5 patients with gliomas, 4 patients with meningiomas, 1 patient with a schwannoma, and 2 patients undergoing temporal lobectomy for seizures. Patients undergoing temporal lobectomy were considered controls since there was no evidence of neoplasm, infection, or injury on pathological examination. Tissue sample size ranged from 0.12 to 1.6 gm. In some patients several biopsies were obtained, including normal adjacent brain tissue, tumor periphery, and tumor core.

After removal, surgical specimens were immediately frozen in pentane that was cooled in dry ice. Samples were stored at $-70°C$ until tissue extraction. Tissue $LTC_4$ levels were determined by radioimmunoassay using the New England Nuclear leukotriene $C_4$ [$^3H$]radioimmunoassay kit (New England Nuclear, Boston, MA). The separation of antibody–antigen complexes from free antigen was achieved by the absorption of the free $^3H$ tracer onto activated charcoal. The tissue samples were extracted using prostaglandin extraction methods previously described [4]. The limit of sensitivity was 3.7 pg per tube. The antibody cross-reacts 55% with $LTD_4$ and 8.6% with $LTE_4$. The cross-reactivity to $LTB_4$, prostaglandins, or unsaturated fatty acids was 0.006% or less.

Brain edema was determined from preoperative contrast-enhanced computed tomograms (CT) by a neuroradiologist (S. S. G.) who was unaware of the brain leukotriene levels. The tumor area was determined by measuring the maximum tumor diameter and the longest diameter perpendicular to the maximum diameter. From these two measurements an averaged diameter was determined and divided by 2 to approximate the average radius. For estimation of area, the tumor was assumed to represent a plane through the equator of a sphere. The averaged radius was squared and multiplied by $\pi$. The total abnormal area (tumor area plus edema area) was also determined by the same method. An edema index was then calculated by dividing the total abnormal area by the tumor area. Since a small neoplasm with a large area of surrounding edema could have the same absolute area of edema as a large tumor with a relatively small area of surrounding edema, we believe an edema index, which rep-

resents the ratio of edema to tumor size, is a better characterization of the edema surrounding a neoplasm. This determination yields similar results whether tumor area or tumor volume is measured.

## Results

Metastatic tumors and gliomas had a significant amount of peritumoral edema (edema index, 5.7 ± 1.8 and 2.1 ± 0.6, respectively) and high $LTC_4$ levels (13.8 ± 8.5 and 6.2 ± 2.3 pg/mg tissue, respectively). There was no edema and no neoplasm in the temporal lobes removed for seizure (controls), and the $LTC_4$ level was 0.4 ± 0.1 pg/mg tissue (Table). One patient with a metastatic tumor had received radiation therapy three months prior to craniotomy. His CT scan prior to operation showed no peritumoral edema. The $LTC_4$ level in this patient was 0.16 pg/mg tissue. A second patient with a metastatic tumor had an edema index of 1.1 and an $LTC_4$ level of 1.9 pg/mg tissue. All other patients with metastatic tumor had an edema index of 1.6 to 12.8 and $LTC_4$ levels that ranged from 2.4 to 72.2 pg/mg tissue. In one patient with a schwannoma, without surrounding edema, an area of normal cerebellum was resected to expose the tumor. This cerebellar tissue had an $LTC_4$ level of 0.7 pg/mg brain and it is included with the control temporal lobes in the Table.

In order to satisfy the statistical assumptions of the analysis-of-variance model, analysis was done on the natural logarithm of the $LTC_4$ levels. There was a significant correlation between edema and the log $LTC_4$ levels with $p < 0.003$ and a correlation coefficient of 0.62185 (Figure). An analysis of covariance with edema as the dependent variable and the log $LTC_4$ as the covariable also revealed a significant relationship between the log $LTC_4$ and edema within the group distribution ($p < 0.02$) but no difference in the log $LTC_4$ levels between the groups.

In two patients with metastatic tumors and two patients with gliomas, nonneoplastic brain tissue immediately adjacent to the tumor was analyzed. The $LTC_4$ level in this tissue was 3.6 ± 1.1 (mean ± SE). This was significantly higher than the level in the control brain tissue at $p < 0.003$ using the two-sample $t$

*Leukotriene $C_4$ Level and Index of Edema Surrounding Brain Tumors and Nonneoplastic Controls (mean ± SE)*

| Tumor Type | $LTC_4$ Level (pg/mg tissue) | Edema Index |
|---|---|---|
| Controls (n = 3) | 0.5 ± 0.1 | 1.0 ± 0.0 |
| Metastatic tumors (n = 8) | 13.8 ± 8.5 | 5.7 ± 1.8 |
| Gliomas (n = 5) | 6.2 ± 2.3 | 2.1 ± 0.6 |
| Meningiomas (n = 4) | 0.6 ± 0.2 | 1.6 ± 0.7 |
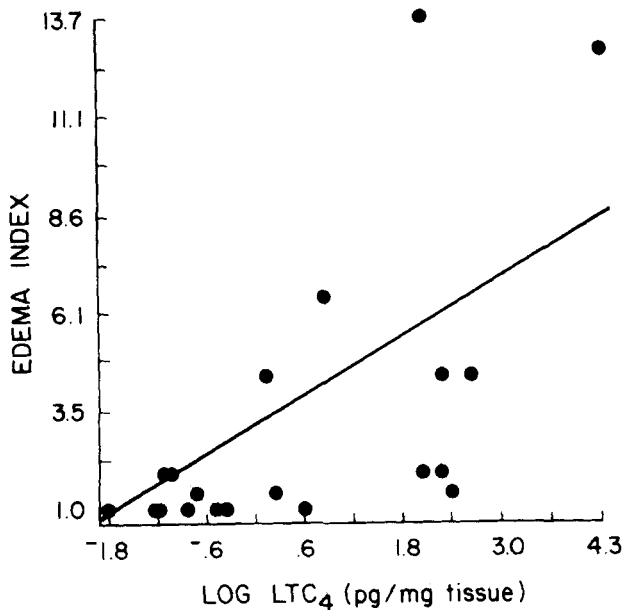
*Graph showing relationship between the natural logarithm of the leukotriene $C_4$ (LTC$_4$) level and the edema index for all patients. There is a significant correlation between log LTC$_4$ and edema at $p < 0.003$. The LTC$_4$ values are expressed as log LTC$_4$ to satisfy the statistical assumptions of the analysis-of-variance model.*

test. We have included in the Table only LTC$_4$ levels for the tumor itself.

## Discussion

Brain lipids are rich in arachidonic acid. During cerebral injury membraine-bound arachidonic acid is released and the tissue content of free arachidonate can increase 20- to 40-fold [12]. Free arachidonic acid has been shown to produce vasogenic brain edema in a dose-dependent manner [6]. Free arachidonic acid is oxidized via two pathways: cyclooxygenase, leading to the formation of prostaglandins; and 5-lipoxygenase, leading to the formation of leukotrienes. We recently demonstrated that vasogenic edema induced by arachidonic acid can be prevented by pretreatment with a 5-lipoxygenase inhibitor, BW755C [3-amino-l-(3-trifluoromethylphenyl)-2-pyrazoline], but not by indomethacin, an inhibitor of cyclooxygenase [3]. This suggests that the ability of arachidonic acid to increase blood–brain barrier permeability is dependent on the formation of 5-lipoxygenase products (i.e., leukotrienes). Furthermore, we have shown that nanogram quantities of pure leukotrienes injected directly into brain significantly increase blood–brain barrier permeability [2, 3]. These observations taken together suggest that leukotrienes may be important in the formation of vasogenic brain edema.

Chan and co-workers [6] suggested that the ability of arachidonic acid to produce brain edema is not specific. They also reported increased edema after injections of linolenic acid, but not saturated fatty acids. Homa and colleagues [8], however, recently reported that polyunsaturated fatty acids, but not saturated fatty acids, stimulate the formation of lipoxygenase products from arachidonic acid. Therefore, theoretically, linolenic acid might increase edema by increasing the conversion of arachidonic acid to leukotrienes. Because the 5-lipoxygenase enzyme accepts a variety of polyunsaturated fatty acids as substrate, fatty acids with 19 to 21 carbons and with 3 or more unconjugated double bonds are converted to leukotrienes by the lipoxygenase enzyme.

Leukocytes and platelets are rich in the lipoxygenase enzyme and are known to accumulate in cerebral injury. The major pathway for arachidonic acid metabolism in leukocytes is via 5-lipoxygenase [13]. Platelets will convert arachidonic acid via 12-lipoxygenase to 12-L-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12-HPETE). The 5-lipoxygenase enzyme is, in turn, activated by 12-HPETE [15] as well as prostaglandin endoperoxides [7], and the rate of arachidonic acid oxygenation by lipoxygenase is increased.

The tissue element or elements responsible for the conversion of arachidonic acid to leukotrienes in brain, however, has not been clearly defined. Moskowitz et al [11] suggested that leukotrienes were formed in brain gray matter. In support of this, leukotrienes $C_4$, $D_4$, and $E_4$ have been isolated after incubation of rat brain tissue with the ionophore A23187 and arachidonic acid in vitro [9]. We have demonstrated that severe leukopenia does not prevent arachidonic acid–induced vasogenic edema, which suggests that leukocytes may not be the major source for brain leukotriene production (unpublished observation, 1985).

Vasogenic edema associated with brain tumors, particularly metastatic and glial tumors, contributes to morbidity and mortality. We report here increased levels of LTC$_4$-like immunoreactivity in brain tumors with peritumoral edema and suggest that one mechanism for peritumoral edema formation may be the elaboration of leukotrienes.

Meningiomas had low LTC$_4$ levels and less edema than did metastatic tumors or gliomas. The LTC$_4$ level in meningiomas, however, appeared disproportionately low. Unlike gliomas or metastatic tumors, meningiomas are extraaxial tumors and leukotriene formation in association with these tumors may occur in the brain parenchyma adjacent to the tumor rather than in the meningioma itself. We did not sample brain tissue adjacent to meningiomas.

Glucocorticoids reduce peritumoral edema [19]. Glucocorticoids also inhibit the release of free arachi-

donic acid, probably through the inhibition of phospholipase $A_2$, and reduce the formation of leukotrienes [5]. One mechanism by which glucocorticoids reduce vasogenic edema, therefore, may be through the reduction of leukotrienes.

Leukotrienes increase blood–brain barrier permeability, and we have observed that brain tumors stimulate the formation of $LTC_4$-like immunoreactivity. Although we believe the formation of brain edema is multifactorial, we suggest that leukotrienes are important in the formation of vasogenic brain edema. Selective inhibition of the 5-lipoxygenase enzyme could reduce peritumoral edema.

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# Kernicterus in an Adult

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A 43-year-old woman was initially seen because of icterus. Clinical investigations revealed severe hepatic damage probably due to non-A, non-B hepatitis. She was treated with extracorporeal charcoal-column perfusion but died two weeks later in a hepatic coma. At autopsy, the brain showed kernicterus with typical discoloration of the hippocampus, the subthalamic nuclei, and the cerebellar dentate nuclei. Kernicterus in an adult is very rare. In this case, extracorporeal charcoal-column perfusion treatment led repeatedly to severe depletion of fibrinogen, with extensive hemorrhages. Overload of the already reduced hepatic glucuronyltransferase capacity resulted in high serum levels of unconjugated bilirubin, an apparent prerequisite for the development of bilirubin encephalopathy.

The term kernicterus refers to bilirubin encephalopathy in children with icteric discoloration of the basal ganglia, especially the globus pallidus, the hippocampus,