Journal of Neuro-Oncology **26**: 125–132, 1995.

Pharmacological blood-brain barrier modification for selective drug delivery

Timothy F. Cloughesy[1] and Keith L. Black[2]
[1] *UCLA Department of Neurology;* [2] *UCLA Division of Neurosurgery, Jonsson Cancer Center, UCLA Neuro-Oncology Program, UCLA School of Medicine, USA*

Key words: blood-brain barrier, drug delivery, leukotrienes, bradykinin, histamine, brain tumors

Abstract

Vasoactive agents have been identified through studies of peritumoral edema and effects on systemic capillaries. Abnormal blood-brain barrier or blood-tumor barrier can develop transient increases in permeability with the intraarterial delivery of vasoactive agents. Normal blood-brain barrier resists the effects of these compounds because of a biochemical barrier that may inactivate or become inert to vasoactive agents. Vasoactive compounds, including leukotrienes, bradykinin, and histamine appear to selectively increase permeability in abnormal brain capillaries. Intracarotid infusion of leukotrienes, bradykinin, and other vasoactive agents can increase drug delivery to diseased tissue.

Malignant gliomas are incurable. Despite current treatments with maximal surgery, radiation therapy and chemotherapy these tumors all eventually lead to recurrence, destruction of normal brain tissue and finally death. Chemotherapy has been shown to be effective in a small but significant percentage of patients [1]. The blood-brain barrier (BBB) is a limiting factor to delivery of antitumor compounds to tumor tissue [2–4]. A variety of approaches have been suggested in order to provide better exposure of antitumor agents to the brain tumor. These have included biodegradable polymer drug [5] delivery (5), liposomal delivery [6], intraarterial delivery, osmotic barrier modification [4] and interstitial drug delivery [7].

Pharmacological manipulation of vascular permeability is a potentially effective way to improve delivery of the anticancer agent to the tumor [8]. An ideal agent for pharmacological alteration of the BBB or blood-tumor barrier (BTB) would: (1) not increase drug delivery to normal brain without tumor; (2) result in selective, significant increase in permeability in the primary tumor mass; (3) increase permeability in distant microscopic tumor foci; (4) be reversible so that an increase in mass effect from edema would not occur.

A number of observations have shown that, in addition to the anatomic capillary barrier, there is also a biologic or enzymatic barrier present [9]. Evidence shows that various vasoactive compounds have a temporary effect on the tumor vasculature or abnormal BBB. Therefore, a receptor mediated or biochemical mediated response may lead to increased capillary permeability. Areas most affected by these vasoactive agents include not only BTB, but also damaged BBB. Some vasoactive compounds are effective in these areas due to a lack of enzymatic protection [9] but others may be caused by the appearance of receptors in these damaged or neovascular capillaries [10]. These biochemical differences become the basis for the pharmacological approach to selectively increasing permeability to brain tumor vasculature for antitumor therapy.

The approach of selective pharmacological ma-

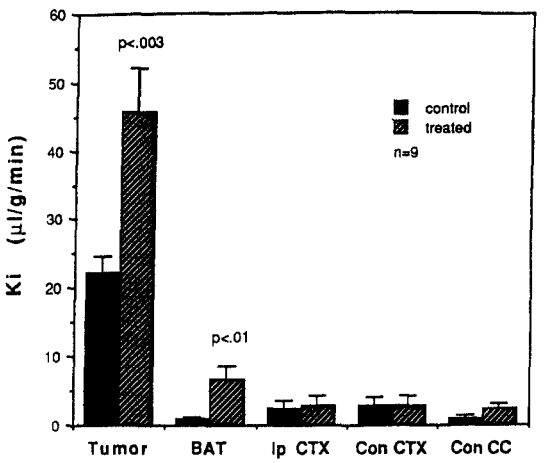

Fig. 1. Effect of intracarotid infusion of either leukotriene C4 (nine rats) or vehicle (nine rats) on blood-brain and blood-tumor barrier permeability. Values for the unidirectional transfer constant, Ki, are shown as the mean (standard error of the mean). BAT (brain adjacent to tumor); Ip CTX (ipsilateral cortex); Con CTX (contralateral cortex); Con CC (contralateral corpus callosum).

nipulation in tumor vasculature came from two areas of investigation: (1) identification of molecules in brain tumor or peritumoral edema which increase capillary permeability; and (2) vasoactive agents which have been shown to produce increased permeability to systemic capillary endothelium. Some of these agents include, leukotrienes [11], prostaglandins [12], arachidonic acid [13], tissue plasminogen activator [14], vascular permeability factor [10], histamine [15, 16], Kallikrein-kinin [17], bradykinin and its analogs [17–19], and eicosanoid [20].

Leukotrienes

Leukotrienes are biologically active compounds formed from the unsaturated fatty acid, arachidonic acid (AA), via the 5-lipoxygenase pathway. Increased levels of leukotrienes are seen in brain tissue during postischemic reperfusion [21] and also in brain tumors [11], subarachnoid hemorrhage, and concussive brain injury [22]. In addition, studies indicated evidence of the tumor expression of genes encoding enzymes (arachidonate 5-lipoxygenase) [23], a process involved in leukotriene production. Finally, 5-lipoxygenase inhibitors decreased vascular permeability both within tumors and in brain adjacent to tumors [24].

The postulated role of leukotriene C4(LTC4) in increased vascular permeability surrounding brain tumors led to speculation that infusion of LTC4 could further increase permeability within tumor capillaries and result in increased transport of antitumor compounds to brain tumors. We tested this hypothesis in a rat experimental brain tumor model using RG-2 gliomas [25, 26]. In this model RG-2 glioma cells (5×10^5 in a 5 µl solution) were stereotactically implanted into the cerebral hemisphere by means of a Hamilton syringe. Ten to 11 days after tumor implantation, a polyethylene 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. Leukotriene C4 was infused at a concentration of 5 µg/0.8 ml at a rate of 53.3 µl/min for 15 minutes. Blood-tumor and BBB permeability were determined by quantitative autoradiography using 14C-AIB. The transfer constant for permeability (Ki) within the tumors was increased twofold by LTC4 infusion compared to vehicle alone (45.58 ± 6.61 vs. 22.33 ± 2.35 µl/gm/min, $p < 0.003$) (Fig. 1). A significant increase in permeability was noted in tissue adjacent to tumor. Importantly, however, the effect of LTC4 was selective, only increasing permeability in tumor or tumor adjacent capillaries. The LTC4 infusion did not increase permeability in normal brain capillaries in the ipsilateral hemisphere. This suggested a unique ability of normal brain capillaries, but not tumor capillaries, to resist the vasoactive effects of leukotrienes.

Intracarotid infusion of LTC4 resulted in increased tumor permeability at doses of 2.5 to 50 µg/0.8 ml infused at a rate of 53.3 µl/min in rat experimental tumors [25]. This effect is completely reversed 30 to 60 minutes after termination of LTC4 infusion. A limiting factor to the use of LTC4 as a method of increased drug delivery is that it appears only to open the BTB to small molecules [27]. When BBB is determined after intracarotid infusion using different sized 14C tracers; AIB (103.1 daltons, radius = 2.8 A), sucrose (342 daltons, radius = 5A), in-

ulin (5000 daltons, radius = 15A), and dextran (70,000 daltons, radius = 60A), permeability was only increased within tumors for AIB, sucrose and inulin. No significant change of dextran was observed. Increased permeability within tumors is dependent upon molecular size. This suggests that LTC4 opens tight junctions rather than increasing vesicular transport through endothelial cells.

The selective effect of leukotrienes on brain tumor capillaries appears to relate to an 'enzymatic barrier' in normal brain capillaries. Unlike systemic capillaries, normal brain capillaries are rich in gamma glutamyl transpeptidase (∂-GTP) [28], an enzyme that metabolizes the peptidoleukotrienes LTC4 to LTD4 [29]. High levels of ∂-GTP were not present in the capillaries of experimental tumors. Theoretically, at physiological concentrations, LTC4 could be inactivated by ∂-GTP in normal capillaries, while tumor capillaries (which lack ∂-GTP) are susceptible to the vasogenic effects of LTC4. Both ∂-GTP and dipeptidase (an enzyme that inactivates LTD4 to LTE4) are enzymes unique to brain capillaries but are not present in systemic capillaries [28]. Interestingly, LTC4 slightly increased BBB permeability in brain adjacent to tumors where ∂–GTP was also moderately decreased [26].

Histamine

Pre-clinical studies have shown that histamine has a dose-related effect on the normal BBB, primarily on small molecular tracers [15]. Some authors have reported that intracarotid histamine infusion selectively increased blood flow in brain tumors and also caused extravasation of Evans blue-albumin within the tumor [30]. This latter finding suggests that the intracarotid histamine infusion increased permeability in tumors without affecting permeability in normal brain [16]. We studied the intracarotid infusion of histamine in a brain tumor model. Ten μg/kg/min of intracarotid histamine was infused, and this selectively increased permeability in brain tumor tissue to small molecular weight tracers. The H2-blocker, cimetidine, prevented the selective increase in tumor permeability after 10 μg/kg/min of histamine, suggesting that the effect of histamine on tumor permeability may be mediated by H2-receptors. We speculated that brain tumor capillaries may not resist the vascular effects of histamine as effectively as do normal brain capillaries. In effect, there is a biochemical mechanism in normal brain capillaries that allows brain capillaries to resist the effects of histamine. This protective mechanism is not as well developed in brain tumor capillaries or systemic capillaries. Histamine can therefore selectively increase permeability in brain tumors without affecting normal brain permeability or systemic physiological parameters.

Bradykinin

Bradykinin is a naturally occurring peptide formed from a plasma protein, high molecular weight kininogen, by the action of kallikrein. Bradykinin increases the vascular permeability of systemic capillaries and has a hypotensive effect that may reduce cerebral blood flow. A high dose of bradykinin will induce breakdown of normal BBB [19]. Bradykinin has been implicated in brain edema and its interstitial concentration is enhanced after experimental trauma. Since kallikrein inhibitors reduce brain swelling, bradykinin has been suggested as a mediator of vasogenic edema. Nagashima *et al.* reported that bradykinin caused increased vascular permeability by activation of B2 receptors of the vascular endothelium [31]. A bradykinin analog, H-Arg-Pro-Hyp-Gly-Thi-Ser-Pro-4-Me-Tyr (psi CH_2NH)-Arg-OH (RMP-7), was designed, proposing that it might increase cerebrovascular permeability by activating B2 receptors on brain microvasculature. Raymond *et al.* [19] reported that high-dose intracarotid infusion of bradykinin caused extravasation of HRP around the normal brain capillary as well as vasodilatation of microvessels and HRP endocytosis in endothelial cells. In a recent series of experiments, the use of low-dose intracarotid bradykinin infusion as a method to selectively deliver high molecular weight agents to brain tumors was examined [32]. These studies demonstrated that intracarotid bradykinin infusion selectively increased the brain tumor permeability for tracers ranging in molecular weight from 100 to 70,000 daltons. The

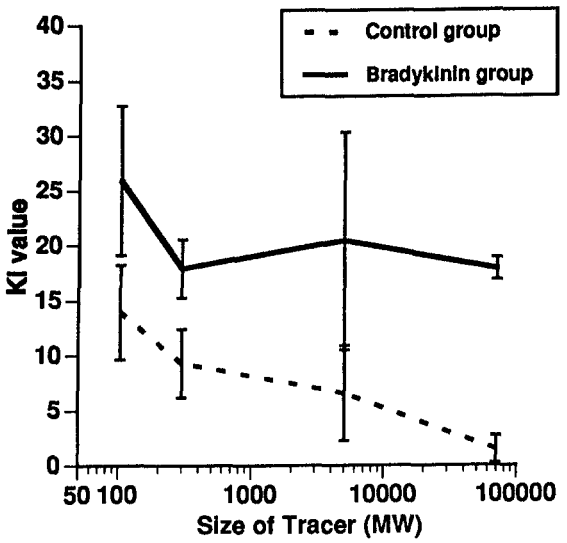

Fig. 2. Ki values (μl/g/min) within RG2 tumors comparing Ki to sizes of tracers with and without intracarotid bradykinin infusions. The values are shown as means SD. The Ki values of the control group (broken line) showed a general decline with increasing molecular weight of radiolabeled tracers. The Ki values in the bradykinin group (solid line) are significantly higher than those of the control group. There was also a decline in Ki with bradykinin between -aminoisobutyric acid and the other tracers. However, Ki did not significantly decline after bradykinin with increasing molecular weights between 342.3 and 70,000.

duration of the effect of intracarotid bradykinin infusion is approximately 20 minutes. It was suggested that low-dose intracarotid bradykinin infusion could be used to selectively deliver antitumor compounds to brain tumors.

The selective increase in permeability in brain tumor capillaries by bradykinin is much greater than the increase by LTC4. Bradykinin results in a 12-fold increase in permeability to high molecular weight dextran. In contrast, LTC4 does not increase permeability in tumors to dextran.

Bradykinin, infused in low doses (10 μg/kg/min) through the carotid artery ipsilateral to RG2 glioma in rats, significantly increased the permeability in tumor capillaries to six different tracers of varying molecular weights compared to the intracarotid infusion of saline alone. In contrast to reports that high dose bradykinin will induce breakdown of the normal BBB [19], permeability in normal brain capillaries was not increased in these studies. Bradykinin (dissolved in 0.9% saline) infused at a rate > 20 μg/kg/min reduced systemic blood pressure in rats when given either intravenously or into the carotid. Therefore, although high dose bradykinin may open the normal BBB, this effect may only occur at doses that result in systemic hypotension.

Table 1. Absolute Ki values for different tracers

	Tumor	BST	Ipsi. Cortex	Contra. Cortex	Ipsi. WM	Contra. WM	Ipsi. BG	Contra. BG
AIB								
Control (n = 11)	13.95 ± 4.29	1.83 ± 1.78	1.11 ± 1.34	0.85 ± 1.10	0.89 ± 0.81	0.63 ± 0.68	1.05 ± 1.43	0.70 ± 0.77
Bradykinin (n = 7)	25.91 ± 6.78**	3.50 ± 1.28*	1.54 ± 2.25	1.03 ± 0.93	1.72 ± 0.99	1.16 ± 1.08	1.04 ± 1.38	1.32 ± 1.74
Sucrose								
Control (n = 6)	9.28 ± 3.12	3.49 ± 1.90	2.50 ± 1.54	2.11 ± 1.38	1.95 ± 1.27	2.24 ± 1.42	1.74 ± 1.14	1.49 ± 1.28
Bradykinin (n = 7)	17.90 ± 2.65**	3.60 ± 1.67	2.38 ± 1.87	1.43 ± 1.42	1.64 ± 1.78	1.60 ± 1.57	1.35 ± 2.02	1.02 ± 1.14
Inulin								
Control (n = 8)	6.55 ± 4.32	0.85 ± 0.99	0.22 ± 0.60	0.13 ± 0.50	0.18 ± 0.52	0.25 ± 0.63	0.12 ± 0.50	0.15 ± 0.58
Bradykinin (n = 9)	20.35 ± 9.85**	1.85 ± 1.45	0.64 ± 1.02	0.71 ± 0.93	1.18 ± 1.30	0.62 ± 0.84	0.39 ± 0.64	0.20 ± 0.61
Dextran								
Control (n = 9)	1.47 ± 1.24	0.25 ± 0.18	0.32 ± 0.32	0.14 ± 0.20	0.14 ± 0.30	0.13 ± 0.19	0.32 ± 0.44	0.11 ± 0.11
Bradykinin (n = 7)	17.84 ± 0.99***	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.05	0.00 ± 0.00

Mean ± SD.

Ipsi. Cortex - ipsilateral cortex; Contra. Cortex = contralateral cortex; Ipsi. WM = ipsilateral white matter; Contra. WM = contralateral white matter; Ipsi. BG = ipsilateral basal ganglia; Contra. BG = contralateral basal ganglia.

Significant values (compared with same region): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Values are mean ± SD. BST (brain surrounding tumor); WM (white matter); BG (basal ganglia); Ipsi (ipsilateral); Contra. (contralateral cortex); for other abbreviations see the text. Significant values (compared with same region): $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$

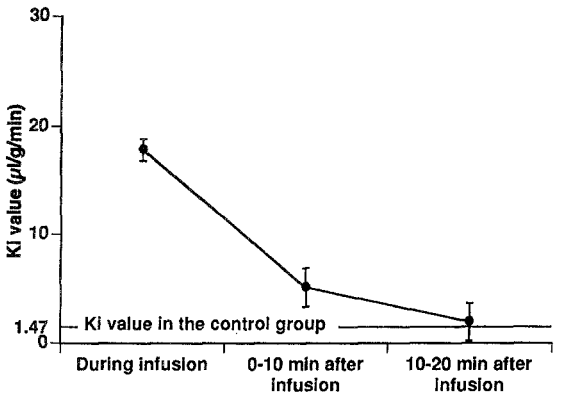

Fig. 3. Time course of bradykinin effects in tumor region. The Ki values in tumors were examined at three different time periods: 0–10 min during intracarotid bradykinin infusion, 0–10 min after the infusion, and 10–20 min after the infusion. Means ± SD of Ki value are shown. The solid line parallel to the x-axis is the Ki value (1.47) in tumors in the control group. The Ki values 20 min after the infusion is not significantly different from that in the control group.

Bradykinin also results in reduced cerebral blood flow [32] and vasodilatation. To determine if bradykinin increased blood volume in rats with experimental tumors after intracarotid infusion, blood volume was calculated with a graphic method using [14C] dextran. The tumor blood volume was almost twice that of normal brain, but the brain and tumor blood volumes were not significantly altered by intracarotid bradykinin infusion.

The absolute Ki values, calculated using the methods of Ono *et al.* [34] and Ziylan *et al.* [35], were obtained for the different substances and are given in Table 1. The permeability, Ki, in tumors to [14C] dextran (MW 70,000) after bradykinin infusion was 12-fold higher than the permeability in controls. The permeability in tumors after bradykinin infusion was also increased using [14C] AIB (MW 103), [14C] sucrose (MW 342.3), [14C] inulin (MW 5,000). When the Ki values in tumors for all tracers were plotted against the size of the tracers after saline infusion, a general decline in Ki was observed as the molecular weight of radiolabeled tracers increased (Fig. 2). The Ki in tumors after bradykinin infusion also showed a decline with increasing molecular weight of tracers, but the slope of the decline after bradykinin infusion was much less than in the control group. The relative effect of bradykinin on increasing permeability is much greater as the size of the tracer increases.

Several mechanisms can account for increased BBB permeability. They include: (a) increased vesicular transport, (b) increased transcellular penetration, and (c) increased opening of the tight junctions of brain endothelial cells [36]. The permeability without bradykinin infusion declines with increasing molecular size of tracers (size dependent). In bradykinin infused animals, the Ki value in tumors decreases between AIB and the other tracers of larger size, suggesting size-dependent transport (between AIB and the other tracers). However, among sucrose, inulin, and dextran, permeability is similar, suggesting size-independent transport (among sucrose, inulin, and dextran). One interpretation of these findings is that increased permeability in control tumors results from the opening of endothelial junctions, and the increased permeability caused by bradykinin might result from both mechanisms.

Because of proteolytic inactivation bradykinin has a short biological half-life [37, 38]. To determine the duration of the bradykinin effect on tumor capillary permeability, we measured the Ki at three different time periods [32]. In these studies, the effect of bradykinin on tumor capillary permeability was diminished 20 minutes after stopping the intracarotid infusion (Fig. 3). The short effect on tumor capillaries is, in fact, desirable for the selective delivery of anticancer drugs in the treatment of brain tumors, since any long term increase in mass effect from edema would be limited.

The extent to which bradykinin selectively increases tumor capillary permeability may be dependent upon specific tumor histology. For example, intracarotid bradykinin infusion increases RG-2 tumor capillary permeability not only to [14C] AIB and [14C] sucrose, but also to [14C] dextran. In contrast, bradykinin increases permeability in 9L and C6 gliomas to [14C] AIB and sucrose, but not to [14C] dextran [39]. The difference in response of tumor capillaries to bradykinin may be related to biochemical differences in the respective tumor capillaries. Investigation of these differences may improve our understanding of the mechanism

by which bradykinin selectively opens brain tumor capillaries.

A synthetic bradykinin analog, RMP-7, is a nonapeptide containing unnatural amino acids at positions 3, 5, and 8 and a reduced peptide bond between positions 8 and 9, and is composed of amino acids with L-configuration. Apparently, the biological half-life of RMP-7 is longer than that of bradykinin. Increased tumor permeability after intracarotid RMP-7 infusion is very similar to that after infusion of bradykinin, except that the effect occurs at 1/100th the dose of bradykinin [18]. The analog RMP-7 has been shown to increase delivery of [14C] carboplatin to brain tumors. The survival of rats with experimental brain tumors is significantly increased with intracarotid infusion of RMP-7 and carboplatin compared to carboplatin without RMP-7. Based on these and other findings, human phase I trials were begun using intracarotid carboplatin and RMP-7 infusion in patients with malignant brain tumors.

Perhaps one of the more important effects of bradykinin opening of the BTB is its ability to increase permeability to large molecules. Cytokines, for example, have been investigated for their anti-tumor effects. Most cytokines have molecular weights between 17,000 and 20,000 daltons and do not readily cross the BTB. Intracarotid infusion of bradykinin, however, has been shown to increase transport of tumor necrosis factor-alpha to tumors by 4- to 6-fold. The ability of bradykinin to increase delivery of this class of antitumor compound to tumor tissue may have important implications in the treatment of brain cancer and other CNS diseases.

For yet unclear reasons, the maximal effect of intracarotid bradykinin infusion on tumor capillary permeability seems to occur during the first 15 minutes of infusion, with the effect being almost completely lost after 2 hours of infusion. This suggests that either tachyphalysis to the bradykinin occurs over time or that a second messenger is exhausted with prolonged infusions. Further studies are required to better understand this phenomenon of decreased response over time.

The argument for the opening of the BBB in normal brain tissue is that tumor cells are known to infiltrate into these normal areas. Therefore, it is important for drug delivery to occur beyond the main tumor mass. These studies demonstrate that infusion with bradykinin or its analogs can increase the permeability of experimental tumors as small as 2 mm. We know that in order for tumors to grow beyond 2 mm it is necessary for angiogenesis to take place. Apparently, this neovasculature is most susceptible to bradykinin or its analogs. Therefore, we feel that bradykinin or its analogs will increase permeability in small microscopic tumor foci that are distant from the main tumor mass. The advantage of this opening by bradykinin is relatively large. There is a tenfold increase in tumor permeability to large molecules, in contrast to a 25% increase of tumor permeability obtained with osmotic barrier opening. In normal brain tissue without tumor, osmotic opening increases the permeability tenfold and exposes the brain tissue to toxic effects of antitumor agents. Infusion with bradykinin does not significantly increase permeability in normal brain tissue; therefore toxicity to normal brain tissue can be decreased using this method. Short effect on tumor capillaries of the intracarotid infusion of bradykinin or its analog is in fact, desirable for selective delivery of anticancer drug in the treatment of brain tumors. Increased opening of the BTB for long periods of time could result in increased brain edema and mass effect.

Clinical Phase I trials are currently underway using both intraarterial and intravenous infusions of the bradykinin analog RMP-7 to increase delivery of carboplatin to malignant brain tumor tissue. The ability to selectively increase transport of drugs and other antitumor compounds to malignant tissue may result in improved outcomes for patients diagnosed with brain tumors.

Acknowledgements

This work was supported by Alkermes, Inc. Grant ALK01-010, NIH grant 1P01NS25554 and NIH Grant 1RO1NS32103.

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Address for offprints: T.F. Cloughesy, Department of Neurology, 710 Westwood Plaza, Room 1–129, Los Angeles, CA 90095, USA