Opening the Blood–Brain and Blood–Tumor Barriers in Experimental Rat Brain Tumors: The Effect of Intracarotid Hyperosmolar Mannitol on Capillary Permeability and Blood Flow

E. M. Hiesiger, MD,* R. M. Voorhies, MD,† G. A. Basler, MA, L. E. Lipschutz, BS, J. B. Posner, MD, and W. R. Shapiro, MD

Using quantitative autoradiography, we investigated the effect of intracarotid infusions of hyperosmolar mannitol solutions on capillary permeability and blood flow. Capillary permeability, expressed in terms of a blood-to-tissue transfer constant (K), was determined in two rat brain tumor models by measuring the entry of ^{14}C-alpha aminoisobutyric acid into brain tumor, into brain tissue adjacent to tumor, and into cortex. Cerebral blood flow was determined by measuring the uptake of ^{14}C-iodoantipyrine in one rat brain tumor model. Blood flow was examined in the same regions as K, as well as in the corpus callosum. Before mannitol administration, K values in both Walker 256 (W256) carcinosarcoma and C_6 gliomas were much higher than those in cortex. C_6 gliomas were about three times more permeable than were W256 tumors. There was a direct correlation between tumor size and increased capillary permeability. Mannitol at a concentration of 1.37 M did not increase the K values for either tumor or adjacent tissue. At 1.6 M, mannitol increased the K values for both tumors (1.7-fold in C_6 glioma and 13-fold in W256) as well as for adjacent tissue. At both concentrations, mannitol markedly increased cortical K values in all groups: by 48- to 72-fold at 1.37 M and by 90- to 105-fold at 1.6 M. The net effect of the mannitol was to reverse the tumor-to-cortex permeability relationship. Cortical blood flow increased modestly after intracarotid mannitol administration on both sides of the brain. These data provide little justification for using intracarotid mannitol during chemotherapy of human brain tumors.

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Chemotherapy is generally only modestly effective against brain tumors. The reasons for this failure may include: the resistance of the tumor cells to cytotoxic drugs at conventional doses, and the failure of the drug to reach the tumor. Two important factors that affect drug delivery are the blood–brain barrier, which impedes the transfer of water-soluble molecules, and brain (or tumor) blood flow, a limiting factor in the delivery of lipid-soluble agents. In brain tumors, the blood–brain barrier (or more precisely, the blood–tumor barrier) is only partially disrupted; the degree of disruption may be tumor dependent [2, 6, 8, 11, 16–19, 22, 30]. Because of the barrier problem, water-soluble drugs have not been used frequently to treat brain tumors [12, 23, 24]. Cerebral blood flow is known to be depressed in and around brain tumors, in both experimental systems and humans [1, 4, 5, 7, 10, 15], making even lipid-soluble agents perhaps less effective.

To enhance drug entry into tumor, attempts have been made to disrupt transiently the blood–brain barrier with intraarterial hyperosmolar agents [13, 14, 22, 23]. Rapoport and associates [27, 28] administered intracarotid 1.6 to 1.8 molal arabinose in rats and observed a 20-fold increase in ^{14}C-sucrose uptake in gray matter. Neuwelt and associates [22] used hyperosmolar barrier disruption to enhance the delivery of methotrexate into avian sarcoma virus (ASV) gliomas

From the George C. Cotzias Laboratory of Neuro-Oncology, Memorial Sloan–Kettering Cancer Center, and the Department of Neurology, Cornell University Medical College, New York, NY.

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Address reprint requests to Dr Shapiro, 1275 York Ave, New York, NY 10021.

*Present address: Department of Neurology, New York University School of Medicine, New York, NY 10016.

†Present address: Ochsner Clinic, New Orleans, LA 70121.

in rats; data from that study indicated that the cortical methotrexate concentration was similar to that of the tumor. Nakagawa and colleagues [20] found that graded hyperosmolar (0.8 to 1.8 molal) barrier disruption failed to increase capillary permeability in rat RG-2 gliomas, although there was a progressive increase in permeability in tumor-free brain tissue. There are thus measurable differences among several brain tumor models regarding the effectiveness of hyperosmolar barrier disruption in the tumor itself.

No studies have examined the effect of intracarotid hyperosmolar agents on blood flow in tumor-bearing animals or humans. Cerebral blood flow has been shown to be depressed 15 minutes after the intracarotid administration of mannitol to tumor-free animals [25].

The available data do not answer two questions: (1) How does barrier disruption with hyperosmolar agents affect the ratio of concentrations between tumor and normal brain of potentially neurotoxic chemotherapy? (2) Does the administration of hyperosmolar agents alter cerebral blood flow in and around brain tumors? To answer these questions, we investigated the effect of disrupting the blood–brain barrier on capillary permeability and blood flow on both brain tumors and brain tissue.

Methods

Permeability was measured in tumor, in brain tissue adjacent to tumor (a 0.5-mm peritumoral zone), and in cerebral cortex. The effects of two different concentrations of mannitol were compared: 1.37 M (a 25% solution, 1.6 molal) and 1.6 M (2.0 molal). Capillary permeability was determined by observing the entry of ^{14}C-alpha aminoisobutyric acid (^{14}C-AIB) over 15 minutes. For studies of cerebral blood flow, we investigated the effects of 1.6 M (2.0 molal) intracarotid mannitol on rats harboring bilaterally implanted C_6 gliomas. By measuring the entry of ^{14}C-iodoantipyrine (^{14}C-IAP), blood flow was determined in tumor, adjacent tissue, and cerebral cortex.

Tumors

Two different experimental tumors were used: Walker 256 (W256) carcinosarcoma (a model of metastatic tumor) and C_6 glioma (a model of primary brain tumor). W256 carcinosarcoma was maintained by subcutaneous transplantation in Wistar male rats weighing 75 to 100 gm. At the 69th transplantation, the tumor was excised, minced in Ham's F-10 nutrient medium with 2.5% fetal calf serum and 15% horse serum, and placed in collagen-coated tissue culture flasks. C_6 glioma was purchased from the American Type Culture Collection (Rockville, MD 20852) and maintained in tissue culture with McCoy's 5A medium containing 10% fetal calf serum at 37°C and 5% carbon dioxide in a humidified incubator. Both tumor lines were prepared for inoculation in the same manner. Cells from confluent flasks were harvested with 0.5% trypsin and 0.2% EDTA in Hank's balanced salt solution, centrifuged, and resuspended in their respective media without serum but containing 0.5 to 1% agar.

Animal Inoculation and Experimentation

Male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA) weighing approximately 350 gm were anesthetized with chloral hydrate (0.35 gm per kilogram, given intraperitoneally) and ketamine (5 mg, given intramuscularly). The skull was exposed by a midline scalp incision, and two shallow 26-gauge needle punctures were made 3 mm to either side of the sagittal suture and 1 mm posterior to the coronal suture. A 2% lidocaine gel was applied to the rat's ears and the head was affixed in a stereotaxic apparatus (Model 900, David Kopf Instruments, Inc, Tujunga, CA 91042).

In the ^{14}C-AIB study, 10 μl of a suspension containing 5×10^5 viable C_6 or W256 tumor cells was inoculated bilaterally to a depth of 7 mm through the pre-bored holes. There were two sets of controls in this study: animals inoculated with C_6 glioma on the right side only (the left hemisphere serving as nontumor controls), and animals inoculated bilaterally with W256 (the left side serving as systemic mannitol controls for the right-side intracarotid mannitol).

For the ^{14}C-IAP study, both controls and animals given mannitol were inoculated bilaterally with C_6 glioma. No nontumor controls were used.

At about day 14, when the animals began losing weight, they were anesthetized with 9% ethrane and oxygen and maintained with 1 to 2% ethrane, 70% nitrous oxide, and 30% oxygen. Both femoral arteries and the right femoral vein were cannulated with V2 vinyl tubing (Bolab, Inc, Lake Havasu City, AZ 86403). In animals receiving mannitol, the right external carotid artery was also cannulated with V2 vinyl tubing in a retrograde fashion, with the cannula tip facing, but not occluding, the bifurcation with the internal carotid artery. The external carotid artery was ligated in rats not receiving mannitol. All animals were allowed to awaken from anesthesia and were studied 2 hours later while awake.

Rats received intracarotid mannitol as an infusion (2.5 ml over 30 seconds) through a 0.2-μm filter into the right carotid artery. The 1.37 M mannitol was purchased from Invenex (Chagrin Falls, OH 44022) as 25% mannitol. The 1.6 M concentration was made from D-mannitol obtained from Sigma (St. Louis, MO 63178). Two minutes elapsed between the end of the mannitol infusion and the start of the isotope administration. Controls received no infusion before administration of ^{14}C-AIB or ^{14}C-IAP.

The ^{14}C-AIB was purchased as alpha-[1-^{14}C]aminoisobutyric acid, specific activity 51 to 53 mCi/mmol, from New England Nuclear (Boston, MA 02118). Radiochemical purity was determined by thin-layer chromatography using silica gel G plates (Analtech, Newark, DE 19711) with *N*-propanol and ammonium hydroxide (70:30, vol:vol) and found to be greater than 99%. ^{14}C-AIB, 100 μCi per rat, was adjusted to physiological pH with 1 *N* sodium hydroxide and injected as a 1-ml bolus into the femoral vein.

The ^{14}C-IAP was purchased from New England Nuclear as 4-[n-methyl-^{14}C]iodo-antipyrine with a specific activity of 50 to 60 mCi/mmol. Radiochemical purity was assayed by thin-layer chromatography on a silica gel G plate with chloroform and methanol (95:5, vol:vol), and found to be

greater than 94.8%. One milliliter of ethanol containing 50 μCi of ^{14}C-IAP was evaporated down to 0.1 ml. The 0.1 ml remaining was brought up to 1.68 ml with 0.1% trypan blue in phosphate-buffered saline at pH 7. The solution was placed in a 3-ml syringe, mounted on a Harvard pump, and attached to the femoral vein catheter.

For the ^{14}C-AIB study, timed blood samples were collected from the left femoral artery every 15 seconds for the first minute, then every minute for 4 minutes, and then at 10 and 15 minutes, for a total of 11 samples (including a pre-isotope sample). For the ^{14}C-IAP experiments, 11 blood samples were drawn at 3-second intervals by continuous sampling from the left femoral artery.

Blood pressure was monitored from the left femoral artery; before intracarotid administration of mannitol it was 114 ± 1 (±SEM) mm Hg. The blood pressure rose to 153 ± 0.8 mm Hg during the first 30 seconds of mannitol infusion, then fell over the next 30 seconds to 97 ± 0.9 mm Hg. The blood pressure then stabilized and was maintained at 116 ± 2 mm Hg for the duration of the experiment. In the ^{14}C-AIB study, 12 of 16 rats required approximately 2.25 ml each of 6% dextran intravenously to maintain their blood pressure. For the ^{14}C-AIB and ^{14}C-IAP studies, body temperature was monitored with a rectal probe and maintained at a mean value of 37.2 ± 0.1°C by mounting the animal on a thermostatically controlled warming block. Other physiological measurements, which did not change significantly during the experiments, were as follows: pH, 7.45 ± 0.01; pO_2, 81.5 ± 3.5 mm Hg; pCO_2, 35.1 ± 1.4 mm Hg; and hemoglobin, 16.3 ± 0.3 gm/dl.

Fifteen minutes after ^{14}C-AIB administration, 22 of the 24 rats in all groups were killed by decapitation. One rat in each of the 1.6-M mannitol experiments (i.e., Group 4 and 6, Table 1) deteriorated early and were killed at 6.7 and 7.7 minutes. The results in these 2 rats did not differ from those in their respective groups, and data from both animals were included in the calculations.

In the cerebral blood flow experiments, the Harvard pump was turned on and began infusing the ^{14}C-IAP 2 minutes after cessation of the mannitol infusion. The animals were killed by decapitation after a 30-second ^{14}C-IAP infusion. Eleven blood samples were immediately centrifuged and 20 μl of the plasma pipetted and weighed in scintillation vials.

Plasma protein was solubilized with 0.30 ml of NCS (Amersham Corp, Arlington Heights, IL 60005), and 10 ml of OCS (Amersham) scintillation counting solution was added to each vial. Plasma radioactivity was measured with a Packard liquid scintillation spectrometer (Packard Instruments Co, Downers Grove, IL 60515). After the animals were decapitated, the brains were dissected within 1 to 2 minutes and placed in Freon-12 (DuPont, Wilmington, DE 19899) on dry ice, mounted with embedding matrix, and stored at −70°C. Frozen coronal sections, 20 μm thick, were cut on a cryostat with a Bright rotary retracting microtome (Hacker Instruments, Inc, Fairfield, NJ 07006).

Autoradiography

Adjacent 20-μm brain sections were either mounted on coverslips and immediately dried at 60°C for autoradiography, or mounted on glass slides and fixed for histological staining with hematoxylin-eosin. The dried tissue sections were placed on mat boards and exposed to single-coated x-ray film (SB-5, MR-1 or OM-1; Eastman Kodak Co, Rochester, NY 14620) in a cassette along with ^{14}C-methylmethacrylate standards (Amsherham Corp) previously calibrated to reference 20-μm brain sections of known radioactivity. After an appropriate interval, the films were developed and the radioactivity determined by quantitative autoradiography as follows.

The autoradiography equipment consisted of an EyeCom II image array processor and Vidicom TV tube (Spatial Data Systems, Inc, Goleta, CA 93116), and a PDP 11/23 computer. Individual coronal brain sections on the autoradiograms were digitized along with the standards. To convert the film images into units of radioactivity, the optical densities of images produced by the ^{14}C standards were determined and a standard curve relating optical density to tissue radioactivity was generated for each film. Based on this curve, the stored optical density data of autoradiographic images could be converted to radioactivity values (nCi/gm of tissue). The Vidicom tube was set at such a height above the film that 1 mm of length in the tissues equalled 24 pixels of length on the 512 × 512 pixel matrix of the image on the display. The minimum resolution was therefore 1 pixel (4.167 μm), but the working resolution of the film was 50 × 50 × 20 μm thickness for each section.

To assure that autoradiographic readings for tumor, tumor necrosis, and adjacent tissue could be verified histologically, a Mylar mask interface was generated by tracing the image of the stained histological section corresponding to the autoradiogram (the adjacent 20-μm section) from the video monitor. Differences beween the autoradiogram and the corresponding histological section, resulting primarily from tissue shrinkage in histological preparation, amounted to less than 5%. These differences could be partially offset by changing the camera's magnification of the stained section to correspond to the autoradiogram.

For estimating the permeability–surface area product of the tissue capillaries, a unidirectional blood-to-brain transfer constant, K (μl/gm–min), was calculated by:

$$K = Ci(T) - 0.01Cp\ (15\ \text{min}) / \int_0^T Cp(t)dt \qquad (Eq\ 1)$$

where Ci is the tissue concentration of ^{14}C-AIB at the end of the experimental time (T) and Cp is the concentration of the labeled AIB in the arterial blood plasma. The tissue concentration was corrected for any ^{14}C-AIB remaining in blood vessels within the specimen by assuming a 1% plasma space and subtracting that fraction of the 15-minute plasma concentration. Based on the resolution of the optical density measurements above x-ray film sensitivity background, the reliable lower limit for K was 0.4 μl/gm–min.

Blood flow was measured by a 30-second timed intraarterial injection of ^{14}C-IAP, a compound that rapidly crosses the blood–brain barrier at a rate dependent on blood flow. The flow in a "region" was determined by use of equations 2 and 3:

$$Ci(T) = \lambda k \int_0^T Ca(t)e^{-k(T-t)}dt \qquad (Eq\ 2)$$

where $Ci(T)$ is the tissue concentration of radioactive ^{14}C-

IAP as determined by autoradiography at a given time (T) after introducing the tracer in the blood; λ is the tissue-to-blood partition coefficient; and t is the variable time. Equation 2 is solved for k using the experimentally determined data for the other variables. k equals a constant that incorporates the rate of blood flow in the tissue as follows:

$$k = mF/\lambda \qquad (Eq\ 3)$$

where F is the rate of blood flow per unit mass of tissue, and m is a constant taken to be equal to 1. λ has been measured as 0.8 for IAP for normal brain tissue [29]. Blasberg [1, 3, 26] reported that λ for ASV-induced tumors ranged from 0.87 to 1.2. Using a value for λ of 0.8 when the "actual" λ is 1.2 will overestimate flow by approximately 4% with flows of 80 ml/100 gm–min. We used the value 0.8 for λ in our measurements. The lower limit of reliable measurement of cerebral blood flow was 0.01 ml/gm–min.

The size of each tumor was determined by measuring the images on the monitor with appropriate calculation of the pixel size. The average diameter and area for each section were measured and combined into a volume estimate by interpolation among adjacent sections. The degree of correlation between the size measurements and the tumor K values was determined.

Results

Figure 1 depicts the computer-generated pseudocolor image of a typical coronal section from a rat bearing implanted brain tumors (C_6 glioma or W256). The disruption of the blood–brain barrier after either 1.37 M or 1.6 M mannitol is readily perceived by the marked increase in radioactivity on the right side of the brains, corresponding to increased entry of the ^{14}C-AIB. Controls received no mannitol. Quantitative results are depicted for the various experimental groups in Table 1 and Figures 2 and 3.

Capillary Permeability in Normal Cortex

Cortical K values among the non-tumor-bearing hemispheres, and tumor-bearing hemispheres in rats not given mannitol and in tumor-bearing hemispheres contralateral to the intracarotid mannitol infusion (Table 1) were similar to K values reported in the literature for several rat brain tumor systems [2, 6, 18, 19]. For example, in Group 2, the W256 nonmannitol experiment, the mean cortical K value for both sides in 4 rats was 0.7 ± 0.4 μl/gm–min. In the left cortices of the rats given mannitol and bearing W256 and C_6 gliomas (Groups 3 through 6), the mean for all 16 rats was 0.6 ± 0.4 μl/gm–min. The K values on the left side of the brain in rats in Groups 3 through 6 receiving contralateral intracarotid mannitol were similar to those obtained in tumor-bearing animals that received no mannitol.

Capillary Permeability in Tumor and Adjacent Tissue

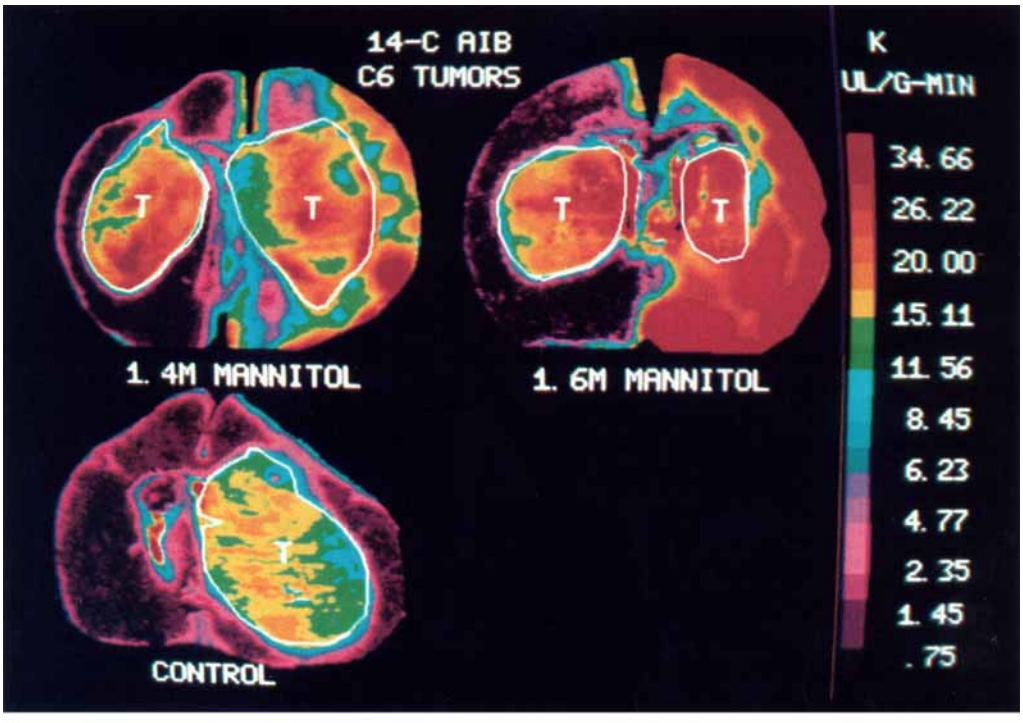
Because there was considerable variation in the size of the tumors, and because previous studies [6, 11, 18, 30] had shown a general relationship between the size of a tumor and the K value, we compared the mean K value with the area and volume of all tumors not exposed to mannitol. For W256 the linear correlation coefficient between tumor K and tumor area was 0.77, with a slope of 0.4 ± 0.02 μl/gm–min/mm^2; there was no correlation between K values and tumor volume. For C_6 tumor the correlation coefficient for tumor area was 0.9, with a slope of 1.1 ± 0.2 μl/gm–min/mm^2 (Fig 2). Unlike W256, there was also a significant correlation between tumor K values and volume; the correlation coefficient was 0.80, with a slope of 0.2 ± 0.1 μl/gm–min/mm^3. Thus, because the K value increased with tumor size, and because the tumors differed in size between the two sides of the brain ($p < 0.05$, paired t test), we elected not to pair the tumors in each rat. Instead, we grouped the left and right sides separately and used grouped statistics for comparison (to be discussed later).

There was a significant difference between K values of the two tumors: overall K for W256 was 5.7 ± 1.0, and for C_6 glioma it was 15.7 ± 3.0 μl/gm–min ($p < 0.025$, grouped t test). For approximately the same tumor cross-sectional area, the capillary permeability in the C_6 glioma was twice that in W256 (compare Group 3 with Group 5 or 6).

The capillary permeabilities in the left-sided adjacent brain tissue for each group are listed in Table 1. As with tumor, the mean permeability of tissue adjacent to both tumor types correlated positively with mean tumor size. We plotted mean K values for adjacent tissue against the mean area for both sides of W256 nonmannitol Group 2 and W256 mannitol Groups 3 and 4. The correlation coefficient was 0.70, with a slope of 0.2 ± 0.1 μl/gm–min/mm^2. The same comparison was made on the combined left sides of the C_6 series (Groups 5 and 6), yielding a correlation coefficient of 0.64, with a slope of 0.2 ± 0.1 μl/gm–min/mm^2.

As can be seen in Table 1 and Figure 3A, in Group 3 (1.37 M mannitol W256) the K values for left-sided tumors (7.9 ± 2.8 μl/gm–min) did not differ from those of the adjacent tissue (4.0 ± 1.1), but both values exceeded those for the cortex (0.4 ± 0.1) ($p < 0.05$ and $p < 0.025$, respectively, grouped t test). In Group 4 (1.6 M mannitol W256 experiment), on the left side, there was a significant difference in K between the tumor (3.3 ± 1.0 μl/gm–min) and cortex (1.1 ± 0.3) ($p < 0.025$, grouped t test), but not between tumor and adjacent tissue (1.5 ± 0.6) or between adjacent tissue and cortex.

In Group 5 (1.37 M mannitol C_6 glioma), the K value for the left-sided tumors (16.8 ± 5.4 μl/gm–min) did not differ from that of adjacent tissue (4.9 ± 1.9), but it was 36 times greater than the K for cortex (0.5 ± 0.3) ($p < 0.025$, grouped t test). For Group 6



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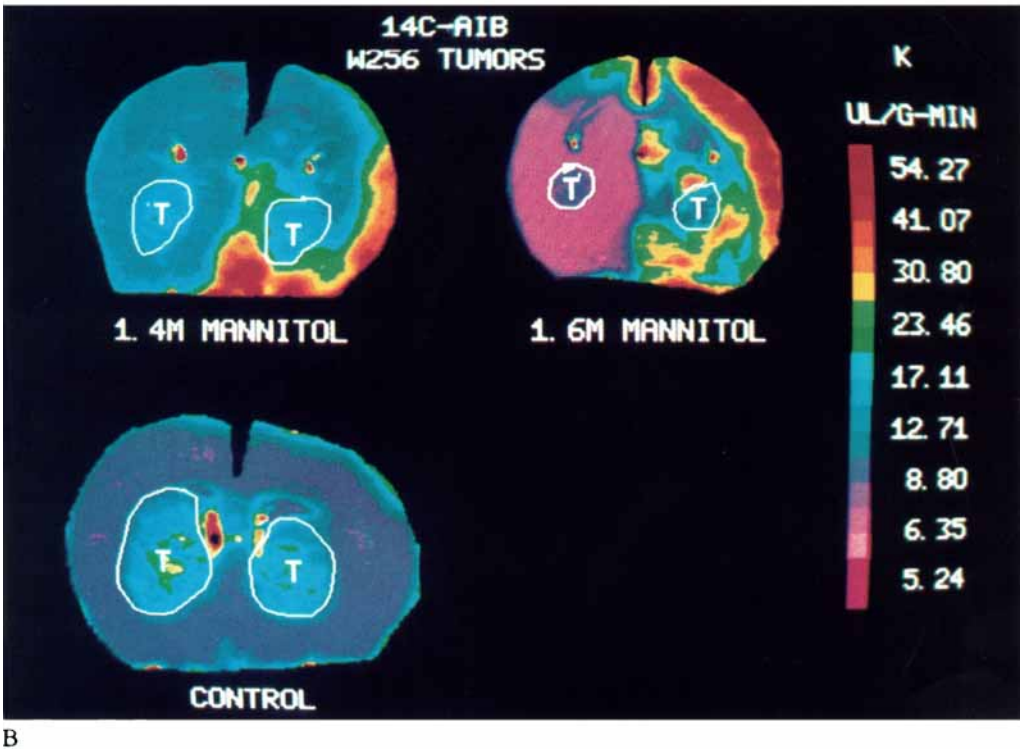

B

Fig 1. Computer-generated television images, color coded for regional tissue permeability of the transfer constant (K), *measured in* μl/gm–min. *The autoradiographic sections shown are of: (A) C_6 gliomas or (B) W256 carcinosarcomas, demarcated by a white border and labeled* T. *The sections are from animals given right intracarotid mannitol at either the 1.37* M *(marked 1.4* M*) or 1.6* M *concentrations, or from controls given no mannitol.* (^{14}C-AIB = ^{14}*C-alpha aminoisobutyric acid.)*

Table 1. Comparison of Averaged Mean Transfer Constant K (μl/gm–min) of ^{14}C-AIB as a Measure of Capillary Permeability for Tumor, Adjacent Tissue (a 0.5-mm Peritumoral Zone), and Cortex

Group	Tumor Type	Mannitol Concentration	Capillary Permeability μl/gm–min						Tumor Size			
			Tumor		Adjacent Tissue		Cortex		Left		Right	
			L	R	L	R	L	R	Area (mm^2)	Volume (mm^3)	Area (mm^2)	Volume (mm^3)
1	C_6	0	...	16.0	...	7.1	1.3	1.4	...	...	27.6	126.0
				±		±	±	±			±	±
			...	1.5	...	1.3	0.1	0.0	...	...	6.7	43.0
2	W256	0	6.9	4.3	4.1	2.6	0.8	0.7	8.9	26.4	4.1	9.6
			±	±	±	±	±	±	±	±	±	±
			2.3	1.0[a]	1.7	0.3[a]	0.4	0.4	3.9	14.0	1.9[a]	6.1[a]
3	W256	1.37 M	7.9	7.4	4.0	8.6	0.4	27.6	16.4	34.8	11.4	33.9
			±	±	±	±	±	±	±	±	±	±
			2.8	0.4	1.1	2.0	0.1	8.8	5.5	11.0	2.4	5.8
4	W256	1.6 M	3.3	42.6	1.5	43.4	1.1	97.2	1.7	3.5	4.8	14.4
			±	±	±	±	±	±	±	±	±	±
			1.0	12.4	0.6	9.2	0.3	13.0	0.6[b]	1.4[b]	1.4	7.4
5	C_6	1.37 M	16.8	19.8	4.9	11.3	0.5	22.6	16.4	61.5	14.3	69.1
			±	±	±	±	±	±	±	±	±	±
			5.4	5.0	1.9	3.6	0.3	5.6	4.7	22.0	5.2	30.0
6	C_6	1.6 M	14.6	24.6	5.4	22.8	0.5	54.7	15.9	59.7	18.5	62.9
			±	±	±	±	±	±	±	±	±	±
			3.5	1.7	1.1	3.5	0.3	15.0	3.4	17.0	3.1	17.0

Data are divided into six groups according to tumor type and manipulations, if any, with right intracarotid hyperosmolar mannitol at either 1.37 M or 1.6 M concentration. (All values are mean ± SEM. M = molar.)

[a] $n = 3$; in all other groups, $n = 4$.

[b] Left-sided tumor area and volume were significantly less ($p < 0.05$) than W256 1.37 M mannitol left-sided tumors. All other tumor areas and volumes were similar.

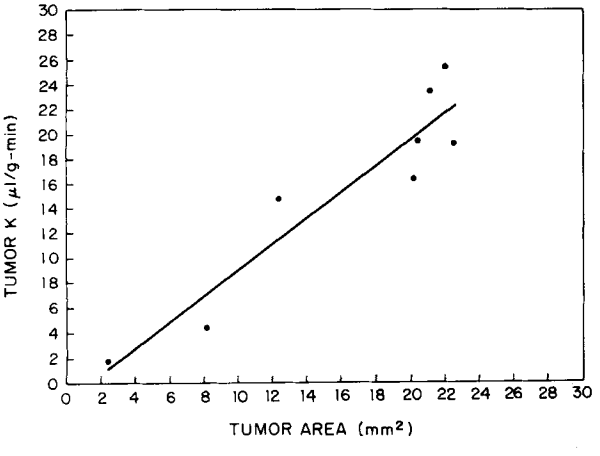

Fig 2. Correlation between mean tumor cross-sectional area (mm^2) and the mean tumor transfer constant (μl/gm–min) for all left-sided (contralateral to the intracarotid mannitol) tumors in Groups 5 and 6. The correlation coefficient was 0.94. The slope of the regression curve was 1.1 ± 0.2 μl/gm–min/mm^2.

(1.6 M mannitol C_6 glioma), left-sided tumor K values (14.6 ± 3.5 μl/gm–min) exceeded those for both adjacent tissue (5.4 ± 1.1) and cortex (0.5 ± 0.3) ($p < 0.05$ and $p < 0.01$, respectively, grouped t test) (Table 1 and Fig 3B).

Relative to the tumor value, the K value for adjacent tissue in the W256 groups was higher than the comparable relationship in the C_6 glioma groups. Thus, for the W256 groups (Groups 3 and 4) the ratio K (tumor):K (adjacent tissue) was 2:1, while for C_6 groups (Groups 5 and 6) the ratio was 3:1. These results indicate that W256 tumor showed a greater increase in the permeability of adjacent tissue than did C_6 glioma.

Effect of Hyperosmolar Mannitol in W256 Tumors

There was no increase in the K values of W256 tumor after an intracarotid infusion of 1.37 M mannitol (Group 3) compared with W256 tumors in the contralateral hemisphere or with W256 tumors in control animals (Group 2). In contrast, after 1.6 M mannitol the K values of tumors in the ipsilateral hemisphere (42.6 ± 12.4 μl/gm–min) were 13-fold greater than those on the left (3.3 ± 1.0; $p < 0.025$, grouped t

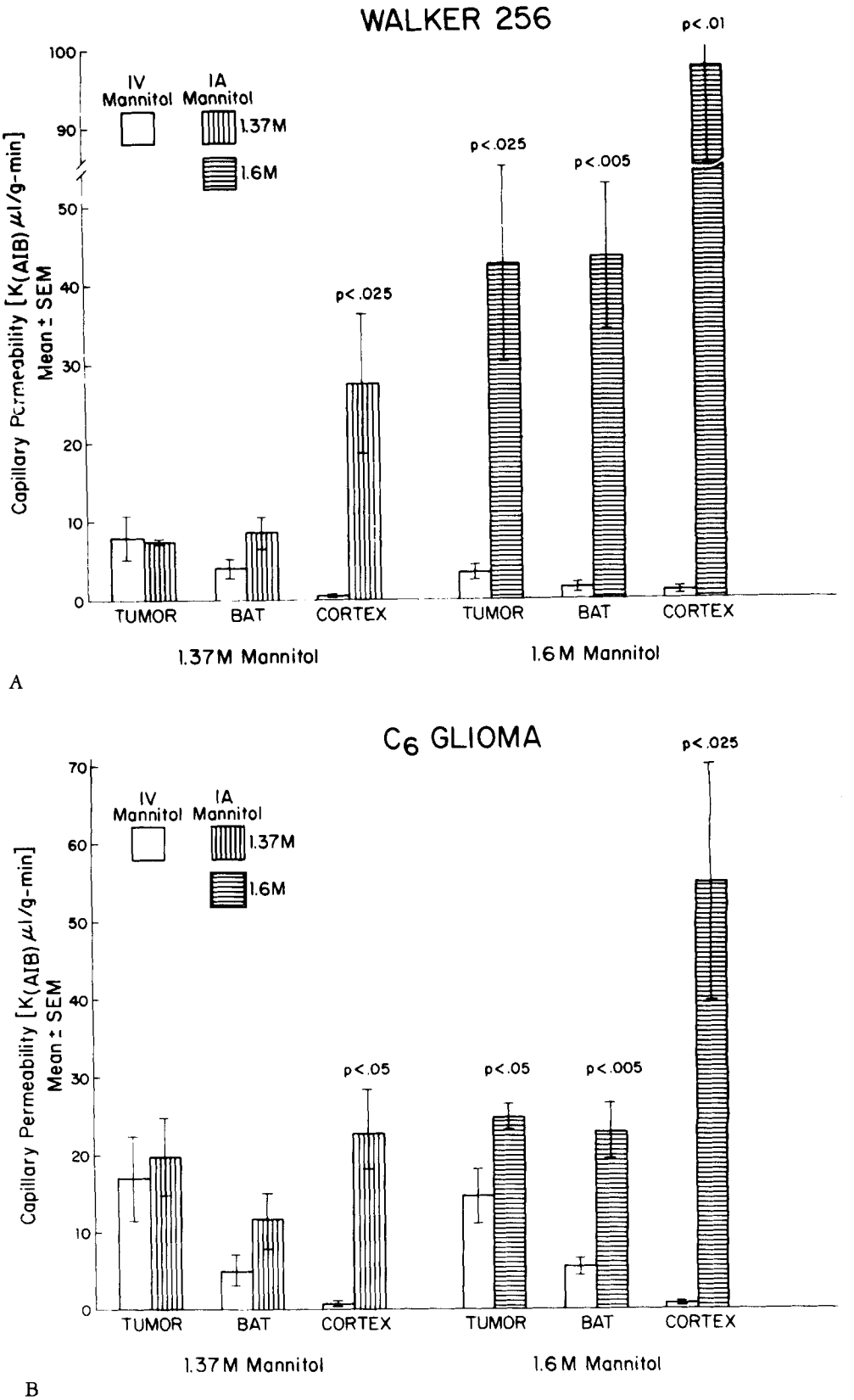

Fig 3. Effects of unilateral intracarotid hyperosmolar mannitol on the transfer constant (K, µl/gm–min) *for* ^{14}C-AIB, *measuring capillary permeability in tumor, adjacent tissue* (BAT), *and cortex, showing (A) the W256 series (Groups 3 and 4), and (B) the C_6 glioma series (Groups 5 and 6).* (IV = *the hemisphere contralateral to the mannitol;* IA = *the hemisphere ipsilateral to the mannitol;* AIB = *alpha aminoisobutyric acid.*)

test). Similarly, 1.37 M mannitol did not increase the K for adjacent tissue significantly, while 1.6 M mannitol increased it about 28-fold, from 1.5 ± 0.6 to 43.4 ± 9.2 μl/gm–min ($p < 0.005$, grouped t test).

A considerably larger increase in K values for cortex ipsilateral to the infusion was seen at both concentrations of mannitol in comparison with W256 tumors. At 1.37 M mannitol, the cortical K values on the left were 0.4 ± 0.1 μl/gm–min, while on the right they were 72-fold greater at 27.6 ± 8.8 ($p < 0.025$, grouped t test). In the 1.6 M experiment, permeability for right-sided cortex was 90-fold greater than that for the left (97.2 ± 13.0 versus 1.1 ± 0.3 μl/gm–min; $p < 0.01$, paired t test). The right-cortical K was approximately twice that of tumor and adjacent tissue on that side.

Effect of Hyperosmolar Mannitol in C_6 Glioma

There was no increase in the K values of C_6 gliomas after 1.37 M mannitol infusion (Group 5, Table 1, Fig 3B). After 1.6 M mannitol infusion (Group 6), the mean K value for tumors in the right hemisphere (4.6 ± 1.7 μl/gm–min) was 1.7 times that of tumors in the left hemisphere (14.6 ± 3.5; $p < 0.05$, grouped t test). The K values for adjacent tissue did not differ at 1.37 M mannitol, but increased 4-fold at 1.6 M mannitol ($p < 0.005$, grouped t test).

The K value for cortex ipsilateral to the mannitol infusions increased markedly at both concentrations. At 1.37 M mannitol, cortical K values increased 49-fold, from 0.5 ± 0.3 to 22.6 ± 5.6 μl/gm–min ($p < 0.05$, paired t test). In the 1.6 M mannitol experiments a 105-fold increase was observed, from 0.5 ± 0.3 to 54.7 ± 15.0 μl/gm–min ($p < 0.025$, paired t test).

Effect of Hyperosmolar Mannitol on Blood Flow

In the three nonmannitol controls (Group A), there was no significant difference in the mean blood flow between the two hemispheres for tumor, adjacent tissue, cortex, and corpus callosum. Therefore, the flow values for these structures from each side were pooled, obtaining respective means of 0.55 ± 0.02, 0.68 ± 0.02, 1.24 ± 0.03, and 0.34 ± 0.02 ml/gm–min (Table 2). Cerebral blood flow in both cortex and corpus callosum is consistent with the range reported for tumor-bearing animals (1.13 to 1.30 and 0.31 to 0.40 ml/gm–min, respectively) [1, 4, 5, 9, 10].

Mannitol (Group B) did not significantly change blood flow in corpus callosum. Cortical flow in the hemisphere contralateral to the mannitol side was increased to 1.65 ± 0.06 ml/gm–min ($p < 0.05$), and on the ipsilateral side to 1.79 ± 0.10 ($p < 0.01$). The difference in the blood flow between the cortices on the two sides was not significant.

Because of the inverse relationship between tumor cross-sectional area and blood flow reported for RT-9 and W256 tumors [9], we examined this relationship ourselves. We compared the mean largest cross-sectional area for each side in each series with corresponding values for the contralateral side (and with both sides compared individually) for the other series. There was no difference in size between the tumors. Overall, there was no significant correlation between the largest cross-sectional area of tumor and blood flow through the tumor or adjacent tissue.

There was no significant difference in tumor blood flow between tumors in opposite sides in either series, or on the same side across series. In comparison with nonmannitol controls (Group A), tumors in the mannitol group (Group B) exhibited no change in blood flow on either side. Mean tumor blood flow for both sides in all series was 0.55 ± 0.02 ml/gm–min, closer to white matter than gray matter flows. As with the tumor blood flows, there was no difference in flow in adjacent tissue between sides in either series, or on the same side across series. When compared with nonmannitol controls (Group A), intracarotid mannitol had no effect on flow for adjacent tissue on either side.

Table 2. Blood Flow (ml/gm–min) Measured with ^{14}C-IAP in Bilateral C_6 Glioma-Bearing Rats

Brain Region	Group A Nonmannitol	Group B Mannitol	
		Left	Right
PRESENT REPORT			
Cortex	1.24 ± 0.03	1.65 ± 0.06	1.79 ± 0.10
Corpus callosum	0.34 ± 0.02	0.24 ± 0.03	0.35 ± 0.11
Tumor	0.55 ± 0.02	0.48 ± 0.09	0.60 ± 0.12
Adjacent tissue	0.68 ± 0.02	0.64 ± 0.03	0.79 ± 0.12

	Tumor-Bearing Rats (range)		Non-Tumor-Bearing Rats (mean ± SEM)
LITERATURE VALUES	Side of Tumor	Contralateral	
Cortex	1.13–1.30	1.30–1.72	1.69 ± 0.13
Corpus callosum	0.31–0.40	0.31–0.39	0.40 ± 0.04

Three rats served as controls (Group A, nonmannitol, 6 hemispheres); 3 other rats were given right intracarotid 1.6 M mannitol. Flow values are mean ± SEM. Values for nonmannitol tumor-bearing rats [1, 4, 5, 9, 10] and non-tumor-bearing rats [28] taken from the literature are shown at the bottom of the table.

^{14}C = ^{14}C-iodoantipyrine.

Discussion

We designed this investigation to quantify the change in blood flow induced by the intracarotid infusion of hyperosmolar mannitol in experimental brain tumors in rats; we also sought indirectly to address the ques-

tion of whether opening the blood–brain barrier in humans with brain tumors is likely to be efficacious. Our results confirm previous observations by Rapoport [27, 28] and Neuwelt [22] and their associates that intracarotid hyperosmolar mannitol opens the blood–brain barrier in the cortex of the rat to a marked degree. Our finding that 1.6 M mannitol increased K significantly more than did 1.37 M mannitol ($p < 0.01$, grouped t test, combined Groups 3 and 5 versus Groups 4 and 6) extends the observations of Nakagawa and colleagues [21] on the response of brain tissue and brain tumor to graded hyperosmolar solutions.

Intracarotid mannitol increased brain tumor permeability in both tumor models, but only at the highest concentration of mannitol; 1.6 M mannitol raised permeability of C_6 gliomas by 1.7-fold and of W256 tumors by 13-fold. Using a natural logarithmic transformation and grouped t test, the mean left-to-right differences in K between the two tumors were significantly different ($p < 0.05$), suggesting that the response to mannitol was tumor-dependent.

In contrast to this modest effect on the tumors, 1.6 M mannitol increased cortical K approximately 100-fold for both tumor series. For both tumor models in the resting state, the capillary permeability relationship between the cortex and the tumor was reversed by mannitol. Before mannitol administration, the W256 tumor was more permeable than the cortex (by a factor of 21 in the 1.37 M mannitol and by a factor of 3 in the 1.6 M series); after mannitol, the cortex became more permeable than the tumor (4 times as permeable in the 1.37 M, and twice as permeable in the 1.6 M series). Before mannitol, the C_6 glioma was more permeable than the cortex (36 times in the 1.37 M and 28 times in the 1.6 M series); after mannitol, cortical permeability increased to equal tumor permeability. Thus, in both tumor models, tumor vessels were less sensitive to osmotic manipulation than vessels in "normal" overlying cortex. These results were similar to those reported by Nakagawa and associates [20] in RG-2 tumor, which also failed to open its barrier to a hyperosmotic load. In contrast, Neuwelt and associates [22] reported increased methotrexate entry into ASV rat gliomas after osmotic manipulation. In that study the exact mannitol concentration was not given, but, at most, it appears that capillary sensitivity to osmotic manipulation is both tumor- and concentration-dependent.

We also examined the effect of hyperosmolar mannitol on blood flow in brain tissue and C_6 gliomas; as noted by others [1, 4, 5, 7, 10], the presence of a brain tumor decreased flow in normal brain structures. Two minutes after mannitol administration there was no effect on flow in tumor, adjacent tissue, or corpus callosum. Unilateral intracarotid infusions of mannitol appear to increase cortical blood flow equally in both hemispheres. There is no good explanation for the singular mannitol effect on cortex, although the transient elevation of blood pressure during the mannitol infusion might have altered cortical vascular autoregulation.

There are no previous studies on the effect of intracarotid mannitol on blood flow in tumor-bearing animals. Our experimental design differs with the only published study that investigated the effect of intracarotid mannitol on blood flow, that of Pappius and colleagues [25], in non-tumor-bearing rats. In that study, disruption of the blood–brain barrier was achieved with mannitol at varying concentrations, but primarily at the 1.37 M concentration, and ^{14}C-IAP was injected 15 minutes after disruption of the barrier. At that point, a 65% decrease in local cerebral blood flow in the perfused hemisphere was seen. There were major differences in experimental design between our study and that of Pappius and associates; however, it is possible that blood flow decreased after the 2-minute point at which we measured it. Because AIB is trapped intracellularly, it is unlikely that such a change in flow would alter AIB egress, but it might increase its extraction. In any event, the effect on AIB was substantially more marked in the cortex than it was in the tumor.

Intracarotid mannitol should have no effect on the delivery of lipophilic chemotherapeutic drugs to brain tumors. Because the delivery of lipophilic agents is flow-dependent, intraarterial mannitol might increase the delivery of lipophilic drugs to normal cortex but not to tumor.

An important implication of our permeability study concerns the use of mannitol pretreatment to enhance delivery of water-soluble drugs. We have shown that intracarotid mannitol at the 1.6 M concentration opens the blood–tumor barrier in C_6 gliomas by 1.7-fold, in contrast to a 105-fold increase in K in the tumor-free cortex. As demonstrated by this study, modification of the blood–brain barrier with mannitol pretreatment could result in delivery of approximately 1.5-times more ^{14}C-methotrexate to cortex than to tumor [13, 14].

These data are relevant to the clinical use of this technique in chemotherapy of brain tumors. The efficacy of hyperosmolar mannitol in a given patient depends on the permeability characteristics of that patient's tumor. In the animals, 1.6 M mannitol (a greater concentration than the 1.37 M used clinically) was required to open the barrier in the W256 and C_6 tumors. While tumors differ, 1.37 M mannitol may be inadequate to open the barrier sufficiently in human brain tumors to permit increased entry of water-soluble chemotherapeutic agents. A 1.6 M mannitol infusion might open the barrier in some tumors, but it would also markedly increase drug entry into surrounding normal brain, thus exposing the brain to potentially

neurotoxic drugs. Furthermore, this concentration produced toxicity in the rat* that would be unacceptable in human subjects. Even in treating invariably lethal intracranial neoplasms, the effects of exposing normal brain to higher concentrations of potentially neurotoxic drugs cannot be justified until such a technique is first proven in animals to produce substantial benefits.

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*The mortality rate in the ^{14}C-AIB experiments was 2 early deaths in 8 rats, or 25%. In the methotrexate experiments [13, 14] 9 of 15 rats (60%) given 1.6 M intracarotid mannitol died prematurely.