Effect of hyperosmotic blood-brain barrier disruption on transcapillary transport in canine brain tumors

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Whether hyperosmotic blood-brain barrier (BBB) disruption is a technique that can be used to increase permeability of brain-tumor capillaries and thereby transiently increase drug delivery to the brain tumor is controversial. Nine virally induced brain tumors were studied in seven dogs, before and after hyperosmotic BBB disruption with 1.4 osmolar mannitol. Each dog was studied with computerized tomography (CT) after administration of the water-soluble tracer meglumine iothalamate. Each study lasted 30 minutes. A baseline CT scan and 35 to 40 additional CT scans were obtained to provide a time-related measurement of the amount of meglumine iothalamate in tissue (A(t)), and 30 plasma samples were collected to provide the time-related measurement of meglumine iothalamate in plasma (C(t)). The data were analyzed by three different methods: 1) a two-compartment model and nonlinear curve fitting were used to calculate K1 (blood-to-tissue or influx constant), k2 (tissue-to-blood or efflux constant), and Vp (plasma vascular space); 2) K1 values were calculated with a two-compartment model, assuming no efflux, at the time point for each CT scan; and 3) a "tissue advantage ratio" was calculated that expressed the ratio of tissue uptake of meglumine iothalamate at each time point, comparing values before and after BBB disruption. Regardless of which method of data analysis was used, there was a marked and significant increase in transcapillary transport of meglumine iothalamate to tumor-free brain regions, while there was only a small, transient, and insignificant increase to the brain tumors. Although there were often marked increases in delivery to cortex in the same hemisphere as the tumors, there was no significant increase to brain immediately surrounding the tumors, perhaps due to altered circulatory dynamics in this region. These data raise serious questions as to the wisdom of using this technique to increase drug delivery to brain tumors in patients and strongly support the continued study of this technique in experimental brain tumors before it is used in patients.

KEY WORDS · brain neoplasm · blood-brain barrier · drug delivery · transcapillary transport · dog

The use of hyperosmotic blood-brain barrier (BBB) disruption (HBBBD) has been proposed as a means of enhancing drug delivery to brain tumors, and has been applied clinically. However, several studies in laboratory animals with brain tumors have suggested that there is little enhancement of the rate at which water-soluble compounds are delivered to brain tumors; in contrast, there are marked increases in the rate at which water-soluble compounds are delivered to normal brain after HBBBD. There is considerable controversy as to whether HBBBD is effective in brain tumors, whether it should be used in patients at the present time, and, if used, whether controlled trials should be undertaken.

When we first studied HBBBD, we assumed that this technique increased blood-to-tumor delivery and we tried to find a way of enhancing drug delivery to brain tumors while minimizing the delivery of potentially neurotoxic chemotherapeutic drugs to normal brain. To our surprise, not even supramaximal hyperosmolar solutions increased the rate of blood-to-tumor delivery in RG-2 rat gliomas. We then applied the technique to a group of ethylnitrosourea (ENU)-induced tumors, which have capillary transport characteristics similar to those of normal brain. Although the interpretation of the results of hyperosmotic disruption in the ENU-induced tumors has itself been a matter of controversy, these experiments showed at most a fivefold increase in the blood-to-tissue transfer constant (K1) of α-aminoisobutyric acid (AIB) in tumor, while a 35-fold increase in K1 occurred in cortex. For us, the issue has slowly changed from one of determining the best way to use HBBBD to increase drug delivery to brain tumors to whether the technique works at all in brain tumors.
and, if not, why not? A series of opinions published recently has raised several issues about techniques, about interpretation of data, and about the use of HBBBD in patients.

In the present series of experiments, we have attempted to gather additional information about the use of HBBBD in brain tumors. We have approached the problem of HBBBD by using a series of virally induced brain tumors in dogs, in which the same tumor was studied before and after the use of hyperosmotic disruption, and by studying the blood-to-tissue transfer of an iodinated contrast agent, meglumine iothalamate (Conray-60), with a computerized tomography (CT) technique.

Materials and Methods

Tumor Induction and Experimental Procedures

Brain tumors were induced in 4- to 8-day-old mongrel puppies by intracerebral inoculation of 0.01 ml Schmidt-Rupin avian sarcoma virus (ASV; batch TCVC-31, 10^5 focus-forming units/ml). The virus was injected freehand through the coronal suture into the left cerebral hemisphere with a gas-tight microliter syringe equipped with an automatic injecting device. At the age of 2 months, the puppies were anesthetized with intramuscular xylazine (0.45 mg/kg) and ketamine (1.8 mg/kg), and were injected intravenously with meglumine iothalamate (3 cc/kg); CT scans were then obtained of the entire brain in adjacent 5-mm slices. Tumor-bearing dogs were identified and used for the experimental studies. All dogs were asymptomatic when studied by CT. Nontumor-bearing dogs were rescanned at 2-week intervals and used for experimental studies when they did develop tumors.

Each tumor-bearing dog underwent an initial experiment in the untreated state; 2 to 4 days later it was subjected to another HBBBD experiment. Tumor-bearing dogs were anesthetized with xylazine and ketamine as described above; anesthesia was repeated as needed to maintain sedation. Unilateral distal femoral arterial and venous polyethylene (PE-90) catheters were inserted, and the wound was irrigated with 2% viscous Xylocaine (lidocaine) and surgically closed. The arterial catheter was used to obtain arterial blood samples during the experiment and to monitor arterial blood pressure and blood gases. The venous catheter was used to administer heparin (1000 U) and for intravenous infusion of meglumine iothalamate. Rectal temperature was monitored periodically throughout the experiment. After the first CT study was completed, the arterial and venous catheters were removed, the vessels were ligated, and the wound was irrigated with Betadine (povidone iodine) and closed surgically.

For HBBBD studies, the dogs were first prepared as just described. In addition, the left carotid bifurcation was exposed and a polyethylene (PE-160) catheter was inserted into the infundibulum of the internal carotid artery and tied into the internal carotid artery; the external carotid and common carotid arteries were ligated. Filtered mannitol (1.4 Osm, 37°C, pH 7.4) was infused at a flow rate of 1.8 ml/sec for 30 seconds. Immediately after completion of the mannitol infusion, an infusion of meglumine iothalamate was started and a CT study was performed as described below.

CT Measurements of Plasma and Tissue Iodine Concentration

Tumor-bearing dogs were placed in the CT scanner in a prone position. A Lucite stereotactic headframe, attached to the CT scanner, was used to hold the head in position. Initial CT scans were obtained to verify that the scanning location was through the maximum cross-sectional diameter of the tumor (based on the previous series of contrast-enhanced scans through the entire brain). When the scanning location was satisfactory, the table position was not changed for the duration of the experiment; the stereotactic headframe was used to assure that the same location was scanned in both studies. In both studies, the same dose of meglumine iothalamate (3 cc/kg) was infused over 5 minutes. Computerized tomography scans were performed using the dynamic mode to program interscan delays, at 5-mm slice thickness, and a technique of 120 kVp, 250 mA, and 2.2-msec pulse width. A total of 40 CT scans were obtained over a 30-minute period, with most CT scans obtained during the first 10 minutes, when blood concentrations were rapidly changing.

Prior to the start of an infusion, two arterial blood samples were obtained. Thirty 1-ml arterial samples were obtained during the 30-minute experiment, with the largest number of samples obtained during the first 10 minutes. At the end of each experiment, the arterial samples were centrifuged and plasma was pipetted into plastic microcentrifuge tubes which were inserted into a Plexiglas phantom and scanned with the same technique as was used throughout the experiment. Overlapping slices at 2.5-mm increments were obtained through each plasma sample, resulting in five to 10 slices from each. Plasma concentrations of iodine (Cp) were obtained by first measuring the value of x-ray attenuation, in Hounsfield units (HU), for each slice of each plasma sample. The mean value of all measurements from each sample was calculated. The plasma value used in calculations (Cp) represented the mean value for a given plasma sample minus the mean value of the plasma in the two baseline samples. Thus, Cp is represented by ΔHU, the change in HU over the baseline value of plasma that did not contain iodine, per unit volume of plasma.

Tissue measurements of iodine (Am), which represent the sum of the intravascular (Ai) and extravascular (Ar) components, were similarly obtained. The mean HU value for each region of interest (ROI) was obtained from each CT scan, and ΔHU was calculated by subtracting the value of the baseline scan for that ROI from the HU for each contrast-enhanced scan. For the calculation of bidirectional transport, the values of Ki,
k₂ (the tissue-to-blood efflux constant), and Vᵰ (the tissue plasma vascular volume) were first calculated for each voxel in the CT scan matrix (as described in the next section) and ROI's were selected from the reconstructed Kᵢ, k₂, and Vᵰ images. For determinations of the time course of a unidirectional blood-to-tissue transfer constant (Kᵢ*, see below) and the "tissue advantage ratio," regional ΔHU values were obtained from each of the series of CT scans. (For the remainder of this manuscript, Kᵢ will be used to indicate a blood-to-tissue transfer constant when efflux, tissue-to-blood, is allowed; and Kᵢ* will be used to indicate a unidirectional blood-to-tissue transfer constant.) In each type of analysis, values were obtained from the following regions: whole tumor (based on the histologically defined margins); tumor center (representing about 20% of the tumor area); tumor periphery (representing 50% to 80% of the tumor area at its margin); brain around the tumor (BAT, a 2.5-mm zone immediately outside the brain-tumor junction); brain surrounding the tumor (BST, a 2.5-mm zone immediately outside BAT); ipsilateral cortex (a region of tumor-free cortex lying within the disrupted area of the same hemisphere as the tumor); and contralateral cortex (an area corresponding in size and location to the tumor, but in the opposite, undisrupted hemisphere).

Correlations Between CT Studies and Tumor Histology

After completion of the second CT study, the dogs were killed with an intravenous overdose of pentobarbital, and the brains were rapidly removed (over 2 to 3 minutes) and frozen in liquid freon. Each brain was serially sectioned 20 μm thick, and selected slides through each tumor were stained with hematoxylin and eosin. All regional tissue measurements were based on histological sections. Grids of corresponding size were placed on the displayed CT image of the contrast-enhanced tumor and upon a magnified image of the corresponding histological section. Measurements were obtained by outlining corresponding areas in the two grids.

Analysis of CT Studies

Three different approaches were used to analyze the data obtained from the CT experiments. In the first approach, the entire set of timed tissue data (Aᵢ(t)) and plasma data (Cᵰ(t)) was used to calculate an influx constant (Kᵰ) for meglumine iothalamate, an efflux constant (k₂), and a plasma vascular space (Vᵰ) according to methods described by Grootuhs, et al. (unpublished data). In the second approach, the time course of Kᵰ for meglumine iothalamate was calculated using equations described by Rapoport, et al., and by Blasberg, et al. In the third approach, we calculated a "tissue advantage ratio" that reflected the delivery advantage over time due to HBBD. Each of these methods of analysis will be described separately.

Mathematical Model for Calculation of Bidirectional Transcapillary Rate Constants. A two-compartment model was used for this analysis of the CT studies. If Aᵢ represents the amount of iodinated contrast material in a tissue volume sampled by the CT scanner, then

\[ Aᵢ = Aₑ + Aᵰ, \]

where Aₑ is the extravascular amount in the tissue (brain or tumor) and Aᵰ is the intravascular amount in the tissue;

\[ Aᵰ = Vᵰ Cᵰ, \]

where Vᵰ is the plasma vascular volume (ml/100 gm) in the tissue and Cᵰ is the arterial plasma concentration. The differential equation expressing the rate of change of the amount of iodinated contrast material in tissue is:

\[ \frac{dAᵢ}{dt} = Kᵰ Cᵰ - kᵰ Aᵰ, \]

where Kᵰ is the blood-to-tissue transfer constant (ml/gm/min) and kᵰ is the tissue-to-blood transfer constant (min⁻¹). Equation 3 can be solved to give:

\[ Aᵢ = Kᵰ \int_0^t e^{-kᵰ(t-τ)} Cᵰ(τ) dτ + Vᵰ Cᵰ. \]

For measurements performed at time t, Equations 1, 2, and 4 can be combined:

\[ Aᵢ = Kᵰ \int_0^t e^{-kᵰ(t-τ)} Cᵰ(τ) dτ + Vᵰ Cᵰ. \]

When the CT studies were completed, there were two sets of data: 1) a timed set of plasma concentration values (Cᵰ(t)); and 2) a timed set of CT scans (Aᵢ(t)). Each CT scan consisted of a 320 × 320 matrix of HU measurements for each 0.8 × 0.8 × 5-mm voxel in a scan (using GE 8800 volume elements). Each voxel represents a timed series of Aᵢ (that is, Aᵢ(t,i)), where i represents the ith voxel in a 320 × 320 matrix and t is the time of the scan. After the CT study was completed, the tape from the GE 8800 CT/T scanner was further analyzed. Nonlinear curve-fitting methods were used to obtain values of Kᵰ, k₂, and Vᵰ for each voxel in the 320 × 320 matrix. For initial evaluation of CT data, we limited k₂ to a range of 0.000 to 0.300 min⁻¹, in 0.003 increments. The calculated values of Kᵰ, k₂, and Vᵰ were then assigned pseudocolors, displayed on a color monitor, and analyzed with an image-processing computer to obtain regional values of Kᵰ, k₂, and Vᵰ for meglumine iothalamate.

Time Course of Kᵰ*. For this analysis, regional values of Kᵰ* were calculated from the CT data for each time point at which a CT scan was obtained. The value of Kᵰ* in each region and at each time point was calculated as previously described by Rapoport, et al., and Blasberg, et al., as follows:

\[ Kᵰ* = \frac{Aₑ}{\int_0^T Cᵰ(τ) dτ}. \]
Fig. 1. Studies in Dog 4.  Left: Histological section from the level of the computerized tomography scans showing a single tumor in inferior frontal cortex. Right: Computed-reconstructed images of the blood-to-tissue transfer constant \( K_1 \) of meglumine iothalamate as calculated with Equation 5, before (upper) and after (lower) hyperosmotic blood-brain barrier disruption. The pseudocolor scale to the right shows the values of \( K_1 \), in units of \( \mu l \cdot gm^{-1} \cdot min^{-1} \). The tumor-free brain in the postdisruption study shows elevated values of \( K_1 \) throughout the hemisphere ipsilateral to the tumor and in much of the contralateral hemisphere, whereas the brain tumor actually shows slightly lower values in the postdisruption study.

### TABLE 1
Summary of values for each tumor*  

<table>
<thead>
<tr>
<th>Tumor No.</th>
<th>Type of Study</th>
<th>Tumor Area (sq mm)</th>
<th>Whole Tumor</th>
<th>Ipsilateral Cortex</th>
<th>Contralateral Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( K_1 )</td>
<td>( k_2 )</td>
<td>( V_p )</td>
</tr>
<tr>
<td>1a H</td>
<td>434</td>
<td>32 ± 0.9</td>
<td>103 ± 2.7</td>
<td>7.4 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>1a C</td>
<td>434</td>
<td>34 ± 1.5</td>
<td>60 ± 2.0</td>
<td>9.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>1b H</td>
<td>165</td>
<td>18 ± 1.0</td>
<td>62 ± 3.8</td>
<td>8.9 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>1b C</td>
<td>142</td>
<td>15 ± 1.1</td>
<td>53 ± 4.6</td>
<td>7.1 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>2a H</td>
<td>88</td>
<td>23 ± 0.9</td>
<td>66 ± 3.1</td>
<td>8.8 ± 0.6</td>
<td>12.0 ± 21</td>
</tr>
<tr>
<td>2a C</td>
<td>73</td>
<td>19 ± 1.1</td>
<td>82 ± 4.5</td>
<td>6.8 ± 0.4</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>2b H</td>
<td>73</td>
<td>26 ± 1.5</td>
<td>73 ± 4.6</td>
<td>5.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>2b C</td>
<td>80</td>
<td>33 ± 2.4</td>
<td>98 ± 5.6</td>
<td>6.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>3 H</td>
<td>121</td>
<td>41 ± 1.7</td>
<td>82 ± 3.3</td>
<td>5.4 ± 0.6</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>3 C</td>
<td>164</td>
<td>36 ± 1.5</td>
<td>97 ± 4.7</td>
<td>11.0 ± 0.5</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>4 H</td>
<td>108</td>
<td>49 ± 2.2</td>
<td>110 ± 4.5</td>
<td>6.7 ± 0.4</td>
<td>7.8 ± 0.4</td>
</tr>
<tr>
<td>4 C</td>
<td>93</td>
<td>32 ± 2.0</td>
<td>89 ± 5.5</td>
<td>4.9 ± 0.6</td>
<td>1.2 ± 4.3</td>
</tr>
<tr>
<td>5 H</td>
<td>249</td>
<td>41 ± 1.0</td>
<td>125 ± 2.8</td>
<td>10.7 ± 0.2</td>
<td>4.8 ± 1.7</td>
</tr>
<tr>
<td>5 C</td>
<td>293</td>
<td>31 ± 0.8</td>
<td>160 ± 2.6</td>
<td>12.7 ± 0.3</td>
<td>4.1 ± 1.8</td>
</tr>
<tr>
<td>6 H</td>
<td>548</td>
<td>14 ± 0.1</td>
<td>65 ± 2.6</td>
<td>6.6 ± 0.1</td>
<td>24.0 ± 0.6</td>
</tr>
<tr>
<td>6 C</td>
<td>424</td>
<td>14 ± 0.4</td>
<td>101 ± 2.2</td>
<td>4.7 ± 0.1</td>
<td>4.0 ± 1.6</td>
</tr>
<tr>
<td>7 H</td>
<td>20</td>
<td>25 ± 2.0</td>
<td>58 ± 10.2</td>
<td>3.7 ± 0.5</td>
<td>21.0 ± 0.8</td>
</tr>
<tr>
<td>7 C</td>
<td>20</td>
<td>34 ± 5.9</td>
<td>64 ± 10.6</td>
<td>2.4 ± 0.5</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>summary H</td>
<td>30 ± 3.9</td>
<td>83 ± 8.1</td>
<td>7.0 ± 0.7</td>
<td>12.4 ± 2.8</td>
<td>50 ± 3.5</td>
</tr>
<tr>
<td>summary C</td>
<td>28 ± 3.0</td>
<td>89 ± 10.6</td>
<td>7.3 ± 1.1</td>
<td>2.8 ± 1.1</td>
<td>35 ± 7.3</td>
</tr>
</tbody>
</table>

* Values of the blood-to-tissue transfer constant \( K_1 \) (\( \mu l/gm/min \)), tissue-to-blood efflux constant \( k_2 \) (\( \times 10^{-3} min^{-1} \)), and tissue plasma vascular space \( (V_p, ml/100 gm) \) for the following regions: whole tumor, a region of cortex in the same hemisphere as the disruption (ipsilateral cortex), and a region of cortex in the hemisphere opposite to the disruption (contralateral cortex). H = hyperosmotic disruption, C = control. The area of the tumor (sq mm) was based on measurements from the histological section. Values are means of values calculated for individual voxels, ± standard error of the means.
Hyperosmotic BBB disruption and transcapillary transport

where \( A_e \) represents the concentration in tissue extracellular space (after a vascular space correction has been applied from Equations 1 and 2 (the value of \( V_p \) used was that from Equation 5)) and \( T \) represents the time of that scan. Note that this method of expressing \( K_t^* \) does not include \( k_2 \); application of this method assumes that tissue-to-blood efflux is negligible during the time course of the experiment. This assumption is reasonable as long as the blood concentration exceeds the tissue concentration at all times following infusion, until time \( T \).

**Tissue Advantage Ratio.** A third method was used to compare the data from the control study with the findings obtained after HBBBD in the same animal. Since a series of 40 CT scans were obtained during the 30-minute period immediately after BBB disruption, it is possible to obtain 40 values of \( A_e \) that represent the time course of the amount of meglumine iothalamate in tissue extracellular space (Equations 1 and 2). Since the animals and tumors were the same in both studies, we assume that the only difference in the amount of meglumine iothalamate that accumulated in the tissue extracellular space was caused by the increased capillary permeability from hyperosmotic disruption. We normalized the \( A_e \) values by the plasma arterial \( C_p \) values at each time point in order to account for differences between plasma concentrations of meglumine iothalamate in the two studies. If \( A_e(t)/C_p(t) \) represents the normalized value at each time point, then the tissue advantage ratio is calculated from:

\[
\frac{A_e(t)/C_p(t) \text{ (after hyperosmotic disruption)}}{A_e(t)/C_p(t) \text{ (before hyperosmotic disruption)}}
\]

(7)

Table 2 presents the values of \( K_t^* \), \( k_2 \), and \( V_p \) calculated for meglumine iothalamate from Equation 5 in the disrupted hemisphere (tumor and ipsilateral cortex) and in the contralateral hemisphere without BBB disruption. Values are not presented for the ipsilateral cortex of Dog 1 because the tumor and surrounding edema occupied most of the ipsilateral hemisphere. The value of \( K_t^* \) in the cortex of the ipsilateral hemisphere with HBBBD was significantly higher than \( K_t^* \) in the ipsilateral cortex of the control (paired t-test, \( p < 0.03 \)) or of the contralateral cortex, either before (paired t-test, \( p < 0.05 \)) or after BBB disruption (paired t-test, \( p < 0.05 \)). The value of \( k_2 \) in the disrupted ipsilateral cortex was similarly and significantly different from the other regions. The value of \( V_p \) was not significantly different among the brain regions, before or after disruption (analysis of variance, \( p > 0.02 \)). Figure 1 illustrates reconstructed images of \( K_t^* \) for meglumine iothalamate obtained in Dog 4 (Table 1), before and after BBB disruption.

Figure 2 illustrates the time course of values of \( K_t^* \) of meglumine iothalamate calculated from Equation 6, for tumor-free cortex and tumor before and after BBB disruption. In tumor-free cortex, the \( K_t^* \) values after disruption are much higher than those from the control study and drop more rapidly, although at the end of the 30-minute experiment the value of \( K_t^* \) of meglumine iothalamate in the hyperosmotically disrupted cortex is still 10 times that of the control value.

We have also presented the data as a time course of normalized tissue concentration ratios, which we have called the “tissue advantage ratio.” The extent to which delivery of meglumine iothalamate to the tissue is enhanced is reflected by a tissue advantage ratio of more than 1.0. These data are summarized in Table 2 and illustrated graphically in Fig. 3 for values from Dog 1. In this dog, the value of the tissue advantage ratio in the ipsilateral cortex peaked soon after the initiation of meglumine iothalamate infusion (corresponding to the

### Table 2

<table>
<thead>
<tr>
<th>Tumor No.</th>
<th>Tissue Advantage Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole Tumor</td>
</tr>
<tr>
<td>1a</td>
<td>1.28 ± 0.1</td>
</tr>
<tr>
<td>1b</td>
<td>1.02 ± 0.4</td>
</tr>
<tr>
<td>2a</td>
<td>0.99 ± 0.3</td>
</tr>
<tr>
<td>2b</td>
<td>0.90 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>1.79 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>0.95 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>1.11 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>0.66 ± 0.4</td>
</tr>
<tr>
<td>summary</td>
<td>1.11 ± 0.1</td>
</tr>
</tbody>
</table>

* Tissue advantage ratio (TAR) for each of the different tumors and tumor-free cortex from the same hemisphere. The TAR represents the normalized value of the concentration of meglumine iothalamate in the tissue extracellular space from the hyperosmotic disruption study, divided by the corresponding value from the control study. If there is no change due to hyperosmotic disruption, the value of the TAR should be 1.0. Values above 1.0 represent enhanced delivery of meglumine iothalamate as a result of hyperosmotic disruption. For each tumor, the value represents the mean ± standard error of the value calculated at the time point of each scan (see Fig. 3).

The tissue advantage ratio will have a value close to 1.0 if there is no difference in the extracellular concentration of meglumine iothalamate between the two studies. This value will be greater than 1.0 if there is an increase in \( A_e \) due to disruption.

### Results

A total of nine tumors were studied in seven dogs. The physiological parameters of all dogs (arterial \( pO_2 \), \( pCO_2 \), \( pH \), arterial blood pressure, and rectal temperature) were within the physiological range at the time the CT studies were performed. Histologically, the tumors represented the typical spectrum of ASV-induced brain tumors: Tumors 1a, 1b, 2a, 6, and 7 (Tables 1 and 2) were anaplastic astrocytomas, Tumors 3 and 4 were polymorphic astrocytomas, Tumor 2b was a fibroillary astrocytoma, and Tumor 5 was a sarcoma.

**Effect of HBBBD in Tumor-Free Brain**

Table 1 presents the values of \( K_t^* \), \( k_2 \), and \( V_p \) calculated for meglumine iothalamate from Equation 5 in the disrupted hemisphere (tumor and ipsilateral cortex) and in the contralateral hemisphere without BBB disruption. Values are not presented for the ipsilateral cortex of Dog 1 because the tumor and surrounding edema occupied most of the ipsilateral hemisphere. The value of \( K_t^* \) in the cortex of the ipsilateral hemisphere with HBBBD was significantly higher than \( K_t^* \) in the ipsilateral cortex of the control (paired t-test, \( p < 0.03 \)) or of the contralateral cortex, either before (paired t-test, \( p < 0.05 \)) or after BBB disruption (paired t-test, \( p < 0.05 \)). The value of \( k_2 \) in the disrupted ipsilateral cortex was similarly and significantly different from the other regions. The value of \( V_p \) was not significantly different among the brain regions, before or after disruption (analysis of variance, \( p > 0.02 \)). Figure 1 illustrates reconstructed images of \( K_t^* \) for meglumine iothalamate obtained in Dog 4 (Table 1), before and after BBB disruption.

Figure 2 illustrates the time course of values of \( K_t^* \) of meglumine iothalamate calculated from Equation 6, for tumor-free cortex and tumor before and after BBB disruption. In tumor-free cortex, the \( K_t^* \) values after disruption are much higher than those from the control study and drop more rapidly, although at the end of the 30-minute experiment the value of \( K_t^* \) of meglumine iothalamate in the hyperosmotically disrupted cortex is still 10 times that of the control value.

We have also presented the data as a time course of normalized tissue concentration ratios, which we have called the “tissue advantage ratio.” The extent to which delivery of meglumine iothalamate to the tissue is enhanced is reflected by a tissue advantage ratio of more than 1.0. These data are summarized in Table 2 and illustrated graphically in Fig. 3 for values from Dog 1. In this dog, the value of the tissue advantage ratio in the ipsilateral cortex peaked soon after the initiation of meglumine iothalamate infusion (corresponding to the
FIG. 2. Dog 4. Time course of the values of the unidirectional blood-to-tissue transfer constant (Kt) of meglumine iothalamate for each time at which a computerized tomography scan was obtained. The values are for whole tumor, before (circles) and after (asterisks) hyperosmotic blood-brain barrier disruption, and for tumor-free brain in the hemisphere ipsilateral to the disruption (ILC), before (x) and after (+) disruption. Note that in tumor between 0 and 4 minutes, the Kt values from the postdisruption study are slightly higher, but that after 4 minutes the two tumor Kt curves are almost superimposed. In contrast, the Kt values in tumor-free cortex are 10-fold higher in the postdisruption study and remain so during the period of the experiment. The scale for the blood-to-tissue transfer constant is in units of µl/gm/min, and is logarithmic.

FIG. 3. Dog 1. Value of the tissue advantage ratio calculated at each time point of the computerized tomography study in tumor (asterisks) and tumor-free cortex ipsilateral to the tumor (ILC, circles). The tissue advantage ratio represents the normalized value of the concentration of meglumine iothalamate in the tissue extracellular space from the hyperosmotic disruption study, divided by the corresponding value from the control study. Note that, similar to the (Kt) unidirectional blood-to-tissue transfer constant curves shown in Fig. 2, there is a slight increase in values in whole tumor during the first 4 minutes, after which the values remain at about 1.0, indicating no delivery advantage from hyperosmotic disruption. The dotted line represents a tissue advantage ratio of 1.0; note that the tumor value is superimposed on the dotted line after 4 minutes.

point of maximal BBB disruption) and then plateaued after 8 minutes at a level of approximately 9.

The Effect of Hyperosmotic Disruption on Brain Tumor. Table 1 presents the values of Kt, k2, and Vp in whole tumor before and after BBB disruption. There was no significant change in these values as a result of disruption (paired t-test, p > 0.25). This is reflected in Table 1 by the fact that in three tumors the value of Kt was actually slightly larger in the control study than in the HBBBD study, and is illustrated in Fig. 1 where the increase in brain Kt is contrasted with very little change within the tumor. An additional indication of the lack of effect of HBBBD on transcapillary transport within the brain tumors is indicated in Figs. 2 and 3. In Figure 2, the time course of Kt is shown for whole tumor before and after hyperosmotic disruption. Except for the values of Kt during the first few minutes of the study, the curves are superimposed. The values of the tissue advantage ratios are presented in Table 2 for each tumor and illustrated in Fig. 3 for Dog 1. The mean tissue advantage ratios for all tumors was 1.1 ± 0.1; in four tumors there was actually a decrease in value, despite considerable increases in tumor-free cortex.

Figure 4 presents a summary from all nine tumors of the mean Kt values of meglumine iothalamate in the tumor center, tumor periphery, BAT, BST, and ipsilateral cortex, both before and after disruption, as calculated from Equation 5. Although the values of Kt for meglumine iothalamate are higher in each area after HBBBD than in the control state, it is only in tumor-free ipsilateral cortex that this difference reaches significance.

Discussion

This study, performed with a different technique and a different tumor model than those we have previously used, confirms our earlier studies. There was no change in the rate of delivery to the brain tumors as a result of HBBBD, while at the same time there was a marked enhancement of delivery to tumor-free brain. As presented in Table 1 and Figs. 1 and 4, there was no significant change in the blood-to-tissue transfer rate constant, Kt, of meglumine iothalamate in this group of ASV-induced canine brain tumors. The data are also presented as a time course of unidirectional Kt values in tumor-free cortex and whole tumor (Fig. 2) and as a time course of tissue concentration values of meglumine iothalamate, comparing studies done before and after HBBBD. The summarized data in Table 2 and the values from single dogs presented in Fig. 3 again demonstrate that there was no enhancement of delivery to brain tumors.

As we have stated before, it is important to empha-
Hyperosmotic BBB disruption and transcapillary transport

![Graph](image)

**Fig. 4.** Mean value of the blood-to-tissue transfer constant ($K_1$) of meglumine iothalamate obtained from all experiments in the tumor center, tumor periphery, brain surrounding the tumor (BST), and ipsilateral tumor-free cortex (ILC), before (crosses) and after (circles) hyperosmotic blood-brain barrier disruption. The $K_1$ values were calculated with Equation 5. The error bar is the standard error of the mean. The vertical axis shows the value of $K_1$ in units of $\mu l/gm/min$. Only in tumor-free cortex is the difference between the predisruption $K_1$ value (control) and the postdisruption study significant ($p < 0.05$, paired t-test).

size that the determination of a transfer rate constant after hyperosmotic disruption is not easily performed and the interpretation of the rate constants presented in any study of HBBBD must be done with care. The purpose of HBBBD is to disrupt the integrity of the capillary wall, which in turn allows water-soluble material in the plasma to cross the capillary wall. In normal brain the capillaries rapidly reconstitute themselves so that the rate that water-soluble material crosses the BBB rapidly decreases during the immediate postdisruption period. As such, any method for measuring a transcapillary transfer rate constant that depends upon a steady-state condition, such as those in Equations 5 and 6, will be in error to some extent, depending upon the tracer used in the study and the methods of data analysis. This error is perhaps less severe when the tracer is AIB. Since AIB is trapped intracellularly once it has crossed the BBB, what is being measured in those types of experiments is a mean $K_1$ value over the time period used in the experiment. When other tracers are used, such as sucrose or meglumine iothalamate, as in the present experiments, the possibility exists for bidirectional movement across the capillary wall. The highest (and most accurate) values of $K_1$ will therefore be reported in those experiments in which AIB is used, the infusion is begun immediately after hyperosmotic disruption, and the experimental time is shortest. The method of analysis used to determine $K_1$ will also influence the results. For example, Equation 6 does not allow for efflux during the time course of the experiment. Some efflux of meglumine iothalamate is almost certainly occurring in the tumors during the 30-minute experiment, since the final plasma concentration ($C_p$) in these experiments was in the same range as the final measured $A_e$ value, and in some instances $C_p$ was less than $A_e$ in tumor near the end of the experiment. Thus, our values of $K_1$ reported in Table 1 and illustrated in Figs. 1 and 4 and our values of $K_1$* in Fig. 2 must be considered "apparent" $K_1$ values. However, as is evident from a comparison of Equations 4 and 6, Equation 6 is the same as Equation 4 if no efflux exists (that is, $k_2 = 0$). If $k_2$ is greater than 0, as is the case with meglumine iothalamate or sucrose, $K_1$* will always be underestimated in Equation 6. The presence of an exponential term containing negative $k_2$ tends to decrease values of $A_e$ for increasing values of $K_1$. Since the term is exponential, and given the expected range of $k_2$ values (even taking into account relatively large changes due to disruption), the effect on $A_e$ is less pronounced. For example, if a $k_2$ of 0.01 min$^{-1}$ is increased tenfold by disruption to 0.1 min$^{-1}$, the effect on $A_e$ will be to decrease it by a factor of 1.09. In the format of Equation 6, if a disrupted value of 0.1 min$^{-1}$ is erroneously assumed to be 0, the resulting error in $K_1$* will underestimate it by 10%. Since the changes measured in $K_1$* are as high as 1000% in disrupted normal brain, a 10% error due to ignoring $k_2$ will not obscure the presence or absence of an effect due to BBB disruption.

The time course presentation of $K_1$* values (Fig. 2) and of tissue concentration values (Fig. 3) illustrates another important point. The marked increase in $K_1$* and tissue advantage ratio in tumor-free cortex immediately after the start of the meglumine iothalamate infusion is clearly shown. The fall of $K_1$ and of the tissue advantage ratio in the period from 2 to 8 minutes probably represents closing, or reconstitution of the BBB. This same pattern was present in all dogs. In the tumor there was a very transient rise in the values of $K_1$* and the tissue advantage ratio during the 1- to 4-minute time period, after which the latter stays very close to a value of 1.0 (Fig. 3) and the curves of the former (from tumor, before and after disruption) are superimposed after 4 minutes (Fig. 2). The early transient rise in $K_1$* and tissue advantage ratio may represent an actual effect of the hyperosmotic disruption on the tumor which dissipates more rapidly than in tumor-free brain. This early effect may be due to action of the hyperosmotic solution on the tumor capillaries, or may represent altered circulatory hemodynamics caused by the infusion of the hyperosmotic solution. In either case, its effect is brief and small.

A major point of confusion about evaluating the effects of HBBBD comes when the effects that are seen in normal brain are extrapolated to brain tumors. This is not an uncommon event. In fact, it was because we extrapolated effects of HBBBD on normal brain to tumors that first led us to perform experiments with HBBBD in experimental brain tumors. This type of extrapolation continues to mislead us. Rapoport ar-
gued that one should not include animals in which the BBB was not disrupted in BAT or BST in the data analysis. This is an issue that has also troubled us, since the effect of HBBBD in BAT and BST has clearly not been as dramatic as seen in more distant tumor-free cortex (Fig. 4). We have previously identified several variables that could explain a difference in the behavior of BAT and BST. Although we do not know which of these variables accounts for the difference in the behavior of BAT and BST vessels in response to HBBBD, it is abundantly clear that this tissue region, like the tumor, behaves quite differently from normal brain. This is clearly illustrated in Fig. 1, which shows the regional $K_t$ values before and after disruption. Despite the fact that most of both hemispheres in this dog were disrupted, the $K_t$ values measured in tumor and in the BAT-BST region were actually slightly lower than in the control state. It appears that one cannot forget that a brain tumor is a growing mass within brain and that it may quite readily disturb the circulatory dynamics in surrounding tissue.

Perhaps the most fascinating question, and one that remains unanswered, is why HBBBD fails to disrupt the vessels of brain tumors. Part of the answer may lie simply in the number of available vessels. In RG-2 experimental gliomas, the density of capillaries is one-fifth that of cortex. However, if that alone were the answer then one would expect to see one-fifth the level of disruption in tumors that is seen in cortex, an interpretation which our studies do not support. The presence of endothelial fenestrations and discontinuities may negate the effect of hyperosmotic solutions, perhaps by serving as an osmotic buffering system. However, at the present time it must be stated that we do not know which factor or combination of factors is responsible for the absence of an effect of HBBBD in brain tumors.

Fishman argued that “published data have not established that osmotic injury is an appropriate adjunct to brain tumor chemotherapy suitable for clinical trial.” Both Rapoport and we stated that randomized trials should be performed in brain tumor patients; however, we now wish to revise our position and agree with that of Dr. Fishman. Since there is almost no objective quantitative evidence to support the contention that this technique works to increase drug delivery to brain tumors, we do not believe that HBBBD should be used therapeutically in patients, even in controlled trials. Additional studies are needed in laboratory models of brain tumors in an attempt to identify why this technique does not work, and perhaps to modify it so that it does work. Any studies in patients should be limited in scope and directed at obtaining quantitative measures of delivery before and after HBBBD, without the concomitant use of chemotherapeutic drugs.

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