In vivo assessment of the window of barrier opening after osmotic blood–brain barrier disruption in humans

TAI SIGAL, M.D., RINA RUBINSTEIN, M.D., FELIX BOKSTEIN, M.D., ALLAN SCHWARTZ, M.D., ALEXANDER LOSSOS, M.D., EDNA SHALOM, R.N., ROLAND CHISIN, M.D., and J. MOSHE GOMORI, M.D.

Neurooncology Center, Department of Nuclear Medicine, and Neuroradiology Unit, Hadassah Hebrew University Hospital, Jerusalem, Israel

Object. Osmotic blood–brain barrier (BBB) disruption induced by intraarterial infusion of mannitol is used in conjunction with chemotherapy to treat human brain tumors. The time course to barrier closure, or the so-called therapeutic window, has been examined in animals but little information is available in humans. The authors, therefore assessed the time course to barrier closure after osmotic BBB disruption in humans.

Methods. Disruption of the BBB was demonstrated using 99mTc-glucoheptonate (TcGH) single-photon emission computed tomography (SPECT) scanning in 12 patients who were treated monthly with combination chemotherapy in conjunction with BBB disruption. The primary diagnosis was primary central nervous system lymphoma in seven patients and primitive neuroectodermal tumors in five. The TcGH (20 mCi) was injected at 1- to 480-minute intervals after osmotic BBB disruption, and patients underwent SPECT scanning after 4 hours. A total of 38 studies was performed. Good-to-excellent BBB disruption was obtained in 29 procedures and poor-to-moderate disruption was seen in the other nine studies.

The TcGH indices correlated with the degree of BBB disruption as measured postprocedure on contrast-enhanced CT scans (r = 0.852). Mean baseline TcGH indices were 1.02 ± 0.07. For the group of patients with good-to-excellent disruptions the mean indices at 1 minute postdisruption measured 2.19 ± 0.18. After 40 minutes no significant change was noted (mean index 2.13 ± 0.2). Then the indices declined more steeply and at 120 minutes after the disruption the index was 1.36 ± 0.02. A very slow decline was noted between 120 and 240 minutes after mannitol infusion. At 240 minutes the barrier was still open for all good-to-excellent disruptions (index 1.33 ± 0.08) but at 480 minutes the mean indices had returned to the baseline level.

Conclusions. Results of these in vivo human studies indicate that the time course to closure of the disrupted BBB for low-molecular-weight complexes is longer than previously estimated. The barrier is widely open during the first 40 minutes after osmotic BBB disruption and returns to baseline levels only after 6 to 8 hours following the induction of good or excellent disruption. These findings have important clinical implications for the design of therapeutic protocols.

KEY WORDS • blood–brain barrier • mannitol • 99mTc-glucoheptonate

The BBB is both a physical and a functional gate that controls the influx and efflux of a wide variety of substances. Because the BBB restricts the entry of many therapeutically useful drugs, researchers have attempted to exploit systems that modulate the BBB to increase delivery of potential therapeutic agents into the CNS. Osmotic BBB disruption by intraarterial infusion of mannitol is used in conjunction with chemotherapy to treat human brain tumors. The time course to barrier closure, or the so-called therapeutic window, has been examined in animals but little information is available in humans. The authors, therefore assessed the time course to barrier closure after osmotic BBB disruption in humans.

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Abbreviations used in this paper: BBB = blood–brain barrier; CNS = central nervous system; CT = computerized tomography; ΔHU = difference in Hounsfield unit; HU = Hounsfield unit; ICA = internal carotid artery; MTX = methotrexate; PCNSL = primary central nervous system lymphoma; PET = positron emission tomography; PNET = primitive neuroectodermal tumor; ROI = region of interest; SPECT = single-photon emission CT; TcGH = 99mTc-glucoheptonate; VA = vertebral artery.
mates of the maximum benefit of barrier opening for drug delivery on one hand and of the rate of return to baseline permeability function on the other carry important clinical implications. The design of treatment protocols should take into account both the optimal duration of drug infusion and the vulnerable period during which usually non-neurotoxic agents may produce unacceptable toxicity secondary to the modified barrier function.

Despite the fact that to date more than 4200 osmotic BBB disruption procedures have been performed at multiple centers in more than 400 patients, the enigma of the time course to barrier closure in humans is basically unsolved. Our goal in this study was to evaluate the time course to barrier closure after osmotic BBB disruption applied in conjunction with triple-agent chemotherapy in 12 patients with malignant brain tumors.

Clinical Material and Methods

Patient Population

Twelve patients (seven women and five men) with a mean age of 45.5 years (range 25–68 years) who harbored histologically verified malignant brain tumors were studied. All patients signed an informed consent document before inclusion in the treatment protocol and this study. Patients’ characteristics are listed in Table 1. Patients received osmotic BBB disruption in conjunction with triple drug chemotherapy at monthly intervals. Seven patients were treated for non-acquired immunodeficiency syndrome PCNSLs and five for PNETs. No more than one session of SPECT scanning was performed during each treatment course, to limit the amount of radiation exposure.

Osmotic BBB Disruption

The BBB disruption procedure has been described previously.5,6,22 Briefly, patients generally received one course of treatment (two sequential BBB disruption procedures 24 hours apart) each month, for a maximum of 12 consecutive months. Ideally, each of the three vascular distributions (two ICAs and one vertebrobasilar artery) was infused eight times during the treatment period, to treat the entire brain. Initial infusions were administered through the arteries supplying the enhancing tumor, based on radiographic studies. The procedure was performed with the patient in a state of general anesthesia. Via a percutaneous transfemoral puncture, catheter placement into either an ICA or a VA was performed for infusion of mannitol. Warm 25% mannitol was then infused at a rate of 3 to 11 ml/second over 30 seconds. One to 2 minutes later, chemotherapy agents were infused intraarterially for 10 minutes by using an angiographic injection pump. Chemotherapy was administered intravenously as well, according to the chemotherapy regimen.

Chemotherapy Regimens

The PCNSL Regimen. An intravenous infusion of cyclophosphamide, 500 mg/m²/day, was administered 30 minutes before BBB disruption to allow hepatic activation of the drug. Etoposide phosphate, 150 mg/m²/day, was infused intravenously 10 minutes before BBB disruption. Methotrexate, 1400 mg/m²/day, was mixed in 200 ml normal saline and infused intraarterially immediately after the

Table 1: Characteristics and procedures in 12 patients treated with chemotherapy and BBB disruption for brain tumors

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Tumor Type</th>
<th>Total Disruptions</th>
<th>Location of Disruptions</th>
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<tr>
<td>1</td>
<td>46, F</td>
<td>PCNSL</td>
<td>6</td>
<td>2 ICA, 4 Lt ICA</td>
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<tr>
<td>2</td>
<td>33, M</td>
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<td>1 ICA, 1 Lt ICA</td>
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<tr>
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<td>45, M</td>
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<tr>
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<td>PCNSL</td>
<td>6</td>
<td>4 ICA, 2 Lt ICA</td>
</tr>
<tr>
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<td>68, M</td>
<td>PCNSL</td>
<td>3</td>
<td>2 ICA, 1 Lt ICA</td>
</tr>
<tr>
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<td>PCNSL</td>
<td>3</td>
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<tr>
<td>7</td>
<td>61, F</td>
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<td>1</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>35, F</td>
<td>PNET</td>
<td>3</td>
<td>1 ICA, 2 Lt ICA</td>
</tr>
<tr>
<td>9</td>
<td>37, M</td>
<td>PNET</td>
<td>3</td>
<td>2 ICA, 1 Lt ICA</td>
</tr>
<tr>
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<td>46, F</td>
<td>PNET</td>
<td>2</td>
<td>2</td>
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<td>PNET</td>
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<tr>
<td>total</td>
<td>38</td>
<td></td>
<td>23</td>
<td>15</td>
</tr>
</tbody>
</table>

* Median age was 45.5 years. — = not done.

FIG. 1. Contrast-enhanced CT scan obtained after BBB disruption in the left ICA territory. The contrast agent was injected 1 minute after the disruption, and the duration of this infusion was 4 minutes. Circles show the selected ROIs on the right (1) and left (2). The average (AV) ± standard deviation (SD) measurements of HU for each ROI are presented on the left side in each panel. The disruption was quantitatively graded by the maximal difference in HUs. A moderate degree of disruption (left) and an excellent disruption (right) are demonstrated. AR = area.
infusion of mannitol. Leucovorin (folinic acid) administration was begun 36 hours after the first dose of intraarterial MTX and was continued until plasma levels of MTX were less than \(10^{-7}\) M.

The PNET Regimen. An intravenous infusion of cyclophosphamide, 330 mg/m\(^2\)/day, was administered 30 minutes before BBB disruption to allow hepatic activation of the drug. Etoposide phosphate, 200 mg/m\(^2\)/day, was infused intravenously 10 minutes before BBB disruption. Carboplatin, 200 mg/m\(^2\)/day, was mixed in 200 ml normal saline and infused intraarterially immediately after the infusion of mannitol. Sodium thiosulfate was given in accordance with a previous report in which an ototprotectant effect was observed against carboplatin-induced hearing loss associated with the procedure of BBB disruption.\(^\text{15}\) Accordingly, patients received intravenous infusions of 20 g/m\(^2\) sodium thiosulfate 4 hours after treatment with carboplatin.

Grading of BBB Disruption on CT Scans

Immediately after BBB disruption, 150 ml nonionic contrast dye (Iopamidol 300) was injected intravenously over 4 minutes. Computerized tomography scans (5-mm-thick sections) were obtained 30 to 45 minutes after BBB disruption. Visual and quantitative grading of BBB disruption was performed on CT scans as described previously.\(^\text{18}\) For quantitative imaging, HU measurements were obtained in the ROIs. For disruptions of the ICA territory, measurements were obtained from bilateral caudate and putaminal areas and from bilateral frontal and temporal cortices (Fig. 1). The maximal difference in HU measurements between the disrupted ROI and the homologous contralateral nondisrupted region was used for the grading of BBB disruption. After disruption of the VA territory, measurements were also obtained from the thalami, superior vermis, and occipital cortex. Measurements of HUs from the VA territory were compared with those obtained from ICA territories, and the maximal difference was used for grading. The disruption was quantitatively graded as poor when the maximal ΔHU was 0 to 3, moderate for ΔHU of 4 to 9, good for ΔHU of 10 to 19, and excellent for ΔHU of greater than 20.

Radionuclide SPECT Imaging

We used TcGH SPECT scanning to demonstrate disruption of the BBB. The 12 patients underwent 38 sessions of BBB disruption (Table 1), after which they received 20 mCi (740 mBq) of TcGH intravenously, at different time intervals after the procedure. The SPECT radionuclide scans were obtained 4 hours after the injection of TcGH by using a dual-headed gamma camera and a low-energy high-resolution collimator. The permeability of the BBB to TcGH was evaluated at the following time intervals after the osmotic BBB disruption: 1 minute postdisruption (eight studies), 15 minutes (four studies), 40 minutes (five studies), 90 minutes (eight studies), 120 minutes (four studies), 180 minutes (three studies), 240 minutes (three studies), and 480 minutes (three studies). An observer (R.R.) working in a blinded fashion determined the disrupted territory by visual inspection. Quantitative evaluation of the disruption was calculated by comparing uptake in both hemispheres. An operator-defined ROI was drawn on an axial slice located 3 to 4 cm beneath the scalp, and the disruption index was calculated. The disruption index was defined as the ratio of mean counts per pixel in the disrupted hemisphere and the homologous contralateral nondisrupted ROI. A ratio of 1:1 represents no increased uptake and a ratio greater than 1.2 is considered abnormal (normal range 1.02 ± 0.074).

Sources of Supplies and Equipment

The angiographic injection pump (Medrad V) was purchased from Medrad, Inc., Pittsburgh, PA. The cyclophosphamide (Cytoxan) and etoposide phosphate were acquired from Bristol Meyers Oncology, Inc., Evansville, IN. The CT scanner (2400 Elite) and the dual-headed gamma camera were obtained from Helix-Elscint, Haifa, Israel.

Results

Correlation Between Quantitative SPECT and CT Measures of BBB Disruption

Quantitative CT assessment of osmotic BBB disruption is in use and has been studied before.\(^\text{18}\) There is, however, less experience with quantitative evaluation of osmotic disruption by TcGH SPECT scanning, although a high level of agreement was shown for planar radionuclide scans. Therefore, an initial step was to confirm the reliability of the calculated disruption index as a measure of patent BBB, in comparison with the routinely used technique of quantitative CT assessment. Figure 2 demonstrates the correlation between the TcGH SPECT disruption index and the maximal difference in HU measured on postdisruption CT scans. For the purpose of these comparisons concomitant injections of both the iodine contrast agent and the radionuclide tracer were administered through two separate intravenous lines at 1 minute after the infusion of mannitol. A high level of correlation was found, with a correlation coefficient of \(r = 0.852\) (Fig. 2).
Visual and Quantitative Assessment of the Time Course to Barrier Closure

The results of TcGH SPECT studies were assessed separately for disruptions graded as poor-to-moderate (nine) and for those graded as good-to-excellent (29) based on postdisruption CT grading. Figure 3 shows the time course to barrier closure derived from the calculated disruption index for poor-to-moderate and for good-to-excellent disruptions. Figure 4 gives examples of the SPECT studies (visual assessment) obtained from the group of good-to-excellent disruptions after early or delayed injections of the tracer dye following the osmotic disruption.

The mean disruption index obtained from injection of TcGH at 1 minute postdisruption differed significantly between those procedures graded on CT scans as poor-to-moderate and those graded as good-to-excellent disruptions (mean ± standard error of the mean, index 1.55 ± 0.13 compared with 2.19 ± 0.18, respectively; \( p = 0.02 \), Student’s t-test). Forty minutes after the disruption the mean indices did not differ from those obtained after 1 minute (poor–moderate 1.48 ± 0.1, and good–excellent 2.13 ± 0.2), indicating that the barrier remained open with little or no recovery of its function. At 90 minutes the barrier was practically closed for patients with poor-to-moderate disruptions (Fig. 3) but was still open for patients with good-to-excellent disruptions (mean index 1.53 ± 0.09). Between 90 and 240 minutes postdisruption, a slow reduction in the disruption index was observed, with almost no changes measured during the interval of 120 to 240 minutes. At 240 minutes the barrier was still open for all patients with good-to-excellent disruptions (index 1.33 ± 0.08), but was found to be closed at 480 minutes after the osmotic disruption in two of three patients.

Discussion

It has been suggested that osmotic opening of the BBB may represent the technique of choice for enhancing the delivery of water-soluble drugs into brain areas infiltrated by tumors and may also prove suitable to treat global CNS diseases.14,21 This concept of enhanced drug delivery, largely investigated in animal models,1,10,12,22 is currently being applied to human brain tumors by using a protocol in which osmotic disruption of the BBB is immediately followed by multidrug chemotherapy regimens.5,6,13 Despite the fact that this technique is used clinically, the therapeutic window, namely, the interval during which delivery of a substance from the blood across disrupted brain capillaries is greatest, is still unclear in humans. The working hypothesis for the design of current treatment protocols assumes that the therapeutic window in humans is similar to the one derived from animal studies. In a rat model, this window lasts a maximum of 15 minutes after infusion of hypertonic mannitol, after which it decreases rapidly and returns to preinfusion levels within 2 hours.16 An early PET study performed in monkeys showed that much of the effect had disappeared after 10 minutes,9 and in another recent PET study in baboons a mean half time to barrier closure of 24 minutes was found.22 Our study showed that the maximum effect in humans lasts up to 40 minutes, after which there is a rapid decline in permeability, with the normal threshold restored between 6 and 8 hours after the osmotic disruption.

Our findings differ from the results of a recent PET study in humans in which rubidium-82 was used to measure blood-to-tissue influx, a measure of vascular permeability.23 That PET study showed that the mean half time for the return of permeability to near baseline values was only 8 minutes for normal brain. These results are mark-
edly different from those obtained in a similar $^{82}$Rb PET study performed in baboons by the same investigators, in which the mean half time for closure of the barrier was 24 minutes. The inconsistency in the estimations of the maximum permeability effect of osmotic BBB disruption between studies most likely reflects variability derived from multiple factors. Hyperosmotic mannitol was used in all studies to disrupt the BBB, yet they differed in the species evaluated, the anesthetic agents, the duration and rate of infusion of the hyperosmotic agent, the tracers and techniques used to evaluate increased permeability, and probably the physiological parameters such as blood pressure, heart rate, and PaCO$_2$. In several studies it was confirmed that factors such as the anesthetic agent, PaCO$_2$, and blood pressure are important determinants that affect the intensity of osmotic BBB disruption in rat models.

The discordance between our study and the previous $^{82}$Rb PET study in humans may indicate that different factors of the disrupted membrane permeability have been evaluated using the various tracers. Rubidium-82 shares pharmacological similarities with K$^+$ and therefore, K$^+$-specific transport mechanisms across the barrier may contribute to $^{82}$Rb uptake in the brain. Indeed, a previous study indicated that K$^+$-specific transport mechanisms across the BBB might contribute to $^{82}$Rb uptake in the brain following mannitol-induced systemic hyperosmolality. In a more recent study it has been shown that reversible osmotic disruption and reconstruction of the BBB is more complex than the suggested simple mechanical...
The shrinkage of endothelial cells. This study demonstrated that the hyperosmolar stress is related to the intracellular Ca++-activated complex mechanism in endothelial cells. The intracellular Ca++ concentration increased and peaked within 10 seconds after the application of mannitol, and a Na+/Ca++ exchanger was the mechanism that most probably pumped out the increased intracellular Ca++ during the returning phase, which lasted approximately 3.3 minutes. It is possible that the 82Rb PET study actually measured the acute recovery phase during which specific pumps that exchange either Na+ or K+ are activated in response to the osmotic stress. This phase is probably short, as demonstrated for the activation of the Na+/Ca++ exchanger, and may account for the mean barrier closure half time of 4 to 8 minutes as measured by the penetration of 82Rb in the study conducted in humans. Current information indicates that osmotic BBB disruption facilitates the penetration of both small and large molecules with a similar relative effect, despite the fact that the BBB exhibits size-dependent differential permeability to agents with different molecular weights. The molecular weight of the TcGH complex is 609, and is in the same order of magnitude as MTX (M, 454), which is administered in conjunction with osmotic BBB disruption to treat patients with brain lymphomas. The TcGH probably penetrates the disrupted barrier by simple diffusion and demonstrates retention in the brain, which is possibly related to a process of binding to proteins, as has been suggested in experimental inflammation. The window of barrier opening for TcGH proved longer than previously observed in our human study, and it probably reflects the status of the barrier function for low-molecular-weight agents such as MTX. Indeed, we have recently demonstrated the enhanced delivery of MTX into the ventricular cerebrospinal fluid in patients treated in conjunction with osmotic disruption of the BBB. It should be noted that the peak concentration of the drug is achieved 2 to 4 hours after the infusion of MTX, although a sharp rise in drug concentration is measured during the first 20 minutes following the infusion of mannitol.

Conclusions

Our findings convey important clinical information regarding the use of potentially neurotoxic agents for either diagnostic or therapeutic purposes. Examples of such agents include gadolinium contrast agents given after osmotic BBB disruption, or sodium thiosulfate, which provides protection against carboplatin-induced ototoxicity in animals and humans treated in conjunction with BBB disruption. When they are given during the susceptible period of incompletely restored BBB function, such agents have the potential to induce significant toxic neurological damage. The unexpected observation that after good-to-excellent osmotic BBB disruption, the barrier function is not fully restored between 2 to 6 hours later (Figs. 3 and 4) indicates that therapeutic protocols should be modified accordingly. This vulnerable period might prove problematic for the clinical application of agents such as sodium thiosulfate, which is chemoprotectant for nephrotoxicity and ototoxicity depending on restricted entry into the CNS. Future clinical trials should evaluate the safety of early administration of such potentially chemoprotectant agents in view of current knowledge related to the time course to barrier closure after osmotic disruption.

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References


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Address reprint requests to: Tali Siegal, M.D., The Neuro-Oncology Center, Hadassah University Hospital, P.O. Box 12000, Jerusalem 91120, Israel. email: siegal@hadassah.org.il.