Osmotic blood-brain barrier disruption in the posterior fossa of the dog

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Transient reversible osmotic blood-brain barrier disruption was produced in the posterior fossa of 33 dogs. A percutaneous catheter technique was used for the infusion of hypertonic mannitol into the vertebral artery. Neither the catheter technique nor the osmotic barrier modification resulted in interference with brain-stem function in most animals. The degree of barrier modification achieved by osmotic disruption in the posterior fossa is similar to that previously described for barrier modification of the supratentorial parenchyma. Methotrexate delivered to the brain via the vertebral artery resulted in a drug concentration of 100 to 300 ng/gm brain tissue. When the same amount of drug was given following osmotic blood-brain barrier disruption, brain tissue contained 1100 to 5000 ng of methotrexate/gm of brain tissue. Finally, the adequacy of the blood-brain barrier modification in the posterior fossa was shown to be quantifiable by the amount of enhancement on computerized tomographic scans.

KEY WORDS • posterior fossa • blood-brain barrier • chemotherapy • methotrexate

The blood-brain barrier limits the access of most drugs to the central nervous system. This barrier is the result of tight junctions (zonulae occcludentes) between the cerebral capillary endothelial cells. It has been clearly demonstrated in experimental models that the blood-brain barrier can be reversibly modified (that is, opened) by the osmotic effect of hypertonic solutions such as mannitol. In order to achieve an effective osmolality at the capillary endothelial cell, the hypertonic solutions must be infused intra-arterially. In our previous studies, we have documented that successful barrier modification of the cerebrum can be achieved, measured, and monitored. However, to apply blood-brain barrier modification reasonably in a clinical therapeutic setting, it is critical that the approach provide accessibility to the structures contained in the posterior fossa.

The present communication describes our experience with modification of the blood-brain barrier in the posterior fossa. We used a canine model, since our previous studies of barrier disruption of the cerebrum had provided parameters that permitted the effective translation of the findings in the dog to man. In addition, the current studies of modification of the barrier in the posterior fossa led to the use of a convenient percutaneous intra-arterial system of agent delivery into the vertebral artery, which allows repeated barrier modification.

Materials and Methods

Canine Model

The initial vertebral artery infusions were done via a cannula inserted into the surgically exposed vertebral artery at the base of the neck. Alternatives were studied seeking technical ease and speed. A method for rapid and simple administration was achieved by percutaneous insertion of the catheter into the femoral artery and cephalad advancement into the vertebral artery with fluoroscopic guidance (Fig. 1). This approach was used for the vertebral artery infusions of saline or mannitol.

Adult mongrel dogs were used in the acute studies, and conditioned hound dogs in the chronic studies.*

* The dogs were obtained from Brink Kennels, Paola, Kansas.
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The dogs were anesthetized with sodium thiopental (20 mg/kg), intubated with an endotracheal tube, and ventilated with a Harvard animal respirator. Anesthesia was maintained with a 60% nitrous oxide-oxygen mixture and supplemental sodium thiopental. A No. 18 intravenous catheter was used for anesthetic drug infusion and fluid management. The left vertebral artery was cannulated using the Seldinger technique; a femoral artery puncture was made with a No. 18 Potts-Cournand needle; a stainless steel guide wire (0.035 in. in diameter) was inserted; and a Cook red catheter was placed in the femoral artery. The catheter was placed in the left vertebral artery at the level of the C-3 vertebra (Fig. 1), and the position was confirmed by fluoroscopy (70 kVp and automatic MA) with a 5- to 10-ml injection of Conray 60 (meglumine iothalamate) contrast medium.

Barrier Modification

Fifteen minutes before barrier disruption, 3 ml/kg of 2% Evans blue dye was administered intravenously. Evans blue was used as a marker dye because it is known to bind tightly, but reversibly, to plasma albumin and therefore does not normally penetrate the tight junctions between cerebral endothelial cells.

In preliminary studies, the amount of mannitol needed was determined by a recognized parameter for successful barrier disruption: transient but complete displacement of all intravascular blood from the area in which barrier modification is desired. The actual criterion of blood "displacement" was direct visual recognition, through a 2-cm suboccipital craniectomy, of complete blanching of cerebellar cortical vessels during the 30-second mannitol or saline infusion.

Mannitol (25%), warmed to 37°C, was filtered through 0.20-μm diameter pores, and infused at 2.47 ml/sec, the minimum rate required to blanch the cerebellar surface as calculated from the visual examination study data. The mannitol was infused over 30 seconds via two infusion pumps. This mannitol concentration and duration of infusion was above the threshold for osmotic barrier disruption. In control studies, normal saline instead of mannitol was infused at an identical rate and volume (2.47 ml/sec over 30 seconds). In the present studies, the sequence of hypertonic mannitol infusion in experimental animals or normal saline infusion in control animals was randomized.

Animal Studies

Acute Studies. Acute studies are defined as those in which the animals were sacrificed 1 hour after intra-vertebral artery mannitol or saline infusion. Methotrexate (100 mg) was infused into the vertebral artery of both control and experimental "acute" animals over a 15-minute period, the delivery beginning 5 minutes after the mannitol or saline infusion. At sacrifice, the brain was removed and computerized tomographic (CT) scanning performed in animals that had received iodinated contrast material. The brain was then sliced to evaluate the distribution of Evans blue staining and grossly examined for pathological changes. Samples were obtained to measure methotrexate concentration.

Chronic Studies. Conditioned dogs were used in the longer-term studies to evaluate toxicity, neuropathological sequelae, and survival. These animals were observed for any signs of neurological deficit for 3 to 14 days. At sacrifice, the brain was removed, evalu-
ated for Evans blue staining, and fixed in Carson's formalin for detailed histopathological examination.

**Computerized Tomographic Studies**

Enhanced CT studies after osmotic barrier disruption with mannitol were performed in some of the acute animals using intravenous Conray 60, 5 ml/kg, infused over a 10-minute period beginning 1 minute after saline or mannitol infusion. One hour after blood-brain barrier disruption, each animal was killed; the brain was removed, suspended in saline in a sealed Lucite container, and placed in an Artronix Neuro-CAT scanner. Scans were obtained in a transverse plane with a 3-mm slice thickness using 120 kVp and 50 MA. The CT images were correlated with the pattern of Evans blue staining, and CT number measurements were obtained for both the posterior fossa and the cerebral hemispheres.

**Radioimmunoassay of Methotrexate**

Assays of methotrexate were performed in duplicate; the procedures, preparation of standards, and development of standard curves were as described previously. Assay at several concentrations yielded a coefficient of variation of less than 10%. The values included the intravascular methotrexate; however, this amount was constant throughout the entire brain (that is, areas in which the blood-brain barrier was and was not disrupted) and therefore was not subtracted.

**Evaluation of the Degree of Blood-Brain Barrier Disruption**

The degree of staining of each hemisphere after the administration of Evans blue was graded as follows: Grade 0, no staining; Grade 1+, just noticeable staining; Grade 2+, moderate blue staining; and Grade 3+, dark blue staining. The degree of enhancement on CT scan was quantitated by comparing the average CT numbers obtained from the cerebellum and a correlative area of the cerebrum. The maximum difference in CT number between the disrupted and undisrupted areas of the same brain was used as a quantitative measure of the degree of enhancement.

**Results**

**Determination of Mannitol Delivery Required to Achieve Barrier Modification**

The initial determination of the rate of mannitol delivery in previous studies which yielded barrier disruption without anatomical or physiological damage was made by direct visual inspection of the cerebrum. Blanching of the cerebellar cortical surface was observed, as in previous studies of the cerebrum, when mannitol (25%) was given at a rate of 2.47 ml/sec. In accordance with our earlier experience in cerebral barrier modification, this was selected as the mannitol infusion rate for studies in nine dogs. Barrier disruption as evidenced by the transudation of Evans blue-albumin into the posterior fossa structures was achieved in all nine animals. However, all four animals that were allowed to awaken from the anesthesia and then serially evaluated over the next 4 days had neurological alterations ranging from torticollis to a tendency to continuously walk in a circle (Table 1). One animal had severe ataxia.

These physiological changes (with subsequent pathological corroboration in one animal) led to an empiric trial of a lower infusion rate (1.08 ml/sec). The critical nature of the threshold infusion rate effect is emphasized by studies in three dogs. When transudation of Evans blue was used as the marker of disruption, only minimal barrier opening was evident in these three animals. Therefore, an intermediate rate of mannitol infusion (2.08 ml/sec for 30 seconds) was selected empirically, and in 12 of 19 dogs barrier disruption was observed by this criterion of Evans blue-albumin staining of structures of the posterior fossa.

**Neuropathological Studies**

Gross neuropathological examination was performed in the 21 dogs (19 infused with mannitol and two with saline) infused at the "intermediate" rate of 2.08 ml/sec. Detailed cytological evaluation in 12 dogs that received mannitol revealed lesions in two animals (Table 1). In one of these two dogs with identifiable lesions, a complication had occurred relating to the anesthesia, and the role of that event in the histological findings cannot be determined. In this dog, a small hemorrhagic lesion was seen in the left medulla adjacent to the pyramid, and a 5 × 8 mm hemorrhagic infarct was seen in the left inferior cerebellum. In the second dog (evaluated 20 hours after mannitol infusion), a zone of hemorrhagic necrosis involved the left half of the medulla, and there was a hemorrhagic area in the hypothalamus and anterior thalamus in the midline measuring 3 × 20 mm. Histological examination revealed focal calcification suggesting a preexistent arteriovenous malformation. Nevertheless, the hemorrhage was primarily within the distribution of the posterior cerebral artery.

Neuropathological studies were also performed at the other infusion rates described above. Of the four dogs given mannitol at the high infusion rate (2.47 ml/sec), one dog with severe ataxia had a hemorrhagic lesion in the left lateral medulla and adjacent superior cervical spinal cord. Finally, it is noteworthy that no
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**TABLE 1**

The neurological sequelae of varying mannitol infusion rates into the vertebral artery

<table>
<thead>
<tr>
<th>Infusion Rate (ml/sec)</th>
<th>Neurological Deficits</th>
<th>Barrier Disruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.47</td>
<td>bilateral hindleg weakness for 24 hrs</td>
<td>+</td>
</tr>
<tr>
<td>2.47</td>
<td>severe torticollis, severe ataxia, hemorrhagic necrotic lesion of medulla &amp; upper cervical cord</td>
<td>+</td>
</tr>
<tr>
<td>2.47</td>
<td>hindquarter spasms at 9 days</td>
<td>+</td>
</tr>
<tr>
<td>2.47</td>
<td>mild torticollis &amp; circling for 6 days</td>
<td>+</td>
</tr>
<tr>
<td>2.08</td>
<td>never regained consciousness, sacrificed after 12 hours; intracerebral hematoma (possible AVM suggested by calcification around hematoma)</td>
<td>+</td>
</tr>
<tr>
<td>2.08</td>
<td>never regained consciousness, sacrificed after 12 hours; hemorrhagic necrotic lesion in cerebellum; necrotic lesion in cervical cord (anesthetic complication may have been a factor)</td>
<td>+</td>
</tr>
<tr>
<td>2.08</td>
<td>none</td>
<td>+</td>
</tr>
<tr>
<td>2.08</td>
<td>none</td>
<td>-</td>
</tr>
<tr>
<td>2.08</td>
<td>none</td>
<td>+</td>
</tr>
<tr>
<td>2.08</td>
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<td>+</td>
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<tr>
<td>2.08</td>
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<td>+</td>
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<tr>
<td>2.08</td>
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<td>+</td>
</tr>
<tr>
<td>2.08</td>
<td>none</td>
<td>-</td>
</tr>
<tr>
<td>1.08</td>
<td>none</td>
<td>+</td>
</tr>
</tbody>
</table>

* Except where noted, all animals in this table were observed for 3 to 14 days and each underwent a careful gross and microscopic neuropathological evaluation by a neuropathologist. AVM = arteriovenous malformation.

† Success of blood-brain barrier disruption was determined by the presence or absence of Evans blue-albumin staining of posterior fossa structures at sacrifice.

Abnormalities were seen in the dogs infused at low mannitol rates (1.08 ml/sec).

**Evaluation of Barrier Modification**

Evans blue-albumin was used as the standard to document barrier disruption, since it provides semiquantitative data and has been the standard reference in previous studies of the cerebrum. In addition, CT analysis was used since, unlike Evans blue, it provides a clinically relevant reference method. Animals were given iodinated contrast agent in addition to the Evans blue dye, and barrier disruption was studied by CT imaging. In the control studies where saline was infused into the vertebral artery, no Evans blue-albumin staining (Grade 0) of the posterior fossa structures was seen, the CT scan showed no enhancement in the posterior fossa (Fig. 2), and the average CT number difference between the cerebrum and the cerebellum was 1. However, when mannitol was infused at the same rate, good barrier disruption was documented by Evans blue-albumin staining and excellent enhancement by CT scan (Fig. 3). In these animals, there was a Grade 2+ to 3+ Evans blue staining, and the average CT number difference between the cerebrum and cerebellum was 25.

**Effect of Barrier Disruption on Drug Delivery to Posterior Fossa Structures**

The effect of osmotic blood-brain barrier disruption in the posterior fossa upon the delivery of drug to its structures was measured. Methotrexate was used as the marker drug. In the control studies, saline at the rate of 2.08 ml/sec was injected into the vertebral artery, followed in 5 minutes by infusion of methotrexate (100 mg). Brain methotrexate concentration ranged from 100 to 300 ng of methotrexate/gm of tissue (Table 2). When mannitol was infused instead of saline, the methotrexate concentration in the posterior fossa structures was increased to a level of 1100 ng/gm brain.

**TABLE 2**

Comparison of saline and mannitol infusions on blood-brain barrier modification

<table>
<thead>
<tr>
<th>Evans Blue-Albumin Staining</th>
<th>Methotrexate Concentration (ng/gm brain)‡</th>
<th>Cerebrum</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cerebrum</td>
<td>Cerebellum</td>
<td>Pons</td>
</tr>
<tr>
<td></td>
<td>Gray Matter</td>
<td>White Matter</td>
<td>Gray Matter</td>
</tr>
<tr>
<td>saline infusion</td>
<td>0</td>
<td>180</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>140</td>
<td>130</td>
</tr>
<tr>
<td>mannitol infusion</td>
<td>3+</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>210</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>230</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>200</td>
<td>180</td>
</tr>
</tbody>
</table>

* Brain methotrexate (MTX) levels and Evans blue-albumin staining 1 hour after vertebral artery MTX infusion.
† Staining grade: 0+ = none; 1+ = just noticeable; 2+ = moderate; 3+ = dark.
‡ Methotrexate (MTX, 100 mg) was administered over 15 minutes via the vertebral artery 5 minutes after infusion of saline or mannitol. The infusion rate for saline or mannitol in these animals was 2.08 ml/sec.

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FIG. 2. Upper: Canine brain 1 hour after vertebral artery infusion of saline. Evans blue was given intravenously before the saline infusion. No staining of the posterior fossa structures can be seen. Lower: Transverse-section computerized tomographic (CT) scan of the same brain. Iodinated contrast material was given just after the intra-arterial saline infusion. No evidence of enhancement was seen. The CT number difference between the cerebellum and cerebrum was 1.

FIG. 3. Upper: Canine brain 1 hour after vertebral artery infusion of mannitol (2.08 ml/min). Evans blue was given intravenously before the mannitol infusion. Marked staining of the posterior fossa structures can be seen. Lower: Transverse-section computerized tomographic (CT) scan of the same brain. Iodinated contrast material was given just after the intra-arterial mannitol infusion. Marked enhancement of the posterior fossa structures due to the penetration of contrast material across the blood-brain barrier can be seen. The CT number difference between the enhanced cerebellum and reference cerebrum was 31.
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to 5000 ng/gm of tissue. In spite of these high drug levels in posterior fossa structures, the methotrexate concentration in the undisrupted cerebrum was the same as that in control animals.

Levels of methotrexate in the brains of animals infused via the vertebral artery at the very high mannitol rate (2.47 ml/sec) were not significantly different from those at an infusion rate of 2.08 ml/sec. Finally, serum methotrexate levels were similar in both the saline- and mannitol-infused animals (in the range of \(2 \times 10^{-5} \) M) at 1 hour after methotrexate infusion.

Discussion

To our knowledge, the use of an osmotic effect to open the blood-brain barrier in the posterior fossa has not been previously explored. However, a fortuitous clinical experience occurred in one of our patients with a brain tumor. During mannitol infusion into the carotid artery to open the cerebral blood-brain barrier, sufficient flow through the posterior communicating artery also caused disruption of the barrier in the posterior fossa. No untoward sequelae resulted, posing the feasibility of barrier disruption in the posterior fossa.

The current studies using vertebral artery cannula demonstrate that blood-brain barrier modification for posterior fossa structures can be performed in the dog. The rate of mannitol infusion was very critical, since slight increases in the infusion rate resulted in significant physiological abnormalities. Although we have previously explored the mannitol threshold levels for cerebral barrier disruption and have defined the risks of therapy, it is quite clear that the posterior fossa is more sensitive to the effects of mannitol. To prevent complications, we used a relatively low rate of infusion. This resulted in only a 63% success rate for barrier disruption, but it markedly reduced the incidence of neurological dysfunction.

The basis for this sensitivity of posterior fossa structures to barrier modification is not certain. Some factors include the extensive vertebral artery collateral blood supply in the dog (Fig. 1) and the spatial constraints of the posterior fossa, making it particularly vulnerable to the 1% to 14% increase in brain water that is known to result from osmotic disruption in the cerebrum. These studies highlight the critical requirement of individual "tailored" dose schedules that are now possible with the use of CT scans to monitor the efficacy of the barrier modification.

Previous studies in rodents, dogs, primates, and recently in humans have shown that osmotic blood-brain barrier disruption is an excellent means of increasing delivery of chemotherapeutic agents to the tumor, brain around the tumor, and the cerebrum. The increase in drug (methotrexate) delivery to the posterior fossa after barrier modification was approximately tenfold that of the controls without barrier modification. This increase in drug delivery is similar to that seen in our studies of osmotic barrier modification of the cerebrum. In addition, the increased drug delivery is limited to the areas of barrier disruption. In terms of clinical therapeutic trials, it is noteworthy that the degree of Evans blue-albumin staining, the degree of enhancement on the CT scan, and the degree of increased drug delivery are closely parallel, making the CT scan a valuable tool as a noninvasive means to monitor barrier modification.

Another measure of the potential applicability involves the sequelae of the procedure. After identifying the relative sensitivity of structures in the posterior fossa to mannitol, an infusion rate was developed as described. Neuropathological examinations revealed hemorrhagic lesions in two animals. Calcification near the hemorrhage in one of the animals suggested that a preexistent arteriovenous malformation may have been present. Nonetheless, the pathophysiology of these two lesions is not clear, but their hemorrhagic character is compatible with emboli. If this is the etiology, clinical neuroradiological techniques and facilities may minimize the likelihood of this complication. It should be noted that no hemorrhagic lesions have been seen in three patients who have come to autopsy after entry into our protocol using osmotic barrier modification to enhance chemotherapy delivery to cerebral malignant tumors.

This technique, if found to be clinically safe, not only may have application in posterior fossa tumors but may be of value in the delivery of antibiotics to cerebellar abscesses, or the delivery of enzymes which are deficient in certain inborn errors of metabolism (as in Tay-Sachs disease). Finally, the present report details application of a percutaneous approach to the vertebral artery that permits repeated infusions in the same animal.

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References


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