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Research Article

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RAPID

PUBLICATIONS

Osmotic Blood-Brain Barrier Disruption

COMPUTERIZED TOMOGRAPHIC MONITORING OF CHEMOTHERAPEUTIC AGENT DELIVERY

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ABSTRACT The present study describes a canine model of transient reversible blood-brain barrier disruption with hyperosmolar mannitol infusion into the internal carotid artery. Studies in this model show that osmotic blood-brain barrier disruption before intracarotid infusion of methotrexate results in markedly elevated (therapeutic) levels of drug in the ipsilateral cerebral hemisphere. Levels in the cerebrospinal fluid correlate poorly and inconsistently with brain levels. Computerized tomograms in this canine model provide a noninvasive monitor of the degree, time-course, and localization of osmotic blood-brain barrier disruption.

INTRODUCTION

A primary limiting factor in the access of drugs or similar agents to the central nervous system (CNS)[1] has been the blood-brain barrier (BBB). The BBB results from the tight junctions (*zonalae occludentes*) between capillary endothelial cells. The BBB is present in most areas of the CNS with a few notable exceptions such as the pineal gland, the area postrema, and the anterior pituitary gland (1).

The nature and significance of the BBB in the presence of primary or metastatic neoplasms in the CNS is not clear. A common view is that the tumor destroys the barrier (2). Thus, it is of particular note that certain parenchymal cancers (testicular, breast, and oat cell) in a variety of metastatic sites have been shown to be quite responsive to systemic chemotherapeutic agents. Yet, metastases from these same tumors to the CNS have been virtually unresponsive to the same chemotherapy (3). Dramatic evidence of this dichotomy was shown in a report of sarcoma patients where adriamycin therapy resulted in progression of brain metastases despite regression of systemic tumor (4). Similarly, chemotherapy has had but trivial effects on primary CNS tumors (5). Pharmacokinetic evidence suggests that an important reason for these failures is an intact BBB at the proliferating edge of tumors (6, 7).

The present study describes transient reversible BBB disruption with intracarotid hyperosmolar mannitol infusion. Studies in a canine model show that osmotic BBB disruption before chemotherapy (methotrexate) results in markedly elevated (therapeutic) levels of drug in the ipsilateral cerebral hemisphere (8). Furthermore, computerized tomograms (CT) provide a noninvasive demonstration of the degree, time-course, and localization of osmotic BBB disruption. Thus, these canine studies demonstrate the feasibility of BBB disruption as a means to deliver chemotherapy

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[1] *Abbreviations used in this paper*: BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computerized tomogram(s); MTX, methotrexate.

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to the CNS, and describe a noninvasive technique to document the localization, time-course, and extent of both BBB disruption and drug delivery.

METHODS

Canine studies. Adult mongrel dogs (20–25 kg) were anesthetized with pentobarbital sodium, intubated with an endotracheal tube and ventilated with a Harvard animal respirator (Harvard Apparatus Co., Millis, Mass.); the blood gases were maintained at a P_{CO_2} of 25–35 mm Hg and a P_{O_2} of 70 mm Hg with supplemental oxygen. Catheters (1.7 mm i.d.) in the femoral artery and vein were used to monitor pressures via a Statham p50 transducer (Statham Instruments Inc., Oxnard, Calif.) connected to a Grass multichannel recorder (Grass Instrument Co., Quincy, Mass.). Blood pressure and urine output were maintained with normal saline. The left internal carotid artery infundibulum was cannulated with a 16-gauge catheter via the common carotid artery. Because mannitol infusion via the common carotid artery resulted in inconsistent barrier disruption, all infusions were into the internal carotid artery.

BBB disruption by hypertonic mannitol intra-arterial infusion was carried out in randomized sequence. Animals receiving intracarotid saline instead of mannitol served as controls. The displacement of all blood flow from the ipsilateral hemisphere during the infusion was documented in initial studies by an ipsilateral craniectomy to create a 2 to 3-cm observation window. Complete cortical blanching during the 30-s mannitol infusion was used as the evidence of displacement. Cerebrospinal fluid (CSF) was obtained by an atraumatic percutaneous Tuohy needle insertion into the cisterna magna. The animal was used only if clear CSF was obtained. Serial serum and CSF samples were obtained before and after BBB disruption.

Mannitol (25%; Merck Sharp & Dohme Canada LTD, Quebec) was filtered and then infused at a rate just sufficient to blanch the cortical surface (1.5 ml/s over 30 s with an infusion pump from Harvard Apparatus Co.). This mannitol concentration and duration of infusion are just above the threshold for osmotic BBB disruption.[2] 5–15 min later, methotrexate (100 mg) was infused over a 15-min period via the internal carotid artery catheter. At sacrifice the brain was removed and sliced before fixation to evaluate the distribution of Evans blue staining and to obtain samples for determination of drug content.

Radioimmunoassay of methotrexate (MTX). The MTX antiserum and a ^{125}I-MTX derivative were obtained from Diagnostic Biochemistry, Inc., San Diego, Calif. Assays were done in duplicate and the procedures, preparation of standards, and development of standard curves were as described in the kit. Assay of MTX at several concentrations yielded a coefficient of variations of <10%.

CT studies. 15 min before the described mannitol osmotic barrier disruption, contrast material (Conray 60, 4–5 ml/kg, i.v.; Mallinckrodt Inc., St. Louis, Mo.) and Evans blue (2%; 3 ml/kg) were administered. Evans blue binds tightly but reversibly to plasma albumin, thereby providing a macromolecular marker of BBB disruption. Evans blue-albumin is normally not able to penetrate the tight junctions between cerebral endothelial cells, the anatomic basis for the BBB. After hypertonic BBB disruption, the tight junctions transiently open as can be seen ultrastructurally (1).

1 h after barrier disruption the animals were sacrificed; the brain was removed and suspended in saline in a sealed Lucite (DuPont, I. E. de Nemours & Co., Inc., Wilmington, Del.) container and placed in the Artronix CT head scanner (Artronix Inc., St. Louis, Mo.). CT scans were obtained in a transverse plane with a 3-mm slice thickness using 120 peak kV and 50 mA. CT images were correlated with the pattern of Evans blue staining and radiodensity number measurements were obtained for both the infused and contralateral hemispheres.

RESULTS

The MTX levels in brain, CSF, and serum of two control and two experimental animals are given in Table I. The values in the control animals receiving isotonic saline instead of mannitol ranged from 130 to 1400 ng MTX/g tissue in the ipsilateral hemisphere and from 60 to 840 ng MTX/g brain in the contralateral hemisphere. The MTX levels in the experimental animals ranged from 1,500 to 28,000 ng MTX/g tissue in the ipsilateral cerebral hemisphere and from 110 to 2,800 ng MTX/g tissue in the contralateral hemisphere. The therapeutic tissue level of MTX is about 300 ng/g tissue (9, 10). The MTX levels were generally highest in the gray matter.

In the control animals the CSF levels were 0.68 and 7 μM, with serum levels being 19 and 26 μM, respectively. The CSF MTX levels in the experimental animals were 3 and 30 μM with corresponding serum levels of 21 and 16 μM, respectively.

TABLE I

Osmotic Blood-Brain Barrier Disruption by Internal Carotid Infusion of Mannitol or Saline Followed by Internal Carotid Methotrexate Infusion*

	Mannitol‡		Saline	
	Dog 1	Dog 2	Dog 3	Dog 4
	ng MTX/g tissue			
Ipsilateral cerebral hemisphere				
Grey matter	10,500	28,000	1,000	1,400
White matter	11,000	20,000	420	690
Basal ganglion	1,500	9,200	130	270
Contralateral cerebral hemisphere				
Grey matter	240	2,800	130	840
White matter	110	710	60	270
Basal ganglion	170	830	160	180
		μM		
Serum‡	21	16	19	26
CSF‡	3.5	34	0.68	7

* MTX (100 mg) infused over 15 min, 5 min after mannitol or saline infusion.

‡ Samples obtained 1 h after methotrexate infusion.

[2] Rapoport, S. I., W. R. Fredericks, K. Ohno, and K. D. Pettigrew. 1979. Quantitative aspects of reversible osmotic opening of the blood-brain barrier. Submitted for publication.

The CT images in the control animals infused intra-arterially with normal saline rather than mannitol demonstrated no extravasation of contrast material (absence of enhancement on the CT scan; no difference in CT number between the two hemispheres) and no staining of the cerebral parenchyma caused by extravasation of Evans blue-albumin (Fig. 1). On the other hand, CT images in the experimental animals revealed clear contrast enhancement of the brain parenchyma in the regions where the barrier had been disrupted (Fig. 2). Comparison with the areas of the brain that were stained with Evans blue dye showed excellent correlation with the enhanced areas on the CT scan (Fig. 3). The attenuation differences between the normal areas of brain and the areas that had been disrupted and enhanced with iodinated contrast material showed a CT number difference of ≅20 U. This corresponds to a 2% difference in attenuation relative to water and is easily detected on the CT scanner. Animals given the contrast material 1 h after osmotic BBB disruption showed little or no enhancement of their brain on the CT scan. Thus, the BBB remains open for less than 1 h after hyperosmolar mannitol injection.

DISCUSSION

The present studies provide evidence that reversible disruption of the BBB by the intra-arterial injection of hyperosmolar mannitol is a means of allowing entry of chemotherapeutic agents into the CNS. In addition, the value of CT as a noninvasive means of identifying and monitoring the BBB disruption is documented.

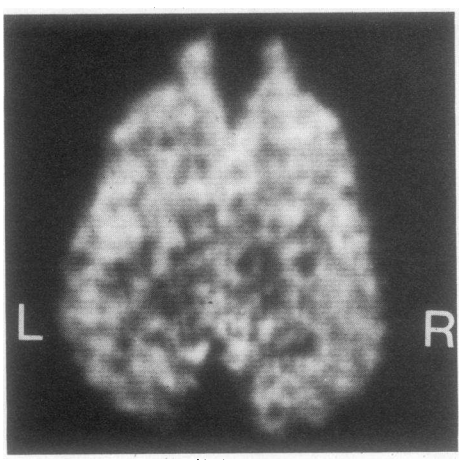

FIGURE 1 CT of canine brain 1 h after infusion of saline rather than mannitol into the left internal carotid artery. Iodinated contrast material and Evans blue were given intravenously before the intra-arterial saline infusion. The image is of a transverse section of the cerebrum. There is no evidence of enhancement (i.e., penetration of iodinated contrast material across the BBB). Inspection of the unfixed canine brain (not shown) showed no staining of the cerebral parenchyma, although Evans blue-albumin was given before the infusion of saline into the internal carotid artery.

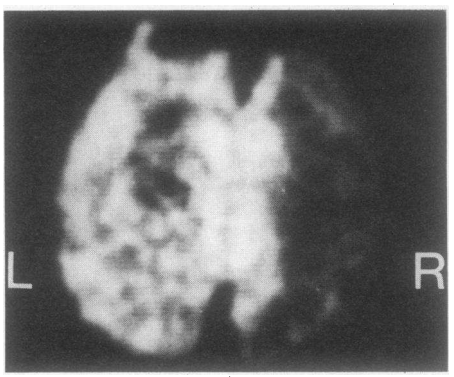

FIGURE 2 A CT of canine brain 1 h after osmotic BBB disruption of left cerebral hemisphere with 25% mannitol. Iodinated contrast material and Evans blue were given intravenously before the intra-arterial mannitol infusion. The image is a transverse section of the cerebrum. The white (enhanced) area is the result of iodinated contrast medium penetrating the BBB. The area that enhances includes the entire left hemisphere and the medial right hemisphere in the distribution of the right anterior cerebral artery. The black areas in both cerebral hemispheres are part of the ventricular system.

The osmotic disruption of the BBB was shown to permit entry of more than therapeutic levels of MTX into the CNS parenchyma. Other attempts to deliver large amounts of MTX to the CNS have been at best marginally successful even where high-dose intravenous infusion of methotrexate was used followed by folinic acid (11) or when the drug was administered directly into CSF (12). As we have demonstrated (Table I), the correlation between brain and CSF MTX concentrations is often poor. The present study demonstrates the feasibility of high-dose delivery of drug to both cortex and white matter and the added advantage of selectivity of the hemisphere treated.

The use of osmotic disruption of the BBB has been shown to be reversible and to have an acceptably low incidence of neurologic complications in rodents and primates (13). Neuropathologic studies in the rat have not demonstrated any parenchymal damage at varying periods up to 2 wk after barrier disruption although damage to the ciliary body of the eye has been observed in primates.[3]

[3] Rapoport, S. I., W. R. Fredericks, J. B. Kirkpatrick, and E. A. Neuwelt. 1979. Osmotic opening of the blood-brain barrier in the rat: studies of brain histology. Submitted for publication.

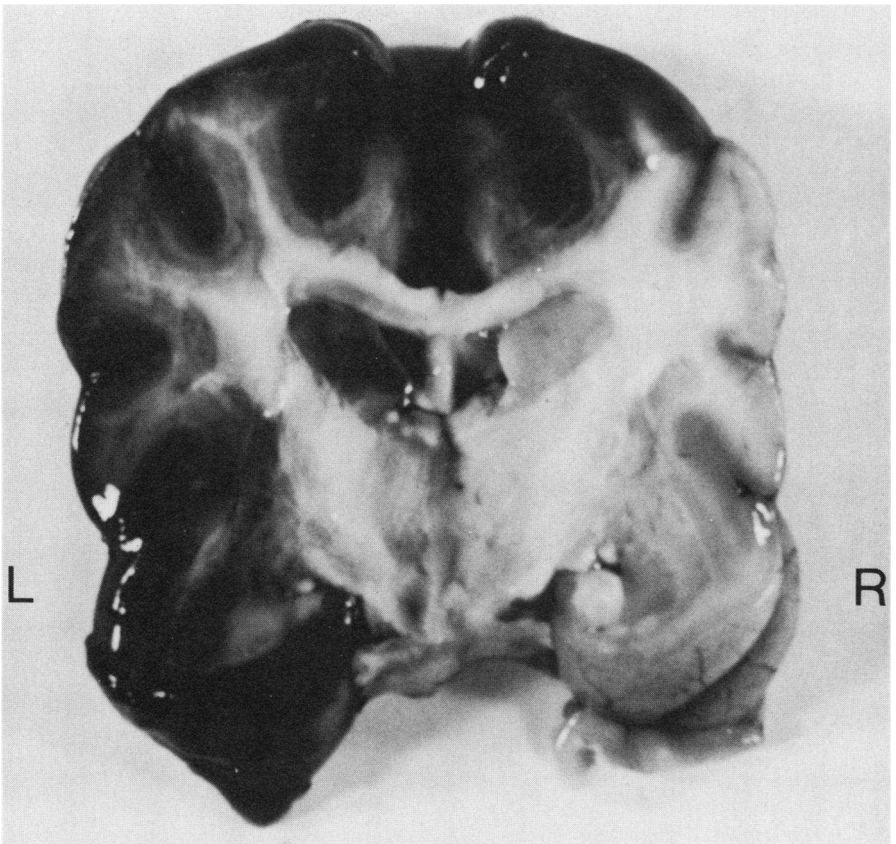

FIGURE 3 Coronal section of unfixed canine brain 1 h after osmotic BBB disruption of left cerebral hemisphere. The dark areas represent penetration across the BBB of Evans blue-albumin (mol wt 68,500). As can be seen, the BBB was disrupted in the entire left cerebral hemisphere and in the distribution of the anterior cerebral artery in the right hemisphere.

The current studies exploit the technology of the CT scan as an important monitor of BBB disruption. Tomographic sensitivity is such that differences in x-ray attenuation of 1% can be seen by scan. CNS lesions, which disrupt the BBB (some tumors, infarcts, or infections), are better identified and localized on the CT scan after the intravenous injection of iodinated contrast material. Normally, these iodinated water soluble agents are unable to penetrate the BBB (14); however, with BBB disruption in a lesion, the contrast agent can extravasate from the intravascular space into the interstitium of the affected tissue. Because the CT scanner can detect these iodine molecules, a significant increase in attenuation of these areas on the CT scan image is seen; that is, contrast enhancement occurs within a lesion. This sensitivity has permitted us to document the presence, extent, and time-course of osmotic BBB disruption. In addition to the qualitative pictures of BBB disruption, the numerical values of CT density given above provide a direct correlate to the degree of barrier disruption. Both the qualitative pictures and the quantitative CT density numbers correlated with the brain MTX concentrations (Table I) and the positive stain with Evans blue dye (Figs. 2 and 3) after BBB disruption. Thus, the CT scan proved to be a sensitive, noninvasive monitor of blood-brain barrier disruption.

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