Reversible Osmotic Opening of the Blood–Brain Barrier in Mice

Wendy R. Fredericks, AB, and Stanley I. Rapoport, MD

Unilateral reversible osmotic opening of the blood–brain barrier can be produced in mice. Infusion of 1.8 molal arabinose in water at a rate of 0.64 ml/min for 30 seconds into the internal carotid artery consistently results in ipsilateral brain staining by intravascular Evans blue dye. Osmotic opening is concentration-dependent (threshold, 1.6 molal arabinose) and reversible within 4 hours. No long-term neurologic deficit occurs. These results suggest that reversible osmotic blood–brain barrier opening can be applied to disease models in mice. (Stroke 1988;19:266–268)

Osmotic blood–brain barrier (BBB) opening has been used in rats, rabbits, dogs, and monkeys to augment brain entry of intravenous water-soluble drugs and proteins normally excluded from the central nervous system. Central nervous system chemotherapy in human patients also has been conducted with this technique. The method has been shown to increase delivery of substances to the brain without causing long-term cerebral damage. However, a number of disease models in which it would be useful to study the central nervous system effects of drugs, enzymes, and antibodies administered systemically have been developed largely in mice. These models include viral and fungal infections, immune deficiencies, experimental tumors, and genetic abnormalities. Therefore, we thought it of interest to extend the osmotic method to unilaterally and reversibly open the BBB in mice. An abstract of this work has been published.

Materials and Methods

BALB/c female mice (Charles River Breeding Laboratories, Wilmington, Massachusetts) weighing 15–22 g, 6–10 weeks of age, were anesthetized with pentobarbital (Somnifer, diluted 1:4 vol:vol with water; Richmond Veterinary Supply, Richmond, Virginia). A PE-50 polyethylene catheter with the end tapered to 0.4 mm diameter with heat was inserted retrograde in the right external carotid artery and secured with 6-0 surgical silk. The catheter tip was placed just above the bifurcation with the internal carotid artery. During surgery, the superior thyroid and occipital arteries were cauterized (Weck, Research Triangle Park, North Carolina), but carotid circulation to the brain never was interrupted. The catheter was filled with 0.04 ml 100 units heparin/ml 0.9% (wt:vol) NaCl, filtered with a 0.22-μm-diameter filter (Millipore, Bedford, Massachusetts). Evans blue dye (Chroma-Gesellschaft, Stuttgart, F.R.G., 2 ml/kg of a 2% [wt:vol] solution in isotonic saline) was injected into a saphenous vein. The dye forms a complex with plasma albumin and is used as a marker of BBB integrity.

Five minutes after Evans blue injection, 41 mice were infused for 30 seconds with a warm (37°C) solution of hypertonic L-(−)-arabinose (Sigma, St. Louis, Missouri) in distilled water filtered with a 0.2-μm filter (Nalge, Rochester, New York). Five control mice were infused with filtered 0.9% (wt:vol) NaCl (isotonic saline). Infusions at a constant rate of 0.64 ml/min were delivered with an infusion pump (Harvard 944, Millis, Massachusetts). The interface between common carotid blood and infusate was observed during infusion. If infusate was seen to pass into the common carotid artery, the experiment was discontinued as it has been shown in rats that brain staining is unlikely in this case. In some experiments, infusion pressure was measured during infusion by connecting the carotid catheter to a strain gauge (Statham, Hato Rey, Puerto Rico) and chart recorder (Gould, Cleveland, Ohio). Thirty minutes to 7 hours after infusion, a mouse was killed with an overdose of sodium pentobarbital i.p., and its brain was examined and graded for staining by Evans blue: 0, no staining; 1+, just-noticeable staining; 2+, moderate blue staining; 3+, dark blue staining.

In experiments designed to examine reversibility of BBB breakdown, 8 mice first were infused with 1.8 molal arabinose at 37°C for 30 seconds at 0.64 ml/min. Two or four hours later, each mouse was reanesthetized with pentobarbital and i.p. and 2% Evans blue in saline (2 ml/kg) was injected i.v.

In long-term experiments involving recovery, six mice underwent osmotic BBB treatment under optimum conditions and were allowed to recover for 2 weeks. They were observed for any signs of neurologic dysfunction, after which the brains were examined for staining with Evans blue.

Results

Mice were infused with a hypertonic arabinose solution, ranging in concentration from 1.0 to 1.8 molal, or with isotonic saline. Table 1 illustrates the effect of infusate osmolality on BBB opening. Staining of the brain by Evans blue was consistently achieved with a retrograde infusion of 1.8 molal arabinose into
the external carotid artery, whereas lower concentrations were minimally or not effective. A typical 3+ grade brain is illustrated in Figure 1. The threshold that produced staining in at least 50% of brains was 1.6 molal arabinose. Occasional minimal staining was produced by 1.2 or 1.4 molal arabinose, whereas infusion of 1.0 molal arabinose or isotonic saline did not result in any staining, indicating that BBB opening with higher arabinose concentrations was not due to infusion pressure.

BBB opening to Evans blue was entirely reversed within 4 hours (Table 2). Under optimal conditions, 2% Evans blue injected i.v. 5 minutes before infusion of 1.8 molal arabinose resulted in consistent staining of the cerebral hemisphere ipsilateral to the infusion. However, when Evans blue was injected 2 hours after arabinose infusion, the ipsilateral hemisphere was very lightly (1+) or not at all (0) stained. Four hours after arabinose infusion, there was no brain staining whatsoever.

All mice in the recovery experiments survived for 2 weeks. At that time, each right hemisphere was still lightly stained, indicating that BBB opening had been successful in each case. None of the mice showed signs of neurologic dysfunction. Animals continue to appear normal at least 6 months after osmotic BBB opening (unpublished observations).

Survival in all experiments was excellent; only one mouse died before completion of an experiment, and its brain was not stained. Most experiments lasted at least 2 hours after arabinose infusion.

**Table 1. Brain Staining by Evans Blue in Relation to Infusate Molality**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Grade of brain staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic saline</td>
<td>0 1+ 2+ 3+</td>
</tr>
<tr>
<td>Arabinose</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>5 0 0 0</td>
</tr>
<tr>
<td>1.2</td>
<td>4 2 0 0</td>
</tr>
<tr>
<td>1.4</td>
<td>4 2 0 0</td>
</tr>
<tr>
<td>1.6</td>
<td>2 3 1 0</td>
</tr>
<tr>
<td>1.8</td>
<td>1 6 5 5</td>
</tr>
</tbody>
</table>

Arabinose solutions were made up in distilled water, molal concentration, and infused at 0.64 ml/min. Data are number of mice.

**Table 2. Reversibility of Osmotic Blood–Brain Barrier Opening to Evans Blue**

<table>
<thead>
<tr>
<th>Time of Evans blue injection</th>
<th>Brain staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>−5 minutes</td>
<td>Yes</td>
</tr>
<tr>
<td>+2 hours</td>
<td>Yes</td>
</tr>
<tr>
<td>+4 hours</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Evans blue was injected i.v. before or after carotid infusion with 1.8 molal arabinose for 30 seconds at 0.64 ml/min.

**Discussion**

Our study shows that unilateral, reversible osmotic opening of the BBB can be achieved in mice. Infusion of 1.8 molal arabinose in distilled water, at a constant flow rate of 0.64 ml/min for 30 seconds into the internal carotid artery, results in reproducible staining of the ipsilateral brain by intravascular Evans blue dye. Survival of the mice is >95%, and no signs of neurologic dysfunction are observed within 2 weeks. Lack of pathology has been reported in rats, dogs, and monkeys.4-7 These results suggest that the method can be used to enhance delivery of drugs, antibodies, and enzymes into the mouse brain.

Whereas osmotic opening of the BBB in mice has been demonstrated qualitatively with Evans blue, previous work in other species has established that the degree of brain staining by Evans blue is highly correlated with the increase in BBB permeability to a number of quantitative tracers. Staining is correlated with enhanced brain uptake of [14C]sucrose in rats,2-3 of x-ray contrast agent in dogs using computed tomography,8-10 and of [125I]albumin and [3H]norepinephrine in rats.1 Therefore, the grade of staining by Evans blue is a semiquantitative measure of the degree of BBB opening to intravascular substances.

![Figure 1. Left: gross view and right: coronal section of 3+ grade mouse brain 4 hours after carotid infusion of 1.8 molal arabinose in distilled water and intravenous injection of Evans blue dye.](http://stroke.ahajournals.org/)

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Osmotic opening in mice is reversible and depends on infused concentration, with a threshold effect at 1.6 molal, as in rats. In mice, the BBB to Evans blue closes within 4 hours after arabinose infusion, as in rats. The degree of staining is related to the concentration of arabinose above the threshold. The higher the arabinose concentration above 1.6 molal, the darker and more reliably the brain is stained. Isotonic saline and 1.0 molal arabinose produce no BBB opening, indicating that the effect is not mediated by elevated infusion pressure. Indeed, net infusion pressures were ≤125 mm Hg (below the threshold of 160 mm Hg shown to be effective in rats), and the common carotid artery was not clamped, reducing the likelihood of a hypertensive effect.

We describe for the first time extension of the well-established method, osmotic opening of the BBB, to mice. A number of disease models, such as viral and fungal infections, immune deficiencies, experimental tumors, and genetic abnormalities, have been established in mice. The osmotic opening method should allow drugs, antibodies, enzymes, or other substances to cross the BBB in the investigation or treatment of these diseases.

References


KEY WORDS • blood–brain barrier • mice • osmotic
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