Effect of drugs on experimental brain edema in mice

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Cold-induced hemorrhagic infarcts in mice caused a spreading decrease in tissue specific gravity around the lesion; the decrease in tissue density represents an increase in edema fluid. The maximum decrease in density in most brain areas had occurred by 6 hours. This time period was used to evaluate the effect of nine drugs on brain edema. Two agents increased edema formation: hexamethonium and meralluride. Metaraminol, cortisone, hydrocortisone, acetazolamide, and dextran did not significantly alter edema formation. Only in the phenoxybenzamine- and urea-treated mice was brain edema less than in the control mice.

KEY WORDS: cerebral infarction • brain edema • drugs • tissue density

Brain edema as a consequence of trauma, tumors, and cerebrovascular accidents continues to be a major cause of death and disability even though a number of drugs are clinically useful in reducing brain edema. Hyperosmotic agents are widely used and have low toxicity; however, their duration of action is short and a rebound increase in intracranial pressure may occur. Glucosteroids do relieve symptoms of intracranial pressure in some patients with brain tumors, but reports differ as to the effectiveness of these drugs in other conditions that cause brain edema. Results from several laboratories indicate edema fluid is less in brain-damaged animals treated with glucosteroids, but other reports reveal that glucosteroids are ineffective in reducing experimental brain edema. Diuretics alone do not appear to alter cerebrospinal fluid (CSF) pressure in normal dogs but acetazolamide prolonged the reduction in CSF pressure after dogs were treated with urea. Reserpine was reported to reduce experimental brain edema, but in another study the results were not conclusive.

Because of the limitations and inconsistencies of drug therapy for treating brain edema, there is a need for continued evaluation of agents that may benefit the brain-injured patient. An obstacle in the assessment of drugs for treating brain edema is the selection of a suitable experimental model. The necessary requirements for testing agents for brain edema therapy were outlined by Benson, et al., and include the production of a reproducible brain lesion, a sensitive measure of brain edema, and the ability to study a large number of animals rapidly. In this report these criteria were largely fulfilled. Brain edema was produced with a cold-induced hemorrhagic infarct, but rather than
dry tissue to a constant weight, tissue density was used to determine changes in edema fluid. This system was used to determine the effect of nine drugs on the formation of brain edema in mice.

Materials and Methods

White male mice* were used. A cold-induced hemorrhagic infarct was made in the mouse cortex by a method similar to that described by Clasen, et al.9 An aluminum probe (4 mm tip), cooled to −150°C in liquid N₂, was placed against the bone overlying the right cerebral cortex of ether-anesthetized mice. The probe was left in place 30 to 40 sec; this produced a sharply demarcated zone of infarction about 1 mm deep and 4 mm in diameter.

Six hours after the cold lesions were made, the mice were decapitated, the brains removed, and duplicate brain samples taken for density measurements. The samples were either small (5 to 10 mg) or large (30 to 40 mg). Small samples were used when the tissue density within the hemorrhagic infarct was measured. The larger samples were used in the drug studies. Since the density of the infarction is less than the surrounding edematous tissue, variability in the size of the large samples could result in inconsistent density values. This inconsistency was minimized by taking care to remove samples of similar size from the same areas of each brain. In addition, all drugs were used throughout the period of an experiment to prevent bias that might occur due to changes in animal age or experimental technique.

The density of tissue samples was determined in a continuous, organic, density gradient. Removal of the mouse brain and dissection of samples were done under a hood at about 95% relative humidity. Tissue handling and preparation of the gradient column have been described previously.16

The drugs used were meralluride (Mercuhydrin), acetazolamide (Diamox), metaraminol bitartrate (Aramine), phenoxybenzamine, hexamethonium (Bistrium), cortisone acetate and hydrocortisone sodium succinate (Solu-cortef), urea, 30% (Urevert), and dextran 75, 6% (Gentran).† Drug doses, except for urea and dextran, are given as milligram of drug, salt form, per kilogram of body weight.

Results

Development of Brain Edema

The time course of edema formation in mice after the production of a hemorrhagic infarct is given in Fig. 1. As edema fluid spreads from the infarct the density of the tissue decreases. The first density change is in the lesion. In this area the fluid accumulation is nearly complete in 3 hrs. Assuming the edema fluid is H₂O, we can calculate the increase in tissue volume required to produce a given decrease in specific gravity (SG) by the following equation:16

\[
\text{percentage of change in tissue volume} = \frac{(\text{SG} - 1) \text{ cont} - (\text{SG} - 1) \text{ exp} \times 100}{(\text{SG} - 1) \text{ exp}}
\]

Based on this equation, the lesion increased about 80% in volume after 6 hrs. Tissue beneath the infarct increased in volume about 17%, contralateral subcortex 11%, and contralateral cortex 5% (Fig. 1).

Since edema was nearly maximal by 6 hrs in all areas, this interval was selected for subsequent experiments; however, the sample size was increased to about 30 mg (3 mm³) in subsequent experiments in order to decrease the risk of error resulting from evaporation during tissue dissection and tissue transfer to the gradient column. In addition, the larger samples improved the probability of obtaining similar samples in control and experimental animals (see Materials and Methods).

†Meralluride (Mercuhydrin) obtained from Lakeside Laboratories, 1707 East North Avenue, Milwaukee, Wisconsin 53201; acetazolamide (Diamox) from Lederle Laboratories, Division of American Cyanamid Company, Pearl River, New York 10965; metaraminol bitartrate (Aramine) from Merck, Sharp and Dohme, West Point, Pennsylvania 19486; phenoxybenzamine from Smith, Kline and French Laboratories, 1500 Spring Garden Street, Philadelphia, Pennsylvania 19101; hexamethonium (Bistrium) from E.R. Squibb & Sons, Inc., Princeton, New Jersey 08540; cortisone acetate and hydrocortisone sodium succinate (Solu-cortef) from Upjohn Pharmaceutical Company, Kalamazoo, Michigan; urea (Urevert) and dextran (Gentran) from Travenal Laboratories, Los Angeles, California 90039.

*White male mice (strain Ha/ICR) obtained from A.R. Schmidt Company, Madison, Wisconsin.
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1.0225
1.0275
1.0325
1.0375
1.0425
1.0475
1.0525

**Fig. 1.** Tissue densities. Hemorrhagic infarctions were made in the cerebral cortex of mice. Tissue samples (5 to 10 mg) were removed from the cortex and subcortex at the times indicated, and the density of these samples was determined from their position in a continuous gradient column. Each point is the mean value for samples from four to six mice. Standard error is represented by the vertical line.

**Urea, Dextran, and Brain Edema**

To determine if SG measurements could indeed be used to detect changes in tissue fluid, a drug known to reduce CSF pressure and brain water was used. Urea, 3 gm/kg, was injected intravenously in mice without a cerebral lesion; 1 hr after the drug injection brain density was significantly increased (p < .05) (less edema fluid) (Table 1); the density of a hemorrhagic infarct also was greater after mice were injected with urea. Dextran, however, did not significantly alter the density of either normal cortex or tissue from the area of the hemorrhagic infarction (Table 1).

**Drugs and Brain Density**

In this experiment, the density of the cortex in normal mice was 1.0483. Six hours after the hemorrhagic lesion was made the density of the lesion and the contralateral cortex was less than the control value (Table 2). The values from the damaged brain served as the control levels for tissue density in the mice given drugs.

The ganglionic blocking agent, hex-

<table>
<thead>
<tr>
<th>Drug</th>
<th>Control Mice</th>
<th>Drug-Treated Mice</th>
<th>Mice With Infarct Only</th>
<th>Mice With Infarct + Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific Gravity</td>
<td>Specific Gravity</td>
<td>Specific Gravity</td>
<td>Specific Gravity</td>
</tr>
<tr>
<td>urea</td>
<td>1.0479</td>
<td>1.0487†</td>
<td>1.0421†</td>
<td>1.0435‡</td>
</tr>
<tr>
<td>(3 gm/kg)</td>
<td>=0.0002</td>
<td>=0.0002</td>
<td>=0.0004</td>
<td>=0.0002</td>
</tr>
<tr>
<td>dextran</td>
<td>1.0468</td>
<td>1.0473</td>
<td>1.0432†</td>
<td>1.0437</td>
</tr>
<tr>
<td>(1.25 gm/kg)</td>
<td>=0.0002</td>
<td>=0.0003</td>
<td>=0.0004</td>
<td>=0.0004</td>
</tr>
</tbody>
</table>

*A cold-induced cerebral infarct was made in mice (40 to 45 gm) and the density of samples from the area of infarction was measured 6 hrs later. Urea was injected intravenously ½ hr before and ½ hr after the lesion was made, which gave a total dose of 3 gm/kg. Dextran (1.25 gm/kg) was injected in the tail vein ½ hr before the lesion was made. Each value is the mean for samples from six mice.

†The value is different from the control value, p < .05.
‡The value is different from that in mice with infarct only, p < .05.
amethonium, increased edema formation (Table 2). Tissue density in the infarct area was significantly less than the density in the same area of control mice. The tissue density in the contralateral hemisphere was similar in the two groups of mice. The diuretic, acetazolamide, did not alter edema formation, but treatment with the mercurial diuretic, meralluride, was associated with a significant decrease in density (increase in edema) in the contralateral hemisphere (Table 2). Cortisone, in one pretreatment dose, did not alter the production of brain edema. Metaraminol, given in both single and divided doses, had no significant effect on the tissue density after cerebral infarction. The alpha adrenergic blocking agent phenoxybenzamine, at 5 mg/kg, did not affect edema formation, but at 20 mg/kg there was a significant decrease in edema in the hemisphere contralateral to the lesion (Table 2).

Hydrocortisone and Brain Edema

Although cortisone in a single pretreatment dose did not alter edema formation (Table 2), a more thorough study was done using hydrocortisone. Hydrocortisone (50 mg/kg) was injected intraperitoneally twice into the brain-injured mice. One group of mice received the first drug injection before the lesion was made, and the other group received both injections after the lesion was produced. In neither group of mice ("pre-treatment" or "post-treatment") was the density of the hemorrhagic infarct or contralateral cortex significantly affected by the drug (Table 3). As can be seen from the differences in standard errors, there was greater variability in the values from the treated mice than in those from controls.

**Discussion**

The use of mice in screening for drug efficacy is advantageous in that mice are inexpensive and easy to work with, but there are also disadvantages associated with their use. The fact that mice are seldom used in brain edema studies prevents a direct comparison

### Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>No.</th>
<th>SG ± S.E.</th>
<th>% Vol Change</th>
<th>Lesion (SG ± S.E.)</th>
<th>% Vol Change</th>
<th>Contralateral Cortex (SG ± S.E.)</th>
<th>% Vol Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>5</td>
<td>1.0483 ± 0.0003</td>
<td></td>
<td></td>
<td></td>
<td>1.0469 ± 0.0003</td>
<td>3.0 (inc)</td>
</tr>
<tr>
<td>lesion (no drug)</td>
<td>10</td>
<td>1.0426 ± 0.0003</td>
<td>13.4 (inc)</td>
<td>1.0410 ± 0.0006</td>
<td>3.9 (inc)</td>
<td>1.0465 ± 0.0004</td>
<td>&lt;1 (inc)</td>
</tr>
<tr>
<td>hexamethonium (20 mg/kg)</td>
<td>5</td>
<td>1.0419 ± 0.0006</td>
<td>1.7 (inc)</td>
<td>1.0408 ± 0.0008</td>
<td>4.4 (inc)</td>
<td>1.0459 ± 0.0003</td>
<td>2.2 (inc)</td>
</tr>
<tr>
<td>acetazolamide (10 mg/kg)</td>
<td>5</td>
<td>1.0426 ± 0.0008</td>
<td>0</td>
<td>1.0426 ± 0.0008</td>
<td>0</td>
<td>1.0465 ± 0.0005</td>
<td>&lt;1 (inc)</td>
</tr>
<tr>
<td>meralluride (5 mg/kg)</td>
<td>5</td>
<td>1.0436 ± 0.0005</td>
<td>2.3 (dec)</td>
<td>1.0416 ± 0.0006</td>
<td>2.4 (inc)</td>
<td>1.0466 ± 0.0004</td>
<td>&lt;1 (inc)</td>
</tr>
<tr>
<td>cortisone (50 mg/kg)</td>
<td>5</td>
<td>1.0422 ± 0.0005</td>
<td>&lt;1 (inc)</td>
<td>1.0428 ± 0.0004</td>
<td>&lt;1 (dec)</td>
<td>1.0477 ± 0.0002</td>
<td>1.7 (dec)</td>
</tr>
<tr>
<td>metaraminol (40 mg/kg, 1 dose)</td>
<td>5</td>
<td>1.0428 ± 0.0004</td>
<td>&lt;1 (dec)</td>
<td>1.0428 ± 0.0004</td>
<td>&lt;1 (dec)</td>
<td>1.0477 ± 0.0002</td>
<td>1.7 (dec)</td>
</tr>
<tr>
<td>metaraminol (40 mg/kg, divided dose)</td>
<td>5</td>
<td>1.0428 ± 0.0004</td>
<td>&lt;1 (dec)</td>
<td>1.0428 ± 0.0004</td>
<td>&lt;1 (dec)</td>
<td>1.0477 ± 0.0002</td>
<td>1.7 (dec)</td>
</tr>
<tr>
<td>phenoxybenzamine (5 mg/kg)</td>
<td>5</td>
<td>1.0422 ± 0.0005</td>
<td>&lt;1 (inc)</td>
<td>1.0422 ± 0.0005</td>
<td>&lt;1 (inc)</td>
<td>1.0464 ± 0.0007</td>
<td>&lt;1 (inc)</td>
</tr>
<tr>
<td>phenoxybenzamine (20 mg/kg)</td>
<td>5</td>
<td>1.0428 ± 0.0004</td>
<td>&lt;1 (dec)</td>
<td>1.0428 ± 0.0004</td>
<td>&lt;1 (dec)</td>
<td>1.0477 ± 0.0002</td>
<td>1.7 (dec)</td>
</tr>
</tbody>
</table>

*Mice were injected with a test drug, and ½ hr later a hemorrhagic lesion was made with a cold probe. Six hours after the lesion was made samples weighing 30 to 40 mg were removed from the area of infarction and from the contralateral cortex. Tissue density was measured in a gradient column. The percentage change in tissue volume (% Vol Change) is given with the direction of this change in parenthesis. SG = specific gravity.†The value is significantly different from the control value, p < .01.
‡The value is significantly different from the value of the control lesion, p < .05.
§The value is significantly different from the value of tissue contralateral to the lesion in nontreated mice, p < .05.
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### TABLE 3

The density of injured brain in mice treated with hydrocortisone*

<table>
<thead>
<tr>
<th>Tissue Sample</th>
<th>Control Specific Gravity</th>
<th>Hydrocortisone (Pretreatment) Specific Gravity</th>
<th>Hydrocortisone (Posttreatment) Specific Gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>1.0506 ± 0.0005</td>
<td>1.0310 ± 0.0002</td>
<td>1.0290 ± 0.0025</td>
</tr>
<tr>
<td>Dors. Hipp.</td>
<td>1.0498 ± 0.0000</td>
<td>1.0460 ± 0.0002</td>
<td>1.0449 ± 0.0007</td>
</tr>
</tbody>
</table>

*Cold-induced cerebral infarcts were made in mice (42 to 44 gm). Six hours later the mice were decapitated, and duplicate samples were taken within the hemorrhagic infarction or cortex. Dissection of brain was then continued deep to the lesion and the lateral ventricle opened. The dorsal aspect of the hippocampus was visualized through the opened ventricle, and samples were removed for density measurement. In the "pretreatment" group of mice, hydrocortisone (50 mg/kg) was injected intraperitoneally 1 hr before the lesion was made, and a second dose of hydrocortisone was given 2 hrs after the lesion was made. In the "posttreatment" group of mice, hydrocortisone was injected 2 and 4 hrs after the hemorrhagic infarct was produced. Control mice received sham operations and normal saline injections (0.44 ml, intraperitoneally). The values are tissue specific gravity ± S.E. Each value is the mean for samples from four mice. Dors. Hipp. = dorsal hippocampus.

†The brain tissue density is less than the density of samples from mice without a cerebral lesion (p < .05).

with results from other laboratories. In addition, the thin layer of white matter in mouse brain makes it impractical to study edema in this tissue. Nevertheless, the cerebral microvasculature in mice is architecturally and functionally similar to larger mammals including man, and the cerebral events after injury are probably the same.

The progression of brain edema from a zone of hemorrhagic infarction was illustrated by the sequential decrease in tissue density (Fig. 1). These findings are comparable to histological findings in which edema fluid has been observed to spread from deeper parts of the lesion into underlying white matter. The finding of decreased tissue density in the contralateral brain hemisphere in both the study on edema development and in the drug experiments was expected since a similar change in density was observed in a previous study. A possible reason for the detection of the density differences in the contralateral hemisphere is that the distance between hemispheres is small in mouse brain and diffusion of fluid to this area may occur before there is appreciable fluid absorption.

The infarcted tissue reached an SG of 1.0250, which represents an 80% increase in volume. The finding that there was small variation in this value, plus the fact that it was constant for at least 24 hours, indicates an 80% volume increase may be the maximum hydration of a hemorrhagic infarction. The retention of this edema fluid correlates with the slow reversal of brain edema observed clinically.

When the percentage increase in tissue volume is estimated from the change in tissue density of brain, edema fluid is assumed to be H2O. This assumption is probably justified since it results in a conservative estimate of the change in tissue volume. If edema fluid had .02 gm/ml of protein, the volume of this fluid needed to change a ml of tissue from 80% to 82% H2O would be about 0.12 ml compared with 0.11 ml if the edema fluid were water.

Intravenous injection of urea increased the density of normal brain as well as edematous tissue. This indicates that urea can remove water from damaged brain. From experiments with brain-injured monkeys Clasen, et al., concluded that the reduction of CSF pressure with urea was due to dehydration of the undamaged hemisphere since no change in H2O content was observed in the damaged hemisphere.

Only two agents used in this study increased brain edema formation. The effect
with hexamethonium, a hypotensive agent, may be explained by a lowered cerebral perfusion pressure, which would decrease oxygenation at the infarct periphery and increase the area of damage. Meralluride also appeared to increase the edema formation in the damaged hemisphere. The failure of this mercurial diuretic to reduce edema supports the concept that the diuretic effect of the osmotic diuretics is not responsible for the brain dehydration observed with these agents. 9

The reason for the increase in edema with meralluride was not apparent, but a toxic effect of the diuretic on cells in the damaged area is possible since the dose used was 5 to 10 times that used clinically as a single injection. Cortisone was not effective in reducing brain edema. Since this single pretreatment dose did not constitute adequate support for the negative findings of others, a more thorough separate study was done with hydrocortisone. This drug also did not alter edema formation. The steroid studies are acute, and while they do not appear to alter leakage of damaged capillaries, it is possible steroids may influence fluid absorption at longer time intervals.

Metaraminol did not alter brain edema formation. Since the drug increases blood pressure, an increase in edema might have been expected. An elevated blood pressure increases hydrostatic pressure, which may increase the extravasation of fluid from capillaries in injured brain. 10,19 Although blood pressures were not obtained in these animals, the dose of metaraminol used (25 mg/kg, base) was believed adequate for producing a significant vasopressor response, the drug dose was half the L.D$_{50}$ for mice and more than 100 times the dose used in treating hypotensive states in patients.

The increase in brain density contralateral to the lesion in phenoxybenzamine-treated mice suggests that there was a reduced amount of edema fluid in this hemisphere. It is doubtful that this response was due to the hypotensive effect of the drug since hexamethonium increased brain edema; nor is alpha-adrenergic blockade of cerebral vessels likely to be the mechanism for improvement since sympathetic influence on cerebral vasculature is believed to be small. Although the drug did not affect edema in the infarct, the favorable effect in the contralateral hemisphere warrants more thorough study with this agent.

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References


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