Glycerol Therapy of Experimental Cerebral Microembolism

BY DENNIS M. WELCH, M.D., REBECCA K. STUDER, M.S., AND BARRY A. SIEGEL, M.D.

Abstract: Glycerol was administered to control rats and to animals with experimental cerebral microembolism as a single intravenous injection (1 gm per kilogram), a single one-hour intravenous infusion (1.5 gm per kilogram), daily one-hour intravenous infusions (1 gm per kilogram), or in multiple oral doses (1 gm per kilogram per four hours). There were no effects of glycerol on the mortality, brain edema or increased brain sodium concentration and 75Se-selenate space due to cerebral microembolism. Brain water was not reduced in the hemispheres contralateral to embolization or in glycerol-treated controls. The results suggest that glycerol, at the dose levels reported to be beneficial in human cerebral infarction, is ineffective for cerebral dehydration.

Additional Key Words: brain extracellular fluid, cerebral infarction, brain edema, therapy, brain water, increased intracranial pressure, brain electrolytes.
Methods

Female rats of Wistar origin, weighing 175 gm, were used for all studies in groups of 6 to 12 animals. Cerebral microembolism was produced as previously described.11 The rats were anesthetized with ether and the right common carotid artery was exposed. After ligation of the external carotid artery, the common carotid artery was punctured and approximately 280 carbonized microspheres (80 μm mean diameter) suspended in 0.05 ml 77% sucrose solution were injected slowly and flushed with 0.45 ml isotonic saline solution. The artery was ligated above and below the puncture site, the needle withdrawn, the wound closed, and the animal allowed to awaken.

Glycerol was administered to microembolized rats in one of four dose regimens.

Group 1: A single intravenous injection over 45 seconds of 10% glycerol (1 gm per kilogram of body weight) was given either 15 minutes, one hour, or two hours before sacrifice at 24 hours after microembolization.

Group 2: A single intravenous infusion over one hour of 15% glycerol (1.5 gm per kilogram of body weight) was completed either ten minutes or one hour prior to sacrifice at 24 hours after microembolization.

Group 3: Daily intravenous infusions over one hour of 10% glycerol (1 gm per kilogram of body weight) were given. The first administration was begun three to five hours after microembolization and the fourth and final infusion was completed 30 minutes prior to sacrifice at 72 hours after microembolization.

Group 4: Multiple oral doses of 20% glycerol (1 gm per kilogram of body weight) were given by orogastric tube every four hours. The first dose was given 24 hours after microembolization and the seventh and final dose was given at 48 hours. The rats were killed two hours later.

In each experiment the following control animals also were studied: (1) normal rats, (2) normal rats treated with glycerol by one of the regimens described above and killed at the same times after completion of drug therapy, and (3) untreated microembolized animals killed at the same times after embolization.

At the designated time, cardiac blood samples were taken and the anesthetized animals were killed by immersion in liquid nitrogen. The brains were carefully removed and the right and left cerebral hemispheres were placed into individual tared counting vials and weighed.

Brain water content was determined by drying the hemispheres at 85 to 90 C to a constant weight. Brain sodium determinations were performed on dilute homogenates by atomic absorption spectrophotometry.

Ten to 30-minute distribution spaces of 59Fe-labeled red cells (an index of cerebral blood volume), 125I-albumin (an index of cerebral plasma volume and/or vascular permeability), and 75Se-selenate (an index of extracellular space and/or vascular permeability) were determined in several groups. The hemispheres and plasma or whole blood samples were counted in a NaI(Tl) crystal well scintillation detector and the various radionuclide gamma emissions were distinguished by pulse height spectrometry and standard Compton scatter crossover corrections. The albumin and selenate spaces were calculated as (cpm/gm brain) ÷ (cpm/gm plasma); the red cell space was calculated as (cpm/gm brain) ÷ (cpm/gm red cells).

Serum glycerol levels at the time of sacrifice were determined in treated animals by a modification of the enzymatic method of Garland and Randle. In addition, groups of normal animals were given a one-hour intravenous infusion of 10% glycerol (1 gm per kilogram of body weight) and serum samples for glycerol assay were taken during and at times up to 24 hours after the end of the infusion. This was done to evaluate the serum concentrations achieved in dosage regimen 3.

Group means for all determinations were considered statistically significant by Student's t-test at P < 0.05.

Results

Mortality following cerebral microembolism was not significantly altered by glycerol administration. In the group treated with daily intravenous infusions of glycerol (group 3), 10 of 14 animals (71%) survived at 72 hours; 9 of 12 untreated animals (75%) survived for this period. In the animals receiving multiple oral doses of glycerol, eight of nine were alive at 48 hours; none of nine untreated animals died.

Brain water was significantly increased compared to the control value in animals killed 24, 50, or 72 hours after microembolization (table 1). Brain edema appeared to be slightly reduced in microembolized animals treated with either a single intravenous dose, a single intravenous infusion, or daily intravenous infusions of glycerol, but the observed changes were not statistically significant. In general, glycerol also had no effect on the brain water content of normal animals; in fact, there was a paradoxical increase in brain water two hours after a single intravenous dose of 1 gm per kilogram of body weight and 30 minutes after the last of three daily intravenous infusions (1 gm per kilogram of body weight).

Similarly, the brain sodium concentration was increased in all microembolized rats (table 2). Brain sodium was not significantly decreased by glycerol therapy and some of the normal animals also showed an unexpected increase in brain sodium after glycerol.

Since the beneficial effects which have been reported with glycerol therapy might be due to dehydration of the normal hemisphere, we also examined the water and sodium contents in the left hemispheres of both treated and untreated microembolized animals. In no instance did glycerol reduce either the brain water or sodium concentration of the left hemisphere.

As we have previously reported,11 the cerebral red cell space was reduced in the microembolized right hemispheres by 17% at 24 hours and had returned to normal at 72 hours. Glycerol resulted in a transient increase in the cerebral red cell space of both hemispheres 15 minutes after a single intravenous dose (group 1). A similar effect was observed in normal animals receiving the same dose of glycerol. The change was no longer apparent one hour after glycerol administration and presumably reflected a transient, generalized intravascular volume expansion (an expected result following a relatively rapid injection of approximately 1.75 ml of 10% glycerol which would
<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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<tbody>
<tr>
<td>Hours after microembolism</td>
<td>Minutes after last glycerol</td>
<td>Hours after microembolism</td>
<td>Minutes after last glycerol</td>
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<td>24</td>
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<td>120</td>
<td>50</td>
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<td>120</td>
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**Brain Water**

Results expressed as gram water/gram dry weight brain (mean ± standard deviation). Numbers in parentheses represent numbers of observations. Determinations are shown for both hemispheres in control and glycerol-only groups; for right hemispheres only in microembolism and microembolism + glycerol groups.

*Statistically significant compared to control group at P < 0.05.

**Brain Sodium**

Results expressed as mEq Na/gm dry weight brain (mean ± standard deviation). Other conditions are the same as those in table 1.
represent about 20% of the rat's blood volume). The red cell space was not significantly increased by glycerol following an infusion over one hour and was not measured in the animals receiving oral glycerol. The $^{131}$I-albumin space paralleled the red cell space; as short equilibration times (10 to 30 minutes) were used in these experiments, we did not see evidence of an expanded albumin space due to extravascular spread which we had encountered in previous studies of cerebral microembolism employing a two-hour equilibration period.$^9$ $^{11}$ The $^{75}$Se-selenate space provides a reliable and sensitive estimate of increased blood-brain barrier permeability and may be increased when the distribution spaces of macromolecular tracers, such as $^{131}$I-albumin, are unaltered.$^9$ The 15-minute selenate distribution space was 4.24 ± 0.50 ml/100 gm brain (n = 6) in normal control animals; 24 hours after microembolism, the corresponding value was 6.91 ± 2.01 ml/100 gm brain (n = 7) (P < 0.05). Fifteen minutes after a single intravenous dose of glycerol of 1 gm per kilogram of body weight (group 1), the selenate space in microembolized animals was 5.85 ± 1.12 ml/100 gm brain (n = 8). This result was not significantly different from that in untreated animals.

The serum glycerol levels during and after a one-hour infusion of 10% glycerol (1 gm per kilogram of body weight) are shown in Table 3. The serum glycerol concentration rose to a peak value of 141 mg/100 ml at the completion of the infusion and fell rapidly after cessation. Somewhat higher levels were achieved in animals receiving 1.5 gm per kilogram of body weight as an infusion of 15% glycerol over one hour; the mean serum glycerol concentration was 253 ± 35 mg/100 ml ten minutes after cessation of the infusion and 176 ± 26 mg/100 ml after one hour. Serum glycerol levels were quite variable after oral administration.

### Table 3

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<tr>
<th>Time after cessation of infusion, minutes</th>
<th>Serum glycerol (mg/100 ml)</th>
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<tr>
<td>5 minutes</td>
<td>95 ± 25</td>
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<tr>
<td>15 minutes</td>
<td>60 ± 13</td>
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<tr>
<td>30 minutes</td>
<td>31 ± 7</td>
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<tr>
<td>2 hours</td>
<td>4.4 ± 2.0</td>
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<tr>
<td>4 hours</td>
<td>2.5 ± 0.5</td>
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<tr>
<td>24 hours</td>
<td>1.4 ± 0.3</td>
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Results reported as mean ± standard deviation for groups of four to six animals.

Discussion

Therapy with hypertonic solutions of glycerol has been shown to be effective in the reduction of experimental cerebral edema due to several different forms of injury.$^{14-17}$ The proposed mechanism of action of glycerol, as with other osmotically active agents, is the withdrawal of fluid from cerebral tissues in response to the increased blood osmolality. This effect appears to be independent of any diuretic action of glycerol administration.$^{18}$ The greatest dehydrating effect is exerted on normal rather than edematous brain tissue;$^{16}$ similar results also have been observed with urea.$^{19}$ $^{20}$

The studies reported here were designed to test the effects of glycerol in an animal model of ischemic brain injury produced by microembolization. The glycerol doses and routes of administration employed were chosen to be comparable to those reported to have beneficial effects in cerebral infarction in man.$^{6}$ $^{8}$ However, we were unable to demonstrate a significant reduction by glycerol of either the mortality or brain edema following experimental cerebral microembolism. Furthermore, there were no significant decreases in brain water in the contralateral hemispheres of control animals receiving equivalent doses of glycerol. Glycerol also had no significant effect, except very transiently following a rapid intravenous injection, on the cerebral red cell space or short-term albumin distribution space. As expected, glycerol did not change the increased blood-brain barrier permeability to molecules such as selenate ion.

The failure of glycerol to reduce cerebral edema in the embolized hemisphere was not a surprising result. Cerebral microembolism, as well as other forms of ischemic brain injury, appears to involve both cytotoxic and vasogenic mechanisms of brain edema induction.$^{9}$ $^{21}$ Abnormal microvascular permeability to a variety of substances is one of the chief characteristics of vasogenic edema, and, as suggested by Klatzo,$^{22}$ hypertonic solutions will be of little benefit under these circumstances since there is no membrane across which the osmotic withdrawal of predominantly extracellular edema fluid could occur.

The serum concentration of glycerol necessary to achieve a reduction of brain water has not been clearly established. McCurdy et al.$^{22}$ have determined that concentrations exceeding 10 mM (92 mg/100 ml) are required to reduce intracocular pressure and it has been assumed that similar levels would be effective in lowering intracranial pressure.$^{6}$ However, Guisado et al.$^{16}$ have recently shown that maintenance of plasma
GLYCEROL THERAPY OF EXPERIMENTAL CEREBRAL MICROEMBOLISM

glycerol concentration at 12 mM by infusion in dogs had no effect on either brain water or cerebrospinal fluid pressure, whereas both were decreased by levels of 32 mM (284 mg/100 ml). These authors recommended that the plasma glycerol concentration must be at least 30 mM in order to sustain cerebral dehydration. Our failure to observe cerebral dehydration in either the glycerol-treated normal rats or the hemispheres contralateral to microembolization would tend to support their conclusion since the doses of glycerol we used did not result in plasma concentrations of 30 mM.

Our results suggest that, in the doses which have been used clinically, the beneficial effects of glycerol in human cerebral infarction are not likely to be due to lessening of brain edema within the region of the infarct or to significant dehydration of normal brain. Meyer et al. noted a mean increase in serum osmolality of only 7.4 mosmoles per liter after a glycerol infusion of 1.2 gm per kilogram of body weight. As suggested by this group, an effect of glycerol on cerebral metabolism may be of importance in its beneficial action. It has been demonstrated that glycerol may act as substrate for brain metabolism. Further investigations of the mechanism of action of glycerol in cerebral infarction appear to be warranted.

References
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