Modification of Acute Focal Ischemia by Treatment with Mannitol

JOHN R. LITTLE, M.D.

SUMMARY A simple implanted device was used to occlude acutely the left middle cerebral artery (MCA) of 16 conscious cats. Eight received no treatment and 8 were given intravenous mannitol (1.2 gm/kg) at the time of occlusion. The initial neurological findings in both groups were similar, that is, agitation, forced circling, and right hemiparesis. The treated cats remained alert but the untreated cats became lethargic and drowsy. Perfusion with a mixture of colloidal carbon and buffered paraformaldehyde was carried out from 30 minutes to 6 hours following MCA occlusion. Results of morphological examination of brains from the treated and untreated groups suggested that mannitol had a protective effect upon cerebral tissue during the primary phase of acute focal ischemia. Light microscopic analysis of neuronal alterations demonstrated considerable preservation of neurons in brains of treated cats. Beneficial effect of mannitol was attributed partly to prevention of capillary narrowing and suppression of ischemic cerebral edema.

PREVIOUS EXPERIMENTAL investigations of focal cerebral ischemia have demonstrated the diphasic nature of ischemic injury. The primary phase is thought to be reversible with preservation of tissue viability if ischemia is relieved. The secondary phase is characterized by tissue necrosis and, therefore, is irreversible.

The treatment of acute focal ischemia ideally should be directed toward protecting cerebral tissue during the primary phase thereby preventing or retarding the transition into the secondary phase. Various hyperosmolar agents have been shown to have a beneficial effect in acute cerebral ischemia when administered at an early stage that is, prior to the breakdown of the blood-brain barrier. Sundt et al. demonstrated substantial preservation of cerebral tissue and control of edema in cats treated with a combination of concentrated serum albumin and low-molecular-weight dextran before middle cerebral artery (MCA) occlusion and with concentrated urea after occlusion.

Hyperosmolar agents also have been shown to have a beneficial effect upon the microcirculation in both acute focal and acute global ischemia. Crowell and Olsson revealed substantial amelioration of microvascular filling using carbon black perfusion in monkeys treated with low-molecular-weight dextran; however, the relationship between the improved perfusion and the evolution of ischemic neuronal changes was not defined. The object of this investigation was to study the effect of mannitol upon the microcirculation and cerebral tissue, particularly the neuronal alterations, during the primary phase of acute focal ischemia.

Methods

Determination of Plasma Osmolality and Hematocrit

One week before implantation of the occluding device, the 8 cats to be used in the treated group were anesthetized with sodium pentobarbital (30 mgm/kg intraperitoneally) and given 20% mannitol (1.2 gm/kg IV). One sample of venous blood (2 ml) was then withdrawn from each cat at a designated time (i.e., 30 minutes, 1 hour, 3 hours, or 6 hours) following mannitol administration corresponding to the time of planned perfusion in the subsequent segment of the experiment. Plasma osmolalities were then determined. Two cats to be used in the untreated group also were anesthetized and venous blood (2 ml) was taken for determination of osmolality.

Four additional cats were anesthetized with pentobarbital and a short 20 gauge polyethylene catheter was inserted into the right femoral vein through a small skin incision. Blood (0.3 ml) was taken for hematocrit determination. Mannitol (1.2 gm/kg IV) was then given during a 15 minute period and hematocrits were obtained 30 minutes, 1 hour, 3 hours, and 6 hours following mannitol administration.

Prophylactic Antibiotics

Each cat received chloramphenicol (30 mgm/kg IM) immediately prior to implantation of the occluding device.

Implantation of Occluding Device

Details of the methods for the implantation of the device used to occlude the middle cerebral artery (MCA) in conscious cats have been described previously. Sixteen adult cats with a mean weight of 3,400 gm were anesthetized with sodium pentobarbital (30 mgm/kg IP). The left MCA of each animal was exposed through a transorbital approach and the slotted housing of the occluding device with the short stylet inserted was applied to its proximal segment. The orbit was sprayed briefly with neosporin aerosol and then filled with rapidly hardening epoxy cement. The incision was closed with 4-0 silk suture.

A 20 gauge polyethylene catheter was inserted into the left femoral vein through a small incision in the groin. The injection nozzle of the catheter was brought out through another small incision on the flank. The wounds were closed with 4-0 silk suture and dressings applied. Each animal was given 30 ml of isotonic saline by intravenous injection.

Occlusion of MCA

The left MCA of the 16 cats was occluded on the second postoperative day by inserting the occluding stylet. Eight cats received 20% mannitol (1.2 gm/kg IV) in a divided dose, half during a 10 minute period before and half during a 10 minute period after MCA occlusion.
Perfusion Technique

The 8 mannitol (i.e., treated) and 8 control (i.e., untreated) cats were perfused in groups of 2 after ischemic periods of 30 minutes, 1 hour, 3 hours, and 6 hours. Each animal was anesthetized with sodium pentobarbital (30 mgm/kg IP) 20 minutes prior to perfusion. A tracheostomy was performed and the animals were ventilated mechanically. Fifteen minutes before perfusion, 10% sodium fluorescein (1 ml) was injected slowly through the intravenous catheter.

A midline thoracotomy was performed. Immediately prior to commencing perfusion the occluding stylet was withdrawn. A large cannula was passed through a left ventriculostomy incision into the ascending aorta and secured with a ligature. The descending aorta was clamped and the right atrium incised. The animals were perfused initially with 50 ml of isotonic saline followed by a mixture of colloidal carbon (250 ml) and phosphate-buffered (pH 7.3) 4% paraformaldehyde (250 ml) at a constant pressure of 120 mm Hg. The brain of each cat was removed carefully 1 hour following completion of perfusion and placed in 50 ml phosphate-buffered 4% paraformaldehyde at 4°C for 48 hours.

Tissue Preparation

The brains were cut into 5 mm coronal slices. These slices were photographed and examined under ultraviolet light using a Kodak-Wratton #21 barrier filter.

The distribution of carbon staining in the left cerebral hemispheres was classified according to the grading system of Crowell and Olsson: grade 0 indicated normal vascular filling; grade 1 referred to a few circumscribed foci of poor filling not more than 3 mm in diameter; grade 2 indicated a large area of improper subcortical filling; and grade 3 referred to an extensive cortical and subcortical region of impaired filling. The intensity of the zones of pallor also was classified.

Thick (75 μ) coronal sections from the left and right hemispheres were cut with a freezing microtome, mounted on glass slides, and examined with a light microscope. Thin (10 μ) semi-serial coronal sections were prepared from paraffin-embedded slices of both hemispheres, stained with hematoxylin and eosin and with cresyl violet, and examined with a light microscope. Neurons were graded according to the severity of the changes present: grade 1, slightly shrunken neurons with or without cytoplasmic vacuolation; grade 2, moderately shrunken neurons with cytoplasmic eosinophilia and increased nuclear basophilia or swollen neurons with pale vacuolated cytoplasm and a pale vesicular nucleus; and, grade 3, severely shrunken neurons with bright cytoplasmic eosinophilia, pyknotic nucleus, and/or incrustations. Coronal sections near the tips of the temporal lobes were given scores according to the distribution and grade of neuronal changes observed. The Kolmogorov-Smirnov two-sample test was used to assess the differences.

Results

Plasma Osmolality and Hematocrit

The results of osmolality and hematocrit determinations are listed in table 1.

Observations Following Left MCA Occlusion

The initial response of the cats in both groups was essentially the same, that is, agitation, circling to the left, followed in 1 to 2 minutes by weakness of the right extremities (table 2). The untreated cats perfused at 6 hours became drowsy and lethargic during the hour preceding perfusion. As well, the right hemiparesis in one of these untreated cats was seen to increase in severity. The treated cats, however, remained alert and active. The right hemiparesis in one cat, perfused at 3 hours, improved, whereas the deficit in the other cats did not change substantially.

Macroscopic Findings

A. Brain swelling.

The cerebral tissue supplied by the left MCA was grossly swollen in the brains of the 2 cats in the untreated group perfused at 6 hours (fig. 1). There was a 3 mm left to right midline shift in both of these brains. Less severe swelling was observed in the brains of the 2 untreated cats perfused at 3 hours. The 30 minute and 1 hour brains in the untreated group did not appear grossly swollen. Slight swelling of cerebral tissue was present in one brain in the treated group perfused at 3 hours. The other 7 brains of the treated cats

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**TABLE 1 Plasma Osmolality and Hematocrit in Cats Following Intravenous Mannitol Administration**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mannitol (1.2 gm/kg)</th>
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<tr>
<td></td>
<td>30 min.</td>
<td>1 hour</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 hours</td>
</tr>
<tr>
<td>Plasma osmolality (mOsm/l)</td>
<td>319 ± 2</td>
<td>334 ± 1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>33 ± 1</td>
<td>31 ± 1</td>
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</table>
B. Changes in the Neuropil

1. Untreated group. Clear spaces with limiting membranes were present around many of the capillaries in the ischemic zone following 30 minutes of occlusion (fig. 3). The spaces were thought to represent swollen astrocytic processes. Perivascular astrocytic swelling became progressively more severe with longer periods of ischemia and appeared to spread in a centrifugal fashion to involve the remainder of the neuropil. The developing ischemic edema often had a patchy distribution and isolated clusters of severely distended cellular processes were identified frequently. Fluid accumulation appeared to be considerable in the pale zones at 6 hours and necrosis of the tissue was evident with rupture of many limiting membranes.

2. Treated group. Swelling of pericapillary astrocytic processes was minimal or absent in the brains perfused at 30 minutes and 1 hour. Mild pericapillary astrocytic swelling and slight vacuolation of the neuropil was seen in one of the brains perfused at 3 hours, whereas, the 3 hour brain with grade 3 filling impairment had a moderate amount of astrocytic swelling. Pericapillary astrocytic swelling generally remained mild in the tissue supplied by the left MCA of the 2 brains perfused at 6 hours. The changes in the neuropil were comparable to those seen at 30 minutes in the untreated, control group. Moderate pericapillary astrocytic
swelling was seen in the circumscribed pale zones in these 2 brains.

C. Capillary Alterations

1. Untreated group. Capillary narrowing in ischemic zones was seen as early as 30 minutes and appeared to increase in severity with longer periods of MCA occlusion. Mean capillary diameters, determined from measurements (500 μm) taken from photomicrographs of anterior and posterior Sylvian cortex, are listed in Table 4. Washout of erythrocytes was essentially complete in tissue made ischemic for 30 minutes, 1 hour, and 3 hours, but after 6 hours of ischemia, numerous erythrocytes frequently remained within the capillaries in the core area of ischemia.

2. Treated group. Capillary narrowing was infrequently demonstrated in the brains perfused at 30 minutes and 1 hour. Mild to moderate capillary narrowing was observed in the pale zones of the brains perfused at 3 and 6 hours; however, in 3 of these, the pale zones were relatively small and restricted to the subcortical tissue. Washout or erythrocytes was essentially complete in all of these brains.

D. Examination of the right cerebral tissue. No abnormalities were identified.

Discussion

A device\(^1\) was applied to the proximal left MCA of 16 adult cats. Following recovery from this initial procedure, the artery was occluded acutely while the animals were conscious. Eight cats received no treatment and 8 cats were given mannitol intravenously at the time of artery occlusion. Perfusion with a mixture of colloidal carbon and buffered paraformaldehyde was carried out from 30 minutes to 6 hours following occlusion. Results of the morphological examination of the brains from the untreated and treated groups suggested that mannitol had a protective effect upon cerebral tissue during the primary phase of acute focal ischemia.\(^1\)

The initial neurological findings in the cats from both the treated and untreated groups were essentially the same; however, the untreated cats perfused at 6 hours became lethargic and drowsy during the hour prior to perfusion. Hayakawa and Waltz,\(^13\) studying a similar model, attributed this depression in the level of consciousness to ischemic cerebral edema, increases in intracranial pressure, and herniation. In our investigation, mannitol appeared to suppress the development of ischemic brain swelling, and the brains of most of the treated cats were slightly shrunken. These animals remained alert and active during the initial 6 hours of MCA occlusion.

The severity and distribution of neuronal alterations in the brains of the treated and untreated cats were compared. The grading system used was derived from previous, combined light and electron microscopic studies of ischemic neurons\(^14\)–\(^17\) (Table 5). Grade 1 changes were thought to represent mild, probably reversible injury and grade 3 changes were considered to be consistent with necrosis.
FIGURE 3. Representative light micrographs of cerebral cortex (calibration = 50μ). A, cortex of right hemisphere demonstrates numerous plump neurons and a capillary loop filled with carbon. B, ischemic cortex after 30 minutes of MCA occlusion in an untreated cat, reveals marked pericapillary astrocytic swelling and mild vacuolation of the neuropil. The neurons appear slightly shrunken. The insert demonstrates neuronal cytoplasmic vacuolation (i.e., grade 1 neuronal change). C, ischemic cortex after 3 hours of MCA occlusion in an untreated cat, shows irregular narrowing of capillaries filled with carbon and marked vacuolation of the neuropil. The neurons are severely shrunken and have bright eosinophilic cytoplasm and pyknotic nuclei (grade 3). D, ischemic cortex at 6 hours in a treated cat, shows capillaries of normal calibre. There is little vacuolation of the neuropil. Many moderately shrunken neurons with eosinophilic cytoplasm (grade 2) are present.

### TABLE 5 Classification of Ischemic Neuronal Alterations

<table>
<thead>
<tr>
<th>Grade</th>
<th>Light microscopic findings</th>
<th>Neuronal alterations</th>
<th>Electron microscopic findings</th>
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<tr>
<td>1.</td>
<td>Slight shrinkage; loss of Nissl substance; and/or cytoplasmic vacuolation</td>
<td>Dispersal of Nissl substance; distension of rough endoplasmic reticulum; swollen mitochondria</td>
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<tr>
<td>2.</td>
<td>Moderate shrinkage; cytoplasmic eosinophilia; increased nuclear basophilia; or Moderate swelling; pale, vacuolated cytoplasm; distended, vesicular nucleus</td>
<td>Increased density of cytoplasm and nucleoplasm; disaggregation of polysomes; or Decreased density of cytoplasm and nucleoplasm; marked distension of endoplasmic reticulum; dispersion of organelles</td>
<td></td>
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<tr>
<td>3.</td>
<td>Severe shrinkage; bright cytoplasmic eosinophilia; pyknotic nucleus; inclusions</td>
<td>Membrane disruption; degradation of cytoplasmic contents; lysosome rupture; synaptic disruption; cellular fragmentation</td>
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</table>
Neurons in the grade 2 category were thought to be transitional, the nature of the alterations suggesting substantial, possibly irreversible injury. In this investigation, light microscopic analysis utilizing the grading system demonstrated considerable preservation of neurons in the brains of the mannitol-treated animals.

A combination of factors is probably responsible for the beneficial effect of mannitol observed in this investigation. The early administration of a hyperosmolar agent has been shown to reduce edema, 5-8 and increase blood flow 6, 7, 9 in areas of acute cerebral ischemia. These actions are thought to be the result of osmolar dehydration 6 and plasma expansion with associated hemodilution 6, 8, 9.

Narrowing of capillaries in the primary phase of acute cerebral ischemia has been demonstrated previously 10-12 and is thought to have a deleterious effect upon tissue perfusion and consequent tissue viability. It was evident in the ischemic cerebral tissue of the untreated cats as early as 30 minutes following MCA occlusion. This narrowing may result from the compression of capillaries by swollen astrocytic processes 10, 11, 13 and the increased tissue pressure produced by the developing cerebral edema. 14 In contrast, narrowing of capillaries and vacuolation of the neuropil generally remained mild in the brains of the mannitol-treated cats. Presumably, the increased plasma osmolality produced by mannitol helped to prevent the migration of fluid from the intravascular to extravascular compartment.

Microcirculatory obstruction, also designated the "no-reflow phenomenon," 22 was not observed in the brains of the treated cats; however, it was well-developed in the ischemic cerebral tissue of the untreated cats perfused 6 hours following MCA occlusion. Leakage of fluorescein into the ischemic tissue of these two cats also was noted. Previous investigations 13, 14, 15 have demonstrated that microcirculatory obstruction and breakdown of the blood-brain barrier characteristically develop in the secondary phase of ischemic injury when tissue degeneration is already advanced. The findings of this study appeared to confirm these previous observations.

Sequential hematocrit determinations following mannitol administration revealed an early, transient hemodilution phase followed by a return to control levels by 3 hours. A hemococoncentration phase subsequently developed and by 6 hours the hematocrits were 9% to 12% above control values. Despite this hemococoncentration, however, microcirculatory obstruction was not observed.

The initial 3 hours following acute MCA occlusion in cats may represent a critical phase with regard to cerebral blood flow (CBF) and consequent tissue viability. Heiss, Hayakawa, and Waltz 23 measuring CBF in a similar model, demonstrated an immediate decrease in CBF following MCA occlusion, but, in most of their cats, there was substantial improvement in CBF after 3 to 4 hours. The increase in serum osmolality and hemodilution produced by mannitol occurred during this critical initial phase and probably were important factors in preventing the development of severe ischemic changes.

Acknowledgment

The author is indebted to Mr. Michael McHugh for his invaluable assistance in the research laboratory.

References

Modification of acute focal ischemia by treatment with mannitol.

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Stroke. 1978;9:4-9
doi: 10.1161/01.STR.9.1.4

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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