Treatment of experimental focal cerebral ischemia with mannitol

Assessment by intracellular brain pH, cortical blood flow, and electroencephalography

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Intracellular brain pH, cortical blood flow (CBF), and electrocorticograms were recorded in regions of severe and moderate ischemia in 10 control rabbits and 10 rabbits given mannitol, 1 gm/kg, after occlusion of a major branch of the middle cerebral artery. Pooling the data from all 20 animals, preocclusion CBF was 46.4 ± 3.6 ml/100 gm/min and intracellular brain pH was 7.01 ± 0.04 (means ± standard error of the means). Although mannitol administration mildly improved CBF in regions of severe ischemia, this increase was not sufficient to prevent metabolic deterioration as assessed by brain pH. However, in regions of moderate ischemia, CBF improved significantly with mannitol and the gradual decline in brain pH observed in control animals was prevented. For example, in the treated moderate ischemia sites 4-hour postocclusion CBF and pH values were 31.8 ml/100 gm/min and 6.89 ± 0.09, respectively, as compared to control values of 14.3 ml/100 gm/min and 6.75 ± 0.06. These results suggest that mannitol may be of benefit in stabilizing regions of moderate, but not severe, ischemia after vessel occlusion.

KEY WORDS • cerebral ischemia • brain pH • cortical blood flow • mannitol • electroencephalography • rabbit

In a model of transient global ischemia, Ames and colleagues demonstrated that after flow restoration there were regions of impaired vascular filling. They postulated that this "no reflow phenomenon" contributed to neuronal death following transient global ischemia. Crowell and Olsson later demonstrated similar microcirculatory obstruction in focal ischemia. Little, et al., showed that this obstruction was related in part to capillary compression by perivascular glial edema.

On the basis of its hyperosmolar characteristics, mannitol has been advocated as an adjunctive treatment for acute focal ischemia. Little and Peerless, et al., demonstrated that mannitol improved neurological outcome and infarct size after middle cerebral artery (MCA) occlusion in the cat. Postulated mechanisms included either a reduction in edema or a decrease in blood viscosity, each of which would facilitate collateral flow after vessel occlusion. However, other investigators have shown that, although oncocytic agents such as albumin improved microcirculatory flow in cats, there were only transient improvements in flow without a reduction in infarct size in squirrel monkeys. This may reflect differences in potential collateral flow between carnivores and primates.

Recently, the rabbit has been investigated as a model of focal cerebral ischemia. The rabbit brain is supplied by the internal carotid artery without contribution from the external carotid artery as in the dog. There is no rete mirabilis as in the cat. Occlusion of the MCA reliably produces cortical infarction similar to that observed in primates. Therefore, assessment of pharmacological regimens in the rabbit may be more analogous to the true clinical setting in which collateral flow is limited after major vessel occlusion. This current experiment evaluates cortical blood flow (CBF), intracellular brain pH, and electroencephalography (EEG) in rabbits receiving mannitol after MCA occlusion.
Materials and Methods

Animal Preparation

Twenty-three New Zealand White rabbits, weighing between 3.5 and 4.5 kg each, were anesthetized with 4% inspired halothane, operated on under 2% halothane, and studied under 0.5% halothane. All animals were given 0.15 mg/kg pancuronium bromide to eliminate respiratory effort and were maintained throughout the experiment on a Harvard respirator. Catheters were inserted into the femoral artery and vein for monitoring blood pressure and arterial blood gases, and for the administration of drugs. A tracheostomy was performed and a PE-50 cannula was inserted into the ligated external carotid artery for delivery of umbelliferone into the internal carotid artery.

The MCA was exposed through a retro-orbital craniectomy similar to that used in a cat. The skin, subcutaneous tissue, and muscle were dissected from the supraorbital ridge and parietal bone. Enucleation of the orbit facilitated removal of the supraorbital ridge with rongeurs. The frontotemporoparietal craniectomy extended medially to the sagittal sinus, laterally to the zygomatic arch, and rostrally to the optic canal. The craniectomy was performed with a high-speed air drill and with the aid of an operating microscope so that no pressure was exerted against the underlying cortex. The dura was removed, and a thin sheet of plastic film (Saran Wrap) was placed on the cerebrum with irrigation to prevent surface dehydration and oxygenation. Total blood loss for the operative procedure did not exceed 5 cc.

The MCA bifurcation was identified at the anterior inferior margin of the craniectomy between the frontal and temporal lobes. Occlusion of one of these divisions will yield three zones of flow: 1) a zone of severe ischemia; 2) a zone of normal flow supplied by the division left intact; and 3) a region of moderate ischemia between these two zones, partially supplied by the division left intact. This border zone of moderate ischemia was located approximately halfway between the occluded and patent branches and could be identified visually by subtle color changes of the cortex under microscopic observation. Suspected border zones were confirmed by measurement of an immediate 60% reduction in postocclusion CBF as compared to preocclusion flows, with only minimal changes in intracerebral brain pH. Immediately following occlusion, severely ischemic regions had an 80% reduction in CBF as compared to preocclusion flows, with a dramatic drop in intracerebral brain pH.

Data Recording

After the operative exposure, the animals were moved to an intravital microscope stage. Measurements of two separate sites on the suprasylvian gyrus were taken at a PaCO₂ of 20, 40, 60, and 80 torr by altering the amount of inspired CO₂ and the respiratory rate. A normal PaCO₂-CBF response curve assured that autoregulation was intact within a normal blood pressure range and that the brain had not been injured. Measurements of a normal intracellular brain pH during this PaCO₂ response curve were further evidence of a physiologically intact cerebrum. After the PaCO₂ response curve was obtained, two measurements of normal CBF and intracellular brain pH at a PaCO₂ of 40 torr were made prior to occlusion of the MCA with bipolar cautery.

Twenty rabbits were equally divided in two groups: 10 animals were treated with mannitol, 1 gm/kg, given as an intravenous bolus after MCA occlusion and 10 formed an untreated control group. Immediately after vessel occlusion and prior to treatment, identification was made of regions of both severe and moderate ischemia. The x and y coordinates were recorded so that these sites could be successively evaluated each hour. Measurements of intracellular brain pH and CBF at all sites were made for 4 hours after MCA occlusion. Results obtained in similar control animals have been reported previously. Three rabbits with a sham craniectomy but without MCA occlusion served as time controls, and intracellular brain pH and CBF were assessed after a normal PaCO₂ response curve during a 4-hour experimental period.

Electrocorticograms were recorded in all animals by placement of gold electrodes at the margins of the craniectomy. Arterial blood gases were measured hourly, and blood pressure was continuously recorded. The animals were sacrificed with a lethal injection of potassium chloride.

Intracellular Brain pH and CBF Measurements

The use of umbelliferone for measuring intracellular brain pH and CBF in vivo has been described elsewhere. Umbelliferone is a lipid-soluble, pH-sensitive fluorescent indicator that freely diffuses across the blood-brain barrier. It is nontoxic and isolated to the intracellular compartment. Its ionic and molecular forms are fluorophors which have different fluorescent characteristics. The ratio of the ionic and molecular forms at 370 and 340 nm excitation, respectively, is pH-sensitive. It is possible to create a nomogram relating pH to the ratio of the 450 nm fluorescent curves of the indicator at 370 and 340 nm excitation.

Cortical blood flow was determined by the washout curve of the molecular form of umbelliferone over a 60-second period, beginning after the arterial spike, using the 1-minute initial slope index. Since the blood-brain partition coefficient of umbelliferone is 1.0 (RE Anderson, unpublished data), its clearance is a measure of perfusion through the intracellular compartment and in effect is focal CBF. It is thought to be dependent on diffusion from capillary to glial cell to neuron. Since this technique measures flow only in the outer 50 μm of the cortex, it eliminates the "look through" phenomenon associated with measurements of blood flow obtained with xenon-133 during ischemia.
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**Instrumentation for Measuring pH**

The microspectrofluorometer used in this experiment was equipped with optics for bright field illumination which permitted: low-intensity excitation energy; high-efficiency recording from an 80-μm avascular area of cortex; a high-speed filter wheel with four interference filters that allowed synchronization of the emission recording system at 450 nm with the 370- and 340-nm excitation bands; and an emission recording system consisting of a high-sensitivity thermoelectrically cooled photomultiplier tube attached to a high-efficiency grating monochromator. Fluorescent emission signals were amplified by a cascaded electrometer amplifier and directed into a photodemodulator synchronized with the filter wheel. The fluorescence washout curves were recorded on a dual strip-chart recorder. The instrumentation is fully detailed in previous reports.22,33

**Statistical Analysis**

Ten sites of severe ischemia in the control animals were compared with 10 sites of severe ischemia in the animals treated with mannitol for each hourly measurement. Likewise, 10 sites of moderate ischemia in the control animals were compared to 10 sites of moderate ischemia in mannitol-treated animals. The statistical significance was calculated by a paired t-test at each hourly measurement. The deviation from mean value is expressed as standard error of the mean.

**Results**

**Stability of the Preparation**

In the three rabbits without MCA occlusion there was no significant decline (paired t-test) in either intracellular brain pH or CBF for 4 hours after a normal PaCO2 response curve. The initial intracellular brain pH was 6.98 ± 0.02 and at 4 hours it was 6.98 ± 0.03. Initial CBF was 48.1 ± 3.7 ml/100 gm/min and at 4 hours it was 43.0 ± 4.0 ml/100 gm/min.

**Intracellular Brain pH and CBF in Severe Ischemia**

Pooling data from all 20 animals with MCA occlusion, preocclusion CBF was 46.4 ± 3.6 ml/100 gm/min and intracellular brain pH was 7.01 ± 0.04 (Table 1 and Fig. 1). In the 10 control animals, CBF at the severe ischemia sites immediately after occlusion was 12.0 ± 2.5 ml/100 gm/min. Within 10 minutes, intracellular brain pH was 6.65 ± 0.05. These sites continued to demonstrate reductions in both intracellular brain pH and CBF over the ensuing 4 hours. Four hours after occlusion, the intracellular brain pH was 6.10 ± 0.10 and CBF was 5.0 ± 1.5 ml/100 gm/min. Compared to the preocclusion values, the reduction in both CBF and intracellular brain pH at each measured interval in the control animals was statistically significant (p < 0.001).

In the 10 mannitol-treated animals, immediate postocclusion CBF at the severe ischemia sites was 13.2 ± 1.6 ml/100 gm/min and brain pH was 6.58 ± 0.05.

**TABLE 1**

<table>
<thead>
<tr>
<th>Time of Measurement (min)</th>
<th>Intracellular Brain pH</th>
<th>Cortical Blood Flow (ml/100 gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>preocclusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-15</td>
<td>7.05 ± 0.04</td>
<td>6.98 ± 0.03</td>
</tr>
<tr>
<td>0</td>
<td>7.02 ± 0.03</td>
<td>7.00 ± 0.04</td>
</tr>
<tr>
<td>postocclusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6.65 ± 0.05</td>
<td>6.58 ± 0.05</td>
</tr>
<tr>
<td>60</td>
<td>6.40 ± 0.07</td>
<td>6.38 ± 0.10</td>
</tr>
<tr>
<td>120</td>
<td>6.15 ± 0.10</td>
<td>6.24 ± 0.10</td>
</tr>
<tr>
<td>180</td>
<td>6.12 ± 0.10</td>
<td>6.23 ± 0.14</td>
</tr>
<tr>
<td>240</td>
<td>6.10 ± 0.10</td>
<td>6.23 ± 0.16</td>
</tr>
</tbody>
</table>

* Data are means ± standard error of the means for 10 rabbits in the control (untreated) group and 10 in the group treated with mannitol.

**Fig. 1.** Graph of intracellular brain pH and cortical blood flow (CBF) in severely ischemic sites in 10 control animals (solid lines) and severely ischemic sites in 10 animals treated with an intravenous (IV) bolus of mannitol (dashed lines). Pooling data from all 20 animals, CBF obtained before middle cerebral artery (MCA) occlusion was 46.4 ± 3.6 ml/100 gm/min and intracellular brain pH was 7.01 ± 0.04. Immediately postocclusion, CBF was 12.0 ± 2.5 ml/100 gm/min in the control group and 13.2 ± 1.6 ml/100 gm/min in the treated group, and brain pH was 6.65 ± 0.05 in the control group and 6.58 ± 0.05 in the treated group. The control group demonstrated a progressive decline in CBF and brain pH over the ensuing 4 hours. Although mannitol treatment stabilized CBF, this improvement was not sufficient to significantly alter intracellular brain pH. Three animals without MCA occlusion (dotted lines) served as time controls.

Results at these sites prior to infusion of mannitol compared well with those in the control group. After the immediate postocclusion measurements, mannitol (1 gm/kg) was given intravenously. One hour after occlusion (45 minutes after treatment) CBF was 15.2 ±
TABLE 2
Measurements of systemic parameters in control and treated groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Preocclusion</th>
<th>Time Postocclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 Min</td>
<td>60 Min</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>control</td>
<td>119.6 ± 1.0</td>
<td>119.8 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>115.0 ± 4.0</td>
<td>115.4 ± 3.3</td>
</tr>
<tr>
<td>PaCO2 (torr)</td>
<td>control</td>
<td>40.1 ± 1.0</td>
<td>39.6 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>41.7 ± 1.0</td>
<td>44.4 ± 1.3</td>
</tr>
<tr>
<td>PaO2 (torr)</td>
<td>control</td>
<td>172.2 ± 7.8</td>
<td>165.2 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>177.6 ± 9.2</td>
<td>160.3 ± 9.0</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>control</td>
<td>7.296 ± 0.015</td>
<td>7.289 ± 0.026</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>7.320 ± 0.060</td>
<td>7.332 ± 0.030</td>
</tr>
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</table>

Intracellular Brain pH and CBF in Moderate Ischemia

In the 10 control animals, initial postocclusion CBF at the moderate ischemia sites was 21.0 ± 2.0 ml/100 gm/min or approximately 40% of preocclusion flows. This was associated with an intracellular brain pH of 6.95 ± 0.05. While the reduction in flow was significant (p < 0.001), the drop in pH was not. For the first 3 hours, CBF was stable but then fell significantly at the 4th hour to 14.3 ± 3.5 ml/100 gm/min (p < 0.01). Intracellular brain pH slowly worsened at each hourly measurement (p < 0.05) and at 4 hours it was 6.75 ± 0.06 (p < 0.01) (Fig. 2 and Table 3).

In the 10 mannitol-treated animals, initial postocclusion CBF at the moderate ischemia sites was 23.8 ± 1.0 ml/100 gm/min, approximately a 60% reduction of preocclusion flows. This was associated with an intracellular brain pH of 6.85 ± 0.04, slightly lower than in the control group. However, both parameters at these sites compared well to those at moderate ischemia sites of the control animals. Mannitol had no effect on CBF at the 1st hour (25.8 ± 3.8 ml/100 gm/min versus 19.5 ± 2.0 ml/100 gm/min in the control group). However, in contrast to the progressive fall in pH observed in the control animals during the subsequent 3 hours, CBF and brain pH remained unexpectedly stable. Thus, at the 4th hour, CBF was 31.8 ± 5.2 ml/100 gm/min in the treated group versus 14.3 ± 3.5 ml/100 gm/min in the control group (p < 0.001), and brain pH was 6.89 ± 0.09 versus 6.75 ± 0.06 (p < 0.01), respectively.

Electroencephalographic Correlates

Baseline electrocorticograms in rabbits consist of a 6- to 10-Hz rhythm. Since the EEG leads were located at the edges of the cranectomy, they summated electrical activity across both normal and ischemic cortex. Of the 10 control animals, eight demonstrated loss of amplitude or a slow 1- to 2-Hz rhythm immediately after occlusion. In these eight rabbits, the EEG continued to demonstrate progressive loss of amplitude and frequency over the next 4 hours. In the 10 mannitol-treated animals, seven showed initial EEG changes after occlusion consisting of a slow 1- to 2-Hz rhythm or loss of amplitude. Although none of these seven animals improved to a normal rhythm, four maintained the postocclusion EEG without further deterioration.

Discussion

Previous evaluation of this model has shown that sites of severe ischemia are evolving infarcts, while moderate ischemia sites appear analogous to ischemic penumbras. Although mannitol improved microcircu-
adjacent moderately ischemic sites were more proximal to deteriorate after the loss of primary perfusion. The would explain why the severe ischemia sites continued dramatic as those reported by Little and others. The circulatory obstruction by attenuating cerebral edema flow after MCA occlusion is less. Prevention of microcirculatory obstruction by attenuating cerebral edema would be expected to have a less positive effect. This would explain why the severe ischemia sites continued to deteriorate after the loss of primary perfusion. The adjacent moderately ischemic sites were more proximal to the source of collateral flow and therefore would demonstrate greater beneficial effects. These results are compatible with early studies by Sundt, et al.,36,37 who treated both cats and monkeys with osmolar dehydrating agents like albumin. In cats, the CBF, infarct size, and neurological outcome were improved. However, albumin did not influence infarct size or outcome in primates. Monkeys, like rabbits, have limited leptomeningeal collateral flow after MCA occlusion. Therefore, attenuating edema-induced microcirculatory obstruction would have less beneficial effects. One other possible explanation is that our rabbit model involved an open-skull preparation as compared to the closed preparation used by Little. The decompression that would occur from a craniectomy might have masked more positive results.

It is important to briefly examine the importance of cerebral edema. Ischemic edema is a combination of both early cytotoxic and late vasogenic edema.14 Cytotoxic edema can be seen within 30 minutes of vessel occlusion under light microscope and first occurs in perivascular glia. However, Little, et al.,20 demonstrated that neuronal injury precedes this glial edema, and that this glial edema then caused microcirculatory obstruction. Therefore, in core regions of ischemia, glial edema...
is a minor factor in infarct evolution. However, in regions of marginal flow where basal metabolism is temporarily intact, progressive glial edema will be a critical factor. This current study supports the contention that mannitol may be of benefit in moderate ischemia regions. The late vasogenic edema is secondary to ischemic endothelial damage with increased capillary permeability. Mannitol would be less beneficial in treating late vasogenic edema since the presence of ischemic endothelial damage would imply the coexistence of irreversible neuronal damage. Furthermore, mannitol could have detrimental effects by leaking across the blood-brain barrier with a rebound increase in edema.

Conclusions

Although mannitol mildly improved CBF in core regions of ischemia, this elevation was not sufficient to alter metabolism as assessed by intracellular brain pH. However, mannitol was beneficial in stabilizing zones of moderate ischemia (ischemic penumbras), presumably by reducing cytotoxic edema that resulted in improved CBF. It should be emphasized that while the present study shows only a mild but significant modification by mannitol in contrast to the reports of others, differences in species and the attendant variations in potential collateral flow, the methods of measuring outcome (CBF, brain pH, infarct size, neurological recovery), and the experimental preparation used render comparison difficult. However, the overall positive findings across this range of preparations support the beneficial effects of mannitol and emphasize the importance of edema in the deterioration observed during acute focal cerebral ischemia.

References

17. Little JR: Modification of acute focal ischemia by treatment with mannitol. Stroke 9:4–9, 1978
31. Sundt TM Jr, Anderson RE: Intracellular brain pH and...
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the pathway of a fat soluble pH indicator across the blood-
32. Sundt TM Jr, Anderson RE: Umbelliferone as an intra-
cellular pH-sensitive fluorescent indicator and blood-
brain barrier probe: instrumentation, calibration, and
33. Sundt TM Jr, Anderson RE, Van Dyke RA: Brain pH
measurements using a diffusible, lipid soluble pH sensitive
34. Sundt TM Jr, Michenfelder JD: Focal transient cerebral
ischemia in the squirrel monkey. Effect on brain adeno-
sine triphosphate and lactate levels with electrocorticog-
graphic and pathologic correlation. Circ Res 30:
703–712, 1972
35. Sundt TM Jr, Waltz AG: Experimental cerebral infarc-
tion: retro-orbital extradural approach for occluding the
middle cerebral artery. Proc Staff Meet Mayo Clin 41:
159–168, 1966
36. Sundt TM Jr, Waltz AG: Hemodilution and anticoagu-
lation. Effects on the microvasculature and microcircu-
lation of the cerebral cortex after arterial occlusion. Neu-
rology 17:230–238, 1967
37. Sundt TM Jr, Waltz AG, Sayre GP: Experimental cerebral
infarction: modification by treatment with hemodiluting,
hemoconcentrating, and dehydrating agents. J Neurosurg
26:46–56, 1967
peutic method for acute brain infarction: revasculariza-
tion following the administration of mannitol and per-
fluorochemicals — a preliminary report. Surg Neurol 17:
325–332, 1982
effect of glucose pretreatment on recovery from diffuse
cerebral ischemia in the cat. II. Regional metabolite levels.
40. Welsh FA, O’Connor MJ, Marcy VR, et al: Factors lim-
iting regeneration of ATP following temporary ischemia
41. Yanagihara T, McCall JT: Ionic shift in cerebral ischemia.
Life Sci 30:1921–1925, 1982
42. Yoshimoto T, Sakamoto T, Watanabe T, et al: Experi-
mental cerebral infarction. Part 3: Protective effect of
mannitol in thalamic infarction in dogs. Stroke 9:
217–218, 1978

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