Effect of mannitol on local cerebral blood flow after temporary complete cerebral ischemia in rats

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The effects of pretreatment with mannitol on local cerebral blood flow (CBF) after permanent or temporary global cerebral ischemia were evaluated with 14C-iodoantipyrine autoradiography in rats under halothane-N2O endotracheal anesthesia. Blood pressure, pulse rate, arterial blood gas levels, and electroencephalographic (EEG) tracings were monitored throughout the experiments. After permanent occlusion of the basilar artery and both external carotid and pterygopalatine arteries, severe global ischemia was induced by permanent occlusion of the common carotid arteries (CCA's) or by a 30-minute temporary CCA occlusion followed by 5 minutes of reperfusion. Intravenous mannitol (25%, 1 gm/kg) or saline solution was administered 5 minutes before occlusion of the CCA's. Cerebral blood flow was measured in 24 anatomical regions.

The EEG tracings flattened within 2 to 3 minutes after the onset of ischemia, and no recovery was observed during reperfusion. In the mannitol-treated rats and the saline-treated controls, autoradiographic studies after permanent occlusion showed no CBF in the forebrain or cerebellum, although brain-stem and spinal cord CBF values were normal. After 5 minutes of reperfusion, CBF in the cortex, basal ganglia, and white matter was 100% to 200% higher in mannitol-treated rats and 50% to 100% higher in saline-injected rats than in the nonischemic anesthetized control group. Heterogeneously distributed areas of no-reflow were seen in all saline-injected rats but were observed in none of the mannitol-treated rats. Pretreatment with mannitol prevented postischemic obstruction of the microcirculation during 5 minutes of recirculation after 30 minutes of severe temporary ischemia, but the EEG signals did not recover. Further studies of the functional and morphological responses to longer periods of postischemic recirculation are needed to verify the extent to which these mannitol-induced effects are protective.

KEY WORDS • ischemia • recirculation • no-reflow phenomenon • hyperperfusion • mannitol • rat

Temporary cerebrovascular occlusion or cerebral circulatory arrest and cardiopulmonary bypass are useful during surgical procedures for complex intracranial aneurysms and arteriovenous malformations. The pathophysiology of circulatory disturbances after temporary ischemia, however, is poorly understood, and an experimental basis for their treatment is needed. Abnormalities of cerebral blood flow (CBF) have been observed experimentally after reperfusion following regional and global ischemia. These abnormalities include the no-reflow phenomenon and delayed hyperperfusion and hyperperfusion, as well as loss of autoregulation and CO2 reactivity. The no-reflow phenomenon was originally described as the absence of microvascular reperfusion after global cerebral ischemia. The recovery of explanted cultured neurons after temporary deprivation of metabolic substrates for 30 minutes in vitro, even though brain function does not recover after 7 to 8 minutes of circulatory arrest in vivo, suggests that vascular endothelium is susceptible to ischemic changes that increase resistance to reperfusion after temporary ischemia. In rats, the no-reflow phenomenon has been observed after complete ischemia of durations greater than 10 minutes, predominantly in the striatum, thalamus, hippocampus, and cerebral cortex. Preventing such reactions is important because optimum reperfusion is required to minimize ischemic brain damage after temporary cerebrovascular occlusion.

Subsequent studies have shown that no-reflow is a complex phenomenon caused by reduction of cerebral perfusion pressure, possibly due to vascular swelling, brain edema and acidosis, and reduced blood viscosity or intravascular coagulation. The extent and reversibility of no-reflow in specific areas depend upon the duration and severity of ischemia. For example, our
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previous studies of reperfusion in rats subjected to
seven-vessel occlusion showed small no-reflow areas in
only two of eight rats after 15 minutes of ischemia, whereas larger lesions were seen in all rats after 30
minutes of ischemia. Less severe no-reflow areas have also been observed after incomplete global ischemia in
rats and after longer periods (3 to 6 hours) of occlusion of the middle cerebral artery in cats and monkeys. The persistence of the no-reflow phenomenon is asso-
ciated with the failure of functional recovery and histo-
logical evidence of ischemic cell damage. Conversely, recovery of normal or hyperemic CBF was associated with metabolic and functional resuscitation,
even after 1 hour of complete ischemia in cats. Thus, preventing the no-reflow phenomenon with phar-
macological therapy could improve the clinical out-
come after temporary cerebral ischemia.

Mannitol, one of the most commonly used drugs in
neurosurgery, is a hyperosmolar diuretic that reduces blood viscosity and increases cerebral microcircula-
tory flow under pathological conditions. In this study, the effects of mannitol on postischemic regional
CBF were evaluated in rats subjected to seven-vessel occlusion. Unlike models of regional and incom-
plete temporary global ischemia, which produce variable effects on CBF and metabolism, this model con-
sistently produces complete temporary global ischemia, as demonstrated previously by autoradiography and in vivo magnetic resonance spectroscopy. Changes in local CBF were measured by 14C-iodoantipyrine (IAP) autoradiography.

Material and Methods

Animal Preparation

Male Sprague-Dawley rats, each weighing 300 to 350
gm, were anesthetized in a halothane inhalation cham-
ber, orotracheally intubated with a No. 16 angiocath-
eter, connected to a rodent respirator, and paralyzed by intraperitoneal injection of pancuronium bromide (1 mg/kg). Anesthesia was maintained with 1.5% halothane in a mixture of 70% N2O and 30% O2 during surgery and with 0.7% halothane during ischemia and reperfusion. Bilateral subcutaneous platinum needle electrodes were placed over the calvaria for continuous single-channel electroencephalographic (EEG) recording with a microcomputer-based spectral analysis sys-

† Both femoral arteries and one femoral vein were cannu-
nulated with polyethylene (PE-50) tubing; one arterial catheter was connected to a pressure transducer and oscilloscope to allow continuous monitoring and recording of pulse rate and systemic blood pressure. Rectal temperature was maintained at 37 to 38°C with a temperature-regulated water jacket. Arterial blood gases were measured during each stage of the experi-
ment. The respiratory rate and volume were adjusted to maintain the arterial pCO2 at 35 to 40 mm Hg and the pO2 at 100 to 120 mm Hg.

Microsurgical dissection and vessel occlusion were
performed through a ventral cervical skin incision. The omohyoid muscles were transected bilaterally. The
external carotid and pterygopalatine arteries were occlud
ed by bipolar cautery. The trachea and esophagus were retracted medially to expose the ven-
tral surface of the occipital bone. A 2 x 2-mm hole was
drilled in the midportion of the clivus, the dura and the arachnoid membranes were opened, and the basilary artery was coagulated at the midpontine level. The bone apertura was packed with Gelfoam sponge.

After the five-vessel occlusion, the common carotid arteries (CCA's) were exposed gently to prevent vaso-
spasms and were then occluded with microclips. The
success of the occlusion was verified by observing the
collapse of the distal segment of the CCA's. Five minutes before the induction of ischemia, intravenous heparin (200 U/kg) was injected to prevent intravascular coagulation. Recirculation was accomplished by removing the microclips from the CCA's and was verified by observing the return of arterial pulsations under the operating microscope.

Cerebral Blood Flow Measurement

The tissue saturation method described by Sakurada, et al., was used to determine CBF in 24 anatomical regions. The radioactive tracer 14C-IAP (100 µCi/kg in 0.85 ml of saline solution) was infused intravenously at a constant rate over a period of 45 seconds with a calibrated pump. Nine 5-second blood samples were withdrawn from the arterial catheter; 0.25-ml aliquots of each sample were counted in a scintillation counter to determine the arterial concentration curve of the isotope. After the blood samples were obtained, the rats were decapitated. The brain was removed quickly, frozen in powdered dry ice, and cut into 20-µm sections. The sections were mounted on glass coverslips, dried on a hot-plate, placed in an x-ray cassette, and exposed to Kodak SB-5 film for 7 days.

The films were analyzed with a videodigitizer image-analysis system to determine regional optical density, from which CBF values were calculated as described by Sakurada, et al. In each anatomical region, CBF was determined from three 1-sq mm areas in three adjacent sections in both hemispheres. The values presented are the average of the 18 determinations in each region. No-reflow areas were defined as those in which the optical density was approximately equal to that of the film background; these areas were not considered in the calculation of local CBF. Areas of hypoperfusion were

† Microcomputer-based spectral analysis system manufactured by Neurotrac, Interspec Inc., Conshohocken, Pennsylvania.
‡ Calibrated pump, Model 901, manufactured by Harvard Apparatus Co., South Natick, Massachusetts.
§ Videodigitizer image-analysis system manufactured by Imaging Research, Peterborough, Ontario, Canada.
defined as those in which CBF was less than 20 ml/100 gm/min.

**Experimental Groups**  
Five experimental groups were studied. In the first two groups, intravenous mannitol (25%, 1 gm/kg) was injected 5 minutes before the induction of ischemia; rats in Group A (four animals) were sacrificed after 30 minutes of ischemia, while rats in Group B (eight animals) were sacrificed after 30 minutes of ischemia followed by 5 minutes of recirculation. Two additional groups of rats received normal saline solution instead of mannitol and were sacrificed after 30 minutes of ischemia (Group C, four animals) or after 30 minutes of ischemia followed by 5 minutes of reperfusion (Group D, eight animals). In six rats (Group E, nonischemic controls), local CBF measurements were obtained after 2 hours of anesthesia without surgery.

**Results**  
There was no significant difference in blood pressure or arterial blood gas levels between rats treated with mannitol and saline controls (Table 1). Mean arterial blood pressure increased by 30 to 40 mm Hg after the induction of ischemia and returned to control levels during reperfusion. The EEG findings did not change significantly during the surgical procedure. After induction of ischemia, the EEG tracings flattened in all rats and remained flat until the end of the experiment; no significant recovery was observed during reperfusion. The hematocrit was not affected by the infusion of mannitol or saline.

In Groups A and C, there was no uptake of $^{14}$C-IAP in the forebrain or cerebellum after 30 minutes of ischemia produced by occlusion of the basilar, external carotid, pterygopalatine, and common carotid arteries. The CBF was normal in the caudal brain stem. The local CBF values in anesthetized control rats and in mannitol-treated and saline-infused ischemic rats are shown in Table 2. After 5 minutes of reperfusion, CBF levels in the mannitol-treated rats (Group B) were 100% to 300% higher than those in the nonischemic controls (Group E). Local CBF increased 50% to 100% over control values in saline-injected rats (Group D) if focal no-reflow regions were excluded. There was no evidence of the no-reflow phenomenon or areas of posts ischemic hyperperfusion in the rats pretreated with mannitol.

The recirculation pattern in the mannitol-treated rats (Group B) was homogeneous and more hyperemic (Fig. 1) than in saline-injected ischemic rats (Group D), in which no-reflow, hyperperfused, and hyperperfused areas were seen in each rat (Fig. 2). Recirculation disturbances were obvious in the neocortex, thalamus, hypothalamus, caudoputamen, and subcortical white matter (Fig. 2 and Table 2). The non-reflow phenomenon was most common in subcortical regions, such as the thalamus, hippocampus, and basal ganglia, and in the neocortical watershed zones between major arteries (Table 3).

**Discussion**  

No-Reflow Phenomenon

These results demonstrate that pretreatment with mannitol prevented the no-reflow phenomenon and augmented posts ischemic hyperperfusion measured 5 minutes after 30 minutes of complete cerebral ischemia. On the basis of previous observations, 10, 18 we assume

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**TABLE 1**  
**Blood pressure and arterial blood gas values in ischemic mannitol-treated (Group B) and saline-treated (Group D) rats***

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Blood Pressure (mm Hg)</th>
<th>pO₂ (mm Hg)</th>
<th>pCO₂ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol-treated (8 rats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>95 ± 4</td>
<td>113 ± 2</td>
<td>36.7 ± 1.8</td>
<td>7.43 ± 0.02</td>
</tr>
<tr>
<td>Recirculation (4 min)</td>
<td>89 ± 3</td>
<td>109 ± 4</td>
<td>35.4 ± 2.3</td>
<td>7.45 ± 0.02</td>
</tr>
<tr>
<td>Saline-treated (8 rats)</td>
<td>98 ± 5</td>
<td>115 ± 2</td>
<td>37.5 ± 2.5</td>
<td>7.44 ± 0.03</td>
</tr>
</tbody>
</table>

**TABLE 2**  
**Local cerebral blood flow values in rats subjected to TGI after pretreatment with mannitol or saline in anesthetized control animals***

<table>
<thead>
<tr>
<th>Structure</th>
<th>Anesthetized Controls (Group E, 6 rats)</th>
<th>Mannitol &amp; TGI (Group B, 8 rats)</th>
<th>Saline &amp; TGI (Group D, 8 rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>179 ± 7</td>
<td>416 ± 28</td>
<td>215 ± 11</td>
</tr>
<tr>
<td>Sensormotor cortex</td>
<td>156 ± 10</td>
<td>515 ± 22</td>
<td>288 ± 15</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>159 ± 11</td>
<td>509 ± 45</td>
<td>253 ± 15</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>150 ± 13</td>
<td>365 ± 16</td>
<td>182 ± 15</td>
</tr>
<tr>
<td>Visual cortex</td>
<td>157 ± 9</td>
<td>345 ± 20</td>
<td>193 ± 16</td>
</tr>
<tr>
<td>Subcortical white matter</td>
<td>54 ± 6</td>
<td>136 ± 18</td>
<td>111 ± 9</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>53 ± 2</td>
<td>129 ± 6</td>
<td>80 ± 16</td>
</tr>
<tr>
<td>Internal capsule</td>
<td>49 ± 1</td>
<td>127 ± 6</td>
<td>96 ± 9</td>
</tr>
<tr>
<td>Caudoputamen</td>
<td>174 ± 16</td>
<td>357 ± 11</td>
<td>206 ± 2</td>
</tr>
<tr>
<td>Septal nucleus</td>
<td>97 ± 5</td>
<td>284 ± 11</td>
<td>152 ± 17</td>
</tr>
<tr>
<td>Thalamus</td>
<td>160 ± 11</td>
<td>430 ± 24</td>
<td>283 ± 34</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>84 ± 3</td>
<td>173 ± 10</td>
<td>127 ± 10</td>
</tr>
<tr>
<td>Amygdala</td>
<td>87 ± 4</td>
<td>261 ± 12</td>
<td>157 ± 18</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>93 ± 5</td>
<td>327 ± 14</td>
<td>198 ± 23</td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>146 ± 4</td>
<td>275 ± 22</td>
<td>183 ± 12</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>174 ± 3</td>
<td>427 ± 19</td>
<td>219 ± 10</td>
</tr>
<tr>
<td>Lateral geniculate body</td>
<td>144 ± 9</td>
<td>498 ± 42</td>
<td>178 ± 20</td>
</tr>
<tr>
<td>Medial geniculate body</td>
<td>132 ± 9</td>
<td>475 ± 34</td>
<td>156 ± 14</td>
</tr>
<tr>
<td>Red nucleus</td>
<td>127 ± 2</td>
<td>334 ± 42</td>
<td>245 ± 20</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>82 ± 6</td>
<td>263 ± 12</td>
<td>137 ± 15</td>
</tr>
<tr>
<td>Pons</td>
<td>107 ± 5</td>
<td>219 ± 18</td>
<td>206 ± 11</td>
</tr>
<tr>
<td>Medulla</td>
<td>108 ± 6</td>
<td>116 ± 10</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>Spinal gray matter</td>
<td>91 ± 5</td>
<td>103 ± 5</td>
<td>107 ± 5</td>
</tr>
<tr>
<td>Spinal white matter</td>
<td>32 ± 3</td>
<td>41 ± 3</td>
<td>37 ± 1</td>
</tr>
</tbody>
</table>

* Values are given as the means ± standard error of the means, in ml/100 gm/min. TGI = temporary global ischemia.
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Fig. 1. $^{14}$C-iodoantipyridine autoradiographic images of representative brain sections obtained 5 minutes after the onset of reperfusion in a mannitol-treated (Group B) rat. These images show diffuse postischemic hyperperfusion in the forebrain and cerebellum and an absence of no-reflow areas.

Fig. 2. $^{14}$C-iodoantipyridine autoradiographic images of representative brain sections obtained 5 minutes after the onset of reperfusion in a saline-treated control (Group D) rat. These images show anatomically heterogeneous patterns of focal no-reflow areas in the neocortex, thalamus, basal ganglia, and subcortical white matter.
TABLE 3

Number of rats and locations in which postischemic no-reflow phenomena were observed*

<table>
<thead>
<tr>
<th>Structure</th>
<th>Mannitol-Treated (Group B. 8 rats)</th>
<th>Saline-Treated (Group D. 8 rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>frontal cortex</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>sensorimotor cortex</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>parietal cortex</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>visual cortex</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>hippocampus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>globus pallidus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>thalamus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>hypothalamus</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Right and Left denote right- and left-sided regions.

Postischemic CBF

The further augmentation of postischemic CBF in the mannitol-treated group 5 minutes after the onset of reperfusion is a striking observation that is difficult to interpret. If this increase in postischemic hyperperfusion is a favorable and temporary physiological reaction that leads to removal of toxic metabolites and restoration of normal cellular metabolism rather than to progressive vasoparalysis, edema, and propagation of infarction, pretreatment with mannitol may have an additional effect that prevents brain damage. Further studies after longer recirculation times are needed to resolve this issue.

Our results in nonsurgical control animals confirm previous reports of relatively high baseline CBF in halothane-anesthetized rats.21 Substantial increases in CBF of up to 300% occurring 5 minutes after 15 minutes of incomplete ischemia due to carotid and vertebral artery occlusion and after complete neck-compression ischemia were also reported in that study.21 Although postischemic hyperperfusion can be followed by recovery of normal CBF and brain function,15 5- to 30-minute episodes of incomplete or complete global ischemia and 60 minutes of regional ischemia are usually followed by delayed hypoperfusion within 10 to 90 minutes.41-43 However, the occurrence of postischemic hyperperfusion, which was seen in the untreated group and was further augmented by pretreatment with mannitol, has not been proven beneficial and therefore cannot be used to predict a favorable outcome.42-43 Further studies of the effects of mannitol on the occurrence of delayed hypoperfusion and ischemic cell injury are needed. Other agents, such as calcium-channel blockers43,44 and cyclo-oxygenase-inhibitors,4 have also been shown to improve CBF after temporary global ischemia, but protective effects upon resuscitation of brain function have been more difficult to demonstrate.

Effects of Mannitol

Several possible mechanisms may explain the action of mannitol in this study. Increased intravascular osmolarity may reduce the formation of endothelial microvilli30 and glial edema33 by preventing diffusion of water from the vascular to the intracellular spaces during ischemic membrane pump failure caused by cessation of energy metabolism.34 Decreased blood viscosity33 and lowered hematocrit as well as increased intravascular osmolarity and blood volume with prevention of systemic hypotension34 may reduce intravascular resistance in the microcirculation, thereby preventing obstruction or no-reflow and improving postischemic CBF during recirculation.39 Our experimental results may substantiate clinical reports suggesting improved tolerance of temporary cerebrovascular occlusion during surgery in patients pretreated with mannitol.35,38

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Ischemic Model

Our seven-vessel occlusion technique reduced blood flow so severely that no isotope uptake was observed in the cerebrum in either the untreated or mannitol-treated permanent occlusion groups. Residual alternative sources of collateral CBF were effectively excluded from access to the cerebral circulation, and detectable levels of isotope did not enter the brain even after treatment of potential microvascular swelling with mannitol. These results provide further evidence that the seven-vessel occlusion method produces complete temporary cerebral ischemia in rats.35 Previous studies have also shown that CBF was normal after five-vessel occlusion with the CCA's remaining patent.
Conclusions

In this study, mannitol prevented areas of postischemic no-reflow phenomenon and augmented hyperperfusion. These findings may explain mannitol's previously reported protective effects. Positive results of future survival studies could be interpreted as justification for the administration of mannitol before cerebral circulatory arrest for intracranial vascular surgery.

Acknowledgments

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