Effects of Mannitol on Microcirculation during Temporary Occlusion of the Middle Cerebral Artery in Cats

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Abstract

Changes in microcirculation during and after 3 hours of middle cerebral artery (MCA) occlusion in cats were studied with the carbon black perfusion technique. The effect of mannitol on these changes was also studied. The impairment of carbon black filling extended to the entire MCA region when transient hypotension was induced during occlusion. The filling defect was reversed with restoration of blood flow following recirculation, but the animals deteriorated with subsequent elevation of epidural pressure. Preocclusional administration of mannitol protected the microcirculation during occlusion and prevented the development of acute brain swelling associated with reperfusion. On the other hand, postocclusional administration of mannitol had an adverse effect. The pathogenesis of dynamic microcirculatory changes in relation to the development of acute brain swelling and the mechanisms of both the beneficial and adverse effects of mannitol are discussed.

Key words: microcirculation, middle cerebral artery occlusion, carbon black, mannitol, hypotension, acute brain swelling

Introduction

Previous experimental work has demonstrated that brain swelling following middle cerebral artery (MCA) occlusion worsens if hypotension occurs during the occlusion period. The swelling is secondary to edema in the infarcted cortex. Mannitol, administered prophylactically, is known to decrease the degree of ischemia resulting from vascular occlusion. However, it has not been proved that these beneficial effects occur if mannitol is administered after the onset of ischemia; the experimental and clinical studies in this area are sparse.

We studied the dynamic microcirculatory changes that occurred during and after 3 hours of MCA occlusion. In addition, we superimposed hypotension on MCA occlusion. To study the microcirculation during these cerebral insults we employed the carbon black perfusion technique. Mannitol was administered prior to and after MCA occlusion, and its effects on the microcirculation were studied.

Materials and Methods

Forty-four adult cats were anesthetized with 25 mg/kg of intramuscular ketamine. The left MCA was clipped via the transorbital approach. Two hours after MCA occlusion, the mean aortic blood pressure was lowered to 50 mmHg for several minutes by withdrawal of venous blood. The aortic and epidural pressures were monitored until the animals expired. Details of this experimental model were described in our previous report.

Carbon black (50 ml) was infused intravenously during the experiment, as described below. The animals were then sacrificed by KCl administration and the brains were removed for detailed histological examination.

Nine animals not given mannitol were divided into three groups based on the time of carbon black infusion and sacrifice. Group 1 animals (n = 3) were infused and sacrificed 2 hours after MCA clipping. Group 2 animals (n = 3) were infused and sacrificed...
after hypotension had been induced. Group 3 animals (n = 3) were infused and sacrificed at the time of maximal intracranial pressure (ICP) elevation after removal of the temporary MCA clip (Fig. 1). In addition, two animals were subjected to MCA occlusion for 24 hours and were then infused and sacrificed.

Twelve animals were given mannitol 2.0 gm/kg intravenously prior to MCA clipping. Carbon black was infused in the same way as in non-mannitol animals, 2 hours after MCA clipping (Group 1M; n = 4), during hypotension superimposed on MCA occlusion (Group 2M; n = 4), and at the time of maximal ICP elevation after release of the clip (Group 3M; n = 4). An additional two animals were given mannitol prior to 24 hours of MCA occlusion and were then infused and sacrificed.

Mannitol 2.0 gm/kg was given intravenously 2 hours after MCA occlusion (Group 1MP; n = 4), during hypotension superimposed on MCA occlusion (Group 2MP; n = 4), or after clip removal (Group 3MP; n = 4). Each group was infused and sacrificed 30 minutes after mannitol administration. Two animals were given mannitol 24 hours after MCA clipping and were infused and sacrificed 30 minutes later.

The brains of sacrificed animals were removed and fixed in 10% formalin. One week later, they were cut into coronal sections. Carbon black filling in the infarcted hemisphere was examined both grossly and microscopically.

**Results**

In all animals that did not receive mannitol, the cortical surface vessels in the infarcted hemisphere were well filled with carbon black, retrodromically in Groups 1 and 2, and anterodromically in Group 3. On the other hand, filling of parenchymal vessels in infarcted cortex was remarkably impaired. Comparatively, filling in the white matter was unaffected. The extent of filling impairment was dependent on the timing of carbon black administration (Fig. 2).

The filling defect was confined to a relatively small area in animals perfused 2 hours after MCA occlusion (Group 1). Only 34.5 ± 5.1% of the hemisphere demonstrated impaired filling. However, much of the area affected by MCA, that is, 64.9 ± 3.5% of the hemisphere, showed impaired filling when carbon black was administered after hypotension had been induced (Group 2). In Group 3, the extent of the filling defect depended on the degree of epidural pressure elevation. A slight defect (13.5%) was noted in one animal in the absence of elevated pressure. The defect increased to 65.7 ± 2.8% in two animals when the pressure was raised to 1340 to 2080 mmH2O (Fig. 2). With 24 hours of MCA occlusion, carbon black filling was only slightly impaired (7.9 ± 1.6%) (Fig. 3).

In animals given mannitol prior to 2 hours of

![Fig. 2](image-url)
MCA occlusion (Group 1M), filling with carbon black was normal. The filling defect in Group 2M was 38.2 ± 4.6%. On the contrary, the filling in Group 3M was exaggerated in the infarcted cortex, affecting 38.5 ± 3.5% of the hemisphere (Fig. 4).

Dilatation of the microvasculature in “hyper-filled” cortex was evident in the microscopic specimen (Fig. 5). There was no pressure elevation after clip removal in Group 3M. The carbon black filling defect was 72.5 ± 3.2% in animals given mannitol and then subjected to MCA occlusion for 24 hours. This rate was markedly higher than that in animals who underwent MCA occlusion for 24 hours but did not receive mannitol (Fig. 3).

In all groups of animals given mannitol after MCA occlusion, the filling defect extended throughout the entire territory of MCA: 69.8 ± 1.9% in Group 1MP, 73.5 ± 2.3% in Group 2MP, and 70.5 ± 2.3% in Group 3MP (Fig. 6). In animals given mannitol 24 hours after MCA clipping, the filling defect was confined to 18.3 ± 1.4% of the hemisphere, an area somewhat larger than that observed in the non-mannitol animals (Fig. 3).

The percentages of filling defects for all groups is summarized in Table 1.
Administered after the onset of ischemia, mannitol impeded carbon black filling. A: 2 hours after MCA clipping; B: hypotension superimposed on MCA clipping; and C: after clip removal.

Table 1 The hemispheric filling defect percentages in all groups

<table>
<thead>
<tr>
<th></th>
<th>2-hour clipping</th>
<th>Clipping plus hypotension</th>
<th>Clip removal</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EDP 140-280 mmH₂O</td>
<td>EDP 1340-2080 mmH₂O</td>
</tr>
<tr>
<td>No mannitol</td>
<td>34.5 ± 5.1</td>
<td>64.9 ± 3.5</td>
<td>13.5 ± 0</td>
</tr>
<tr>
<td>Preocclusion mannitol</td>
<td>0</td>
<td>38.2 ± 4.6</td>
<td>38.5 ± 3.5*</td>
</tr>
<tr>
<td>Postocclusion mannitol</td>
<td>69.8 ± 1.9</td>
<td>73.5 ± 2.3</td>
<td>70.5 ± 2.3</td>
</tr>
</tbody>
</table>

Asterisk indicates "hyper-filling."

Discussion

The competence of collateral circulation is said to be an important determinant of the severity of regional ischemia due to arterial occlusion, and it can be compromised by systemic hypotension. Therefore, ischemia is most likely the result of disruption of the collateral supply from other arterial trunks and subsequent lowered perfusion of the infarcted cortex. Ralston et al. and Crowell et al. have observed this aggravating effect of hypotension in experimental cerebral infarction.

In this study, transient hypotension induced during MCA occlusion extended the carbon black filling impairment to the entire occluded territory. If the carbon black filling defect represents obstruction of the microvasculature, the persistence of an extensive filling defect following recovery of blood pressure after hypotension can be interpreted as reflecting the failure of microvascular reopening rather than a failure of restoration of collateral circulation. It would constitute a so-called "no reflow" phenomenon. The recovery of filling after the cessation of occlusion suggests that the once obstructed microvasculature reopened and that perfusion increased. The vascular obstruction following ischemia is thought to be an organic change caused by erythrocyte aggregation or compression by swollen endothelial cells and perivascular glia, and to be reversible over time, with reestablishment of perfusion pressure.

Our findings show that the microcirculatory changes in the infarcted cortex are dynamic and closely related to the change in perfusion pressure. The ischemic injury to the cerebral tissue and the microcirculatory impairment are accentuated by transient hypotension during occlusion. The permeability of the microvasculatures in the non-carbon-filled areas is reportedly increased. Therefore, it is conceivable that the increased perfusion after arterial occlusion is stopped reopens the obstructed microvasculature, and reflow through these damaged vessels facilitates transduction of the plasma into the parenchyma with increased permeability.

The development of cerebral edema in the infarcted cortex is progressive, and acute brain swelling occurs rapidly. In this event, the reopened microvasculature will again close as a result of compression by cerebral edema. Aggravation of the filling impairment in animals in which the epidural pressure was markedly elevated, may reflect this secondary obstruction of microcirculation.

The preocclusional administration of mannitol significantly protected the microcirculation in the infarcted cortex, and this also prevented the development of acute brain swelling after recirculation. Little has experimentally demonstrated that mannitol has a protective effect in acute focal...
ischemia, and attributes the beneficial effect to prevention of fluid migration from the intravascular to the extravascular compartment by means of increased plasma osmolarity. In the clinical situation, preoperative administration of mannitol reportedly made it possible to occlude major cerebral arteries for 80 minutes during surgery for aneurysm.\(^5,6\)

The filling defect after 24 hours of MCA clipping was less extensive than after 2 hours of MCA occlusion. This indicates that the microvascular impatency can be reversed by the passage of time, and that restoration of perfusion pressure is not the only means of reversal.

The efficacy of mannitol in the treatment of acute cerebral ischemia has not been proved, although alleviation of ischemic symptoms following subarachnoid hemorrhage was reported.\(^7\) Mannitol was not found to affect the clinical course of acute stroke.\(^8\) Kagawa \textit{et al.} \(^5\) demonstrated that the protective effect of mannitol on cerebral infarction was variable when it was administered within 60 minutes following arterial occlusion, and was absent when administered 120 minutes after occlusion. However, its protective effect was unequivocal with preocclusional administration.

The pathogenesis of the aggravating effect of mannitol observed in this study is not clear. However, it is widely known that hyperosmolar agents do not affect cerebral edema but dehydrate normal brain, providing additional space for edema.\(^9\) Furthermore, such agents are believed to penetrate edematous brain regions through the disrupted blood-brain barrier; thus, the intra- and extravascular osmotic pressures are reversed.\(^10\) The breakdown of the blood-brain barrier, demonstrable with fluorescein staining, is seen as early as 90 minutes after occlusion in the areas of impaired microcirculation.\(^11\) Therefore, it is conceivable that mannitol enhances brain edema in infarcted cortex and secondarily aggravates microvascular patency. Little\(^12\) suggested that early administration of mannitol is particularly important, as its dehydrating action in ischemic tissue depends upon the integrity of the blood-brain barrier. On the other hand, mannitol is known to enhance cerebral blood flow in the normal brain.\(^13\) Access of mannitol to the ischemic area may be limited by obstruction of the microvasculature.\(^14\) Therefore, another possibility is that blood is withdrawn to the normal brain tissue from the ischemic tissue, leading to worsening of the ischemia and impaired microcirculation.

Preocclusional administration of mannitol exacerbated the filling impairment in cats subjected to 24 hours of MCA clipping, in contrast to those subjected to 2 hours of occlusion. Assuming that the animals had excellent filling early on, as suggested by the results of 2 hours of clipping, the impairment must have worsened over time. Watanabe \textit{et al.} \(^22\) showed that preocclusional administration of mannitol prevented cerebral infarction when administered within 60 minutes, but was ineffective 180 minutes following occlusion. In Little’s study,\(^16\) administration of mannitol at the time of MCA occlusion prevented the development of ischemic cerebral edema, capillary narrowing, and severe neuronal alterations during the initial 6 hours, but had no effect 12 hours following occlusion. Little\(^17\) suggested that mannitol’s effectiveness temporally corresponds to the period of plasma osmolality elevation that it produces, and that its failure thereafter is attributable to the edema that develops once plasma osmolality has returned to the pretreatment level.

\textbf{References}

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