Cerebral protection by intermittent reperfusion during temporary focal ischemia in the rat

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Temporary arterial occlusion has been routinely used as an adjunct in intracranial aneurysm surgery. This has commonly been performed using a protocol of multiple short periods of occlusion alternating with periods of restoration of normal circulation. Recently, the logical basis of this method has come under scrutiny. There is extensive experimental evidence to suggest that repetitive, brief periods of global ischemia may cause more severe cerebral injury than an equivalent single period of global ischemia. Only recently has this issue begun to be addressed with regard to focal ischemia. Hence, despite the common use of temporary clipping, little experimental data are available regarding the ischemic consequences of temporary arterial occlusion with periods of reperfusion versus uninterrupted temporary occlusion.

To investigate this issue, a protocol of occlusion/reperfusion that simulates the temporal profile that occurs during surgery was performed in a rat model of focal ischemia. Sixteen anesthetized Sprague–Dawley rats were divided into two groups. The animals in Group I underwent 60 minutes of uninterrupted middle cerebral artery occlusion and the animals in Group II were subjected to six separate 10-minute occlusion periods with 5 minutes of reperfusion between occlusions. Histopathological analysis was performed 72 hours postischemia. Group I had significantly increased mean infarction volumes (50.0 ± 12.1 mm³) compared to Group II (8.7 ± 3.1 mm³) (p = 0.008). Injuries in Group I occurred in both the cortex and striatum, whereas Group II showed only striatal injuries. Furthermore, the extent of the injuries in Group II was less severe, characterized by ischemic neuronal injury rather than frank infarction.

The results indicate that intermittent reperfusion is neuroprotective during temporary focal ischemia and support the hypothesis that intermittent reperfusion is beneficial if temporary clipping is required during aneurysm repair.

KEY WORDS • aneurysm surgery • cerebral ischemia • cerebral protection • reperfusion • temporary clipping • transient focal ischemia • rat

The temporary arrest of local arterial circulation by occlusion of a parent vessel has been used as an adjunct in intracranial aneurysm surgery since its introduction by Pool20 in 1961. Increasingly, this technique has been used in the management of both routine and complex aneurysms. Proponents of temporary clipping cite the decreased risk of intraoperative rupture, safer dissection and delineation of perforating vessels, and the ability to control hemorrhage during premature intraoperative rupture as advantages afforded by this procedure.5,9,11,14,19,21,25–29 Despite these advantages, temporary clipping can have disastrous ischemic sequelae in the territory of the temporarily occluded vessel. To lessen this risk of ischemia, temporary arterial occlusion during aneurysm surgery has been commonly performed using a protocol of multiple short periods of occlusion interspersed with periods of restoration of normal circulation.19 Recently, Samson, et al.,21 have suggested that increasing episodes of temporary arterial occlusion may not be as well tolerated as a single protracted ischemic period.

Considerable experimental evidence exists establishing that repetitive episodes of global cerebral ischemia enhance the extent of neuronal injury when compared with an equivalent single period of ischemia.1,10,12,13,30,31 However, little experimental data are available on repetitive episodes of focal ischemia. Furthermore, most studies have used protocols that do not simulate the temporal profiles that are likely to occur during surgery. Considerable disagreement continues to exist regarding the use of brief periods of reperfusion during temporary clipping versus a single protracted period of temporary occlusion. To investigate this issue, a protocol of occlusion/reperfusion that simulates the temporal profile that occurs during surgery was performed in a rat model of transient, focal cerebral ischemia.

Materials and Methods

Animal Preparation

Sixteen adult male Sprague–Dawley rats, each weighing between 350 and 450 g, were used for the study. The rats were denied food and water the evening before the experiment. Anesthesia was induced with 3% halothane and a mixture of 70% nitrous oxide and 30% oxygen. The rats were intubated and maintained on 0.5% to...
TABLE 1
Physiological parameters in rats that underwent uninterrupted (Group I) and interrupted (Group II) MCA occlusion

<table>
<thead>
<tr>
<th>Physiological Parameters</th>
<th>Control Group (Group I)</th>
<th>Reperfusion Group (Group II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head temp (°C)</td>
<td>36.4 ± 0.1</td>
<td>36.1 ± 0.1</td>
</tr>
<tr>
<td>Rectal temp (°C)</td>
<td>37.4 ± 0.2</td>
<td>37.2 ± 0.2</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>97.3 ± 1.6</td>
<td>96.5 ± 1.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 ± 0.01</td>
<td>7.39 ± 0.01</td>
</tr>
<tr>
<td>Pao2 (mm Hg)</td>
<td>40.7 ± 0.7</td>
<td>40.0 ± 0.7</td>
</tr>
<tr>
<td>Glucose (g/dl)</td>
<td>121.4 ± 6.9</td>
<td>122.1 ± 5.7</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>—</td>
<td>43.1 ± 1.3</td>
</tr>
</tbody>
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* Values are expressed as the mean ± standard error of the mean. Abbreviations: MABP = mean arterial pressure; MCA = middle cerebral artery; temp = temperature; — = not applicable.

1% halothane and a mixture of 70% nitrous oxide and 30% oxygen. Femoral arterial and venous catheters were inserted for the measurement of arterial blood pressure and sampling for arterial blood gas levels, serum glucose, and hematocrit determinations. The rats were given pancuronium bromide (0.6 mg/kg initial dose, 0.2 mg/kg maintenance doses) and mechanically ventilated. Temperature probes were inserted into the rectum and the left temporal muscle (as an indirect measure of brain temperature), and heating lamps were used to maintain rectal and temporalis temperatures at 37°C to 38°C and 36.5°C to 37.5°C, respectively. Measurements of mean arterial blood pressure, blood gas levels (pH, PCO2, and PO2), hematocrit, serum glucose, and temperature were made immediately before the onset of ischemia and again just before the end of ischemia (before the final reperfusion in the intermittent ischemia group). At the end of the experiment, the catheters and probes were removed and the wounds were sutured closed. The animals were allowed to recover from anesthesia in a warmed oxygen-enriched environment and then transferred to a cage. The animals were allowed free access to food and water for 72 hours.

Middle Cerebral Artery Occlusion

The operative exposure was performed according to the model of Shiraiishi and Simon.23 Briefly, a 2-cm vertical incision was made just posterior to the lateral canthus of the right eye. The temporalis muscle was incised and reflected superiorly, thus exposing the zygomatic arch and squamosal bone. The zygomatic arch was carefully incised and a spatula retractor was inserted to retract the zygoma and mandible inferiorly, thus exposing the infratemporal fossa and foramen ovale. Using the operating microscope (Carl Zeiss, Inc., Thornwood, NY) the foramen ovale was enlarged to 3.5 × 3.0 mm using a high-speed dental drill. The field was irrigated frequently with cooled saline to avoid thermal damage. The dura was carefully incised using a small blade, and 1-mm diameter cotton balls were placed between the cortex and the base of the skull. This allowed visualization of the most proximal middle cerebral artery (MCA) by elevating it slightly within the subarachnoid space and avoiding any brain retraction. Using a blunt needle, the MCA was freed from its surrounding arachnoid to facilitate clip occlusion.

At this point, physiological parameters were measured in the manner described above. Using a specially designed 15-g microclip (Zen temporary clip; Ohwa Tsusho, Tokyo, Japan), the proximal MCA was occluded and the flow interruption was observed. Reperfusion was performed by removing the clip and visually verifying blood flow in the MCA. Occlusion/reperfusion was performed as described in the experimental protocol. Just prior to the completion of ischemia, physiological determinations were again made. At the completion of the experiment, the zygomatic fracture was reduced and the wound closed.
Reperfusion during temporary focal ischemia

The main objection to temporary clipping has been the risk of ischemic injury to the brain. The risk of ischemic injury is highly dependent on the duration of arterial occlusion; this relates to a critical threshold at which ischemic tissue progresses to infarction. This critical time period is highly variable for different vascular territories; it is a function of the actual vessel occluded and the extent of collateral blood flow available to the vascular territory in question. Batjer, et al. investigated this critical threshold for different cerebral vascular territories in a retrospective study of patients undergoing aneurysm repair. They reported that 60 minutes of internal carotid artery occlusion, 35 minutes of MCA occlusion, and 4 to 19 minutes of basilar artery occlusion were well tolerated. Although these values were obtained with the patient under deep etomidate anesthesia, which is a known neuroprotective agent, this study does demonstrate that there are limits to the duration of temporary occlusion that can safely be used.

In view of these limits, many neurosurgeons have advocated the use of neuroprotective agents, such as etomidate and induced hypertension, during the period of temporary occlusion. In addition, two different and conflicting technical paradigms of temporary occlusion have been advocated in an effort to reduce the risks of ischemic sequelae. Some neurosurgeons have suggested allowing short periods of reperfusion during the arterial occlusion rather than a single protracted period of occlusion. This opinion is based on the intuitive assumption that multiple shorter periods of ischemia will preserve energy stores and prevent or blunt the initiation of deleterious metabolic cascades such as acidosis, excitotoxicity, altered membrane permeability, and generation of free radicals. Furthermore, periods of reperfusion will allow for repletion of the energy stores and possibly ameliorate any detrimental reactions that may have begun. However, other neurosurgeons argue that these periods of reperfusion are unnecessary and may even exacerbate the degree of ischemic injury in accord with the “reperfusion injury” concept. Proponents of this view suggest using a single temporary occlusion period within the limits of tissue vulnerability. In fact, Samson, et al. recently reported that intermittent reperfusion did not appear to prevent ischemic injury and when used in the older population may perhaps be detrimental. Despite compelling arguments for both methods, it remains controversial whether or not to use short periods of reperfusion during temporary clipping.

Experimental Perspectives

Using a rat model of proximal MCA occlusion, we have shown that six 10-minute periods of ischemia interrupted by 5 minutes of reperfusion produce less ischemic injury than a single 60-minute continuous period of ischemia. Thus it appears that interrupted focal ischemic insults are better tolerated than a single uninterrupted transient focal insult. Of interest, we also found that the interrupted peri-
ods of focal ischemia resulted in a lesser degree of injury when compared to the uninterrupted group, that is, selective neuronal injury versus frank necrosis and infarction, respectively.

Three other studies have addressed this question in a manner that approximates the temporal profile that is likely to occur during surgery. Steinberg and associates reported less ischemic damage in rabbits undergoing three 20-minute ischemic periods interrupted by 10 minutes of reperfusion than in rabbits undergoing 60 minutes of uninterrupted ischemia. However, these authors assessed histological injury at 6 hours and could not rule out the possibility that interrupted occlusion may merely delay the onset of irreversible damage. Goldman and colleagues have also reported decreased infarct volume in rats that underwent interrupted MCA occlusion. Using the intraluminal suture model, these researchers performed six, nine, and twelve 10-minute ischemic periods separated by 5-minute reperfusion intervals and compared the effects to those of a single occlusion having the same total ischemic duration. Histological data were assessed 72 hours after ischemia. Although these researchers found neuroprotection with intermittent reperfusion, their model has the potential drawback of not simulating what occurs to a vessel during clip occlusion. This model may also be associated with significant intimal injury during a multiple occlusion paradigm. In contrast, a well-designed and controlled study by Selman, et al., reported no significant difference in the volume of infarction between normotensive rats that underwent single and multiple ischemic episodes. However, these authors found significant worsening with reperfusion in spontaneously hypertensive rats that underwent the same experimental paradigm.

The discrepancy between the results of Selman, et al., and the findings of other researchers including us is of significant interest. The larger volume of infarction obtained in the spontaneously hypertensive rats may be partially understood in terms of the known effects of hypertension on the vascular system. As Selman, et al., point out, a greater degree of ischemia and its impact may be present in spontaneously hypertensive rats due to inadequate collateral circulation and decreased functional compliance.
Reperfusion during temporary focal ischemia

which limit the overall vascular dilation (autoregulation) in response to an ischemic insult.

The variance in results obtained with normotensive rats is not as clear. One may speculate as to the possible factors related to this variability. Selman, et al.,22 raise the possibility of a species-specific response to ischemia and reperfusion. Along these lines a strain-specific response may be entertained. Selman, et al., and Goldman and colleagues8 studied Wistar rats, whereas in our study we used the Sprague-Dawley strain. Duverger and MacKenzie6 examined the influence of strain as well as other parameters including arterial pressure on infarction volume following focal ischemia in rats. They noted that the most reproducible volume of infarcted tissue was seen in Fischer 344 rats. The volume seen in the Sprague-Dawley strain was large but had considerable variation, whereas the infarcted volume in Wistar rats was small but the variability was extreme. These researchers also found spontaneously hypertensive rats to be associated with significantly larger infarction volumes that in normotensive rats.

Differences in occlusion and reperfusion time periods must also be considered. Focusing only on rats, Goldman and colleagues8 found neuroprotection using an experimental paradigm similar to ours: 10 minutes of ischemia separated by 5-minute periods of reperfusion. Selman, et al., used three 20-minute periods of ischemia separated by 10 minutes of reperfusion. Whether the depth of the ischemia or the effects of the reperfusion significantly differ between these two protocols is a matter of speculation. It is reasonable to propose that there may be critical time periods for occlusion and reperfusion that may influence the response to intermittent reperfusion during focal ischemia. A time-course study of these variables may further elucidate the basis for the different results obtained.

A significant limitation of our study, as well as those of Steinberg, et al.,26 and Goldman and colleagues,8 is the lack of cerebral blood flow measurements during the periods of occlusion, which would have confirmed the ischemia. Although a visually confirmed interruption in the blood column in the MCA was made in our animals, one may wonder whether a lesser degree of ischemia was present in the intermittently occluded vessel. Despite this limitation, we believe our model is a good approximation of the intraoperative setting. We used short ischemic periods (10 minutes) with brief periods of reperfusion (5 minutes), similar to the typically used 2 to 10 minutes of ischemia and 5 to 10 minutes of reperfusion typically recommended during surgery.19,28 In addition, we used a specially designed vascular clip for MCA occlusion and an anesthetic technique typical of most intracranial operations. Furthermore, our study was conducted under normothermic conditions, which is important in view of the fact that mild brain hypothermia has been shown to be protective during focal ischemia.

Global Versus Focal Ischemia

Studies in evaluating the effects of reperfusion during global ischemia have provided strong evidence that reperfusion worsens the extent of ischemic injury when compared to an analogous period of global ischemia without reperfusion.1,10,12,13,15-17,30,31 Multiple mechanisms have been proposed to explain these results. These include breakdown of the blood-brain barrier, allowing for the formation of vasogenic edema and extravasation into the brain parenchyma of deleterious substances such as ions, neurotransmitters, and neuroactive substances that would normally be excluded by an intact barrier.3 Postischemic hypoperfusion, progressive lactic acidosis, platelet activation with subsequent thrombosis, and late inflammatory responses have also been implicated.4 One mechanism undergoing intense study is the role of excitatory amino acids in postischemic brain injury. Massive rises in extracellular concentrations of glutamate have been documented in global ischemia.3,13 The elevation in glutamate levels is believed to give rise to activation of N-methyl-D-aspartate receptors with resultant changes in intracellular calcium ion concentrations and conductance.

Investigations regarding reperfusion during focal ischemia have been few and contradictory.3,10,18,22,26 Ohtaki and associates19 found no difference in ischemic injury resulting from interrupted or uninterrupted arterial occlusion. However, with ischemic periods lasting 3 hours, one must question whether the reperused tissue had already progressed to infarction. The present study and those previously mentioned (Goldman and colleagues,3 Selman, et al.,22 and Steinberg and associates20) have addressed this issue in a manner analogous to the intraoperative setting. Our results concur with those of Goldman and colleagues and Steinberg and associates, indicating that periods of reperfusion during transient focal ischemia are neuroprotective.

What are the possible mechanisms for this protection? It has been suggested that brief ischemic periods accompanied by reperfusion might prevent critical depletion of adenosine triphosphate stores and, in turn, might prevent energy-dependent ion pump failure with the resultant initiation of biochemical cascades leading to infarction.8 This protection may also relate to blunting or decreased release of detrimental neurotransmitters or, alternatively, result from intact or still functioning uptake mechanisms and better metabolic recovery. One must also investigate the possibilities of less blood-brain barrier disruption, less cumulative acidosis, and better microcirculatory recovery. Clearly, further study of the underlying mechanisms of reperfusion following focal ischemia are needed.

Conclusions

We investigated the role of intermittent reperfusion in a rat model of transient focal ischemia. Less histopathological evidence of ischemic injury was found in the reperfused group than in the nonreperfused control group. Because of the obvious differences between experimental conditions and those occurring during intracranial surgery, the clinical relevance of these results must be viewed with caution. Notwithstanding, these results support the hypothesis that intermittent reperfusion is beneficial if temporary clipping is required during aneurysm repair.

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References


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