Cerebral damage caused by interrupted, repeated arterial occlusion versus uninterrupted occlusion in a focal ischemic model

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Temporary intracranial arterial occlusion is often utilized during the surgical treatment of intracranial aneurysms. Although numerous experimental studies have suggested that repetitive, brief periods of global ischemia cause more severe cerebral injury than a similar single period of global ischemia, this issue has not been extensively studied in relation to focal ischemia. It remains controversial whether it is safer to use brief periods of interrupted, temporary occlusion separated by reperfusion periods, or a more prolonged, single temporary occlusion. This question is addressed in studies on a rabbit model of transient, focal cerebral ischemia.

Sixteen anesthetized rabbits underwent transorbital occlusion of the left internal carotid, middle cerebral, and anterior cerebral arteries, with one of two paradigms: uninterrupted occlusion (1 hour of temporary occlusion followed by 5 hours of reperfusion in eight rabbits), or interrupted occlusion (three separate 20-minute periods of occlusion, with 10 minutes of reperfusion between occlusions, followed by 4 hours, 40 minutes of reperfusion in eight rabbits). Histopathological evaluation for ischemic neuronal damage and magnetic resonance imaging studies for ischemic edema were conducted 6 hours after the initial arterial occlusion.

The animals in the interrupted, repeated occlusion group showed a 59% decrease in the area of cortical ischemic neuronal damage (mean ± standard error of the mean 10.0% ± 1.7%) compared with the uninterrupted occlusion group (24.4% ± 5%, p = 0.016). There was no difference between the groups in the extent of striatal ischemic damage or area of ischemic edema. These results suggest that interrupted, repeated focal ischemia causes less cortical ischemic injury than uninterrupted transient ischemia of a similar total duration. Although caution should be exercised in extrapolating from these results to the clinical situation, they may have important implications for temporary arterial occlusion during intracranial surgery.

KEY WORDS: temporary clipping · intracranial arterial occlusion · intracranial aneurysm · cerebral ischemia · cerebral protection · reperfusion

DELIBERATE temporary occlusion of intracranial arteries is often used in the treatment of complex cerebral aneurysms to decrease the risk of intraoperative aneurysm rupture, reduce aneurysm bulk, facilitate safe dissection of the aneurysm neck, and permit accurate final clip placement without compromise to critical vessels. While numerous experimental studies have suggested that, under certain circumstances, repetitive brief periods of global ischemia cause more severe cerebral injury than a single period of uninterrupted global ischemia,1,2,10,12−14,16,17,21,22,24−27,35,37 this issue has not been extensively investigated in relation to focal ischemia. It remains controversial among cerebrovascular surgeons whether it is safer to use brief periods of uninterrupted temporary occlusion separated by reperfusion periods, or to employ a more prolonged single temporary occlusion.11,20,29,34 We examined this question using a rabbit model of focal cerebral ischemia.32,33

Materials and Methods

Experimental Protocol

Sixteen male New Zealand White rabbits, each weighing 2.5 to 3.9 kg, were sedated and anesthetized with 3% halothane delivered by mask. A femoral arterial catheter and ear-vein catheter were placed and the animals underwent tracheostomy. Artificial ventilation and anesthesia were maintained using 1% halothane in a mixture of 0.5 L/min O2 and 4.5 L/min air. The rabbits underwent transorbital clip occlusion of the left internal carotid, middle cerebral, and anterior cerebral arteries with one of two paradigms (eight rabbits each): uninterrupted occlusion (1 hour temporary occlusion followed by 5 hours of reperfusion) or interrupted occlusion (three separate 20-minute periods of occu-
Interrupted, repeated arterial occlusion in ischemic model

Fig. 1. Photomicrographs of comparable regions of cortex within the middle cerebral artery distribution (at the coronal level of anterior commissure, Level 2) in uninterrupted occlusion (left) and interrupted occlusion (right) animals (see Materials and Methods section). Severe early ischemic neuronal injury is present in uninterrupted occlusion cortex with relative neuroprotection in interrupted occlusion cortex. H & E, × 50.

sion, with 10 minutes of reperfusion between occlusions, followed by 4 hours, 40 minutes of reperfusion). Brain temperature was controlled at 38°C with a heating lamp and fan. Mean arterial pressure was kept at 55 to 70 mm Hg using phenylephrine as needed, and end-tidal CO₂ was controlled at 35 to 40 mm Hg. Arterial blood gas, hematocrit, and plasma glucose levels were measured periodically throughout the experiment and any arterial base deficit was corrected as necessary.

Six hours after the initial arterial occlusion, the animals were sacrificed with a lethal dose of intravenous sodium pentobarbital (100 mg/kg) and perfused transcardially with normal saline (300 cc) followed by 300 cc of 10% buffered formalin (pH 7.4). The brains were left in the skull overnight, then removed and fixed in formalin for an additional 4 to 6 days.

Magnetic Resonance Imaging

The intact fixed brains were evaluated with magnetic resonance (MR) imaging for ischemic edema using a 1.5-tesla system. We obtained spin-echo, T₂-weighted images 3 mm thick using a repetition time of 2500 msec and an echo time of 80 msec. In our laboratory, MR studies of rabbits with similar ischemic lesions have demonstrated no significant differences in relative signal intensity and area between premortem and post-fixation images, with overlap values of 80% to 85%. Magnetic resonance imaging is a sensitive measure of early cerebral edema and the high-intensity signal on T₂-weighted images represents an increase in the water content or state of hydration of the ischemic brain. Three standard coronal MR images (at the levels of the anterior commissure and of the optic chiasm) were analyzed. The areas of high-intensity MR image signal, representing ischemic edema in the left cortex and hemisphere, were delineated on a tracing of the image. A computer image-analysis system was used to measure the areas of ischemic edema; the results were expressed as a percentage of the total area of left cortex or left hemisphere for each coronal level. An average percentage of ischemic edema on MR imaging for each animal was calculated as the total area of high signal for both coronal levels divided by the total area of cortex for both coronal levels.

Histopathological Studies

The rabbit brains were processed for paraffin embedding, cut at 10 μm, and stained with hematoxylin and eosin. Ischemic neuronal damage was evaluated with light microscopic examination of five standard coronal sections: Level 1, 3 mm anterior to the anterior commissure; Level 2, at the anterior commissure; Level 3, 3 mm posterior to the anterior commissure; Level 4, 6 mm posterior to the anterior commissure; and Level 5, 9 mm posterior to the anterior commissure. Early ischemic neuronal damage was defined as neurons showing moderate-to-severe shrinkage, increased nuclear basophilia, and a shrunken or pyknotic nucleus. These histological criteria have been used previously to characterize severely injured ischemic neurons.

All areas containing neurons with ischemic neuronal damage in the cortex and striatum were delineated on a tracing of the microscopic section at × 40 to × 200. The computer image analysis system was used to measure the areas of ischemic neuronal damage within the left cortex or left striatum; the results were expressed as the percentage of the total area for these corresponding structures. An average percentage area of cortical or striatal ischemic neuronal damage for each animal was calculated as the total area of ischemic neuronal damage in the left cortex or left striatum for all five coronal levels divided by the total area of the left cortex or left striatum for all five coronal levels.

Statistical Analysis

Statistical analyses utilized the two-tailed, unpaired t-test. All values are given as the mean ± standard error of the mean. Differences were considered significant at p values less than 0.05.

Results

Histopathological Findings

The animals in the group with interrupted, repeated occlusion showed a 59% decrease in the area of cortical ischemic neuronal damage (10.0% ± 1.7%) compared with the uninterrupted occlusion group (24.4% ± 5%) (Figs. 1 and 2). This benefit of interrupted arterial occlusion against cortical injury was found most prominently at coronal Levels 2, 4, and 5 (p < 0.05), but did

* Computer image-analysis system manufactured by Jandel Scientific Sigma-Scan, San Rafael, CA.
not quite reach statistical significance for Level 3 (p = 0.063) or 1 (p = 0.095) (Fig. 2). There was no difference between groups in the extent of striatal ischemic damage (38.1% ± 11.4% in the interrupted occlusion group vs. 37.4% ± 7.8% in the uninterrupted occlusion group).

**Magnetic Resonance Imaging Findings**

There was no difference in the area of cortical or hemisphere edema between the two groups. The group with interrupted, repeated occlusion demonstrated a 14.6% ± 3.9% area of cortical edema and an 11.6% ± 3.2% area of hemispheric edema, compared with a 12.5% ± 3.5% area of cortical edema and a 9.9% ± 2.7% area of hemispheric edema in the group with uninterrupted occlusion (p > 0.6).

**Systemic Parameters**

There were no significant differences between groups in mean arterial pressure, heart rate, brain temperature, rectal temperature, pH, PaO₂, PaCO₂, hematocrit, volume of saline or phenylephrine infused, or body weight (Table 1). Blood glucose was slightly lower during occlusion in the uninterrupted occlusion group (106 ± 8 gm/dl) compared with the group with interrupted, repeated occlusion (148 ± 7 gm/dl, p = 0.0009).

**Discussion**

Intraoperative temporary arterial occlusion is frequently used by cerebrovascular surgeons in the treatment of intracranial aneurysms, since it affords several advantages: 1) for early surgery after subarachnoid hemorrhage it allows for final aneurysmal neck dissection and clipping with decreased risk of rupture; 2) for giant, large, or complex aneurysms, it facilitates complete dissection of important arterial branches or critical perforating vessels arising near the aneurysmal neck, and placement of clips across a broad neck without compromising the parent vessel; 3) for partially thrombosed aneurysms, it permits opening of the thrombosed sac, evacuation of clot and atherosclerotic plaque, and repair of the residual aneurysmal neck or reconstruction of the parent vessel without excessive bleeding; 4) for situations where an intracranial aneurysm has prematurely ruptured intraoperatively, it reduces hemorrhage from the aneurysm and allows the final dissection to be completed before definitive clip placement.

Despite the benefits of temporary arterial occlusion in the treatment of intracranial aneurysms, there is still a disadvantage associated with the technique: namely, the risk of ischemic injury in the territory of the temporally occluded artery. This risk is related to the duration of arterial occlusion, the particular vessel occluded, and the collateral blood flow available to the ischemic territory. Even with induced hypertension and various neuroprotective agents, there are limits to the total duration of temporary occlusion that is tolerated without incurring neurological deficits, due to the initiation of detrimental processes including excitotoxic neuronal injury, activation of phospholipases, influx of calcium, and generation of damaging free radicals.

In an effort to decrease the ischemic injury associated with temporal arterial occlusion, some cerebrovascular neurosurgeons have recommended interrupting the arterial occlusion with intervals of reperfusion rather than utilizing a single, more prolonged period of arterial occlusion. The rationale underlying this approach is that brief periods of ischemia will preserve energy stores and reduce excitotoxic and other detrimental enzymatic cascades compared with longer periods of ischemia, while the reperfusion interval will allow for recovery of energy stores and reversal of damaging reactions. Typically, arterial occlusion periods of 2 to 10 minutes are interrupted by reperfusion intervals of 5 to 10 minutes. Advocates of this method of interrupted occlusion argue that the technique prolongs the total interval of arterial occlusion that is safely tolerated. However, other cerebrovascular neurosurgeons maintain that not only is interrupted arterial clipping technically more difficult than a single, prolonged occlusion, but it also worsens the extent of ischemic damage due to reperfusion injury or other cumulative ischemic insults compared with a single ischemic period of similar total duration. These surgeons recommend a single temporary occlusion period of 2 to 60 minutes, the time required to complete the final aneurysm neck dissection and clipping. It remains controversial whether it is safer to use brief periods of interrupted, temporary occlusion separated by reperfusion periods or to use a more prolonged, single temporary occlusion.
Interrupted, repeated arterial occlusion in ischemic model

A number of experimental studies examining global ischemia suggest that repetitive brief periods of sublethal global ischemia worsen the extent of cerebral injury compared with a similar single period of global ischemia.1,2,6,12-14,18,21,22,24-27,35,37 These studies are most commonly based on studies of bilateral common carotid artery occlusion in the gerbil2,6,12-14,21,24,27,35,37 and two- or four-vessel occlusion in the rat10,16,32 or cat.1 They demonstrate that two to four ischemic periods of 2 to 5 minutes each, interrupted by 1 hour of reperfusion, cause more selective neuronal cell loss, accelerated ischemic damage, larger infarcts, and a greater extent of edema than with a single continuous ischemic period of the same total duration. After such global ischemia, the ischemic areas include hippocampus, striatum, thalamus and, to a lesser extent, cortex. Some evidence suggests that the cumulative damage from interrupted occlusion is secondary to the following: postischemic hypoperfusion (maximum at the time of subsequent ischemic insults), postischemic protein synthesis inhibition, postischemic worsening in glutamate excitotoxicity (from increased glutamate release, alterations in the glutamate receptor, or changes in calcium conductance), progressive acidosis during repeated occlusion or alkalinosis during the reperfusion phase, worsening cerebral edema, delayed thrombotic occlusion of the clipping site, or reperfusion injury to the microvasculature.1,2,6,10,12-14,18,21,22,24-27,35,37

It is also clear from these global ischemia studies that the extent of cumulative injury with repeated, sublethal ischemic episodes (of 2 to 5 minutes each) is highly dependent on the duration of the reperfusion interval, with 1 hour of reperfusion causing the most severe damage. In fact, repeated brief ischemic periods of 2 to 5 minutes each, interrupted by reperfusion periods of 3 to 10 minutes, do not result in more ischemic injury than a single continuous occlusion period of the same total ischemic duration.12,35 Four episodes of repeated global ischemia (3 minutes each in duration), followed by 27 minutes of reperfusion between ischemic periods, do not increase subsequent glutamate release or have a cumulative deleterious effect on pCO2 or extracellular tissue acidosis.20,36 Furthermore, extending the reperfusion interval to 6, 12, or 24 hours, or to 2, 4, or 7 days may have a protective effect compared with uninterrupted ischemia,12,14,16,27 possibly due to altered gene expression and the synthesis of beneficial heat stress proteins.16

Until recently, the issue of interrupted, repeated periods of occlusion versus uninterrupted continuous arterial occlusion during focal ischemia has not been rigorously investigated. Using a rabbit model of transient focal ischemia and comparing 1-hour continuous ischemia with three separate 20-minute ischemic periods interrupted by 10 minutes of reperfusion, we have shown that interrupted, repeated focal ischemia causes less ischemic injury than uninterrupted transient ischemia of a similar duration. Goldman, et al.,6 have also reported a decreased cortical infarct volume in rats undergoing interrupted middle cerebral arterial occlusion (six, nine, or 12 10-minute periods of occlusion separated by 5 minutes of reperfusion compared with a single arterial occlusion of similar total duration). They used the intraluminal suture model and examined histopathology 72 hours following the onset of ischemia. Kimura and his colleagues15 recently presented some pilot studies using a rat model of focal cerebral ischemia to evaluate ischemic edema with diffusion-weighted MR imaging. They found that a single 40-minute occlusion period or two 20-minute occlusion periods separated by 20 minutes of reperfusion allowed diffusion-weighted imaging hyperintensity to resolve, but two 20-minute occlusions separated by 60 minutes of reperfusion resulted in persistence of the ischemic edema on diffusion-weighted images. A preliminary report by Ohati, et al.,20 on rat suture model described no protection from cortical infarct (assessed with 2,3,5-triphenyltetrazolium chloride), with interrupted arterial occlusion compared with continuous occlusion. However, their paradigm employed a longer occlusion time of 3 hours, with a more prolonged reperfusion interval of 1 hour between occlusions. The longer reperfusion period may have had a detrimental effect on ischemic pathophysiological processes, emphasizing the critical importance of the reperfusion duration between arterial occlusions.

The mechanism of relative neuroprotection for interrupted arterial occlusion versus continual occlusion

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is unknown. It may relate to decreased total release of excitatory amino acids, preserved adenosine triphosphate stores, improved microcirculatory regulation, prevention of severe acidosis, or attenuation of other pathophysiological processes. The lack of benefit in the striatum and densest cortical ischemic regions in our model suggests that neuroprotection may be effective in the penumbral zones but not in the core ischemic areas where blood flow is severely compromised. In our ischemia model other neuroprotective strategies, such as the administration of N-methyl-D-aspartate antagonists or a selective alpha-2 adrenoreceptor agonist (dexmedetomidine) are also protective in penumbral areas but not in core ischemic regions. Differences in underlying pathophysiology between focal and global ischemia might also explain the lack of neuroprotective benefit for interrupted occlusion in global ischemia, even with short reperfusion intervals.

Blood glucose levels were slightly lower during ischemia in the uninterrupted occlusion group (106 g/dl) compared with the interrupted, repeated occlusion group (148 g/dl); however, we do not believe that this can account for the protective effect of interrupted, repeated occlusion. Both of these blood glucose values are within the normal physiological range, and small differences such as these have not been shown to influence the degree of ischemic injury. While interrupted occlusion with repeated periods of reperfusion might be expected to cause more ischemic edema than a single occlusion, we found no difference between our groups. Both experimental paradigms resulted in relatively small areas of cortical ischemic edema (14.6% and 12.5%). It is not known if interrupted, repeated occlusion would worsen the extent of ischemic edema compared with a single occlusion using a longer total arterial occlusion time.

There are some limitations to the present study. Since we assessed histological injury at 6 hours following the onset of ischemia, we cannot exclude the possibility that interrupted occlusion simply delays the onset of irreversible damage. The findings of Goldman, et al., suggest that the protection of interrupted occlusion against infarct persists for at least 72 hours but, clearly, further experiments assessing unequivocal infarct at several days and even weeks after ischemia are needed to address this issue. Second, although the total occlusion time was similar for our two groups and we assessed histological injury at the same time point following initiation of ischemia in both groups (6 hours), the group with interrupted occlusion experienced 10 minutes less time between the end of the final occlusion period and sacrifice (4 hours, 40 minutes) than did the uninterrupted group (5 hours). We think it unlikely that this difference in final reperfusion time explains the histological protection, but the possibility remains that there was less maturation of the ischemic histological changes in the interrupted occlusion group.

The clinical relevance of the present findings are also unclear. There are many differences in collateral blood flow, anesthetic agents, various drugs and neuroprotective agents administered, brain temperature, and time of arterial occlusion and reperfusion between our rabbit ischemic model and temporary arterial occlusion utilized during intracranial vascular surgery. Extreme caution should be exercised in generalizing from these experimental results to intraoperative surgery in patients. Further experimental studies should investigate the optimum periods for temporary occlusion and for reperfusion in terms of achieving maximum neuroprotection, and should examine the limits of total occlusion that are tolerated. We should also critically analyze our clinical series undergoing temporal cerebral arterial occlusion to discern trends in benefit for particular paradigms of temporary occlusion, acknowledging that this perfuse will be an uncontrolled and a retrospective analysis.

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References

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damage following secondary ischemic insult in the gerbil: cumulative damage and protective effects. Brain Res 553: 238–242, 1991

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