Temporary Experimental Intracranial Vascular Occlusion

Effect of Massive Doses of Heparin on Brain Survival

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It is widely held that the maximum permissible period for total cerebral ischemia is in the range of 5 minutes. Total absence of perfusion for a longer duration is felt invariably to result in an infarction. This propensity of cerebral tissue to ischemic damage remains an overwhelming problem in neurosurgery directed toward or concerned with intracranial vascular structures. Not infrequently in the operative attack of cerebrovascular problems such as vascular malformations, occlusive disease (endarterectomy), and especially intracranial aneurysms, it becomes necessary to occlude the vascular inflow temporarily. The danger of irreversible damage has led to the commonly accepted practice of performing multiple brief occlusions with intermittent periods of flow restoration. This is surgically undesirable as it greatly shortens the effective periods of operative manipulation and leads to technical error.

Various means have been tried in an effort to prolong the allowable ischemic period so that more precise and time-consuming procedures might be performed. These have included efforts to lower the rate of cellular activity by hypothermia or barbiturate administration. Other investigators have tried to add excesses of physiological compounds to delay the onset of cellular damage. However, more impressive evidence gradually began to accumulate that the cerebral tissue could survive longer periods of ischemia if vascular patency was maintained. This concept gathered further support with the demonstration of microthrombus formation in capillaries during circulatory arrest; these thrombi can prevent perfusion of tissue even when the gross circulation is restored within a tolerable period.

Several agents have been administered in an effort to maintain capillary patency during temporary arrest of the circulation. These included the anticoagulants heparin and coumarin derivatives as well as osmotic agents such as Mannitol, urea, low molecular weight dextran, and hypertonic glucose. Fibrinolytic substances have been administered to obtain dissolution of the microthrombi in situ.

After review of the accumulated evidence from experimental studies, the fact emerges that heparin has been the only substance that has consistently provided prolonged brain survival. Even this protection has not been guaranteed by the standard amounts of heparin but remains highly dependent on massive doses.

With this fact in mind, we undertook the present experimental study to establish the efficacy of temporary and reversible massive anticoagulation therapy in the prevention of cerebral infarction from transient middle cerebral arterial occlusion during intracranial operations.

Materials and Methods

Twenty-five Rhesus monkeys (Macacus mulata), which weighed 2.5 to 4 kg each, were selected for this study. All animals were subjected to identical operative procedures. All were anesthetized with intravenous pentobarbital (30 mg/kg) and endotracheal tubes were passed to assure a patent airway. The Harvard pump respirator was

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present should be the need for respiratory assistance arise; however, it was required only on rare occasions.

As the initial step, the anterior neck was prepared and the carotid sheath contralateral to the intended craniectomy exposed through a short, oblique skin incision 1.5 cm superior to the clavicle. The common carotid artery and jugular vein were identified and isolated. The artery was doubly ligated. A polyethylene catheter was inserted into the jugular vein and positioned so that the tip lay in the superior vena cava. Patency of this central venous catheter was maintained by very slow infusion of Ringer's lactate. This catheter served both as the route for administration of all drugs and for withdrawal of blood specimens.

After closure of the cervical incision, the animals were positioned with the head rotated 45° toward the side of carotid ligation and slightly flexed. Craniectomy was carried out essentially as described by Sundt and Waltz with certain modifications. It was not found necessary to incise the temporalis muscle nor to remove the coracoid process of the mandible. A small trephine opening in the anterior margin of the temporal bone was widened by rongeurs. The orbit was entered at its lateral margin by rongeuring away the lateral sphenoid wing. The orbit roof was removed medially to the margin of the anterior clinoid and posteriorly to include the greater wing of the sphenoid. After adequate bony removal had been accomplished, the operating microscope was positioned (Zeiss Model 25124) and set to a magnification of 10. A spatula was inserted into the orbit and by retracting its contents downward only minimal retraction was necessary on the inferior surface of the frontal lobe. A No. 11 knife blade was used to make a small opening in the dura just lateral to the optic nerve. Small angle microscissors could then be used to enlarge this opening. The middle cerebral artery is quite apparent under the 10 magnification as it runs in the cleft between the frontal lobe and temporal tip. This was easily followed medially several millimeters until the internal carotid and anterior cerebral arteries were visualized. A sharp hook was used to strip the overlying arachnoid from the middle cerebral artery just distal to its origin. A small Mayfield clip was then applied to the vessel at this point and allowed to remain in place for the specified period. No attempt was made to suture the dura but the opening was covered with a small gel foam patch. The temporalis muscle was tacked to the pericranium to achieve stability and the scalp was closed in layers.

Blood was drawn on all animals for Lee-White clotting time determinations at the following stages of the operation: 1) at commencement of the craniectomy, 2) while the clip was in position, and 3) after completion of the surgical procedure. All animals were then allowed to recover from surgery. They were evaluated for alertness, appetite, and evidence of neurological dysfunction.

At 7 to 10 days postoperative the animals were sacrificed with large doses of pentobarbital. The brains were immediately removed, suspended in 10% buffered formalin, and allowed to fix. Following this the brains were coronally sectioned and evaluated for the presence and degree of infarction. Grading of infarction was performed according to the following scale:

Grade 0 = no infarction
Grade 1 = 1 cu cm or less brain tissue infarcted
Grade 2 = approximately 2 cu cm infarcted brain tissue
Grade 3 = approximately 3 cu cm (Fig. 1 A) infarcted brain tissue
Grade 4 = greater than 3 cu cm infarction or death as a result of infarction (Fig. 1 B).

After grading, representative sections were embedded sectioned at 6 μ and stained with hematoxylin and eosin. The stained slides were then examined microscopically to confirm the gross diagnosis of infarction.

Prior to surgery the animals were divided randomly into groups as follows:

Group 1 (Occlusion for 15 minutes, controls). Nine monkeys comprised this group. These animals had the standard operative craniectomy with temporary occlusion of the middle cerebral artery for 15 minutes. No anticoagulation was given.

Group 2 (Occlusion for 15 minutes, treated). Eight monkeys were included. In these animals heparin was administered through the central venous catheter, in a dosage of 5 mg/kg, 1 to 2 minutes prior to mid-
Middle cerebral artery occlusion. This allowed complete perfusion of the heparin before occlusion. After 15 minutes of occlusion, the Mayfield clip was removed. Immediately thereafter protamine sulfate was given intravenously until the clotting time was again within normal range. Initially protamine titrations were performed on each animal but as the heparin effect in this short period was uniformly reversed by milligram equivalent doses of protamine, this titration procedure was discontinued. The final clotting time was performed on all animals to assure that it had returned to a normal range (Table 1).

**Group 3 (Occlusion for 30 minutes, treated).** In these 8 monkeys heparin (5 mg/kg) was administered and reversed by protamine exactly as in Group 2. In this group, however, the occlusion of the middle cerebral artery was maintained for a period of 30 minutes.

**Results**

**Clinical Evaluations.** Two animals from Group 1 (no treatment) died as a result of massive infarction, one late on the first postoperative day and the other on the third postoperative day. Prior to death both animals displayed a dense hemiplegia on the side contralateral to the middle cerebral artery occlusion. They were unable to stand or to feed. Examination of both of these animals revealed massive acute infarctions with much swelling of the involved hemisphere and midline shift.

One animal in the 15 minute heparinized group (Group 2) had evidence of a third nerve palsy on the side of craniectomy. This was manifested by a ptosis and dilated pupil with retention of at least some ocular adduction. No infarction was found in this animal on pathological examination and the etiology of the nerve paresis was assumed to be the intraorbital surgical trauma.

Generally, the degree of clinical deficit paralleled the degree of infarction seen on pathological examination. Three animals in the non-heparinized group (Group 1) had significant degrees of hemiplegia and lethargy. They were found to have infarctions of grades 4, 4, and 2 respectively.

There were significant exceptions to this generalization. One control animal, subsequently proved to have a grade 3 infarction, and one animal in the 30-minute treated group, at postmortem examination, had a grade 4 infarction (Fig. 1B), but neither had more than a very minimal hemiparesis and both were quite alert postoperatively.

Due to these rather remarkable recoveries, with pathologically demonstrable large infarctions, it was concluded that clinical grading was not sufficiently accurate. Thus, final conclusions drawn from this study are based on pathological rather than clinical data.

**Pathological Evaluation.** In the untreated animals (Group 1), only one brain failed to show some degree of infarction (Table 2). Four of the animals in Group 1 sustained infarctions of grade 4. As mentioned earlier, two of these animals died as a result of their infarction. The mean infarction grade for these nine animals was 2.55 with a standard error of \( \pm 0.52 \). Examples of grade 3 and grade 4 infarctions are illustrated in Fig. 1A and B.

In the heparinized animals undergoing occlusion for 15 minutes, only one brain of the eight revealed any infarction and this was rated as grade 1. In this group the mean infarction grade was only 0.12.

The difference in the two groups (15 minutes occlusion without treatment versus 15 minutes occlusion with heparin at 5 mg/kg) was highly significant. Based on a non-paired \( t \) test this difference was significant at a level of \( p < 0.01 \). Since all the control animals experienced some infarction, it seemed apparent that animals having occlusion for periods longer than 15 minutes would simply manifest larger areas of infarction and previous studies have shown this to

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**TABLE 1**

*Range of clotting times (minutes)*

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Pre-operative</th>
<th>During Occlusion</th>
<th>Post-operative</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-min controls</td>
<td>4÷9</td>
<td>6–8½</td>
<td>6–7½</td>
</tr>
<tr>
<td>15-min treated</td>
<td>7–8½</td>
<td>&gt;1 hour</td>
<td>8–10</td>
</tr>
<tr>
<td>30-min treated</td>
<td>6–9</td>
<td>&gt;1 hour</td>
<td>7–10</td>
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</tbody>
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be true. However, the maximum period of time that such massive anticoagulation would offer significant protection is not known; therefore, a treated group with occlusion for 30 minutes was studied.

In the heparinized animals subjected to 30 minutes of occlusion (Group 3) only two of the eight brains showed infarctions. One of these, however, was grade 4 in magnitude (Fig. 1 B). This led to a mean infarction grade of 0.62±0.49 standard error. If this group is compared with the group of controls at 15 minutes, the difference is significant, with p<0.02. Also to be emphasized is the fact that there were no deaths in this group.

**Distribution of Infarction.** Prior to sectioning, all brains were grossly inspected for evidence of contusion at the operative site. Care was taken that operative damage was not mistakenly classified as infarction. The brain in Fig. 1 B demonstrates the large infarction that became obvious when the brain was sectioned.

In nearly all cases the infarction was primarily in the distribution of the deep perforating branches of the middle cerebral artery. Thus the putamen, head of the caudate nucleus, internal capsule, and insula were the structures most often infarcted rather than the convexity cortex (Fig. 1 A and B).

Exceptions to this did occur; one infarction (Fig. 1 C) extended primarily in the cortical distribution zones of the distal branches of the middle cerebral artery.

**Effects of Anticoagulation on Clotting Times.** The normal range of Lee-White clotting time on these monkeys fell in the range of 6 to 9 minutes as demonstrated by preoperative determination (Table 1). No significant change occurred in the clotting times when the middle cerebral artery was occluded without heparin administration. On the other hand, clotting times after heparin administration were tremendously prolonged and no specimen clotted within the first hour (Table 1). At the conclusion of the operative procedure, after protamine sulfate administration, all animals had clotting time determinations essentially within normal range (6 to 10 minutes).

**Effects of Anticoagulation on Surgical Blood Loss.** No problem was encountered with excessive bleeding in the anticoagulated animals. Care was taken that prior to heparinization all bone and scalp bleeding was controlled. No animals subsequently demonstrated bleeding from these previously controlled areas. As the effects of heparin were reversed prior to wound closure, no excessive bleeding was noted during this stage.

At the time of sacrifice all subgaleal, epidural, and subdural spaces were inspected for hematoma formation. No significant accumulation was found in any of the animals.

**Effects of Anticoagulation on Infarction Type.** It is interesting to note the absence of hemorrhage into the infarctions which did occur in the animals receiving heparin. All areas of infarction in both control and heparinized animals were entirely ischemic in nature rather than hemorrhagic.

**Discussion**

Various experimental models have been developed to produce cerebral infarction. Harvey and Rasmussen performed both temporary and permanent middle cerebral artery occlusions on Rhesus monkeys, and made extensive clinical and pathological evaluation of these animals. They found that occlusion of the middle cerebral artery for longer than 15 minutes produced at least microscopic infarction and clinical evidence of damage. Our pilot studies confirmed these findings, but in an effort to reduce times of
occlusion necessary to produce gross infarction, the contralateral carotid artery was ligated immediately prior to craniectomy. This served to decrease the over-all cerebral blood flow available via collateral channels and to make these studies more applicable to man. By utilizing this method we were able to produce gross infarction with temporary middle cerebral artery occlusion for only 15 minutes.

It was formerly felt that the marked susceptibility of cerebral tissue to ischemic damage was a quirk of its metabolism. The nervous tissue was thought to be incapable of performing anaerobic metabolism or utilizing substances other than glucose even in the presence of oxygen. Geiger, et al., however, demonstrated brain survival in cats after perfusion for 90 minutes with glucose-free solutions.

Various reports then began to appear suggesting that nervous tissue could survive longer periods deprived of oxygen, glucose, or even blood. In the usual clinical situation, however, ischemia of longer than 5 minutes duration continued to produce irreversible changes. This is thought to occur because in an acidic milieu as in circulatory arrest, the blood rapidly sludges and coagulates thus obstructing capillaries and permanently depriving the affected area of perfusion. This kind of intravascular coagulation has been shown to play an important role in the pathogenesis of several otherwise unrelated conditions such as endotoxin induced Schwartzman reaction, incompatible blood transfusions, and amniotic fluid embolization to mention only a few. More recently the concept was developed that intravascular coagulation is the determinant factor in irreversible shock or poor perfusion states regardless of its etiology. This mechanism, namely, hemoconcentration, sludging, and subsequent coagulation of capillary blood, has been demonstrated following complete circulatory collapse. More important, it has been found distal to arterial occlusion in several organs including the cerebral cortex. Thus, recent evidence strongly indicates that the initial step towards irreversible brain damage has been intravascular coagulation rather than metabolic failure.

Crowell, et al., by utilizing prior heparin anticoagulation, were able to increase the sur-

Fig. 1. Coronal brain sections which illustrate infarctions. A. Grade 3 infarction in a 15-minute untreated animal involving the basal ganglia and white matter with relative cortical sparing. B. Grade 4 infarction in a 30-minute heparinized animal with involvement of cortex as well as deep structures. Note the absence of hemorrhagic changes. C. Grade 4 infarction in a 15-minute untreated animal illustrating mainly cortical involvement with sparing of deeper structures.
vival of dogs after complete circulatory and cardiac arrest, presumably by thus preventing cerebral intravascular coagulation. They obtained similar results by use of a fibrinolytic. However, the many reported post-operative bleeding complications would not encourage clinical trials with this agent. Recent work by Ames, et al., emphasizes the importance of this microvascular occlusion in the pathogenesis of cerebral infarction. By utilizing a technique of perfusion with a carbon suspension they demonstrated the non-patency of cerebral capillaries immediately following ischemic episodes. Prior anticoagulation with heparin, in their series, seemed to offer protection to the cortical surface but not to deeper areas. Also electron microscopy in these areas suggested that bleb formation by the capillary endothelial cells rather than intravascular coagulation may be the mechanism of obstruction. As they note, however, it remains a very real possibility that these changes represent fixation artifacts. This appears quite likely since, whatever the underlying pathology, perfusion of these areas was delayed and thus hindered fixation.

Further evidence that infarction represents primarily a microthrombotic problem has been added by Neely and Youmans. They produced total exsanguination of the nervous system in dogs by elevation of cerebrospinal fluid pressure to a level of 400 mm of mercury. Animals subjected to this complete ischemia for 25 minutes or less all recovered and demonstrated fairly good cerebral function. While this does not offer conclusive proof, it does suggest that the capillaries do not become permanently occluded in the absence of blood. While observing the micro-circulation, Meyer noted the previously described hemoconcentration, sludging, and thrombus formation in the small capillaries. According to his observations, anticoagulants such as heparin and coumarin tended to prevent the adherence of erythrocytes and platelets both to themselves and to the vessel wall. All of these studies indicate that anticoagulation serves to delay thrombus formation in the small vessels and thus prevents the initial step towards irreversible brain damage.

For application in neurosurgical problems the coumarin derivatives remain entirely too cumbersome. Some of the obvious problems are the delay in onset of anticoagulation and in reversibility. Anticoagulation with heparin may be immediately attained and just as rapidly reversed with protamine sulfate. As shown by our experiences this can be done without troublesome bleeding from concurrent surgical wounds. Thus by administering heparin, temporary occlusion could be more safely utilized. This would allow such procedures as control of aneurysmal bleeding, encaement of an aneurysm, or endarterectomy of a cerebral vessel to be carried out in a dry surgical field where temporary clips have been applied to the vessels under operative attack. The protamine necessary to reverse the effects of heparin before closing the craniotomy was constantly found to be equivalent in milligrams to the heparin dosage given. The milligram-to-milligram relationship holds for short periods (less than 1 hour) because there is no significant metabolism of the drugs in such brief periods. Even though in the experimental animals there were no coagulation deficits nor bleeding problems, we feel that in the clinical situation protamine titrations should be available.

The dosage of heparin necessary to prevent intravascular coagulation effectively was previously shown to be quite high. It was found that amounts less than 5 mg/kg of body weight were not effective. This was, consistently found to be the smallest effective dosage in our initial studies and was thus chosen as the standard level for the purposes of this study. The usual clinical dosage necessary to achieve routine anticoagulation is approximately 100 to 150 mg, administered each 6 to 8 hours. This is about 1.5 to 2.0 mg/kg. For the purposes discussed in this study the required clinical dosage would then be 350 to 400 mg (5 mg/kg) given intravenously. Following restoration of flow in the operated vessel, 350 to 400 mg of protamine intravenously would then serve to return the coagulation to normal immediately.

When the intravascular thrombosis occurs, the initial event seems to be a hemoconcentration secondary to loss of fluid from these capillaries. On this basis others have tried to reverse the entire process by utilizing osmotic agents such as Mannitol,
urea, and hypertonic glucose. The literature reveals conflicting information pertaining to the use of Mannitol. Earlier studies seemed to indicate that this substance did not offer any protection. Our current studies seem to indicate less infarction in animals given Mannitol. This is in keeping with current information obtained from other investigators indicating some benefit from both Mannitol and hypertonic glucose. This protection is not, however, as significant as that afforded by heparin.

The protection by massive heparin anti-coagulation against infarction from temporary vascular occlusion seems definite. In the present experimental model, which consistently produced infarction, heparinized animals experienced much less infarction than controls after 15 minutes of occlusion (p < 0.01). After 30 minutes of occlusion, the heparinized animals again showed significantly less infarction (p < 0.02) when compared to even the 15-minute controls. Despite this improvement, in instances of prolonged occlusion serious infarction can occur, as shown by a grade 4 infarction in one animal. It is also of considerable importance that even when infarction did occur in the temporarily heparinized animals this was not associated with hemorrhage. All infarctions, both in controls and treated animals, were entirely ischemic in nature.

Heparin is easily administered, and has been previously shown to be safe in other surgical situations. Its use for certain neurosurgical problems may well provide the extra minute occasionally required to save cerebral tissue. In view of the encouraging experimental findings, the clinical application of this method deserves serious consideration.

Summary

By using a well-defined and proven experimental model of middle cerebral artery occlusion without trauma to the brain, we found that at 15 minutes of temporary occlusion, cerebral infarction regularly occurred. Massive doses of heparin given just prior to temporary occlusion, however, can prevent infarction. This protection extends up to 30 minutes in many animals. The anti-coagulation effect can always be safely reversed by the appropriate use of protamine sulphate.

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