The effect of heparin reversal after carotid endarterectomy in the dog

A scanning electron microscopy study

WILLIAM F. CHANDLER, M.D., MARK S. ERCIUS, M.D., JOHN W. FORD, VIRGINIA LABOND, AND WILLIAM E. BURKEL, PH.D.

Section of Neurosurgery and Department of Anatomy, University of Michigan Medical Center, Ann Arbor, Michigan

The purpose of this study was to determine if total reversal of heparin immediately after carotid endarterectomy would have an adverse effect on the thrombogenicity of the endarterectomized vessel wall. After systemic heparinization, unilateral common carotid endarterectomies were performed under the operating microscope on 14 dogs. Half of the animals were given protamine sulfate to reverse the heparin. Three hours after resumption of blood flow, these arteries, as well as contralateral vessels used as controls for fixation technique, were perfused with glutaraldehyde and prepared for scanning electron microscopy (SEM). Thrombin clotting times were measured throughout the experiments. Sections of the endarterectomized portions viewed by SEM showed nearly total coverage of the exposed collagen of the media with flattened platelets. There were scattered leukocytes, but few erythrocytes, little fibrin, and no true thrombus. There were no differences between the animals that received heparin reversal and those that did not. A group of five additional arteries underwent the same procedure except that no heparin was given. As expected, large amount of thrombus had formed within the lumina of these control vessels by 3 hours. Since previous studies suggest that arterial thrombosis usually occurs within 3 hours of endothelial injury, the authors conclude that total reversal of heparin does not increase thrombogenicity of the endarterectomized vessel. This suggests that heparin may be safely reversed in patients to help maintain postoperative hemostasis.

KEY WORDS • carotid artery • endarterectomy • heparin • thrombosis • scanning electron microscopy

Carotid endarterectomy is a widely used and generally accepted surgical procedure for the treatment of transient ischemic attacks and the prevention of stroke. Although early postoperative mortality (0.6% to 7%) and morbidity (0.6% to 14%) are low, when they do occur they are usually due to cerebral ischemic events. Many of these events are related to early postoperative thrombus formation at the site of endarterectomy, causing either arterial emboli or occlusion. Anticoagulation with heparin is now widely used intraoperatively and, as is the practice in most vascular operations, is usually reversed with protamine sulfate at the end of the procedure. In an attempt to reduce postoperative thrombus formation and subsequent morbidity and mortality, some authors have recently advocated continued postoperative anticoagulation by not reversing the heparin at the termination of the procedure. Since there are no convincing data, either clinical or experimental, in vessels the size of the human internal carotid artery to support this contention, and since this clearly increases the risk of postoperative hemorrhage and wound hematomas, we believe that more evidence is necessary before inactivating the natural coagulation process in the early postoperative period.

To provide this evidence, we have used the scanning electron microscope to study the early healing process of the arterial wall after an endarterectomy of the
canine common carotid artery. A group of dogs was first studied without heparinization as a control group. The remaining dogs were fully heparinized before endarterectomy. Half of those dogs then had the heparin reversed with protamine sulfate following the endarterectomy and half did not.

**Materials and Methods**

Seventeen dogs, weighing 29 to 40 kg, were anesthetized with intravenous pentobarbital, 25 mg/kg, and placed on controlled endotracheal ventilation with room air. Of the carotid arteries available from the animals, 19 underwent endarterectomy and were divided into the following groups:

Group A: Non-heparinized control arteries: five arteries from three animals in which no heparin was given.

Group B: Heparin without reversal: seven arteries from seven animals that were fully heparinized with 145 units/kg of heparin prior to endarterectomy, and the heparin was never reversed.

Group C: Heparin with reversal: seven arteries from seven additional animals that received full heparinization, which was reversed with protamine sulfate, 1 mg/100 units of heparin administered beginning 10 minutes after the endarterectomy.

Several of the remaining normal unoperated vessels were harvested simply to serve as controls for our fixation techniques.

The common carotid artery was exposed and heparin was given if appropriate for that animal's group. A 3-cm portion of artery was isolated with vascular clamps, and a 2-cm arteriotomy was performed. Two transverse incisions were then made in the intima, and a subintimal plane was developed using the operating microscope at low magnification. The intima and a portion of the underlying media were carefully excised. The surface was then irrigated with heparinized saline, and the vessel was closed with 6-0 Proline suture. The artery was then unclamped, blood flow resumed, and protamine sulfate given to the group with heparin reversal. Three hours after resumption of blood flow, 8-cm sections of the vessels were isolated with 2-0 silk sutures. Proximal and distal No. 14 catheters were placed and the isolated segments flushed with buffered normal saline. Phosphate-buffered (pH = 7.4) 2.5% glutaraldehyde was then perfused for 30 minutes at 100 mm Hg pressure. The vessels were then harvested and stored overnight in cold buffered 2.5% glutaraldehyde. They were then dehydrated in a graded series of ethanols and critical-point dried with CO₂. After placing these specimens on aluminum stubs, they were coated with a 300 to 400 Å layer of gold using a Polaron sputter coater* and examined with the AMR 1200 scanning electron microscope (SEM).†

Thrombin clotting times (TCT's) were measured to determine heparin units at two dogs with reversal and three dogs without reversal to insure adequate anticoagulation and reversal. Femoral vein samples

---

† AMR 1200 scanning electron microscope manufactured by Amray, Bedford, Massachusetts.
were drawn before and immediately after heparinization and at regular intervals thereafter.

Results

Group A: Non-Heparinized Controls

All of the endarterectomized vessels from the animals without heparin had gross thrombus within their lumina. The thrombus was easily identifiable without magnification, and a number of the vessels were nearly occluded. The composition of the thrombus was confirmed with SEM and consisted of fibrin, platelets, red blood cells, and some leukocytes.

Group B: Heparin Without Reversal

The SEM's revealed that adequate removal of the intima exposing the collagen of the media was achieved in all vessels operated on. All of the vessels were patent. Their typical appearance was a monolayer of flattened platelets adherent to the endarterectomized surface (Fig. 1 left). Occasional areas of medial collagen were visible between the platelets (Fig. 1 right); however, nearly total coverage was typical. The platelet monolayer was essentially devoid of fibrin and thrombus, although small focal areas of red thrombus were occasionally seen near the suture line. Leukocytes were regularly found on the platelet carpet (Fig. 2).

Group C: Heparin With Reversal

These surfaces appeared identical to the vessels in Group B. Once again, a monolayer of flattened platelets with essentially no fibrin or thrombus was present. Leukocytes were again found on the platelet carpet.

It should be emphasized that no difference was detected between the groups with and without heparin reversal. Fibrin and thrombus were rarely seen in either group.

The unoperated vessels as well as the endothelium proximal and distal to the endarterectomized surfaces served as controls for our fixation technique. Normal endothelium without thrombus was seen (Fig. 3). Leukocytes were sparsely adherent to the endothelium.
Anticoagulation Studies

Measurement of TCT's was performed to determine heparin units by the method of Penner in five heparinized dogs. Samples drawn immediately following heparinization revealed a range of heparin units between 0.5 and 2.7, well above the therapeutic level of 0.2 heparin units. These values decreased steadily, and the animals without heparin reversal remained therapeutically heparinized for 1.88 to 3 hours after administration. Animals given protamine sulfate were shown by TCT to have immediate reversal to zero heparin units.

Discussion

Various authors have reported early arterial occlusion rates after carotid endarterectomy ranging from 0.3% to 11%, with most reported rates distributed throughout the lower half of that range. Although these occlusions were not always associated with morbidity, they were often linked with neurological deficit or death. There is considerable clinical and experimental evidence that postoperative thrombus formation and occlusion occur very early after blood flow is resumed through an artery injured by endarterectomy. Much of the initial reaction occurs within minutes (as discussed below), and thrombosis rarely occurs after the first few hours. Because of this predictable reaction to arterial injury, we selected a time of 3 hours after endarterectomy to observe with the SEM the hematological response to the injured vessel. We anticipated that the prolonged anticoagulation effect of unreversed heparin in Group B arteries would provide a cleaner arterial wall with less fibrin and thrombus formation than would the immediate neutralization of heparin with protamine sulfate in Group C. In fact, the results showed no difference between the two groups. None of the heparinized animals exhibited any significant formation of fibrin or thrombus. There was only the homogeneous appearance of flattened platelets with scattered leukocytes. This is similar to the SEM appearance after endarterectomy described by others, with the exception of less fibrin attached to the platelets. Our non-heparinized control (Group A) arteries confirmed what has been shown previously in the dog, that heparinization is necessary during the endarterectomy to prevent thrombus formation.

The canine common carotid artery was selected for two reasons. First, it is comparable in size to the human internal carotid artery, which is where most postoperative occlusions occur. Second, the temporal profile of the hematological response from 30 minutes to 3 months after endarterectomy has been studied extensively by Dirrenberger and Sundt. Their studies showed that no new thrombus was formed after 4 hours postendarterectomy, forming the basis for our hypothesis that the hematological reaction seen at 3 hours is indicative of the long-term response. It should be pointed out that the surface of the normal canine carotid artery may differ in thrombogenicity from the same surface in an atherosclerotic human carotid artery. We believe, however, that this model does allow exposure of the highly thrombogenic collagen of the media, which is similar to the clinical situation.

The initial hematological response to an injured or endarterectomized artery has been well studied and is reasonably predictable. After exposure of the media, thrombus formation is immediately initiated by platelet adhesion. Collagen has been shown to be one of the most potent platelet stimulators present in the vessel wall. Platelet adhesion is followed by a change in platelet morphology called "platelet transformation." During this process, the usually discoid platelets become swollen spheres and begin extending pseudopodia (Fig. 4). Pseudopods lengthen and the platelets spread or flatten, forming a monolayer closely opposed to the surface beneath.

A sequence of events collectively called the release reaction or degranulation occurs during transformation. Substances including calcium, serotonin, fibrinogen, lysozyme enzymes, and most importantly adenosine diphosphate (ADP) are expelled. The ADP induces platelet aggregation or the clumping together of sticky platelets. Besides ADP, other aggregation inducers include Factor Xa, thrombin, serotonin, and epinephrine. The transformation process also unmaskes platelet Factor 3, a powerful catalyst which along with Factors V and Xa induces thrombin formation, ultimately producing fibrin. Fibrin stabilizes platelet aggregation, promoting thrombus.
Heparin reversal after endarterectomy in dogs

The platelet carpet or monolayer covers the exposed medial collagen rapidly. Baumgartner and Muggli,3,6 using rabbit subendothelimum, demonstrated that the platelet monolayer begins forming immediately and is complete within 10 minutes of injury. They called this platelet carpet a "pseudoendothelimum;" it renders the surface nonthrombogenic or, as stated by Moseley, et al.,28 antithrombogenic.

Thrombin induces fibrinogen to form fibrin. Antithrombin 3 is a natural inhibitor of thrombin and, therefore, inhibits fibrin formation. The anticoagulant, heparin, accelerates antithrombin activity 50- to 100-fold,37 decreasing fibrin production. There is also evidence that heparin inhibits Factors IX, X, XI, and perhaps VII as a function of their serine moieties.36 Heparin may have an antiplatelet effect,32,46 but it has also been reported to have platelet-enhancing activity.47

Our specimens from the heparinized arteries in Groups B and C, as previously mentioned, had very little fibrin and few platelet aggregates. It has been suggested that if fibrin is not present to stabilize platelet aggregates, they are washed away.6 Heparin, therefore, seems to allow the platelet carpet to form but inhibits platelet aggregate stabilization. The non-thrombogenic pseudoendothelimum remains, allowing the vessel surface to reendothelialize without further risk of thrombosis.

The duration of action of heparin is dose-dependent. Collins, et al.,10 reported that in humans the half-life of an intravenous bolus of 150 units of heparin/kg is 65 minutes, and it is 50 minutes for a bolus of 100 units of heparin/kg. Commonly, heparin reversal is achieved with 1 mg protamine sulfate/100 units of heparin administered. Protamine sulfate, a strong base, combines with the strongly acidic heparin to achieve neutralization. Protamine sulfate is an anticoagulant itself, and, therefore, overdosage is undesirable. Collins, et al.,10 correctly advised that, within 60 minutes of heparin administration, 0.5 mg of protamine sulfate/100 units of heparin administered easily suffices for complete reversal.

In conclusion, we believe that there is convincing experimental and clinical evidence that systemic heparinization should be used during a carotid endarterectomy.10,11,16,24,32,33 Our current study does not support the contention that intraoperative reversal of heparinization results in increased thrombus formation in an artery the size of the internal carotid artery. There appears to be a brief critical period of 10 to 15 minutes following resumption of blood flow when heparin may be helpful,36 and we plan to study this time period in more detail.

References


22. Hattori A, Watanabe T, Izumi T: SEM study on he-
mmostatic reaction. Mural thrombus after the removal of endothelium, with special references to platelet behavior, site of fibrin formation and microhemolysis. Arch Histol Jpn 41:205–227, 1978


Manuscript received December 1, 1980.
Accepted in final form August 11, 1981.
Address reprint requests to: William F. Chandler, M.D., University Hospital, Section of Neurosurgery, Outpatient Building, C5068, 1405 East Ann Street, Ann Arbor, Michigan 48109.