Effect of Anticoagulants and Inhibitors of Platelet Aggregation on Thrombotic Occlusion of Endarterectomized Cat Carotid Arteries

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SUMMARY Cat carotid arteries measuring 1.8 to 2 mm in diameter were endarterectomized under the operating microscope over a 1-cm segment and the arteriotomy was closed with a 9-0 monofilament nylon suture. Vessels exhibiting significant narrowing of the lumen due to faulty closure of the arteriotomy were excluded from the study. The vessels were divided into six groups according to the method of treatment of the animals: control, aspirin, Coumadin, Coumadin plus aspirin, heparin for less than four hours, and heparin for four to eight hours. All vessels in the untreated group subjected to simple arteriotomy and closure remained patent. Only heparin demonstrated an apparent beneficial effect after endarterectomy with 100% of the vessels treated more than four hours and 30% of those treated less than four hours remaining patent. This is contrasted to a 0% patency in other endarterectomized vessels.

THE USE OF THE OPERATING MICROSCOPE with attention to the details of techniques, including meticulous handling of the vessel intimal lining, has permitted successful surgery on vessels as small as 1 mm in diameter. However, experimental stripping of the intima and internal elastic lamina (endarterectomy) of arteries with an external diameter of less than 2 mm has been followed by an exceedingly high incidence of thrombosis. There are no known agents that have been demonstrated to be totally effective in blocking arterial thrombosis; however, varying degrees of protection have been afforded by a variety of agents including anticoagulants, fibrinolysin, clinical and low molecular weight dextran, and, more recently, compounds that inhibit platelet function. Each of these in theory might afford protection against thrombosis by interfering with a distinct phase of the thrombotic process.

The purposes of this study are (1) to examine the occurrence of thrombosis after endarterectomy in arteries less than 2 mm in diameter, (2) to evaluate the protection against thrombosis by using several select agents including Coumadin, aspirin, Coumadin in combination with aspirin, and heparin, and (3) to determine, if possible, with the aid of histological evidence, the effect of these agents on the thrombotic process.

Methods

Male and female cats weighing 2 to 4 kg were used. By direct measurement in many animals the carotid arteries in the neck consistently measured 1.8 to 2 mm in external diameter. All endarterectomies were performed by one of us (D. G. P.).

Technique of Endarterectomy

With the animals under anesthesia from pentobarbital administered intraperitoneally, the carotid artery was exposed in the neck and measured with calipers; a 2-cm segment was then isolated between miniature Mayfield vascular clips. An operating microscope was used to perform longitudinal arteriotomy measuring 1.3 to 1.5 cm and the vessel walls were separated by three or four traction sutures of 9-0 monofilament nylon.

Two transverse incisions, 1 cm apart, were then made through the intima with a No. 11 surgical blade. A plane of subintimal dissection was established and the intima, internal elastic lamina, and some of the media were removed from the entire 1-cm segment between the transverse incisions with a small dissecting spatula. This technique gave a relatively constant plane of dissection, verified by histological examination, which split the medial layer and left a reasonably smooth endarterectomized surface. Proximal or distal intimal flaps were not created by this method of endarterectomy.

The vessel was then irrigated with physiological saline solution and the arteriotomy closed with a running suture of 9-0 monofilament nylon. Care was taken to avoid compromising the arterial lumen, and after removal of the clip with resumption of flow, the diameter of the endarterectomized segment frequently was greater than that of the unoperated artery. Bleeding at the suture line was usually minimal and controlled with temporary application of a Gelfoam or cotton pledget. Those vessels showing a significant degree of stenosis after closure due to faulty technique were excluded from the study.

Vessel Examination

Immediately after resumption of flow the vessels were observed closely for evidence of thrombosis. The color and turgid state of the operated segment usually made it readily apparent when thrombosis with occlusion had occurred. If the segment appeared definitely thrombosed immediately after the operation, it was removed at that time. Those segments that appeared to remain patent were reexamined and removed at a later time — up to four weeks after endarterectomy. In each segment, patency at the time of removal was determined by the presence or absence of proximal to distal flow on incising the artery above the site of endarterectomy (after placement of a distal clip to prevent backflow). All segments were saved for histological examination and stained with hematoxylin and eosin (H & E), Lawson's elastic van Gieson (ELVG), phosphotungstic acid-hematoxylin (PTAH), and toluidine blue.

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This investigation was supported in part by Grant NS-6663 from the National Institutes of Health, Public Health Service.
Experiments

Group 1 (Simple Arteriotomy)

Simple longitudinal arteriotomy and closure were performed in six vessels. Endarterectomy was not done. The vessels were reexamined for patency after intervals of one to ten days.

Group 2 (Controls)

Twelve arteries were included in the control group after satisfactory endarterectomy and closure. No medications were administered before or after the operation.

Group 3 (Aspirin Pretreatment)

Endarterectomy was performed in seven vessels in which the animals had been pretreated with aspirin (acetylsalicylic acid USP, Aldrich). Dosage was 10 mg per kilogram of body weight and was administered orally in a single dose 12 hours before endarterectomy. Blood specimens were drawn from two of the aspirin-treated animals at the time of endarterectomy for in vitro studies of platelet aggregation by collagen.9

Group 4 (Coumadin Pretreatment)

Three animals were treated with orally administered sodium warfarin (Coumadin, Endo) in dosages proportional to adult human beings by body weight. Prothrombin times were measured daily and, when two to four times the control level, endarterectomy was performed on the six carotid arteries. With all animals, treatment extended over three to seven days before operation and included two to five doses of Coumadin.

Group 5 (Aspirin-Coumadin Pretreatment)

Three animals were treated with Coumadin according to the previously outlined schedule. In addition, 12 hours before operation they were given a single oral dose of aspirin (10 mg per kilogram of body weight). Endarterectomy was then performed on the six carotid arteries.

Groups 6 and 7 (Heparin Treatment One to Four Hours and Four to Eight Hours)

Sixteen animals were treated with heparin (sodium heparin, 1,000 USP units per milliliter, Fellows) administered intravenously via a femoral vein catheter in a dosage of 100 U per kilogram of body weight. The first dose of heparin was given in the late stage of arterial repair after endarterectomy and approximately five minutes before removal of the vascular clips. Clotting times were measured by a modified Lee-White technique before administration of the first dose and every half hour thereafter for the duration of anticoagulation. To maintain anticoagulation, the heparin dose was repeated when clotting times fell below three to five times the control level, the latter ranging from two to five minutes in all animals.

Duration of heparin anticoagulation varied from one to several hours, and Group 7, those in which anticoagulation was maintained for four to eight hours.

Results

Simple Arteriotomy (Group 1)

The numbers of patent and thrombosed vessels in each experimental group are depicted in figure 1. Of the six vessels subjected to arteriotomy, all were patent when examined one to ten days after operation. Microscopically, the specimens removed after one and two days revealed some accumulation of platelet and fibrin (thrombus) at the site of arteriotomy and suture but this was not enough to occlude the lumen. Specimens removed seven and ten days after arteriotomy showed little or no thrombosis.

Endarterectomy (Groups 2, 3, 4, and 5)

The occurrence of thrombosis in the control and experimental groups exclusive of those treated with heparin (i.e., endarterectomy alone and endarterectomy after treatment with aspirin, Coumadin, or aspirin plus Coumadin) was virtually 100%. Only three vessels were flow-patent (two in the control group and one in the aspirin-pretreated group), each on examination in the first 15 to 30 minutes after the completion of endarterectomy. The state of these vessels in relation to the time of this examination after endarterectomy and restoration of flow is presented in table 1. It is immediately apparent that the number of patent vessels in these experimental groups is small, 3 of 31, with these three occurring only in the first 30 minutes after restoration of flow. Furthermore, subsequent microscopic examination of these three flow-patent vessels demonstrated them to be occluded with masses of platelets, an evidence of impending thrombosis.

Histological examinations of the endarterectomized segments in each of these groups demonstrated uniform
findings. The depth of endarterectomy was similar in all vessels with the intima, internal elastic lamina, and part of the media excised. All vessels, including the three aforementioned flow-patent vessels, demonstrated occlusion of the lumen by typical thrombi with platelet masses adhering to the denuded vessel wall and arteriotomy site. Fibrin likewise was present in all of the thrombi, even in those animals pretreated with Coumadin and adequately anticoagulated as determined by elevation of the prothrombin time.

In essence, thrombosis was a uniform occurrence after endarterectomy in Groups 2, 3, 4, and 5 with obstruction to flow in all but three vessels, and in those it was impending. Furthermore, thrombotic occlusion occurred rapidly, within the first half hour after restoring flow through the endarterectomized segment. No protection against thrombosis was afforded by pretreatment with aspirin, Coumadin, or a combination of aspirin and Coumadin, nor did these drugs alter the histological picture of the thrombus. That the dosage of aspirin used in the experiment was effective in blocking in vitro collagen-induced platelet aggregation was confirmed in two animals pretreated with aspirin.

### Endarterectomy (Groups 6 and 7) (Heparin Treated)

Of 13 vessels subjected to endarterectomy and heparin anticoagulation for one to four hours, four (30%) were patent when examined 1 to 30 days later. The patency rate of the 13 vessels from animals anticoagulated with heparin for four to eight hours after endarterectomy was 100% on examination five hours to 20 days after operation. The state of the vessels, duration of heparin therapy, and time of examination are illustrated in figure 2.

Microscopic examination of these vessels showed that the depth of endarterectomy was similar to that of the previously mentioned groups. The thrombi in vessels of animals anticoagulated with heparin for four hours or less were composed largely of platelets with varying amounts of fibrin present. One vessel from an animal receiving heparin anticoagulation for four hours and having sluggish flow when opened six hours after endarterectomy was essentially thrombosed microscopically.

In the first hours after endarterectomy, a typical finding in the patent vessels was a thin circumferential deposit of platelets on the endarterectomized surface of the lumen (fig. 3). In subsequent days, an amorphous layer developed over the exposed media; subsequently, this layer became organized and two to three weeks after endarterectomy the media was covered with a new endothelial lining (fig. 4). Also, by the end of the third week a definite circumferential elastic lamina could be identified below the neointima (fig. 5).

### Discussion

Abnormalities in blood flow, changes in the vessel wall, and changes in the blood itself (Virchow's triad) have long been recognized as factors predisposing to thrombosis. Thrombosis rarely results unless more than one of these changes exist.

In the normal vessel with undisturbed flow, platelets carried by the blood come into frequent contact with one another, other cells, and, in time, the vessel wall, but such contact does not lead to adherence or aggregation. In vitro studies by Yu and Goldsmith and by Leonard et al. indicate that platelets interact with one another both in flowing whole blood and in platelet-rich plasma. Whereas these interactions most probably also occur in vivo, a common observation is that aggregation or adhesion does not occur in the presence of normal intima. Disturbed flow (e.g., vortices, eddies), produced by mechanical disarrangements such as atherosclerotic stenosis or surgical alteration of geometry, potentiates adherence. This potentiation may be caused by local accumulation of activated clotting factors, by enhanced residence times of platelets trapped in these regions of relatively slow flow, or by both.

The changes in the vessel wall, particularly when as severe as produced with endarterectomy, appear to be paramount in predisposing to thrombosis. An intact vessel wall with normal endothelium will not initiate either coagulation of blood or adhesion of platelets. A disrupted endothelial surface, however, exposes subendothelial elements, namely, collagen fibers, basement membrane, and microfibrils of elastic tissue, to which platelets adhere. Adherence is associated with or initiates the release from platelets of various components, the most important of these being adenosine diphosphate (ADP), which induces circulating platelets to cohere to one another and to those already adherent.

Simultaneous activation of the coagulation system by tissue thromboplastin, also released from the site of vascular injury, leads to deposition of fibrin strands, which permeate
and encapsulate the accumulating platelet aggregates, thereby strengthening them against the eroding forces of the circulating blood.

Based on this understanding of the genesis of thrombus formation, attempts at preventing thrombosis after endarterectomy, as in this study, might logically be directed toward prevention of the initial platelet adhesion and aggregation at the endarterectomy site, or inhibition of

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**Figure 3.** Left: Carotid artery of cat nine hours after endarterectomy and heparin anticoagulation maintained for six hours. Elastic fibers of media are exposed to lumen and covered with a poorly stained deposit of platelets. (ELVG, X 400.) Right: Same specimen stained to demonstrate better the platelet deposits (arrows). (PTAH, X 400.)

**Figure 4.** Left: Carotid artery of cat two days after endarterectomy and heparin anticoagulation maintained for five hours after operation. Arrow indicates amorphous layer developing over previously exposed media. (ELVG, X 400.) Right: Carotid artery of cat two weeks after endarterectomy, after which heparin anticoagulation was maintained for six hours. Note thick organized layer of new intima covered with neointima (arrow). (PTAH, X 400.)
fibrin deposition and stabilization of platelet aggregates, or both.

As indicated by the 100% patency rate in those vessels subjected to arteriotomy alone, the presence of a foreign surface, such as the suture, and minimal narrowing of the lumen caused by closure were not sufficient to produce an occluding thrombus. Endarterectomy, however, was followed by thrombosis in virtually 100% of the control vessels. This was not unexpected. Noteworthy was the intensity with which this process occurred, occlusion being complete within 15 to 30 minutes after resumption of blood flow. From this observation alone it appears that if therapeutic agents are to be successful in preventing thrombosis in endarterectomized small arteries, their effect must be present from the moment of, or even prior to, the restoration of blood flow.

The thrombi formed in the endarterectomized control vessels were histologically similar to those experimentally produced by other investigators, being composed primarily of platelet masses. Based on such findings and the knowledge that the initial phase of arterial thrombosis is platelet adhesion and aggregation, certain investigators have attempted to afford protection against thrombosis through the use of various inhibitors of platelet function. Some success has been reported with aspirin, dipyridamole, sulfinpyrazone, and prostaglandin E. The mechanism of action of each of these compounds on platelets differs. Aspirin inhibits collagen-induced platelet aggregation; it also blocks the platelet-aggregating effects of antigen-antibody complexes and low concentrations of thrombin. However, it does not prevent aggregation by exogenous ADP, suggesting that its effect on platelets is due to its inhibition of the release of endogenous platelet ADP. A single small oral dose of aspirin quickly produces this inhibition, which is maintained throughout the life of the platelets. The dose of aspirin used in this study was equivalent to an average dose for humans and was of a magnitude known to have the desired inhibitory effect on platelet aggregation. The latter was documented in our study by demonstrating in vitro inhibition of collagen-induced platelet aggregation in two animals pretreated with aspirin.

Aspirin has been and is being widely used in clinical trials for the prevention of both arterial and venous thromboembolism but results have been variable. Results with sulfinpyrazone or with aspirin-dipyridamole or warfarindipyridamole have been more promising than those with aspirin alone. Aspirin alone failed to reduce the incidence of arterial thrombosis or of pulse reduction in patients undergoing catheterization of the brachial artery. The failure of aspirin to protect against thrombosis in this study probably was due to the magnitude of the injury imposed by endarterectomy relative to the size of the vessel lumen. Such an extensive area of denuded vessel wall exposes a large area of media with components — particularly collagen — that are known to initiate platelet reaction. If this reaction were indeed blocked in vivo by the aspirin pretreatment, platelet adhesion and aggregation still might proceed due to ADP liberated from the injured tissue. In addition, liberated tissue thromboplastin
would induce activation of the unaltered coagulation mechanism with fibrin deposition to stabilize accumulating platelets.

Coumadin sufficient to prolong prothrombin times two to four times control also showed no protective effect against thrombosis. Hladovec had similar results in electrically provoked thrombosis in rats. This is not surprising because at these prothrombin time levels Coumadin has been a relatively ineffective antithrombotic agent.21-23 has no effect on platelet function,29 and, at lower levels, may enhance formation of thrombi.28

In theory, an agent or combination of agents that inhibit both platelet aggregation and coagulation should be effective against arterial thrombosis.29 However, in this study, pretreatment with combined aspirin and Coumadin was totally ineffective in preventing thrombosis after endarterectomy. Endarterectomy seemed to provide concentrations of ADP, collagen, and tissue thromboplastin (relative to platelets and plasma-coagulation factors flowing over the subendothelial surface) in excess of the counteracting capacities of aspirin and Coumadin in the doses and on the schedules they were given.

In contrast, heparin, especially when anticoagulation was maintained for four to eight hours after operation, was highly effective in preventing thrombosis in our study. Heparin inhibits fibrin formation by sharply accelerating the rate at which plasma antithrombin neutralizes thrombin. Heparin also increases the level of inhibitor to Factor Xa (a potent thrombogenic intermediate in the coagulation cascade) and does so at doses far less than those required to enhance the neutralization of thrombin. This fact has provided the rationale for therapy with small doses of heparin to prevent postoperative venous thrombosis.30 Theoretically, such a regimen should provide antithrombotic activity for many hours after each small dose of heparin even though the coagulation time remains normal during most of the period of therapy. The current concept is that heparin activates antithrombin, which neutralizes not only thrombin and Factor Xa but also Factor IXa, Factor XIa, and perhaps all the other serine proteases of the clotting mechanism as well.29 Heparin’s inhibition of coagulation at so many steps in the cascade (which Coumadin does not affect) could explain in part its prolonged antithrombotic action long after the coagulation time has returned to normal, as in our experiments.

Heparin also possesses inhibitory activity against platelet aggregation induced by collagen,31 thrombin,32 and Factor Xa.33 In low doses, heparin enhances fibrinolysis34,35 and in very high doses it depresses platelet adhesiveness (retention by a column of glass beads).36 Coumadin possesses none of these effects.

In this study the modest degree of protection against thrombosis afforded by heparin therapy of four hours or less contrasted sharply with the 100% patency rate when heparin anticoagulation was maintained for more than four hours. It is unlikely that the protection against thrombosis was due to heparin’s inhibitory effect on platelet adhesiveness and aggregation as described above, inasmuch as these phenomena have been observed only with high concentrations of heparin30,44 that would not be reached by the dosage used in this study. Furthermore, Spaeet and Zucker44 have demonstrated platelet adhesion and aggregation even at high concentrations of heparin.

A more likely mechanism would be related to the multiple effects of heparin on the coagulation mechanism. In the control and nonheparin-pretreated animals, thrombotic occlusion was an early event. However, when heparin anticoagulation was continued for more than four hours, all vessels remained patent, many of them over prolonged periods of observation. It appears that, in this model, the first several hours comprised a critical period, after which thrombosis, if it had not occurred, was unlikely to occur at all. Effective heparin anticoagulation during this period probably prevented thrombosis by inhibiting fibrin stabilization of platelet aggregates.

To explain the existence of the critical period after endarterectomy when thrombosis is likely to occur, we can implicate the local release of ADP and tissue thromboplastin from injured tissues.17,25-26 The amounts available would depend on the severity or extent of vessel wall injury. On release, ADP is rapidly dephosphorylated to adenosine monophosphate by enzymes in plasma.27,28 After depletion of the ADP supply at the site of injury we would anticipate that its thrombogenicity would be lowered and that the critical period for thrombosis would be past.

An alternative or coexistent protective process might be an alteration of the denuded vessel wall in the hours after endarterectomy to reduce its thrombogenicity. Such a phenomenon might be the deposition of one or more plasma proteins, particularly albumin, which has been demonstrated to render a surface less attractive to platelets.39 Salzman et al.40-42 suggested the same phenomenon to account for the reduced adherence of platelets to heparinized surfaces. Such a protein coat completely covering the endarterectomized surface during an adequate period of anticoagulation could render it inert to thrombosis. Subsequent reendothelialization after two weeks would complete the healing process and offer permanent protection.

Laboratory experiences as well as clinical experiences with endarterectomy and microvascular procedures have reinforced our impressions, as in this study, that when thrombosis occurs after operation it most frequently occurs as an acute rather than as a delayed phenomenon. As a result of knowledge gained in this study, in certain clinical vascular surgical cases we have elected not to reverse the heparin that was administered before vessel clamping so as to provide additional protection against postoperative thrombosis or excessive platelet accumulation at the surgical site. We have not found it necessary to employ the routine use of anticoagulants in the postoperative period as suggested by some.43-46

Conclusion

Using carotid endarterectomy in cats as a model we have evaluated aspirin, Coumadin, Coumadin plus aspirin, and heparin for their protective effect against thrombosis in endarterectomized vessels. No protection was found with aspirin, Coumadin, or these two in combination. Heparin anticoagulation, when maintained for one to four hours, offered modest protection and when maintained for more than four hours prevented thrombosis in all cases. The possible reasons for such an effect are discussed.
Acknowledgment

The authors gratefully acknowledge the assistance of Dr. R. Jean Campbell in preparation and interpretation of the histological specimens; Dr. Eric F. Grabowski for valuable discussions; Ms. Martina R. Briones, Mr. William L. Donovan, and Mr. Donald C. Lilja for technical assistance; and Ms. E. Lucy DeSerré for her secretarial assistance.

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