The Effect of Combined Aspirin and Dipyridamole Therapy on Thrombus Formation in an Arterial Thrombogenic Lesion in The Dog

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SUMMARY We investigated the potential of aspirin and dipyridamole in combination to inhibit thrombus formation by comparing endarterectomized segments of 20 dog carotid arteries in animals treated with pre- and post-operative aspirin and dipyridamole to 20 arteries from untreated animals and 20 arteries from animals receiving intra-operative heparin. The temporal profile of thrombus formation was assessed by means of angiography, light microscopy, and scanning electron microscopy at time intervals ranging from 30 minutes to three months from the time of surgery. All of the aspirin-dipyridamole vessels remained patent and only one had significant gross thrombus formation. This contrasted to six occlusions and six significant gross thrombi in the control group and one occlusion and six significant gross thrombi in the heparin group. The combination of oral aspirin and dipyridamole minimizes thrombus formation in the highly thrombogenic lesion created by carotid endarterectomy in the dog.

PLATELET PHYSIOLOGY and the pharmacology of platelet-affecting drugs have been the subject of considerable recent interest. Several major clinical trials have been recently completed or are currently in progress to evaluate the potential of drugs inhibiting platelet activity to lower the morbidity and mortality method in analyzing survival. J Chronic Dis 8: 699-712, 1958


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Materials and Methods

Animal Preparations

We selected, from a homogenous population of 10 to 15 kg healthy male mongrel dogs, 30 animals for this study; after having completed the procedure in ten operated solely for standardizing the technique. One of us, H.G.D., with the aid of an operating microscope, performed bilateral 1 cm long common carotid artery endarterectomies in each animal and then closed the vessel primarily with a running 8–0 prolene suture. We have previously described preparation of this lesion in detail. We assigned animals randomly to one of three groups on the basis of drugs administered: control, heparin, and aspirin-dipyridamole. In order to prevent a bias that might have developed from minor changes in surgical technique, the numbers of animals in each group were sequentially balanced throughout the duration of the study.

We gave no drugs with anticoagulant properties to the control group; 100 units/kg of heparin intravenously immediately prior to occlusion of the carotid arteries for endarterectomy, supplemented by 10 units/kg/hr starting four hours after surgery and continuing for four hours, (unless sacrificed earlier) to the heparin group; and 325 mg of aspirin and 50 mg of dipyridamole daily, beginning two weeks prior to surgery and continuing until death, in the aspirin-dipyridamole group. All dogs scheduled to survive longer than four hours after surgery received 600,000 units of long acting penicillin during the operation.

Vessel Evaluation

Preparations were terminated at intervals of 30 minutes; one, two, and four hours; one, two, and three days; one week; and one and three months after endarterectomy. There was one dog (two vessels) from each group studied at each of these time intervals. Prior to death, each vessel was re-exposed and retrograde common carotid angiography was performed via a catheter placed in the carotid artery distal to the surgical site. After angiography was completed, the common carotid artery was cannulated proximal to the site of endarterectomy with a No. 15 needle. With the catheter used for angiography still in place, the segment of artery containing the endarterectomy was thus isolated from the circulation. This isolated segment was gently flushed with normal saline to clear the vessel lumen of blood, then perfused with 250 cc glutaraldehyde (2% in 0.1 mol phosphate buffer, pH 7.4, at room temperature) over 30 minutes at a pressure of 100 mmHg. A transverse section from the middle of the endarterectomy was taken for light microscopy and stained with hematoxylin and eosin. The remainder of the specimen was opened longitudinally, photographed, and prepared for scanning electron microscopy (SEM). The specimens for SEM were dehydrated with ethanol and dried with liquid carbon dioxide using the Sorvall critical point drying system. The specimens were mounted on aluminum stubs, coated with carbon and gold-palladium, and examined with an ETEC scanning electron microscope operating at 20 KV. Photographs were made on Polaroid type 55 film.

Results

Angiography

Angiographic findings are summarized in table 1. Vessels were classified as occluded, patent with clot (> 25% stenosis) or patent without clot. There were no occluded vessels in the antplatelet group compared with six (30%) in the control group (p < 0.05) and one in the heparin group. Considering only the patent vessels, nineteen (95%) in the aspirin-dipyridamole group were completely free of thrombus at the endarterectomy site compared to 8 of 14 vessels (57%) in the control group and 13 of 19 vessels (68%) in the heparin group. Thus the antplatelet group proved superior to both the control (p < 0.01) and the heparin group (p < 0.05) in terms of minimizing gross thrombus formation at the site of endarterectomy. There was no statistical difference between the antplatelet and heparin groups in terms of patency but each group was significantly better than the control group (p < 0.05).

The angiogram of a control animal sacrificed one hour after surgery, showing a large amount of soft clot compromising the vessel lumen at the surgical site, is compared to angiograms of one hour heparin and aspirin-dipyridamole arteries showing minimal narrowing at the suture line but no intravascular thrombus, in figure 1.

Histology

The findings on light microscopic review of vessel cross sections corroborated the angiographic estimate of patency in each case. The one hour control vessel showed a large amount of soft thrombus (fig. 2a), consistent with the angiographic appearance of this vessel (fig. 1a). The one hour heparin and aspirin-dipyridamole vessels (fig. 2b and 2c) showed scant thrombus formation that was not appreciated on angiography.

Scanning Electron Microscopy

Endarterectomy resulted in removal of the intima, the internal elastic lamina, and varying amounts of the innermost media. The exposed denuded surface was

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of vessels</th>
<th>Number occluded</th>
<th>Patency rate</th>
<th>Patent with clot</th>
<th>Patent without clot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin-dipyridamole</td>
<td>20</td>
<td>0</td>
<td>100%</td>
<td>1</td>
<td>19 (95%)</td>
</tr>
<tr>
<td>Heparin</td>
<td>20</td>
<td>1</td>
<td>95%</td>
<td>6</td>
<td>13 (68%)</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>6</td>
<td>70%</td>
<td>6</td>
<td>8 (57%)</td>
</tr>
</tbody>
</table>
EFFECT OF ASPIRIN AND DIPYRIDAMOLE ON THROMBUS FORMATION

primarily a rough irregular layer of elastic and collagen fibers. SEM studies highlighted the extreme thrombogenicity of this surface. Within 30 minutes of completion of endarterectomy in the control animal, the entire endarterectomized surface was covered with a fibrin-platelet layer of varying thickness. Thrombus formation resulted as red blood cells (RBCs) became enmeshed in the developing fibrin-platelet network. Some white blood cells were also found in the evolving thrombus.

When examined two hours after surgery, mural thrombus formation was quite prominent in the control vessel (fig. 3a), while being more moderate in the heparinized vessel (fig. 3b). The two hour antiplatelet vessel showed only a sparse fibrin-platelet complex with essentially no thrombus formation (fig. 3c). Thus at this acute post-operative stage the protective effect of aspirin-dipyridamole therapy was readily apparent.

By two days after surgery, mural thrombus in the control vessel was less impressive than it had been in the more acute animals, i.e. 30 minutes to 24 hours. By comparison the antiplatelet vessel had a much cleaner surface at this stage, with a very thin and incomplete fibrin-platelet-RBC coating. There were no qualitative differences between the aspirin-dipyridamole and heparin vessels at the two-day stage. Mural thrombus resolution was well underway by one week and was essentially complete by one month after surgery. There were areas in control and heparin vessels in which mural thrombi organized and remained adherent to the vessel wall instead of lysing.

Discussion

A large body of knowledge has recently evolved regarding the role of the platelet in intravascular thrombosis. Aspirin, dipyridamole, and other drugs which inhibit platelet activity have been extensively studied in clinical situations, but results to date have not been conclusive regarding their clinical effectiveness. We found the combination of oral aspirin and dipyridamole to have a beneficial effect in limiting thrombus formation on a highly thrombogenic surface and, therefore, believe the present study lends strong support to the use of these agents clinically in thromboembolic disorders.

The pharmacology of these agents and the role of the platelet in thrombus formation have been the subject of several recent detailed reviews and will only be briefly outlined here. The initial event in thrombus formation involves adherence of a layer of platelets to subendothelial constituents, such as collagen, basement membrane, and microfibril, which are exposed by removal of the endothelium. This is followed by release of platelet granules and the aggregation of am-

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**Figure 1.** Retrograde carotid angiograms of animals sacrificed one hour after surgery. a: The control vessel demonstrating extensive thrombus formation at the site of endarterectomy. b: Vessel from the heparin group showing no clot. Minimal narrowing is present at the suture line. c: Antiplatelet vessel showing a normal appearing lumen without clot.

**Figure 2.** Cross sections of vessels sacrificed one hour after surgery. a: Control vessel showing significant luminal occlusion by soft thrombus (T). b: Heparinized vessel and c: Antiplatelet vessels are widely patent with minimal intravascular thrombus (T). X 16, H & E.
FIGURE 3a. Scanning electron micrographs of vessels from the three different groups sacrificed two hours after surgery. Control vessel demonstrating prominent mural thrombus formation.

FIGURE 3b. Heparinized vessel shows a thinner, more homogenous fibrin-platelet matrix with fewer RBCs than the control vessel.
Elevation of cyclic AMP levels leads to inhibition of a variety of platelet functions including: 1) platelet adherence to damaged vessel walls, 2) platelet aggregation, and 3) release of platelet granular contents. Variable results have been reported in clinical and animal studies of this drug. In most studies in which dipyridamole has been found to be beneficial, it has been used in combination with another platelet-active drug or an anticoagulant.

Further studies comparing different platelet affecting agents, used in differing combinations and dosage schedules, would be of value. With increasing understanding of the pharmacology of these drugs, it would be possible to select combinations of agents that inhibit multiple different aspects of platelet function.

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CSF Serotonin Concentrations and Cerebral Arterial Spasm in Patients with Ruptured Intracranial Aneurysm

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SUMMARY In 26 patients with recent rupture of an intracranial saccular aneurysm the CSF concentrations of serotonin (5-HT) were measured repeatedly by a radioimmunoassay. The 5-HT level in ventricular CSF collected between the 2nd and 15th day after SAH ranged between <2 and 5 nmol/l. These did not differ from the levels found in the ventricular CSF (<2-3 nmol/l) and lumbar CSF (<2-3 nmol/l) of control patients. 5-HT concentrations did not correlate with the severity of angiographical vasospasm, nor with CSF pressure or clinical grade. In two patients with severe postoperative vasospasm, however, cisternal CSF concentrations exceeding 25 nmol/l were observed. Thus, although these results do not support the conception that 5-HT plays a major role in sustaining delayed vasospasm, they suggest that 5-HT liberated from platelets may be operative in the initiation of cerebral arterial spasm.

PERSISTENT CONstriction of the major cerebral arteries is a common phenomenon in patients with subarachnoid hemorrhage (SAH). The deleterious cerebral ischemia that accompanies severe vasospasm significantly increases the mortality and morbidity in patients with ruptured intracranial aneurysm. However, the etiology of cerebral vasospasm remains obscure, and no satisfactory treatment has yet been devised.

Serotonin, or 5-hydroxytryptamine (5-HT), is a potent vasoconstrictor which has been implicated in the etiology of cerebral vasospasm following aneurysms rupture. Our knowledge of the possible role of 5-HT in vasospasm has mainly been achieved by experimental work. In 1961 Raynor, McMurtry, and Pool demonstrated that 5-HT applied to the cerebral cortex of the cat caused spasm of the pial vessels. Since then, several studies have pointed to 5-HT as a causative agent and it has been proposed that the sustained release of 5-HT from shed blood platelets in the subarachnoid space is responsible for the prolonged arterial constriction. Other investigations do not support the conception that 5-HT plays a major role in the production of vasospasm.

These inconsistent results have not been clarified by human studies. Althoug elevated levels of 5-HT in cerebrospinal fluid (CSF) from patients with various cerebrovascular diseases have been reported, the risk of measuring closely related indoles in CSF instead of 5-HT with insensitive or nonspecific methods has been stressed.

In the present study we used a specific and highly sensitive radioimmunoassay for the determination of 5-HT in CSF. The purpose of this investigation was to obtain information of the concentration of 5-HT in CSF of patients with SAH, and to study the relation between 5-HT and cerebral vasospasm.

Patients and Methods

Clinical Series

The series studied included 26 patients (13 women and 13 men) with recent rupture of an intracranial saccular aneurysm, admitted to the Department of Neurosurgery in Aarhus between April 1980 and February 1981. The mean age of the patients was 50 years (range 27-72). The clinical condition of the patients was assessed daily and graded according to the system of Hunt and Hess. Cerebral angiography was performed in all patients between the 1st and 7th day after the initial bleeding and was repeated in 10 patients postoperatively. The degree of vasospasm was measured on angiograms and defined as follows: a reduction in arterial diameter of less than 25% was no
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