Endothelial Cell Ischemic Injury: Protective Effect of Heparin or Aspirin Assessed by Scanning Electron Microscopy

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Abstract: Scanning electron microscopic observations of the luminal surface of the rabbit common carotid artery subjected to occlusion for 30 minutes or two hours revealed crater-like and balloon-like defects in the endothelial surface. The frequency of occurrence of these abnormalities was significantly decreased by pretreatment with heparin or aspirin in doses considered to have antiplatelet aggregating activity.

Introduction

When examined by scanning electron microscopy (SEM), ischemic endothelium reveals numerous "crater-like" defects as well as outpouchings which balloon into the vessel lumen (fig. 1). These changes are observed infrequently in the endothelium of the contralateral, sham-operated (unoccluded) carotid artery, and never in carotid arterial segments of unoperated animals. Such alterations are irregular in distribution, appearing in single or contiguous endothelial cells within vascular regions subjected to ischemia, while other cells in the same area may appear unaffected. Formed blood elements, including platelets, are occasionally noted in the vicinity of "craters" and "balloons." These observations suggested a possible explanation for these lesions which is compatible with the results of recent investigations emphasizing the role of blood cells, especially platelets, in the initiation of vascular injury. The present study was undertaken to examine the effects of heparin and aspirin in doses having significant antiplatelet aggregating activity.

Methods

Thirty New Zealand white rabbits were lightly anesthetized with sodium pentobarbital (Nembutal® 35 to 40 mg per kilogram IV) and both common carotid arteries were surgically exposed. A single Heifetz clip was used to occlude the right carotid artery at approximately the middle of its length. Ten animals received no treatment prior to occlusion for 30 minutes (five animals) and two hours (five animals). A second group of ten animals was pretreated with heparin (Panheparin® 1,400 units per kilogram IV) five minutes prior to occlusion for 30 minutes (five animals) and two hours (five animals). A third group of ten animals was pretreated with aspirin (acetylsalicylic acid, 50 mg per kilogram, administered orally) two hours prior to occlusion for 30 minutes (five animals) and two hours (five animals). Following arterial occlusion, the clip was removed and the carotid arteries were immediately fixed by perfusion of 2.5% glutaraldehyde in 0.1 M Sorensen's phosphate buffer (pH 7.4, 4°C) through an aortic cannula. Arterial segments were then removed from three areas: 5 mm distal to the clip, 5 mm proximal to the clip on the right side, and the contralateral sham-operated control vessel. All specimens were prepared for scanning electron microscopy by the critical point drying technique. The frequency of occurrence of endothelial cell alterations was determined by counting abnormalities (craters or balloons) in a series of 20 random microscopic fields, at l,000X magnification, from each of the three arterial segments of all animals. P-values were calculated by a two-way analysis of variance about the mean.

Results

Animals having carotid artery occlusion for 30 minutes without pretreatment showed a highly significant increase in the average numbers of craters in the ischemic arterial segment (distal to the clip), as compared to proximal (P < 0.01) or sham-operated control segments (P < 0.0005) (table 1). The average number of balloons also was statistically greater in the distal segment as compared to proximal (P < 0.0005) or sham-operated control segments (P < 0.01). Animals pretreated with heparin showed an 80% decrease in frequency of occurrence of craters (P < 0.001) and a 30% decrease in the numbers of balloons (P < 0.1) in the distal segment as compared to distal segments of untreated animals. Aspirin
pretreatment caused a similar reduction in the numbers of craters and balloons in the distal arterial segment as compared to the untreated group. The number of craters and balloons in proximal and sham-operated control segments of heparin-treated and aspirin-treated animals was approximately the same or somewhat less than that of the untreated group.

Animals having carotid artery occlusion for two hours without pretreatment or with heparin or aspirin pretreatment showed results similar to those subjected to occlusion for 30 minutes (with the exceptions indicated in table 2). However, the effect of heparin or aspirin pretreatment was less marked than that of the 30-minute group.

The diminution in numbers of endothelial craters and balloons was the only detectable morphological difference between arteries from animals pretreated with heparin or aspirin and specimens from animals having occlusion without prior treatment. Figure 2 illustrates the normal appearance of the endothelial surface in the distal segment of an aspirin-treated animal.

**Discussion**

Animals pretreated with heparin or aspirin prior to carotid occlusion for 30 minutes showed a marked decrease in the frequency of occurrence of craters and balloons distal to the occluding clip as compared with proximal and sham-operated control segments of untreated animals (table 1). Animals pretreated with the same doses of heparin or aspirin prior to carotid occlusion for two hours also showed a decrease in numbers of craters in the distal segment as compared with the untreated (table 2); however, this effect was not as marked as in the 30-
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In microcirculatory beds, such as the cerebral capillary network, craters or balloons, such as those demonstrated in this study, might result in diminished blood flow. In larger arterial vessels, a sequential relationship of platelet aggregation, similar endothelial cell injury, and thrombogenesis, or atherogenesis, seems compatible with known facts and is amenable to further experimental study.

This investigation illustrates an endothelial abnormality which appears to be influenced by two pharmacological agents considered possibly beneficial in preventing thromboembolic phenomena related to heart attacks and strokes in man. This supports the hypothesis that platelet-endothelial cell interaction may be of importance in intimal damage. If, as it appears, the craters or balloons are, indeed, nonspecific but significant reactions of the endothelial cell to various injuries, this ischemic model, with correlative studies utilizing light and transmission electron microscopy, may be useful for evaluating mechanisms of intimal damage. Additional physiological and pharmacological studies, using this model, may provide information of relevance to therapeutic intervention in a variety of human vascular disorders.

Acknowledgment

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References

10. Flaherty JT, Ferrans VJ, Pierce JE, et al: Localizing factors in minute group. This may be attributed to prolonged mechanical trauma incurred as a result of the two-hour arterial occlusion. Alternatively, this effect may be due to a decline from the maximum blood levels of these drugs and hence in their effectiveness in preventing the formation of such lesions. Studies in which the level of antiplatelet aggregating activity of these drugs is maintained through continuous administration may resolve this question.

Similar endothelial lesions have been seen by us following mechanical trauma and in the normal untreated carotid artery and aorta near the ostia of branching vessels, sites of known Theological trauma. Craters also have been described in arteries of rabbits maintained on high cholesterol diets or following intravenous epinephrine treatment.

In addition to the indirect pharmacological evidence obtained from these experiments, several other investigations support the hypothesis of a possible primary role of platelets in the production of endothelial injury. Geissinger et al. reported the accumulation of platelet aggregates at intercostal artery orifices in swine aortas. Further, the rate of endothelial cell turnover has been shown to be greater in this location. Frost, utilizing scanning electron microscopy, demonstrated the adherence of platelets to the aortic endothelium of rabbits following feeding of a high cholesterol diet. Furthermore, Jorgensen et al. described, with light and transmission electron microscopy, the concurrence of platelet aggregation and endothelial cell damage.


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