Increased fibrinolytic activity in the intima of atheromatous coronary arteries: protection at a price

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Objective: The aim was to quantify and compare the fibrinolytic activity of normal blood vessels (saphenous vein, internal mammary artery, and aorta) and atheromatous arteries (coronary endarterectomy specimens).

Methods: Fibrinolytic activity was measured by fibrin plate and colorimetric assays on fresh samples of coronary endarterectomy core, internal mammary artery, human aorta, and saphenous vein from patients undergoing coronary artery bypass surgery. Results: Fibrinolytic activity on fibrin plates ranked in the order endarterectomy cores > internal mammary artery > saphenous vein. The increased activity of endarterectomy cores was associated with an increased content of extractable tissue plasminogen activator and was suppressed by monoclonal antibody to tissue plasminogen activator. Paired comparisons of tissues from the same patients confirmed this increased activity in endarterectomy specimens relative to normal artery or vein. Urokinase activity was also increased in some endarterectomy specimens, but was more variable than tissue plasminogen activator. Conclusions: The increased fibrinolytic activity of endarterectomy cores may help preserve patency in atheromatous vessels, but at the possible price of increased intimal instability and fibrous proliferation.

Cardiovascular Research 1993;27:882-885

The association of fibrinolytic activity with the walls of blood vessels has been widely recognised since the pioneering work of Todd and Astrup. The discovery that vascular endothelial cells synthesise and secrete tissue plasminogen activator has tended to focus attention on the luminal surface of vessels, and on the role of tissue plasminogen activator in preventing local thrombus formation. This has perhaps tended to overshadow the observation that fibrin gel lysis associated with transverse plasminogen activator in preventing local thrombus formation. In the course of work aimed at assessing the role of local fibrinolytic activity on the patency of vascular grafts we have measured the fibrinolytic activity of biopsy specimens from human internal mammary arteries and the long saphenous vein prepared for coronary bypass grafting and compared them with the fibrinolytic activity of fresh samples of aorta and endarterectomy cores obtained during the course of coronary artery surgery.

Methods

Samples of saphenous vein, internal mammary artery, human aorta, and endarterectomy cores from atheromatous coronary arteries were obtained from patients undergoing coronary artery surgery. In a subgroup of seven patients endarterectomy samples were obtained simultaneously with samples of non-coronary vessels (saphenous vein (n=3), internal mammary artery (n=2), and aorta (n=7)) and these were analysed separately as paired specimens. Samples were taken after patients had been heparinised and all specimens were obtained after the patients had been placed on cardiopulmonary bypass. Samples were immediately placed in 0.5% polyethylene glycol (PEG) 6000 solution and transported to the laboratory. The specimens were washed in the PEG solution and excess adventitia carefully dissected and discarded where appropriate. Samples were then opened and carefully pinned on to cork boards, intimal surface uppermost. Punch biopsies (2 mm) were taken and placed on plasminogen sufficient fibrin plates to quantify the area of fibrinolysis produced by the specimens. The diameter of the area of fibrinolysis was determined as previously described by Haverkate. The vertical and horizontal axes were measured and the mean taken as the diameter.

Fibrin plates were prepared by dissolving 100 mg fibrinogen grade L (Kabi) in 65 ml TRIS buffered saline pH 7.6. Aliquots of this solution (1 ml) were added to 9 ml TRIS buffer and coagulated with 10 U/ml thrombin. Gels 1 mm thick were poured into plastic plates and allowed to set for 20 minutes. After adding biopsies the plates were incubated in a humid sealed box at 37°C for 24 hours. Plates were stained with Coomassie blue and the average diameter of the area of fibrinolysis was calculated as described.

For quantification of tissue plasminogen activator and urokinase activity with chromogenic substrates, samples were prepared as described but 1-2 cm portions of each sample were dried on blotting paper, weighed, and finely minced in 1 ml 0.5% PEG 6000. These samples were left at 20°C for 60 minutes. The samples were then centrifuged at 3000 g for 10 minutes and 0.1 ml aliquots of the supernatant extracted for analysis. Activity of tissue plasminogen activator was measured with chromogenic substrate S2251 (Kabi Diagnostics). Working standard curves were prepared from 580 000 IU/mg 1 tissue plasminogen activator (Roethinger Ingelheim). Confirmation of specificity was obtained by blocking tissue plasminogen activator activity with monoclonal tissue plasminogen activator antibody (Serotec). Activity of urokinase was assessed with chromogenic substrate S2444 (Kabi Diagnostics). The working standard curve was prepared from Ukidan 5000 (Serono Laboratories, UK). Absorption at 405 nm was measured with a spectrophotometer. Aliquots of each sample (0.1 ml) were also taken for estimation of protein content by Bradford’s method. The standard curve for protein estimation was prepared from bovine serum albumin solution (1 mg/ml).

Results were analysed by Mann-Whitney U test and Wilcoxon test for paired data.

Appropriate ethics committee approval was obtained before this study.

Results

Figure 1 shows fibrinolytic activities as assessed by diameter of fibrinolysis. The mean diameters-mm 1 (SEM) were: endarterectomy (n=11) 10.4 (1.04), Internal mammary artery (n=8) 7.0 (0.87), and saphenous vein (n=10) 4.9 (0.69). Endarterectomy cores produced significantly greater areas of fibrinolysis than compared with saphenous vein (p<0.002) and internal mammary artery (p<0.04). No difference was seen between the internal mammary artery and saphenous vein (p>0.13).

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Figures 2 and 3 show the activity of tissue plasminogen activator and urokinases, respectively, in extracts of the vascular tissues, expressed as IU·mg⁻¹ tissue. The mean amounts of tissue plasma activator in IU (10⁴)·mg⁻¹ tissue (SEM) were: endarterectomy (n=11) 22.9 (3.1), saphenous vein (n=7) 9.6 (0.4), internal mammary artery (n=9) 7.7 (2.1), aorta (n=7) 8.4 (0.9). Endarterectomy cores yielded significantly more extractable tissue plasminogen activator than the aorta (p<0.005), internal mammary artery (p<0.004), and saphenous vein (p<0.02). No other significant differences were found [internal mammary artery v saphenous vein, (p=0.29), internal mammary artery v aorta (p=0.52), aorta v saphenous vein (p=0.75)].

Anti-tissue plasminogen activator antibody (Serotec) incubated with endarterectomy core supernatant significantly reduced the amount of detectable tissue plasminogen activator from 8500 IU·mg⁻¹ tissue to 625 IU·mg⁻¹ tissue (averages of two samples). Endarterectomy core was also stored in PEG solution at 4°C. Tissue plasminogen activator activity as determined by chromogenic assay was reduced by 80% after 24 hours and by 95% after 48 hours storage.

The mean amounts of urokinase in IU (10⁴)·mg⁻¹ tissue were: endarterectomy (n=7) 7.7 (1.3), internal mammary artery (n=5) 3.0 (0.5), saphenous vein (n=8) 5.0 (0.7), and aorta (n=7) 3.5 (0.6). Endarterectomy specimens produced significantly more urokinase activity than aorta (p<0.02) or internal mammary artery (p<0.05). The saphenous vein produced more urokinase than the internal mammary artery (p<0.05). No other significant differences were found [internal mammary artery v aorta (p=0.93), endarterectomy v saphenous vein (p=0.08), aorta v saphenous vein (p=0.09)].

Endarterectomy specimens produced significantly more tissue plasminogen activator than normal vessels in the paired sample group [mean amounts of tissue plasminogen activator in IU (10⁴)·mg⁻¹ tissue (SEM): endarterectomy (n=7) 29.92 (1.71) normal vessel (n=7) 12.08 (1.62). (p<0.05; Wilcoxon test) (fig 4)].

Histological examination of the endarterectomy specimens showed, as expected, typical human fibrous atherosclerosis. Cell content was variable but usually included α-actin positive smooth muscle cells. En face examination of the endothelium after silver nitrate staining showed it to be intact, with polygonal cell outlines. We have not yet been able to localise tissue plasma activator or urokinase production to individual cell types by immunohistochemical techniques.

Discussion

We have shown the presence of unexpectedly large amounts of fibrinolytic activity in fresh endarterectomy cores from atheromatous human coronary arteries. This is largely attributable to tissue plasminogen activator with a variable contribution from urokinase. Fibrinolytic activity has been previously reported in atherosclerotic human coronary⁵ and peripheral arteries⁶ by fibrin slide techniques, although the use of postmortem material would have led to an underestimation of the activity present. Loss of tissue plasminogen activator activity with time may also explain the low levels of fibrinolytic activity described earlier by Noordhaek.¹ Demonstration of fibrin or fibrinogen epitopes in the vessel wall¹ does not exclude mural fibrinolytic activity, because they may be present on degradation products.

Endarterectomy specimens obtained during coronary atherectomy are representative of human atherosclerotic plaque⁷ ⁸ and contain abundant amounts of connective tissue.
with numerous associated cells including smooth muscle cells and macrophages. Morphological study of the surface of atheromatous plaques in the coronary arteries of recipient hearts removed at the time of transplantation shows a variety of patterns, in some areas desquamated endothelium with adherent leucocytes and platelets and in others apparently intact endothelium albeit with cells abnormal in size, shape, and arrangement. Surface microscopy of our own specimens showed similar appearances.

Endothelial cells, whether derived from the luminal surface of a vessel or from the vasa vasorum, are an obvious source of both tissue plasma activator and urokinase. Smooth muscle cells also produce plasminogen activators, and the expression of binding sites for urokinase on these cells has recently been demonstrated. Plasminogen to plasmin conversion accomplished by tissue plasminogen activator, or urokinase, may be important in the matrix conversions that accompany smooth muscle cell division and migration. Recent reports document increased urokinase activity during smooth muscle cell mitogenesis and increased activity of tissue plasminogen activator during cellular migration after injury to the arterial wall. In vitro studies also suggest that increased urokinase activity associated with smooth muscle cells may be pathophysiological in atherosclerotic vascular lesions. We have so far been unable to localise tissue plasminogen activator or urokinase production to individual cell types in endarterectomy specimens by immunohistochemical means, possibly because these proteins are diffusely bound to the matrix immediately after release from cells.

Intraluminal coronary thrombosis is associated with acute myocardial infarction or unstable angina, and studies in young infarct survivors have shown a reduction in plasma tissue plasminogen activator activity, largely as a result of increased concentrations of plasminogen activator inhibitor. Activity in plasma does not, however, necessarily reflect localised activity within the vessel wall. It is interesting that both older and recent accounts of plaque appearance reflect localised activity within the vessel wall. It is probably more relevant to note that fibrin often fails to stabilise the ruptured plaque than to speculate on whether the haemorrhage originates from the lumen or a vas vasi. Conversely, thrombus is uncommonly found in association with the lesions of patients with stable angina referred for percutaneous coronary atherectomy, and more recently epidemiological studies on a wider patient basis have shown that some patients with extensive coronary artery disease have unexpectedly high plasma concentrations of tissue plasminogen activator. The finding of higher concentrations of basal antigenic tissue plasminogen activator at rest in patients with coronary artery disease is not new, and a decreased release of tissue plasminogen activator after exercise has also been reported in these patients. This reduction has been explained on the basis that there is a slow but continual release of tissue plasminogen activator from the vascular wall reducing the vascular deposits of the compound in patients with coronary artery disease and resulting in a decreased release after exercise. There is no direct evidence to support this hypothesis and consequently it may be that the atheromatous vessel wall has high concentrations of tissue plasminogen activator locally but this is not released systemically in response to exercise because of its important local protective anti-thrombotic effect within the diseased vessel. Such a hypothesis would accord with our findings.

In conclusion, we believe that our findings of increased fibrinolytic activity in the atheromatous intimal layer of human coronary arteries may reflect a genuine pathophysiological state in which the abnormal vessel wall produces locally increased amounts of tissue plasminogen activator and urokinase relative to their inhibitors. A teleological advantage is protection from local thrombosis; the price, perhaps, is accelerated intimal proliferation and increased intimal instability.

Key terms: atheroma; endarterectomy; fibrinolytic activity; vessel wall.

Received 8 September 1992; accepted 19 October. Time for primary review 14 days.

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