Fibrinolytic Activity in Human Atherosclerotic Coronary Arteries

By Hau C. Kwaan, M.D., F.R.C.P. (Edin.), and Tage Astrup, D.Ph. (Copenhagen)

ABSTRACT
The localization of fibrinolytic areas in normal and atheromatous human coronary arteries was studied by the histochemical fibrin slide technique. Fibrinolytic activity was caused by a plasminogen activator. The activator, occasionally observed at sites in the normal endothelial lining, was abundantly present in relation to vessels in the normal adventitia. The pathological adventitia contained an increased number of active sites and endothelium covering an atherosclerotic plaque was richer in fibrinolytic sites than the normal endothelium. Within the vascularized plaque, fibrinolytically active capillaries could be traced from their origin in the vasa vasorum or, occasionally, in the endothelium. The results confirm and extend previous observations obtained in assays after extraction. They present patterns similar to those observed in normal tissue repair.

ADDITIONAL KEY WORDS
vessels capillaries tissue repair

Assays of thromboplastic and fibrinolytic activities of extracts of the layers of the normal arterial wall of animals and man have been reported (1-4). In man the aortic intima is rich in thromboplastin with little or no fibrinolytic activity suggesting that fibrin formation may occur readily while fibrin resolution is delayed. The intima of the atherosclerotic aorta contains less thromboplastin than the normal intima (5-7), while the media and adventitia have higher fibrinolytic activity (assayed as plasminogen activator) than the normal layers (7). However, separation of the layers of the wall is more difficult in the pathological samples, and the limited amounts available make determinations less accurate. With the histochemical fibrin slide technique, plasminogen activator has been localized to the vascular endothelium, especially in veins and venules (8-11), but occasionally also in small arteries (11). Plasminogen activator is particularly abundant in newly formed capillaries, by means of which it is brought into an area of tissue repair (12, 13). In a human thrombosed vein fibrinolytic activity was observed in relation to the sites of recanalization but was absent where the thrombus was attached to the vessel wall (9, 14). A similar pattern of distribution appeared in experimentally produced venous thrombosis in rats (15). In human atheromatous arteries fibrinolytic activity has been localized to vessels in the plaques (9). It is the purpose of this study to describe in detail the localization of plasminogen activator in the atherosclerotic human coronary artery.

Methods
Human hearts were obtained by autopsy less than 48 hr after death. The ages ranged from 36 to 82 yr. The proximal parts of both coronary arteries were isolated, and a segment removed from the right coronary artery and the anterior descending branch of the left coronary artery, each at a distance of 3 to 5 cm from the aorta. Specimens were embedded in a preparation of glycols and resins suitable for cutting at temperatures between −15 to −30°C ("O.C.T.II," Lab-
Normal coronary artery (left) sectioned 3 cm from its origin. Fibrinolytic activity localized to the vasa vasorum and, less commonly, to sites at the endothelial lining (arrow). Fibrin slide incubated for 30 min, Harris' hematoxylin.

Sections were cut at 6 μ with a Lab-Tek Cryostat Microtome, and collected on microscope slides covered with a 0.06-mm thick layer of bovine fibrin (rich in plasminogen). After a brief fixation for 1 min in 50% (v/v) methanol in saline, the slides were incubated in a moist chamber at 37°C for periods ranging from 3 to 60 min. After fixation in 10% formaldehyde the sections were stained with Harris' hematoxylin and, if needed, counterstained with oil red O. Fibrinolytically active sites appear as clear zones of lysis. Of each specimen seven slides, with two to four sections on each, were prepared and incubated for different periods of time. To test for unspecific protease activity, slides were also prepared with plasminogen-free bovine fibrinogen (16). Details of the technique are described elsewhere (8, 11, 17).

Results
None of the sections produced lysis on plasminogen-free fibrin; lysis was observed only
FIGURE 3
Atheromatous coronary artery with thickened intima and fatty deposits (black spots). Fibrinolytic areas related to the endothelium covering the atheroma. There are active sites within the vascularized plaque. Fibrin slide incubated for 30 min, Harris’ hematoxylin and oil red 0.

FIGURE 4
Atherosclerotic coronary artery with a fibrous plaque (left) and calcification (C). Small areas of fibrinolytic activity (arrows): located to the endothelial lining (top), adjacent to the calcified area, and in the vasa vasorum growing into the plaque from the adventitia (bottom). Fibrin slide incubated for 45 min, Harris’ hematoxylin.

FIGURE 5
Atherosclerotic coronary artery. Extensive lysis related to the adventitia (bottom and right). Lytic areas related to vasa vasorum (V) leading from the adventitia into the fibrous plaque. Active site at the endothelium (top). Fibrin slide after 45 min incubation, Harris’ hematoxylin.
FIGURE 6
Atherosclerotic coronary artery. Marked lysis in the adventitia and related to vasa vasorum (V) originating in the adventitia (V). Active sites are also present at a capillary, shown by serial sections to grow into the plaque from the endothelial lining (arrow). Fibrin slide after 45 min incubation, Harris' hematoxylin.

FIGURE 7
Atherosclerotic coronary artery. Capillary growing into an atherosclerotic plaque from the endothelial surface. Fibrinolytic activity present at a site in this capillary (bottom). Fibrin slide after 45 min incubation, Harris' hematoxylin.

FIGURE 8
Atherosclerotic plaque with recent hemorrhage (H) into a fibrous area, showing fibrinolytic activity (arrow) related to a capillary adjacent to the hematoma. Many active areas in the adventitia. Fibrin slide after 45 min incubation, Harris' hematoxylin.
on plasminogen-rich fibrin, indicating that fibrinolytic activity is caused by a plasminogen activator. No significant differences were observed in the fibrinolytic activity of specimens collected at different periods within 48 hr after death.

**NORMAL SAMPLES**

In the normal coronary artery (20 samples) plasminogen activator is located to vessels in the adventitia with only an occasional active site at the endothelial surface. There was no activity in the media (Figs. 1 and 2).

**ATHEROSCLEROTIC SAMPLES**

Fibrous plaques occurred in all of the 25 specimens of atherosclerotic coronary arteries studied. Among these, 22 were partly calcified. In 23 specimens, organized and vascularized fatty plaques were present. Areas of subintimal hemorrhage occurred in 4 specimens.

The endothelium covering an atheromatous plaque presents numerous, partially confluent, sites of fibrinolytic activity (Fig. 3). Fibrinolytic sites are more numerous in the adventitia behind an atheromatous plaque than in the normal adventitia. Active sites are also related to capillaries within the vascularized plaque (Fig. 3). Sites of activity were absent from fibrous plaques and in calcified lesions, but appeared adjacent to such areas (Fig. 4). In the atheromatous plaques with cellular organization and marked vascularization, fibrinolytic activity was related to the capillaries. By serial sectioning, it was possible to trace such vessels from the adventitia through the media (15 samples) (Figs. 5 and 6), or occasionally (4 samples) to their origin in the endothelial lining (Figs. 6 and 7). Fibrinolytic sites were present only in parts of such abnormal vessels and not throughout their whole lengths. In the plaques with recent intimal hemorrhage, the area of hemorrhage was fibrinolytically inactive, but capillaries involved in the process of organization were active (Fig. 8). The results obtained in this series were confirmed in an additional series of 10 normal and 20 pathological samples studied later.

**Discussion**

The localization of plasminogen activator in normal and atherosclerotic human coronary arteries, as here observed, substantiates the relation of fibrinolytic activity to vascular structures as well as its involvement in tissue repair (12, 13, 15). In contrast to the sporadic occurrence of fibrinolytic sites at the normal endothelium of the coronary artery, there were numerous active, sometimes confluent, zones along the endothelium covering an atheromatous lesion. Previous assays by the extraction method had demonstrated small amounts of plasminogen activator in the human coronary intima (3). These assays had been performed on unselected, apparently normal or only slightly pathological samples, and it is quite possible that the presence of early atheromatous changes could explain the observed fibrinolytic activity.

The intimal plaque is often well vascularized with capillaries extending into the thickened intima either from the vasa vasorum or the endothelial lining (18). In the present study fibrinolytic activity could be related to both of these types of vessels. Fibrinolytic activity was absent from areas of recent subintimal hemorrhage but there were active capillaries in adjacent areas undergoing organization. An increased number of active sites in the adventitia behind an atheromatous lesion confirmed previous results obtained by the extraction method (7). The absence of fibrinolytic activity in the avascular fibrous plaques or in calcified lesions should be noted.

The results of the present study of the distribution and localization of plasminogen activator in the atheromatous coronary artery substantiate the concept that the fibrinolytic system participates in tissue repair and open up speculations about the possible role of localized fibrinolytic activity in the natural history of the atheromatous plaque.

**References**


