Fibrinolytic (Plasmin) Therapy of Experimental Coronary Thrombi with Alteration of the Evolution of Myocardial Infarction

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To explore the possibilities of fibrinolytic therapy of coronary thrombosis, experimental studies were carried out to document lysis of coronary thrombi and to investigate the effect of fibrinolytic blood upon myocardial infarction. Serum-induced coronary thrombi were produced by a new technic and were followed by serial coronary arteriography. Control animals were compared to animals in which significant fibrinolytic activity had been induced by systemic infusions of plasmin. Tissue studies suggest that plasmin may change the evolution of early infarction. Whether these changes will ultimately result in salvage of ischemic tissue will be determined by studies now in progress.

RESTORATION of coronary circulation by dissolution of an obstructing thrombus could change the clinical course of myocardial infarction. Human plasmin (fibrinolysin) is an enzyme that has been shown to be effective in the dissolution of clots in peripheral vessels. No data relative to the action of plasmin on experimental or clinical coronary thrombi have been reported.

In this first report the method of producing intracoronary thrombi in dogs and of documenting their presence and size by direct serial arteriography will be described. It will be shown by these same objective criteria that these thrombi can be lysed by plasmin (fibrinolysin) in adequate dosage. The effect of the fibrinolytic state and restoration of coronary blood flow upon the histologic structure of early infarction will be discussed.

Methods and Materials

Adult mongrel dogs weighing 15 to 25 Kg. were anesthetized with intravenous sodium pentobarbital (25 mg./Kg.) and placed in a right lateral position on the operating table. Ventilation was maintained by a Harvard respirator pump through an endotracheal tube with a tidal volume of 150 to 300 ml. at a rate of 12 per minute. The heart was exposed by precordial fenestration, which included resection of 2 rib segments (fig. 1). The pericardial sac was opened and sutured to the margins of the thoracotomy opening. Cyanosis and overbreathing with respiratory alkalosis were avoided by adjusting the tidal volume for each animal to maintain the blood pH at 7.2 to 7.35. The heart action was kept under continuous electrocardiographic surveillance. To prevent ventricular fibrillation or other paroxysmal arrhythmias, premature contractions were treated with 50 to 100 mg. of procaine amide intravenously, and 5 to 10 mEq. of potassium chloride was added to the intravenous or intracoronary infusion. Drying of the heart surface was minimized by saline irrigation and covering the chest opening with a transparent plastic sheet.

A tiny proximal side branch of the left anterior descending coronary artery was dissected free and a no. 160 polyethylene catheter inserted into it. Slow infusion of 5 per cent glucose solution in saline with a constant infusion pump assured patency of this system up to 18 hours and permitted serial coronary arteriography through this catheter. Delineation of the coronary tree was obtained by injection of a radiopaque mixture of 50 per cent sodium diatrizoate (Hypaque) and whole blood in a ratio of 3:1. Admixture of blood was found necessary to prevent ventricular fibrillation. Two to four milliliters of these nonclotting mixtures were required for radiographic visualization of these vessels. Complete patency of the coronary arteries prior to occlusion is shown by the arteriogram in figure 2.
Coronary thrombi were produced consistently by inducing a "hypercoagulable state" in a dissected segment of a coronary artery. The distal end of this segment was narrowed by a stenosing ligature, indicated by a radiopaque metal marker, to prevent slipping of the clots to be formed. The control arteriograms revealed that these ligatures merely narrowed and did not totally occlude the artery (fig. 2). The arterial segment was isolated between 2 clamps for 10 minutes. Bulging intra-coronary clots were formed within 2 minutes in this isolated segment after rapid intraluminal injection of a mixture of blood and freshly prepared serum from the operated animal in a ratio of approximately 0.5 ml. of serum to 2 ml. of freshly drawn blood. The clotting time of this mixture was tested in vitro prior to injection. Admixture of serum was found to reduce the Lee-White clotting time from 5 to 8 to 1 to 2 minutes. To produce large clots consistently, it was found necessary to prevent escape of injected material prior to clotting by clamping the finest side branches originating from the occluded segment. This modification of the method described by Wessler\(^7\) resulted in firm thrombi with varying proportions of fibrin, platelets, and red cells. To prevent hemorrhage from the puncture site, electropolished no. 30 hypodermic needles\(^8\) were used to enter the lumen.

\(^{*}\)Stainless no. 30 hypodermic needles purchased from Vita Needle Co., Needham, Mass., were electropolished by courtesy of Dr. E. Knuth-Winterfeldt, Polytechnical Institute, Copenhagen, Denmark.

\(^{†}\)Obtained as plasmin from Merck, Sharp, and Dohme Co., West Point, Pa., and as fibrinolysin from Ortho Research Foundation, Raritan, N.J.

FIG. 1. Fenestration method for the production of serum-induced coronary thrombi and serial coronary arteriography. A medium-sized coronary artery is occluded by a thrombus wedged into a stenosing ligature (indicated by a radiopaque metal marker).

FIG. 2. Control arteriogram delineating the patent coronary arteries prior to occlusion.

After production of coronary occlusion, the animals were kept under anesthesia by injection of 30 to 65 mg. of sodium pentobarbital as needed for observation periods up to 15 hours.

During the experiment the change in clot size was determined at 1 to 2 hour intervals by measurement of the length of the filling defects in serial arteriograms. The final size and configuration of these thrombi were noted at autopsy and the hearts were then fixed in formalin for histologic examination. Tissue sections were chosen from the central and marginal zones of the infarcts and from the uninvolved wall of the left ventricle.

The clots were observed for at least 8 to 15 hours in the control group and until lysis occurred in the treated animals. The blood of the treated and the untreated animals was examined periodically to determine fibrinolytic activity by the eu- globulin method and whole clot lysis time.\(^1\)

In the treated animals an intravenous infusion of plasmin (fibrinolysin)\(^†\) was started from 1 to 8 hours after production of the coronary thrombus. Infusion of 4,000 units per Kg. per hour of plasmin was sufficient to induce and maintain a fibrinolytic activity in the range of 15 to 60 minutes lysis time of a standard fibrin clot.\(^1\) The process of clot lysis was judged by alterations in the length
FIBRINOLYTIC THERAPY OF EXPERIMENTAL THROMBI

Fig. 3. Serial coronary arteriograms depicting the lysis of a coronary thrombus by systemic plasmin treatment. A. Arteriographic filling defect (between arrow point and metal marker) indicating size of thrombus untreated for 8 hours. B. Partial lysis. C. Complete lysis after 4½ hours of intravenous plasmin therapy. Patency of coronary arteries appears restored. The small residual filling defect is caused by the stenosing ligature.

of the arteriographic filling defects (fig. 3). The treated animals were killed within 1 hour after restoration of vascular patency in the arteriograms, the vascular tree was dissected, and the hearts were preserved for tissue studies.

RESULTS

Technical Aspects

Forty-four animals were operated upon to develop the technics for production of coronary thrombi and serial arteriography. Twenty-eight animals were lost prematurely from complications of the various procedures with termination usually in irreversible ventricular fibrillation.

Of these 28 animals 15 died prior to coronary occlusion: 5 during the dissection of coronary arteries, 5 after the first arteriogram, 2 probably from respiratory alkalosis due to overbreathing, and 3 from unknown causes. After coronary occlusion fatal ventricular fibrillation occurred in 13 animals due to excessive extent of infarction in 6, Hypaque injection in 1, hemorrhage from arterial puncture in 1, unknown causes in 3, and slipping of a coronary thrombus in 2 of the earlier experiments. Analysis of these complications and subsequent technical improvements permitted control of the predominant sources of mortality.

Arteriography as a cause of fibrillation was practically eliminated by admixture of blood to Hypaque in a ratio of 1:3 prior to intracoronary injection. This observation may be of importance in clinical angiography.

Intermittent occlusion of the coronary arteries during dissection was carefully avoided after fibrillation had been observed following sudden release of accidentally compressed vessels.

The mortality due to excessively large infarcts was greatly reduced by obstructing smaller vessels.

Effects of Plasmin

In 16 experiments coronary thrombi were produced successfully and the survival periods were sufficient for prolonged observation by serial coronary arteriography. The initial size of these thrombi and their subsequent variation in time was estimated from the length of the arteriographic filling defects. Immediately after coronary occlusion the range of clot size was 8 to 20 mm. Early retrograde extension up to the next proximal
side branch was often observed. The alterations of coronary thrombi and the microscopic changes in early myocardial infarction will be described separately for each group.

Control Group. In 7 untreated animals coronary thrombi could be demonstrated by persistent filling defects for periods up to 15 hours (fig. 4). From 7 to 15 hours after coronary occlusion an average decrease of 20 per cent was observed in the size of the filling defects, which can be accounted for by clot retraction and possibly minimal lysis. Two of these control animals developed fatal ventricular fibrillation 2 and 9 hours after occlusion. Dissection of coronary vessels at autopsy confirmed the presence of cylindrical thrombi in all 7 animals. One additional untreated animal showed spontaneous fibrinolytic activity, which resulted in disappearance of the filling defect after 7 hours.

The control animals were autopsied 10 to 15 hours after coronary occlusion or earlier in case of fatal complications. The histologic findings consequently refer to the early changes in the infarcted myocardium. The infarcts showed marked interstitial edema, dilatation and congestion of the capillary vessels, scattered focal necrosis of muscle fibers, and marked fibrinous epicarditis with subepicardial leukocytic infiltration. Microthrombi were frequently observed. Figure 5 shows the marginal zone of a 12 hour old infarct with capillary microthrombi and vascular congestion. More severe changes were observed in the center of the same infarct with heavy edema, shrunken muscle fibers, and marked platelet aggregation in dilated vessels (fig. 6).

Plasmin-Treated Group. In the group of 8 animals treated with intravenous or intracorony infusions of plasmin, objective evidence of lysis was obtained in all (fig. 4). Six were treated intravenously and 2 by intracoronary infusion. Fibrinolytic activity was induced 1 to 8 hours after coronary occlusion. Within 2 hours after the start of plasmin treatment progressive shortening of the filling defects became apparent. Complete lysis of coronary thrombi was achieved in 4 animals within 3 to 7 hours. Partial lysis (60 per cent or more) was observed in 4 dogs. One was killed and revealed a tiny residual clot at autopsy. Three animals succumbed to complications 3 to 5 hours after beginning of treatment (ventricular fibrillation in 1, shock due to excessive oozing from the operative wound in 1, and injection of pentobarbital in 1).

The progress of lysis of a coronary thrombus is depicted in the series of arteriograms in figure 3. The filling defects extending from the arrow point to the metal marker indicate the size of the thrombus. In this experiment total lysis of an 8 hour old thrombus was achieved after 4½ hours of systemic plasmin infused at a rate of 4,000 units per Kg. per hour.

The animals were autopsied after restoration of patency as shown by the arteriograms or earlier in case of fatal complications. The microscopic appearance of the treated hearts was in striking contrast to the control hearts of similar duration. Treated infarcts showed less edema in the marginal areas and much less capillary dilatation and epicardial
FIBRINOLYTIC THERAPY OF EXPERIMENTAL THROMBI

Fig. 5 Left. Photomicrograph of a 12 hour old untreated infarct showing the marginal zone with thrombi in capillaries and venules.

Fig. 6 Right. Photomicrograph showing the central zone of the infarct in figure 5 with heavy interstitial edema, shrinkage of muscle fibers, and massive platelet aggregation in a dilated vessel.

fibrin deposits in both central and marginal areas. No microthrombi could be detected in the treated group, suggesting that they may have been either prevented or dissolved. These changes occurred even without complete lysis of the primary thrombus, indicating that plasmin may penetrate the infarcts by collateral perfusion.

Figure 7 is considered representative of a 13 hour old infarct, where coronary circulation had been restored after 6 hours of plasmin treatment. Capillary thrombi and vascular congestion are completely absent. There is moderate edema and some focal muscle cell necrosis with cell invasion.

The predominant tissue changes were graded according to frequency and severity to show the contrast between the treated and the control group (fig. 8). We were unable to find any histologic evidence of damage in the animals treated with plasmin. There was no hemorrhage or evidence of increased necrosis.

DISCUSSION

These experiments prove the dissolution of intracoronary thrombi by the fibrinolytic enzyme, plasmin, within 4 to 8 hours. The composition of these thrombi approximates the constituents of clots in clinical thrombosis. The speed of lysis is the same as for experimental peripheral venous and arterial clots. In man peripheral thrombosis has been successfully treated by plasmin although the speed has not been accurately measured. Since successful lysis of thrombi located at various sites of the circulatory system has been proved in animals and man, the feasibility of fibrinolytic therapy in human coronary thrombosis appears established.

The objective of fibrinolytic therapy is restoration of blood flow to organs by lysis of vascular blockade. The chances for success depend upon the tolerance of the organ to ischemia. It is well known that the time limits of viability are quite different in various organs. Earlier studies suggested that temporary coronary occlusion for more than 30 minutes would result in irreversible myocardial damage similar to permanent occlusion. However, more recent reports indicate that the duration of ischemia may not be the sole factor determining the time limits of viability. Myocardial anoxia may be tolerated for periods up to 2 hours during hypothermia or during perfusion of the heart with heparin-containing solutions.

The mechanism of ischemic injury has been studied in detail by Bing and his associates in terms of survival time of excitability, energy production, and energy utilization of
the heart muscle. It was found that each of these vital functions deteriorates at a different rate that depends in all probability on the resistance of biochemical and biophysical processes to anoxia.

The experimental evidence seems to indicate that the time limit of reversible ischemic injury or myocardial viability is very flexible and related only in part to the length of ischemia. Preservation of the patency of the capillary bed by heparin and slowing of cardiac metabolism by hypothermia appear to extend the myocardial viability.

No data have been reported on the effect of the fibrinolytic state upon the ischemic myocardium. In our studies, the early stages of infarction showed a different histologic structure after induction of the fibrinolytic state. Compared to controls, the plasmin-treated infarcts showed less vascular congestion, less edema, decreased fibrinous epicarditis, and absence of capillary thrombi. These changes sometimes appeared within 1 hour of plasmin therapy, before lysis of the thrombus occluding the main coronary vessel.

These observations suggest that fibrinolytic blood may affect not only the initial occluding thrombus, but penetrate into the infarct itself by collateral perfusion. Studies are now in progress to determine whether rapid restoration of the patency of the capillary bed may influence the viability of the ischemic myocardium by improving the supply of oxygen and metabolic fuels.

Since no deleterious effects of plasmin, such as myocardial rupture or intramyocardial hemorrhage, were observed, we think that our experimental evidence offers a sound rationale for a trial of fibrinolytic therapy of human myocardial infarction. With the recent development of purified preparations of plasmin for human use, such a study is now possible.

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Summary
Forty-four dogs were used to develop a technic for production of serum-induced coronary thrombi. The fate of these thrombi was followed by serial coronary arteriography.
In an untreated control group of 8 animals the coronary thrombus persisted in 7. In 1 animal with spontaneous fibrinolytic activity the thrombus disappeared.

Significant fibrinolytic activity was induced in 8 dogs by systemic infusions of plasmin. Total lysis of the coronary thrombus was achieved in 4 within 3 to 7 hours. Partial lysis of 60 per cent or more was observed in 4.

In comparison to controls of similar duration, the plasmin-treated infaracts showed less vascular congestion and edema and decreased fibrinous epicarditis. Also striking was the complete absence of microthrombosis, which were frequently seen only in the control hearts.

**SUMMARIO IN INTERLINGUA**

Quaranta-quatro canes esseva usate pro disveloppar un technica de inducere thrombos coronari per medio de sero. Le destino del thrombos esseva studiate per arteriographia coronari in series.

In un grupo de 8 animales que recipieva nulle tractamento, 7 monstraveva persistencia del thrombos coronari. In le octave, spontane activitate fibrinolytic resultava in le disparition del thrombo.

In un grupo de 8 canes, grados significative de activitate fibrinolytic esseva inducite per le infusion de plasmina in le circulation systemie. Lyse total del thrombo intra 3 a 7 horas esseva effectuate in 4 de iste animales. Lyse partial de 60 pro centro o plus esseva observate in le altere 4.

In comparation con infarcimento de simile duration in animales de controlo, le infarcimento in animales tractate con plasmina esseva associate con minus sever grados de congestion vascular e de edema e con reduce grados de epicarditis fibrinose. Frappante esseva etiam le complete absentia de microthrombosis in le animales tractate con plasmina. In le animales de controlo, microthrombos esseva vidite frequentemente.

**REFERENCES**


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