SOME OBSERVATIONS ON THROMBOLYSIS in vitro

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(Republic of Haematology, Radcliffe Infirmary, Oxford) The widely accepted intrinsic clot lysis theory (Alkjaersig, Fletcher, and Sherry, 1959) as a primary mechanism of thrombolysis postulates that during formation of thrombi plasma plasminogen adsorbed on to the fibrin/clot surface is activated by circulating plasminogen activators with resultant autodigestion of the thrombus.

Studies in this laboratory have revealed that the plasminogen content of plasma, before and after the formation of artificial thrombi by Poole's modification of the Chandler tube method (Poole, 1959), was not altered, more than 90% of the original plasminogen content of the plasma being recoverable in the defibrinated plasma. Furthermore, the plasminogen content of washed and drained artificial thrombi was uniformly low, and any exogenously added plasminogen was easily washed out of the thrombus.

Similarly, the plasminogen content of human thrombi formed spontaneously over plastic surfaces in contact with a circulation in vivo, and that of fresh and unorganized native (in vivo) thrombi obtained by operative intervention, without exception, was low.

In an artificial circulation, lysis of Chandler tube thrombi prepared from native blood, or plasma, or from recalcified platelet-rich or platelet-depleted citrated human plasma could not be readily achieved by perfusion with a wide variety of concentrations of plasminogen activators in saline-buffer or plasma. On the other hand, perfusion with human plasmin (grade A, Kabi) in saline led to predictable thrombolysis, the rate of lysis being the function of the enzyme concentration used.

From this study, it has been possible to place recent and unorganized native (in vivo) thrombi and emboli into three broad categories: (1) thrombi and emboli, which were deficient in intrinsic plasminogen; (2) thrombi and emboli which, apart from containing only trace amounts of plasminogen, were also rich in an antiplasmin-like activity; and (3) thrombi and emboli which were deficient in plasminogen but rich in a plasminogen activator.

In an artificial circulation, thrombi of the first category behaved similarly to the Chandler tube thrombi, definite lysis only occurring when perfused by human plasmin. Thrombi in the second category failed to lyse with both plasminogen activators and with low concentrations of plasmin; however, when treated by agents known to induce a 'peptone effect' (Astrup, 1968), successful thrombolysis could be achieved with low concentrations of plasmin. Thrombi in the final category lysed slowly but spontaneously, and it was possible to enhance the rate of lysis by perfusion with plasminogen activators.

The physico-chemical properties of the plasminogen activator isolated from saline extracts of thrombi of the latter category were different from those of tissue activators of plasminogen, leukoproteases, urokinase, and streptokinase (Dalal, Shah, Allington, and Sharp, 1969).

Recent unorganized thrombi and emboli of all categories were found to be permeable to various blood components, including plasma plasminogen and antiplasmins, and it was possible to flush red cells out of such thrombi by prolonged gentle perfusion finally leaving a pearly white coralline network of fibrin.

On the basis of these and our other findings, it is suggested that the selective thrombus activator of plasminogen binds with a globulin fraction of plasma and that this complex activates the plasminogen permeating through the thrombus mass to an enzymatically active plasmin-globulin complex. This complex could not be inhibited by circulating antiplasmins. Thus this enzymatically active plasmin-globulin complex could digest the fibrin core of a thrombus in the presence of circulating antiplasmins and it is suggested that this may be the mechanism responsible for thrombolysis in vivo (Dalal, P. M., Shah, P. M., Sharp, A. A., and Allington, M. J., 1969).

THROMBUS DISSOLUTION

A. A. SHARP (Department of Haematology, Radcliffe Infirmary, Oxford) Thrombi have been assumed by many to be standard units made up of fibrin interspersed with platelets, red cells, and white cells and we tend to assume that all thrombi are equal in the eyes of God. Yet it must be obvious to all that there are thrombi and thrombi and that the nature of the fibrin matrix must vary depending on the site of thrombus formation.

Studies in our laboratory have shown that there are considerable differences between fibrin clots formed in a test tube, those formed artificially in a Chandler tube in vitro, and those native thrombi formed in vivo. Further, there are considerable differences between a thrombus formed on the wall of a vessel, and a thrombus occluding an artery or vein. There are also differences between fresh and old thrombi, age having a variable influence on morphology. When considering thrombolysis there appear to be thrombi which can lyse spontaneously, those which can be lysed by extraneous factors, and those which can never lyse or be lysed (Dalal, Shah, Sharp, and Allington, 1969). Also when certain thrombi are perfused, the perfusate can be shown to pass easily through the interstices of the fibrin, washing out the red cells and trapped plasma. Therefore, it is reasonable to assume that even occluding thrombi may allow the
slow passage of blood through the thrombus allowing access of plasma and its contents and thus the agents promoting thrombolysis to the fibrin of the thrombus.

This latter observation leads one to speculate that thrombus perfusion by blood may happen in certain circumstances but not in others. The way the fibrin is formed, in an artery or vein, in smooth or turbulent flow, quickly or slowly, or the time elapsed since it has formed, may alter the size of the interstices between the fibrin strands, and, therefore, the ability of the plasma factors or activator to gain access to the fibrin. Further, one knows that in some instances blood cannot flow through small vessels by virtue of a subtle change in the red cell-plasma ratio, the plasma protein concentration, or due to the formation of 'sludge'. Such changes from the normal would tend to render the fibrin mesh more impervious to perfusion and prevent the access of those factors necessary for fibrinolysis. The relationship of platelet aggregates to the fibrin and their size may also interfere with the passage of blood through a thrombus. White cells may also influence the situation but their role is impossible to define at present. The phenomenon of clot retraction may also play its part by altering the characteristics of the fibrin mesh and again platelets play their part in this phenomenon. The, as yet, ill-defined effect of factor XIII on the pattern of the fibrin mesh must also be considered. The relationship of the thrombus to the pattern of blood flow must also influence the chances of efficient perfusion. An isolated thrombus in a main vein may produce sufficient resistance to promote flow through a developing collateral circulation and so reduce the chance of blood passing through the thrombus, but if large numbers of multiple thrombi form which obstruct both the main and collateral circulation, then the perfusion pressure through all thrombi would be expected to increase and the chances of thrombus dissolution should be increased. All these variables alone or together must play their part in determining the access of proteolytic activity to fibrin in a thrombus and so influence thrombolysis.

The mechanism by which thrombus dissolution takes place once an active proteolytic enzyme comes in contact with fibrin must also be considered. The term fibrinolysis or thrombolysis invokes the dissolving of fibrin by enzymatic action (perhaps beginning at the periphery of a thrombus and gradually eating towards the centre), the digested fibrin being washed away in the blood stream as submicroscopic fragments. However, recent studies in this laboratory have shown that the dissolution of a thrombus in a known lytic system does not result in the fibrin going into solution like salt does in water. While there is some weight loss and radioactivity can be released from fibrin, thrombus still retains its identifiable mass and shape and actual dissolution appears to be the breaking up of the thrombus into multiple fragments of varying size, some big, some very small.

Thrombi have been shown to retain their solid nature for a very long time after exposure to a known effective lytic agent, unless they are exposed to mechanical agitation, when the tendency to fragmentation is enhanced.

In an attempt to see if it was possible to determine how lysis or dissolution takes place, fibrin thrombi have been studied by phase contrast microscopy before and after exposure to strong concentrations of plasmin. This study is only in its early stages, but so far it has not revealed any obvious difference between a freshly-formed fibrin thrombus perfused with buffer and a thrombus perfused with a high concentration of plasmin for several hours. In the latter the fibrin strands could still be seen forming a strong mesh binding a mass of apparently unchanged contrasting material. No holes appeared nor could other differences be detected, and its mass appeared to be unaltered. However, this plasmin-treated fibrin could be more easily disrupted by mechanical trauma than the original. Phase contrast microscopy can be criticized on the grounds of resolution, but parallel electron microscopy studies, while showing alteration in the banding of the fibrin strands, have not revealed how lysis actually takes place.

These observations may be explained by likening a fibrin thrombus to a three-dimensional chain mail such as was worn by our forefathers in battle. If one breaks one link in a single chain the chain must separate into two parts. If two chains are cross-linked, side by side, the problem becomes more complex, and in a three-dimensional situation, such as is found in thrombosis, the number of links that have to be broken increases considerably before a separation into two parts can take place. If one considers that some bonds are stronger than others, then another variable is introduced.

Therefore our concept of lysis may be incorrect and the word dissolution or disintegration may be a better description of what actually takes place. If plasmin acts by breaking linkages between molecules of formed fibrin, it can only attack where those sites can be reached. In a complex mesh this process must be a random phenomenon but once plasmin has broken any one bond it is more likely to get at the next above, below, or alongside. However, before a thrombus can disintegrate into fragments, a very large number of interlinking bonds must be disrupted, and it is easy to imagine how considerable 'lysis' may have occurred within a thrombus without any significant change being apparent in that thrombus. Physical agitation would be expected to aid the action of a proteolytic process by separating the bonds already digested, thus improving the access of the enzyme to the remaining unaltered links or bonds.

Thus, when considering the efficiency of lytic therapy in any given situation, thrombolysis could be taking place in a thrombus, yet, at the end of a period of therapy, an apparently unaltered mass might remain which could still maintain its structure and still be capable of blocking the passage of blood or radio-opaque media. This situation would constitute a failure of therapy, and yet after an hour, a day, or a week later this thrombus might break up allowing partial or complete restoration of blood flow, a delayed clinical success not supported by venography or arteriography, and therefore an objective failure of therapy.

Thus either spontaneous or induced thrombolysis is unlikely to be successful in removing thrombi in every instance. Many factors influence any given situation, and the impression that some but not all thrombi disintegrate
In evaluating the usefulness of one or other type of therapy to promote thrombolysis, a significant percentage of failures is to be expected.

REFERENCES

RECENT PROGRESS IN THROMBOLYTIC THERAPY

A. AMERY, J. VERMYLEN, AND M. VERSTRAETE (Leuven University, Belgium) This study compares the effect on fibrinogen and plasminogen levels of the infusion of a high (1,200,000 units) and medium (250,000 units) standardized initial dose of streptokinase. In both groups the administration of the initial dose was followed by a continuous infusion of 100,000 units of streptokinase per hour.

Immediately after the initial dose of 1,200,000 units of streptokinase, the plasma plasminogen was already below 1% of the normal value in half the patients. No significant further change in the distribution was found in samples withdrawn after 24 hours of maintenance therapy; at that time the plasma plasminogen concentration was below 1% of the normal value in 48% of the patients and below 5% of the normal value in 93% of the patients.

Immediate after the initial dose of 250,000 units of streptokinase the plasma plasminogen level was below 1% of the normal value in only 13% of the patients. Nevertheless, after 24 hours of maintenance therapy, the plasminogen level had reached values which were practically identical to those observed following the high initial dose; in 55% of the patients the plasma plasminogen level was less than 1% and in 86% of the patients less than 5% of the normal value.

The changes of the fibrinogen levels, as observed with the fibrin polymerization time test, also showed some interesting differences. When 1,200,000 units of streptokinase were given as the initial dose, there was a marked drop of the fibrinogen level but after 24 hours' maintenance therapy there was a significant increase. When 250,000 units of streptokinase were infused there was a smaller immediate fall of the fibrinogen level but after 24 hours' maintenance therapy it had decreased still further. It should be emphasized that the biological fibrinogen assay used in this study is influenced not only by the actual fibrinogen level but also by the presence of those fibrinogen degradation products which interfere with polymerization.

PLASMIN, PLASMIN INHIBITORS, AND DEGRADATION PRODUCTS OF FIBRINOGEN IN HUMAN SERUM DURING AND AFTER INTRAVENOUS INFUSION OF STREPTOKINASE

INGA MARIE NILSSON (Coagulation Laboratory, University of Lund, Malmö, Sweden) The variation of plasminogen, plasmin, plasmin inhibitors, fibrinogen, and its split products has been studied in sera from patients before, during, and after a 24-hour intravenous infusion of streptokinase. The amounts of α₂-macroglobulin, α₂-antitrypsin, and degradation products of fibrinogen in the serum have been estimated by immunological techniques. To separate plasminogen from an assumed complex between inhibitors and plasmin, sera were fractionated by gel-filtration in Sephadex G 200 and the eluted fractions were examined immunologically with specific antisera for the presence of plasminogen, plasmin, α₂-macroglobulin, or α₂-antitrypsin. The thrombin inhibiting capacity of the fractions was also estimated.

It has been found that practically all plasminogen activated to plasmin within the first hours of the infusion. The plasmin was recovered mainly as a complex with the 'immediate inhibitor', i.e., α₂-macroglobulin. This complex was almost completely eliminated within 24 hours, during which time the α₂-macroglobulin in serum decreased by about 50%. Alpha1-macroglobulin did not recover its initial level until three to four weeks after the infusion. The plasminogen recovered its original concentration within about 48 hours. No complex formation could be demonstrated between plasmin and the slow inhibitor, i.e., α₁-antitrypsin. Alpha₁-antitrypsin increased markedly after the infusion of streptokinase. The thrombin-inhibiting capacity of the macroglobulin fractions after gel filtration varied mainly with the concentration of α₂-macroglobulin, while the inhibiting capacity of the fractions containing albumin was almost unchanged during the infusion of streptokinase. The concentration of the degradation products was highest four hours after the beginning of the infusion. Only trace amounts were demonstrable 24 hours after the end of the infusion.

In our earlier investigations we questioned whether the 'intrinsic clot lysis' theory alone can explain the dissolution mechanism of thrombi in vivo (Hedner, Nilsson, and Robertson, 1966). We found no difference between the plasminogen content of plasma and that of serum, and our experiments argue against plasminogen being adsorbed to the fibrin during clot formation. Also in thrombi, we found the plasminogen values to be very low. Most workers administer large doses of streptokinase in order to suppress the plasminogen to a very low level and thereby prevent plasmin formation but we wonder whether it is not possible to get active plasmin. Plasminogen is synthesized very rapidly, and even if it is suppressed to almost nil, plasminogen will continuously be produced and there will be at least some plasminogen present during therapy. The most important antiplasmin, α₂-macroglobulin, decreased rapidly in the beginning of the streptokinase infusion and unlike most other proteins it is synthesized very slowly, in the course of three to four weeks. This means that even if the plasminogen level is low during therapy, the plasmin formed on activation is not inactivated by α₂-macroglobulin and may help to cause thrombolysis by its adsorption to fibrin.

REFERENCE