Thrombolytic therapy: pharmacology

Thrombolysis

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Intravascular thrombosis is one of the most significant problems in clinical medicine and surgery today. While prophylaxis is the obvious goal, thrombus formation will occur without warning in apparently healthy men and women and this will continue to present a therapeutic challenge for many years to come. Thus, a discussion of the mechanism of thrombolysis is worthwhile and important.

The basic experiment that best illustrates the problem is as follows: if streptokinase (SK) or urokinase (UK) is added to blood and clot or thrombus formation is then allowed to take place rapid lysis of the fibrin will occur. Yet, if fibrin formation is promoted first and then activator activity induced, fibrinolysis will be very slow or may never take place at all.

Over the years, research into this problem in our department has revealed many divergent and puzzling results.

Frozen sections of thrombi obtained following surgical intervention in man were found to be difficult to lyse: SK or UK had no direct effect while pure plasmin produced a dramatic digestion of the fibrin. Mixtures of activator with plasma or serum had variable, slow, and sometimes no effect.

When blood clots, artificial Chandler thrombi, or thrombus material obtained by surgical intervention were perfused in a low pressure system (5 mm Hg), a similar pattern of results was obtained. Yet, other studies (McNicol, Bain, Walker, Rifkind, and Douglas, 1965) obtained very different results. Using SK alone in saline in a high pressure system, they were able to induce fibrinolysis in artificial thrombi.

Two theories have been advanced in the past to explain the phenomenon of thrombolysis. Alkjaersig, Fletcher, and Sherry (1959) suggested that lysis was proportionate to activator activity and if the latter was kept constant, to the plasminogen content of the fibrin mass. They believed that plasminogen must be adsorbed to the fibrin and activated *in situ* to form plasmin and so promote lysis of fibrin. While this theory is almost universally accepted, evidence has been accumulating to suggest plasminogen is not, in fact, adsorbed to fibrin when it forms and that the only plasminogen present in a thrombus is that contained in the trapped plasma (Hedner, Nilsson, and Robertson, 1966; Ogston, Ogston, and Fullerton, 1966). Recent experiments in our department have also shown that this would appear to be the case and that demonstrable antiplasmin is also present in trapped plasma within a thrombus. The second theory suggests that when activator converts plasminogen to plasmin, the plasmin is bound to the excess antiplasmin present and the complex disassociates on contact with fibrin to release plasmin and promote lysis of fibrin (Ambrus and Markus, 1960).

On present evidence, neither theory by itself can explain all the phenomena observed and perhaps a compromise between the two may be nearer the truth (Konttinen, 1968).

If it be accepted that plasminogen is not bound to fibrin and that in a fibrin mass, plasminogen and antiplasmin are contained only in the trapped plasma, how can activator induce thrombolysis?

In high pressure situations, SK in a saline perfusion alone may induce progressive and complete lysis. This observation essentially supports the theory of Sherry's hypothesis of bound plasminogen as any trapped plasma-plasminogen and antiplasmin must be diluted and removed by the perfusion fluid. However, this discrepancy can be explained by suggesting that the trapped plasminogen is activated immediately on contact with activator and the activator-plasminogen complex or the plasmin binds to the fibrin. Further, there is evidence that once this linkage is established, lysis will progress uninterrupted by changes in the perfusate or the blood (Weiner, Redisch, and Weisberg, 1959).

This tends to support the Ambrus concept, yet experimental systems would suggest that perfusion of fibrin with plasma with a high plasmin-antiplasmin content is far less effective than

the rapid activator induced lysis system. Similarly, plasmin added to plasma is ineffective unless added in sufficient amounts to overcome the antiplasmin.

Another variation of this mechanism can be proposed. Dalal, Shah, Allington, and Sharp (1969), studying thrombi *in vivo*, discovered two such thrombi which lysed progressively when perfused by buffer alone. It was possible to isolate an 'activator complex' from these thrombi which appeared to bind with α_2 globulin. This complex added to plasminogen released plasmin which experimentally could not be neutralized by antiplasmin or aprotinin (Trasylol). Therefore, an alternative hypothesis can be put forward, namely, that activator can link with an α_2 globulin in the plasma or in the trapped plasma of a thrombus and generate from the plasminogen a plasmin that is protected from the action of antiplasmin, the receptor linkage for antiplasmin apparently being blocked by the α_2 globulin. Further proof of this concept has been in the work of James, Taylor, and Fudenberg (1967) and by the often observed fibrinogenolysis after the induction of activator activity in blood during therapy. This latter phenomenon is linked to the activator plasminogen inter-reaction and does not take place after plasminogen depletion. Yet, the amounts of free plasmin detected in circulating blood obtained at the time of fibrinogenolysis are variable. While this tends to disprove the hypothesis, consumption of the complex during lysis or by SK could provide an explanation.

So far, confirmatory evidence to prove this hypothesis has eluded us. Yet, one interesting observation can be reported: in a study made in our department (Berry, Sharp, and Allington, 1970) on postmortem lytic activity a significant and powerful activator was isolated from the cadaver blood. This activator had properties similar to exercise and endothelial activator. When fractioned through a Sephadex G200 column, the fraction containing a significant proportion of this activity appeared to be linked to a α_2 globulin and, in fact, on an immune basis this appears to be α_2 M globulin.

These arguments suggest several hypotheses for discussion and experimentation: (1) that activator + plasminogen or the resultant plasmin selectively bind and act on fibrin, antiplasmin having no effect on this mechanism; or (2) activator may link with a protein (α_2 globulin) and, reacting with plasminogen, promote a plasmin-α_2 globulin complex with high affinity for fibrin or fibrinogen and which is protected against α_2 M or α_1 antiplasmins. (3) The activator-plasmin α_2 complex could explain the fibrinogenolysis that occurs during lytic therapy. Thus, it would appear that there are two types of thrombolysis: (a) Intrinsic immediate, progressive, and rapid and likely to be the most important. This is induced by the action of activator within a fibrin mass. (b) External or 'frontal' slow and relatively inefficient and due to an external action by plasmin released from the circulating plasmin antiplasmin complex.

These hypotheses do, of course, require further work, but if the new, vast literature is studied, one can find that these are not all original ideas and there are clues which suggest that they are not entirely fanciful.