Inhibitors of fibrinolysis

Inhibitors of fibrinolysis: evidence of some instances of mutual potentiation

SERGIO COCCHERI AND V. DE ROSA

From the Institute for Occupational and Social Medicine, University of Bologna Medical School, Bologna, Italy

Inhibitors of fibrinolysis may be divided into naturally circulating inhibitors and antifibrinolytic agents. Natural inhibitors are present in plasma and are components of blood cells, especially platelets. Several types of antifibrinolytic activity can be distinguished: antiplasmin activity, and anti-activator activity against streptokinase, urokinase, and tissue activator induced plasminogen activation.

Antiprotease activities in the blood are mainly attributed to α_2 macroglobulin (mw 800 000), an immediate plasmin inhibitor, and to α_1 antitrypsin (mw 40-71 000), a progressive plasmin inhibitor. Progressive antiplasmin activity seems also to be localized in the zone of 'inter- α ' antitrypsin.

Recent investigations have shown that antitrypsin activity in plasma is a very complex system with genetically controlled individual variations (Fagerhol, 1971).

Anti-activators againsts treptokinase, urokinase, and tissue activator certainly represent three different inhibiting 'activities' but there is still some question whether they are really three different substances or rather the result of peculiar interactions between antiplasmins. Accordingly anti-urokinase and anti-tissue activator activities could be a result of the interaction of α_2 macroglobulin and α_1 antitrypsin, while anti-streptokinase activity could be a function of α_2 macroglobulin alone (Spöttl, 1970). In contrast, the work of Dr Nilsson's group reported at this meeting suggests the existence of anti-activators as individual substances differing from antiplasmins.

Antifibrinolytic activity in platelets is not more than 10% of the whole plasma activity. Both α_1 antitrypsin and factor XIII or a similar protein seems to be involved in the antifibrinolytic activity of platelets.

The patho-physiological significance of the naturally circulating inhibitors of fibrinolysis is still under discussion. It may be recalled that an increase of antiplasmin activity has been observed

in human atherosclerosis and in thrombosis, and a decrease in liver cirrhosis. A specific decrease of a fraction of α_1 antitrypsin has been found in cases of pulmonary emphysema and in related diseases. An increase of anti-activators can be associated with multiple thrombosis, while a selective inhibition of urokinase surprisingly associated with a decrease of anti-streptokinase activity was observed in the last trimester of pregnancy (Brakman and Astrup, 1963). It is interesting to note in this respect that we observed a higher mortality from pulmonary embolism in pregnant mice, following tissue thromboplastin injection, compared to normal mice, an effect which could not be attributed to variations in antithromboplastic activity (Coccheri, Ollendorf, and Astrup, 1969).

Coming now to antifibrinolytic agents we should at first consider extractive inhibitors.

Soya bean and peanut inhibitors form highly dissociated complexes with plasmin. They also act against plasminogen activation.

Bovine lung trypsin inhibitor (pulmin), aprotinin (trasylol or kallikrein inhibitor), and pancreatic inhibitor seem to be similar in structure and activity; they all have a high affinity to plasmin, and also inhibit plasminogen activation, especially when induced by streptokinase (Egeblad, 1967).

Mingin, a urinary trypsin inhibitor, is a weak inhibitor of plasmin and of plasminogen activation. Mingin is characterized by a mutual potentiation with ϵ -amino caproic acid (EACA), an interesting feature when we consider that mingin might be an excretion form of a spontaneously circulating inhibitor (Astrup and Nissen, 1964; Astrup, 1968).

Among synthetic inhibitors, EACA, tranexamic acid (AMCHA) and p-amino-methyl-benzoic acid (PAMBA) have low affinity to plasmin, and predominantly inhibit plasminogen activation. EACA in fact combines three main properties: inhibiting effects, activating effects at small doses, and potentiation with other inhibitors.

When administered *in vivo* antifibrinolytic agents enhanced atherogenesis in rabbits fed a high cholesterol diet (Coccheri, Borgatti, Loreti, and Gennari, 1964) and increased fibrin deposition in the kidney after thrombin infusion (Beller, Mitchell, and Gorstein, 1967). Pulmin treatment, however, does not increase the death rate in mice with pulmonary embolism due to tissue thromboplastic injection, although the rate of thrombolysis in the lungs is significantly impaired (Kwaan, Coccheri, and Astrup, 1967). In fact, inhibition of thrombolysis seems to be a slow process with little influence in acute phenomena.

Our investigations *in vitro* have been directed at pharmacological potentiation between antifibrinolytic agents.

A simple lysis time system with bovine or human fibrinogen, plasminogen activators (urokinase, streptokinase), thrombin, and inhibitors has been used.

We have confirmed the mutual potentiation between EACA and mingin in experiments with bovine fibrinogen and urokinase activation. The effect is dose-dependent, being decreased when reducing the concentration of either inhibitor. EACA and mingin seemingly behave as co-factors of each other. The potentiation effect of EACA with mingin is still observed when EACA is present in very small concentrations which would act alone as an activator.

Small amounts of EACA also potentiate the effect of pulmin, and vice versa, but the effect disappears when higher concentrations of either inhibitor are used.

Between aprotinin and AMCHA, in a bovine fibrinogen system activated by urokinase, no potentiation was observed. However, when traces of human plasma were added to the system, the

Fig. Kallikrein inhibitor effect on (a) SKA activated human fibrinogen; (b) potentiation in presence of AMCHA.

inhibitory effect of AMCHA was greatly enhanced while that of aprotinin was not affected.

Thereafter, we tested the two inhibitors for potentiation in a system containing human fibrinogen and streptokinase. A very striking potentiating effect was detected when increasing amounts of aprotinin were added in presence of a low concentration of AMCHA (see Figure). The observed phenomena of potentiation seem therefore to differ in bovine and human systems.

It is our opinion that these phenomena should be carefully investigated in systems *in vitro* as close as possible to human conditions, and afterwards confirmed *in vivo*. The results may be a great help in the treatment of hyperfibrinolytic conditions.