In Vivo Fibrinolytic Activity and Pharmacology of Various Plasmin (Fibrinolysin) Preparations


A quantitative method based on the disappearance of $^{111}$I labeled fibrin has been used to study the in vivo effect of fibrinolytic agents. Various preparations of human and bovine plasmin were found to lyse clots effectively. The minimal effective dose of human plasmin prepared by the method of Kline was 15 Loomis units/Kg. Plasmin preparations given intravenously produced hypotension and leukopenia followed by leukocytosis. Doses above 30 Loomis units/Kg caused decrease in fibrinogen level and clotting index.

The in vivo fibrinolytic activity of human plasmin (fibrinolysin) has been demonstrated by Clifton, Grossi and Cannamela, and Sherry, Titchener, Gottesman, Wasserman and Troll. Essentially qualitative methods have been used in all these studies. In the previous paper methods have been described for the in vivo quantitative testing of fibrinolytic agents, and for the differentiation between fibrinolytic and anticoagulant activity. It was thought that the fibrinolytic effect of various doses of different plasmin preparations should be compared and minimal effective doses established. At the same time, some pharmacologic responses to human plasmin have been studied. Guest and associates have studied the physiological effects of bovine plasmin.

Methods and Materials

Materials, methods, and procedures have been described in the previous paper. Only $^{111}$I labeled clots and emboli were used in this study. In addition to human plasmin prepared by the methods of Kline, (fraction A), and Fletcher, bovine plasmin prepared by the procedure of Loomis was studied. Human plasminogen was activated in vitro for 10 to 20 minutes before starting the infusion with doses of streptokinase-streptodornase indicated in the tables. In experiments where blood pressure effects of plasmin were tested, either rapid intravenous injections or 15 minute infusions were used. In all other experiments, the plasmin was infused intravenously in 500 ml saline during a four hour period.

Results

Figure 1 shows an experiment in which a dog with three thrombosed vessels (1 artery and 2 veins) first received 500 units/Kg of nonactivated human plasminogen (prepared by the Kline method). This had practically no fibrinolytic effect. Subsequent to this infusion and daily thereafter for five days, 500 units/Kg of activated plasmin was infused. Within 24 hours significant lysis was observed. During the subsequent days, the same doses became less and less effective. Nonactivated plasminogen did not alter clotting index or fibrinogen levels; activated plasmin greatly reduced both. Prothrombin tests with added fibrinogen indicated that decrease in fibrinogen level is responsible.
for only part of the clotting index changes. On the following days biochemical changes induced by the same dose of plasmin became less and less pronounced. This diminishing of biochemical response corresponded with the decrease in degree of fibrinolysis. Treatment was continued in spite of apparent refractoriness. Often (as in this example) the ability of plasmin to induce biochemical changes returned after a few days of treatment. We know of no explanation for this. A study of the mechanism of these phenomena is in progress.

Figure 2 shows the effect of a single infusion of plasmin prepared according to the method of Fletcher. Significant fibrinolysis and the above mentioned biochemical changes can be noted. Figure 3 illustrates the important fibrinolytic effect of 30 units/Kg. of plasmin, activated with 1100 units/Kg. of streptokinase. Definite lysis occurred during the first 24 hours, but further doses were ineffective. This dose did not alter significantly the clotting index. Fibrinogen level increased, probably due to intercurrent infection. A control experiment is shown in figure 3 of the previous paper.
Table 1D§ analyzes the significance of all results obtained within 96 hours with various dose levels of human plasmin as compared to controls. It appears that doses from 15 to 1000 units/Kg. of plasmin induced significant lysis. Table 2D§ summarizes the detailed data, including results obtained with peripheral emboli. Tables 3D§ and 4D§ analyze the significance of the degree of lysis at 4 and 24 hours after the first infusion.

Table 5D§ analyzes the significance of the experiment in which 1000 units/Kg. human plasmin was infused into normal dogs. Subsequent to this, clots were produced by the usual methods. Thus, plasmin became incorporated into the clot. This resulted in almost complete lysis within 4 hours without further treatment. Tables 6D§ and 7D§ demonstrate the effectiveness of human plasmin prepared according to the method of Fletcher. 11 Tables 8D§ and 9D§ summarize results obtained with bovine plasmin prepared and activated according to the method of Loomis. 8 Table 10D§ tabulates the biochemical and hematologic changes observed in the above experiments. It appears that all plasmin preparations tested are able to lyse thrombi and emboli in vivo. In high doses, all preparations decrease clotting index and fibrinogen level.

Pharmacology. As illustrated in figures 1D§, 2D§ and 3D§, human plasmin prepared according to the Kline method exerts a hypotensive effect if rapidly infused in doses greater than 30 units/Kg. The hypotension produced was found to be proportional to the dose (fig. 1D§). As illustrated in figure 3D§ blood pressure effects varied in an experiment in which plasminogen corresponding to 500 units/Kg. plasmin, or streptokinase-streptodornase sufficient to activate the former or 500 units/Kg. plasmin activated with streptokinase, was injected intravenously. Streptokinase-streptodornase had little effect on blood pressure. Plasminogen produced only slight hypotension, while the same dose of activated plasminogen (plasmin) induced a pronounced fall in blood pressure. No consistent, specific changes were seen in the electrocardiogram during and after infusion of plasminogen or plasmin in doses up to 1000 units/Kg. The hypotensive effect of 125 units/Kg. plasmin on the first, second, and fifth day of daily treatment is illustrated in figure 3D§. The hypotensive effect diminishes with decrease in fibrinolytic activity and reduced ability to produce biochemical changes.

Hematologic changes are recorded in Table 10D§. Plasmin in all instances rapidly induced leukopenia. However, while the infusion of plasmin was still in progress, the white cell count started to increase and occasionally surpassed the original level before completion of the infusion. Red cell counts and hematocrit values did not change significantly, except in some chronic experiments in which anemia gradually developed.

Discussion

It appeared that doses of plasmin can be found which cause no important pharmacologic or biochemical changes, yet are able to lyse clots under the conditions of the experiments described. It should be remarked that in these experiments plasmin treatment was started shortly after the clots had formed. Histologic studies revealed little organization in control clots. It is likely that highly organized clots would be more resistant to treatment with plasmin.

Guest and associates 9 found that bovine plasmin stimulates intestinal smooth muscle of rabbits, decreases cardiac output in perfused turtle hearts, produces hypotension in dogs and guinea pigs, and leukopenia in dogs. After inactivating the fibrinolytic activity of bovine plasmin by incubation with plasma, cysteine, or glutathione, the above pharmacologic effects were found to be unaltered. It was suggested that another plasma factor, termed vascularine may be responsible for these changes. In our studies with purified human plasmin in dogs, hypotension and leukopenia followed by leukocytosis was noticed. On the other hand, it appeared that inactive plasminogen is less effective in this respect than plasmin. This may suggest that plasmin or products of its reaction with proteins may be directly involved in bringing about the pharmacologic changes observed.
SUMMARY

Human plasmin prepared by the methods of Kline or Fletcher or bovine plasmin prepared by the method of Loomis was found to effectively dissolve radioactive arterial or venous thrombi or emboli.

The minimum effective dose was 15 Loomis units/Kg. of plasmin, fraction A, prepared according to Kline.

Doses above 30 units/Kg. produced a fall in fibrinogen level and clotting index, and induced hypotension proportional to the dose of plasmin. No specific changes were seen in the electrocardiogram. Plasminogen was found to be less hypotensive than corresponding doses of plasmin.

Leukopenia occurred rapidly after starting the infusion. Subsequently, leukocytosis ensued, often before the end of the infusion of plasmin.

SUMMARY IN INTERLINGUA

Esseva constatate que plasmina human in preparatos secundo Kline o Fletcher o plasmina bovin in preparatos secundo Loomis dissolveva efficacemente radioactive thrombos o embulos arterial o venose.

Le minimal dose efficace esseva 15 unitates Loomis de fraction A de plasmina per kg. preparate secundo Kline.

Doses in excesso de 30 unitates per kg. produceva un reduction del nivello de fibrinogeno e del indice coagulatique. Illos induveva hypotension proportionalmente al dose de plasmina. Nulle specific alterationes electrocardiographie esseva observate. Plasminogeno se mostrava minus hypotensive que doses correspondente de plasmina.

Leucopenia occurreva rapidemente post le initiation del infusion. Postea il habeva leucocytosis, frequentemente ante le fin del infusion de plasmina.

REFERENCES

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