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Two Forms of α_2-antiplasmin: Post-traumatic Changes in the Rat

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ABSTRACT

The plasminogen-binding (PB-AP) and non-plasminogen-binding (NPB-AP) forms of α_2-antiplasmin (AP), were assayed in rat plasma by a modified rocket immunoelectrophoretic technique before and up to 48 h after turpentine-induced trauma, using an intermediary gel containing kringles 1-3 from plasminogen. The concentration of PB-AP was significantly elevated by 22 % 24 h post-traumatically, while NPB-AP was decreased at that point in time, leaving the total AP level unchanged. Total AP increased by 57 % during the period 24 - 48 h after trauma, mainly on account of increases in the NPB-AP form.

It is concluded that the plasma level of AP can remain unchanged in spite of increased fibrinolysis inhibition, owing to a relative increase in the functionally more active PB-AP.

INTRODUCTION

The important fibrinolysis inhibitor α_2-antiplasmin (AP) has been found to exist in a plasminogen-binding (PB-AP) and a non-plasminogen-binding (NPB-AP) form in man (1, 2, 3, 4, 5, 12, 14, 15). We have previously reported on the presence of AP in the rat and described some of its changes after induction of trauma (8, 9, 10). The purpose of the present investigation was to determine the effects of turpentine trauma on the two forms of the inhibitor in the rat.

MATERIALS AND METHODS

Chemicals: French turpentine oil (Kebo AB, Sweden), CNBr-Sepharose^R 4B, Sepharose^R 4B (Pharmacia Fine Chemicals, Sweden); Agarose M^R

(LKB, Sweden). Anti-rat α_2AP-IgG was prepared as described previously (8).

Animals: Male Sprague-Dawley rats (300 - 380 g) from the Anticimex Farm, Stockholm, were used. They had free access to food (Ewos rat pellets) and tap water throughout the experiment. All surgical procedures were carried out under ether anaesthesia.

Turpentine trauma was induced by an intramuscular injection of 0.5 ml of turpentine into each hind leg. Animals, five at each time, were killed 24, 36 and 48 h after the turpentine injection.

Preparation of plasma: Four millilitres of blood was drawn from the aorta into a plastic tube containing 1 ml of 3.8 % trisodium citrate solution. The tubes were immediately centrifuged and the plasma was aspirated with siliconised pipettes and stored at -20°C until analysed. Plasma from five untreated animals was pooled and used as a control.

Assay of the different forms of α_2-antiplasmin: An electroimmunoassay technique mainly as described by Wiman et al (15) was employed, using an intermediary gel containing kringles 1-3 from human plasminogen coupled to CNBr-activated Sepharose 4B (~5 mg kringles/ml settled gel). In this gel the PB-AP form is absorbed, while the NPB-AP form runs through and forms rockets in the subsequent anti-α_2-AP-IgG, containing agarose gel. The intermediary agarose gel contained 9 % "kringle-Sepharose". Total AP was measured on the same plate, using pure Sepharose 4B in the intermediary agarose gel to equate any loss of antigen due to unspecific adsorption. The difference in height between the rockets was considered to represent PB-AP. Pooled normal rat plasma, 0.5 - 1.25 µl, diluted to 5 µl in 0.024 M Veronal buffer pH 8.6, was used for standard curves. The samples contained 0.83 µl of plasma and were diluted to 5 µl in Veronal buffer. All results are expressed in per cent of total AP found in pooled normal rat plasma.

Statistical analysis: Differences between groups were tested by Wilcoxon's rank sum test. A p value below 1 % was considered significant.

RESULTS

The results are presented in Fig. 1. PB-AP amounted to 63.1 % of the total AP in control animals.

After 24 h PB-AP was significantly increased by 22 % to 77 ± 4 %. At the same time NPB-AP was significantly decreased to 22 ± 7 % while the total AP remained unchanged. Between 24 and 48 h the total AP increased to 156 %, and this was mainly referable to an increase in the NPB form.

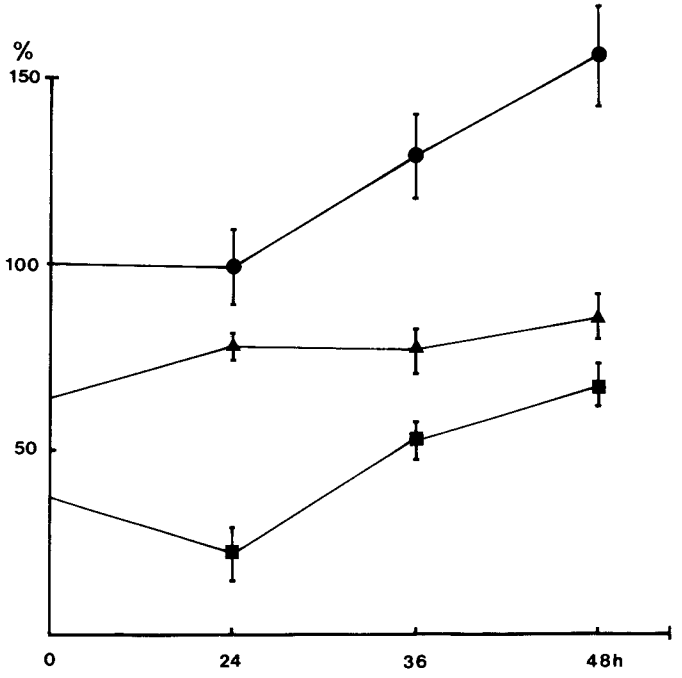

Fig. 1

Total α_2-antiplasmin (●), plasminogen-binding (▲) and non-plasminogen-binding (■) forms of α_2- antiplasmin 24, 36 and 48 h after induction of trauma with turpentine. Values are expressed in per cent (mean ± SD; n = 5) of total α_2-antiplasmin in normal rat plasma.

DISCUSSION

In this study the concentration of the plasminogen-binding form of α_2-AP was increased in rat serum 24 h after turpentine trauma. There was a concomitant decrease in the non-plasminogen-binding form.

In a previous investigation it was found that the fibrinolysis inhibition activity in serum, which involves not only plasmin inactivation but also inhibition of plasminogen activation and changes in plasmin(ogen), fibrin interactions, was increased by 20 % 24 h after turpentine trauma even though no changes were observed in the plasmin inhibition activity in plasma or in the immunologically determined α_2-antiplasmin concentration (9). These results may be explained by the present finding of a 22 % increase in PB-AP but no change in the total AP - provided that both

forms of α_2-AP are potent plasmin inhibitors and that the plasminogen--binding form also interferes with plasminogen activation and with the plasminogen uptake by fibrin. Earlier investigations with use of human α_2-AP have yielded results supporting this view (5, 13, 14).

Preliminary studies (11) have indicated that PB-AP is synthesized by the liver, in which case NPB-AP should be a metabolite, although the present findings do not preclude the reverse.

A known metabolite of AP is its complex with plasmin, which in humans and in mice is rapidly removed from the circulation (6, 7). Preliminary attempts to measure plasmin-antiplasmin complexes by using Lysine Sepharose instead of plasminogen kringles in the intermediary gel failed to show evidence of such complexes in the present study.

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REFERENCES

1 Bagge, L., Jacobsson, H. & Saldeen, T.: Post-traumatic variation of the primary fibrinolysis inhibitor α_2-antiplasmin. In: Progress in fibrinolysis. pp 307-312, Volume V. Churchill Livingstone, 1981.

2 Bagge, L. & Saldeen, T.: The primary fibrinolysis inhibitor and trauma. Thromb Res 13:1131-1136, 1978.

3 Christensen, U. & Clemmensen, I.: Purification and reaction mechanisms of the primary inhibitor of plasmin from human plasma. Biochem J 175:635-641, 1978.

4 Clemmensen, I.: Different molecular forms of α_2-antiplasmin. In: The physiological inhibitors of blood coagulation of fibrinolysis (ed. D. Collen, B. Wiman & M. Verstraete), pp 131-136. Biomedical Press. Elsevier (North Holland), 1979.

5 Clemmensen, I., Thorsen, S., Mullertz, S. & Petersen, L.C.: Properties of three different molecular forms of the α_2-plasmin inhibitor. Eur J Biochem 120:105-112, 1981.

6 Collen, D. & Wiman, B.: Turnover of antiplasmin, the fast-acting plasmin inhibitor of plasma. Blood 2:313-324, 1979.

7 Gonias, S.L., Fuchs, H.E. & Pizzo, S.V.: A unique pathway for the plasma elimination of α_2-antiplasmin-protease complexes in mice. Thromb Haemostasis 48(2):208-210, 1982.

8 Högstorp, H., Jacobsson, H. & Carlin, G.: Studies on α_2-antiplasmin in the rat. Thromb Res 21:247-253, 1981.

9 Högstorp, H., Jacobsson, H. & Saldeen, T.: Effect of hepatectomy on the posttraumatic fibrinolysis inhibition and the primary fibrinolysis inhibitor in the rat. Thromb Res 18:361-368, 1980.

10 Högstorp, H. & Saldeen, T.: Synthesis of α_2-antiplasmin by rat liver cells. Thromb Res 28:19-25, 1982.

11 Högstorp, H. & Saldeen, T.: Rat hepatocytes synthesize the plasminogen-binding form of α_2-antiplasmin. Abstr. Xth International Congress on Thrombosis and Haemostasis. San Diego July 14, 1985. Thrombosis and Haemostasis No 1, Vol 54, p 1581.

12 Kluft, C. & Los, N.: Demonstration of two forms of α_2-antiplasmin in plasma by modified crossed immunoelectrophoresis. Thromb Res 21:65--71, 1981.

13 Moroi, M. & Aoki, N.: Inhibition of plasminogen binding to fibrin by α_2-plasmin inhibitor. Thromb Res 10:581-586,1977.

14 Wiman, B.: Affinity-chromatographic purifications of human α_2-antiplasmin. Biochem J 191:229-232, 1980.

15 Wiman, B., Nilsson, T. & Cedergren, B.: Studies on a form of α_2-antiplasmin in plasma which does not interact with the lysine-binding sites in plasminogen. Thromb Res 28:193-200, 1982.

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