Immunological comparison between human and rat plasminogen activators in blood and the vessel wall

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SUMMARY To evaluate the rat as an experimental model for plasminogen activator research, the ability of antibodies specific for human tissue type plasminogen activator and urokinase to suppress the plasminogen activator activity in whole plasma and in the vessel wall was studied in both rat and man. Plasminogen activator activity in plasma was assayed on fibrin plates containing plasminogen. Plasminogen activator in the vessel wall was shown by the fibrin slide technique. Antibodies against human tissue type melanoma cell activator and urokinase were raised in goats and mixed into the fibrin film or the fibrin plates. In both species antibodies to melanoma cell activator were able to suppress the plasminogen activator activity completely in plasma and in the vessel wall. Anti-urokinase, however, had no suppressing effect. In rat plasma the inhibitory effect on the fibrinolytic activity was seen only with high concentrations of antibodies against melanoma cell activator, which suggests that rat plasminogen activator in plasma and vessel walls is similar to, but not identical with, human tissue type plasminogen activator.

Plasminogen activators are specific proteolytic enzymes which convert the inactive plasminogen into active plasmin, thereby initiating the fibrinolytic system.¹ In tissues and body fluids two types of plasminogen activator have been identified: tissue like and urokinase like plasminogen activators. In blood the tissue like plasminogen activator is the main type present, and there are only small amounts of urokinase like plasminogen activators.^{2,3} The fibrinolytic system can also be activated by an additional mechanism that is dependent on factor XII, high molecular weight kininogen, and prekallekrein.⁴

Most malignant tumours produce urokinase like plasminogen activators.^{5,6} A melanoma cell line has been found to produce a tissue like plasminogen activator, which is similar to and cross reacts with the tissue like plasminogen activators from the human uterus.^{7,8} Cultures of this melanoma cell line have made it possible to obtain plasminogen activator in sufficient amounts for immunisation and further research. In human plasma, the plasminogen activator activity was suppressed by antibodies directed against tissue like plasminogen activators

from human uterus, porcine heart, or human melanoma cells.⁹⁻¹¹ In tissues, such as the ureter and the vein wall, the plasminogen activator activity was suppressed by antibodies specific for tissue like plasminogen activators, whereas antibodies to urokinase had no inhibitory effect.^{12,13} In fetal organ cultures, however, urokinase like plasminogen activators as well as tissue like plasminogen activators were secreted. Cultures of fetal aorta were an exception as they produced only tissue like plasminogen activators.¹⁴

Variations in the fibrinolytic activity similar to those recorded in man have been reported in rats after administration of hormones and vasoactive drugs.^{15,16} In vivo, complete lysis of fibrin deposits or occlusive thrombi has been described after the administration of tissue like plasminogen activators.^{17,18} Rat clots, however, in comparison to human ones, were less susceptible to the lytic activity of plasminogen activator in vitro.¹⁸

The aim of this comparative study on man and rats was to characterise the blood and vascular plasminogen activators immunologically in order to evaluate the rat as an experimental model for plasminogen activator research. Antibodies against human low molecular weight urokinase and against melanoma cell activator were used.

Material and methods

COLLECTION OF BLOOD AND VESSEL SPECIMENS

Human blood was obtained by venepuncture from two volunteers after venous occlusion for 3 and 10 min. Rat blood was collected after decapitation of two Wistar and two Sprague-Dawley rats. In both species, 6 ml blood was collected in plastic tubes containing 1 ml 0.1 M sodium citrate and immediately centrifuged at 2500 rpm for 10 min. Biopsy specimens, about 1 cm long, from two dorsal foot veins in man and from the vessels of the hind foot from four rats were sealed and immediately frozen in liquid nitrogen.

DETERMINATION OF PLASMINOGEN ACTIVATOR ACTIVITY

Fibrinolytic activity in whole plasma was assessed by the fibrin plate technique.¹⁹ Citrated whole plasma (30 μ l) was applied to unheated fibrin plates containing plasminogen rich human fibrin. After 18 h incubation at 37°C the diameter of the lytic zones was measured; the results were expressed in mm.²⁰ *Fibrinolytic activity in the vessel walls* was determined by a modification of Todd's histochemical fibrin slide technique.²¹ Using a cryostat microtome, cross sections (8 μ m thick) were collected on glass slides and covered with a thin plasminogen rich fibrin film obtained by the addition of thrombin (Topostatin, Roche) to human fibrinogen (KabiVitrum). After a preincubation period of 30 min at 21°C, the fibrin slides were incubated in a moist chamber for 30, 60, and 120 min and then fixed and stained. Plasminogen activator activity in the sections was evident by zones of clearing, surrounded by a darkly stained fibrin film.

ANTIBODIES TO MELANOMA CELL ACTIVATOR

Pure melanoma cell activator was obtained from the medium of Bowes melanoma cell line. The melanoma cell activator was absorbed by immunosorbent chromatography using antibodies against porcine tissue activator, eluted with potassium thiocyanate, and finally purified by chromatography on arginine-Sepharose and gel filtration as described previously.¹²

Antiserum to melanoma cell activator was raised in a goat by twice injecting subcutaneously at an interval of three weeks 150 μ g of purified, electrophoretically homogeneous melanoma cell activator, emulsified with Freund's complete adjuvant. The antiserum gave a single precipitation line with melanoma conditioned culture medium in the gel diffusion test. IgG from the serum of immunised goat and normal goat was purified by ammonium sulphate precipitation, DEAE-Sephadex chroma-

tography, and Sephadex G-200 gel filtration as described previously.¹² One milligram of total IgG completely suppressed the activity of 0.07 mg pure melanoma cell activator.

ANTIBODIES TO UROKINASE

Urokinase (10 000 Plouq units), obtained from Lövens, Copenhagen, was further purified by p-aminobenzamidine-Sepharose chromatography.²² High and low molecular weight urokinase were separated by gel filtration. IgG from antiserum raised to low molecular weight urokinase was prepared as described for melanoma cell activator.

INCORPORATION OF IgG INTO THE FIBRIN SLIDES AND PLATES

Normal goat IgG (control IgG), antibodies to melanoma cell activator, and antibodies to low molecular weight urokinase were incorporated into the fibrin by mixing the purified IgG fractions with the human fibrinogen before the addition of thrombin. In the fibrin plates the IgG fractions were diluted 1/100, 1/1000, and 1/6000, while the final dilution in the fibrin slides was 1/100. Fibrinolytic activity in whole plasma on the fibrin plates containing IgG were determined in duplicate, with the controls determined in triplicate.

Results

The spontaneous fibrinolytic activity in plasma applied to fibrin plates with and without antibodies is shown in the Table. In human plasma antibodies to melanoma cell activator completely suppressed the fibrinolytic activity after venous occlusion for 3 min. After 10 min occlusion, the fibrinolytic activity was hardly measurable on addition of melanoma cell activator at the 1/6000 dilution. In all rat plasma samples the fibrinolytic activity was completely suppressed by antibodies to melanoma cell activator in the 1/100 dilution. No inhibitory effect of antibodies to melanoma cell activator was seen at greater dilutions. In both species the fibrinolytic activity in plasma samples applied to fibrin plates containing normal IgG or anti-urokinase varied within the range of the control plates as shown in the Table.

In the control fibrin slides the lysed areas over the vessel sections increased with incubation time. No inhibitory effect on the plasminogen activator activity in the vessel wall was seen after the addition of anti-urokinase or control IgG to the fibrin film. On the contrary, even at the shortest incubation period the fibrinolytic activity of human and rat vessel walls was completely suppressed in the fibrin films containing antibodies to melanoma cell activator. The absence of lysis in the fibrin films with antibodies to

Fibrinolytic activity in man after venous occlusion (VO) and in active rats as determined on fibrin plates with and without antibodies and control IgG incorporated in the dilutions 1/100 (A), 1/1000 (B), and 1/6000 (C).

Plasma samples	No	Control		Control IgG			Anti-urokinase			Antibodies to melanoma cell activator		
		Mean	(range)	A	B	C	A	B	C	A	B	C
Man												
VO (3 min)	1	6.5	(5.0-7.5)	7.0	6.5	6.0	5.0	6.5	5.0	0	0	0
VO (10 min)	1	9.0	(8.0-11.0)	10.5	11.5	10.0	7.5	7.0	8.5	0	0	2.0
Rat												
Wistar	2	5.5	(4.0-6.0)	6.0	6.0	6.0	5.5	6.0	6.0	0	5.0	5.0
Sprague-Dawley	2	5.5	(4.5-6.5)	7.0	5.5	6.0	5.5	4.5	4.5	0	3.5	5.0

Values are the diameters of the lytic zones (in mm)

Fibrinolytic activity reflected by zones of clearing in human veins (a-d) and rat vessels (e-h). a + e = controls; b + f = with IgG from non-immunised goats after incubation for 120 min; c + g = with IgG for immunised (anti-urokinase) goats after incubation for 120 min; d + h = with IgG from immunised (antibodies to melanoma cell activator) goats after incubation for 120 min.

melanoma cell activator is illustrated in the Figure, which shows the fibrinolytic activity of the vessel specimens after 120 min incubation with and without antibodies added to the fibrin film.

Discussion

A recent report on the immunological characterisation of the plasminogen activator in the human vein wall showed that the fibrinolytic activity was initiated by tissue like plasminogen activators.¹² This observation was confirmed in the present study, in which antibodies to melanoma cell activator completely suppressed the plasminogen activator activity in the vessel wall of man and also of the rat. Anti-urokinase had no inhibitory effect even after a prolonged incubation period of 2 h. In both species, the plasminogen activator activity in citrated whole plasma could be completely suppressed by antibodies to melanoma cell activator. No significant inhibitory effect of anti-urokinase was shown, although small amounts of urokinase in

human plasma have been reported.²³ A slight inhibitory effect of anti-urokinase could be explained by similarities of the urokinase and melanoma cell activator molecules.²⁴ Immunological similarity was thus shown between the vascular and blood plasminogen activator in man and rat. This observation implies that the relatively high fibrinolytic activity seen in active rats is probably caused by tissue like plasminogen activators being released from vessels by the same mechanism as in man. Dilution of the IgG fraction containing antibodies to melanoma cell activator resulted in the loss of its inhibitory capacity on the rat plasma, while considerably lower concentrations still suppressed the plasminogen activator activity in the human plasma samples which had higher initial fibrinolytic activity. Moreover, in a previous comparative study on plasminogen activator from human uterus and from a rat brain tumour cell line, the rat plasminogen activator was less susceptible to the inhibitory effect of antibodies specific for human uterine plasminogen activator, although these antibodies at high concent-

rations completely suppressed the rat plasminogen activator activity.²⁵ Hence, antibodies to melanoma cell activator seem to bind more firmly to human than to rat plasminogen activator.

In conclusion, the rat tissue like plasminogen activators are similar to, but probably not identical with, the human tissue like plasminogen activators. In rats, as in man, the tissue like plasminogen activators are present in plasma as well as in the vessel wall. The firmer binding of antibodies against melanoma cell activator to human plasminogen activator indicates minor species related differences in the structure of the otherwise closely related plasminogen activators of these two species. In man vascular plasminogen activators are important regulators of the fibrinolytic activity in blood. Absence of immunological differences between the rat plasma plasminogen activator and the vessel wall plasminogen activator indicates a similar central position of tissue like plasminogen activators in the fibrinolytic system of the rat.

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