Immunological characterisation of plasminogen activators in the human vessel wall

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SUMMARY A histochemical technique was used to identify the activity of the plasminogen activator (PA) in the vessel wall of veins. Antibodies against melanoma cell activator and urokinase (UK), both raised in goats, were mixed into the fibrin film. The PA activity was quenched by the antibodies against melanoma activator but remained unchanged when antibodies against UK, or an IgG preparation of normal goat serum, was mixed in the fibrin film. The results of this study show that the PA activity in the vein vessel wall is immunologically similar to or identical to the PA derived from melanoma cells which has previously been shown to cross-react with the tissue-like PA. No UK-like activity was present in the vessel wall.

The fibrinolytic system is activated by plasminogen activators localised in tissues, in blood and in different fluids of the human body. Two types of plasminogen activator (PA) have been recognised, blood/tissue-like and urokinase-like.

The blood vessels are considered to be the main source of blood and tissue PA.^{1,2} Using histochemical techniques, it has been demonstrated that PA is confined mainly to vasa vasorum and to the intima,¹⁻³ from which the release of this activator occurs.⁴⁻⁶ There are also inhibitors to the fibrinolytic system in the vessel wall,⁷ which mainly inhibit urokinase.⁸

Urokinase (UK), the main activator in urine, is produced not only in the kidney and urinary outflow pathways,⁹ but is also present in carcinomas and tumour derived cell-lines^{10,11} in normal uterine endometrium¹² and in various fetal organs.¹³

Blood and tissue PA differ from UK in terms of immunological reaction, enzymatic activity and molecular weight.¹³⁻¹⁵ Blood PA can be increased by venous occlusion, physical exercise and various substances such as adrenaline, nicotinic acid, vasopressin and its synthetic analogue DDAVP.¹⁶ Tissue PA is responsible for the fibrinolytic activity induced in human blood by the above mentioned stimuli.^{6,17} However, UK might be responsible for some of the spontaneous fibrinolytic activity in plasma.^{18,19}

We report here the quenching of PA activity in the vein vessel wall by monospecific antibodies against UK and PA of the blood/tissue-like type.

Material and methods

DETERMINATION OF PA ACTIVITY IN THE VESSEL WALL

Biopsy specimens, about 3 cm long, obtained from the great saphenous vein during surgery, were hermetically sealed in parafilm and immediately frozen to -70°C. PA activity was determined with a modification of Todd's histochemical fibrin slide technique¹ as described in detail by Pandolfi *et al.*² Briefly, sections 8 µm thick, were cut in a cryostat microtome, collected on glass slides and covered with a thin fibrin film rich in plasminogen. The film was obtained by mixing bovine fibrinogen (1%) in phosphate buffer (pH 7.8) which was coagulated with thrombin (Topostasine, Roche). After a preincubation period of 30 min at a temperature of 19°C, the fibrin slides were incubated at 37°C in a moist chamber for 20 and 30 min and after that fixed and stained. PA activity in the sections was reflected as clear zones of lysis, surrounded by dark stained fibrin film.

TISSUE PA AND ANTISERUM TO THE MELANOMA CELL ACTIVATOR

Pure tissue PA was obtained from the culture

medium of a cultivated melanoma cell line (provided by Dr D Collen). It is very similar to or identical with human tissue PA from uterine tissue.¹⁵ The cells were first grown to confluence in medium M 199 containing 10% vol/vol fetal calf serum and then for several passages in serum-free medium, which was harvested. The activator was purified by immunosorbent chromatography using antibodies against porcine tissue activator essentially as described for the purification of human tissue PA. The melanoma activator was absorbed from the medium to the immunosorbent and eluted with KSCN. The eluted material was purified further by chromatography on arginine-Sepharose and by gel filtration.²⁰ The final product was electrophoretically homogeneous.

ANTISERUM TO TISSUE PA

Antiserum was raised in a goat by two injections, the second three weeks after the first, of 150 µg purified activator, emulsified with Freund's complete adjuvant. The injections were given subcutaneously at different sites in the back. The antiserum gave one precipitation line with melanoma conditioned culture medium in gel diffusion. The melanoma activator showed an immunological reaction of complete identity with tissue PA purified from human uterine tissue. IgG from the antiserum and normal goat serum was purified by ammonium sulphate precipitation, DEAE-Sephadex chromatography and Sephadex G-200 gel filtration as earlier described.¹⁷ The IgG completely neutralised plasminogen activator activity in plasma and ordinary euglobulin precipitates. One milligram total IgG completely quenched the activity of 0.07 mg pure melanoma activator.

ANTISERUM TO UROKINASE

UK was obtained from Løvens, Copenhagen (10 000 Ploug units) and further purified by *p*-aminobenzamidine-Sepharose chromatography²¹ and low molecular weight (M_r 31 000) and high molecular weight (M_r 54 000) urokinase separated by gel filtration.²² Antiserum against low molecular weight urokinase was raised in a goat as described for tissue PA and IgG from the antiserum prepared in the same way.

QUENCHING OF PA ACTIVITY

Quenching of PA activity was studied using IgG antibodies against melanoma activator (anti-MA), urokinase (anti-UK), and an IgG preparation from a control goat (control IgG). Antibodies were incorporated into the fibrin film by mixing the same concentration of the IgG fractions of anti-MA, anti-UK or control IgG with the fibrinogen solution before

PA activity determined according to a histochemical fibrin slide technique as seen by the naked eye

the addition of thrombin.

Results

As seen from the Figure, the PA activity was quenched by the antibodies against melanoma activator (anti-MA). Neither anti-UK nor IgG from the control goat (control IgG) inhibited the activity, and the results were independent of the length of incubation periods. The activity was the same in the fibrin slides coated with anti-UK, or control IgG, as well as in the control slides without any IgG. PA activity was solely localised to the adventitia of the vessel wall. Similar results were obtained from one corresponding vessel in three different individuals.

Discussion

The two main PA of the organism are blood/tissue PA, and UK. In blood there is an additional mechanism activating the fibrinolytic system, which is dependent on factor XII, pre-kallikrein and high molecular weight kininogen (reviewed by Murano²³). PA derived from tissues has a strong affinity for fibrin¹⁴ and is immunologically related to the vascular PA, which has been partly purified from cadaveric vein perfusates.²⁴⁻²⁶

UK-like material has been found in several tissues and fluids.¹²⁻¹³ In rabbit endothelial cultures Loskutoff and Edgington⁵ found a release of tissue-like as well as UK-like PA. Certain human cell lines produce either tissue-like or UK-like PA, or both.²⁷⁻²⁸ Holmberg *et al*¹³ found a release of both UK-like and tissue-like PA in cultures of most human fetal organs, but only of tissue-like PA in cultures of aorta explants. It is, therefore, debatable whether or not UK-like PA is partially responsible for the spontaneous fibrinolytic activity of the blood.

Recently Rijken *et al*⁶ demonstrated that PA activity, released into the blood after venous occlusion or physical exercise, is related to PA of the tissue-like type. These results have been corroborated by Holmberg *et al*¹⁷ who demonstrated that the fibrinolytic activity of human blood induced by venous occlusion or DDAVP was quenched by antibodies to the melanoma plasminogen activator. UK-like material measured by radioimmunoassay, was not released by DDAVP, nor was the intrinsic fibrinolytic system of plasma affected. Furthermore, Åstedt²⁹ showed that there was no release of UK-like activity after venous occlusion. In the present study the PA activity of the vein vessel wall was exclusively tissue-like PA because it was completely quenched by anti-MA but not by anti-UK, nor by goat IgG. This is in agreement with the above mentioned studies that PA released from the vessel wall is of the tissue-like type.

Releasing the plasminogen activator content in the vein vessel wall into the blood stream is the most important function of the fibrinolytic defence system, and is one which has been shown to be defective in many patients suffering from recurrent thrombosis.³⁰ The present results bear this out and demonstrate that the plasminogen activator contained in the vessel wall is of the same type as that released by a variety of stimuli.

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