Immunological characterisation of plasminogen activators in the human vessel wall

H LJUNGMÉR,* L HOLMBERG,† A KJELDGAARD,‡ IM NILSSON,§ B ÅSTEDT**

From the *Department of Surgery, the †Department of Paediatrics, University of Lund, Malmö General Hospital, Malmö, the ‡Department of Obstetrics and Gynaecology, Huddinge Hospital, Huddinge, the §Department for Coagulation Disorders, University of Lund, Malmö General Hospital, Malmö, and the **Department of Obstetrics and Gynaecology, University Hospital, Lund, Sweden

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. Using histochemical techniques, it has been demonstrated that PA is confined mainly to the intima, from which the release of this activator occurs. There are also inhibitors to the fibrinolytic system in the vessel wall, which mainly inhibit urokinase.

Urokinase (UK), the main activator in urine, is produced not only in the kidney and urinary outflow pathways but is also present in carcinomas and tumour derived cell-lines in normal uterine endometrium and in various fetal organs.

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

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 paraffin and immediately frozen to −70°C. PA activity was determined with a modification of Todd's histochemical fibrin slide technique as described in detail by Pandolfi et al. Briefly, sections 8 μm thick, were cut in a cryostat microtom, 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 (Topostase, 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. 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. 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. 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 Lövens, Copenhagen (10 000 Ploug units) and further purified by p-aminobenzenamidine-Sepharose chromatography and low molecular weight (M, 31 000) and high molecular weight (M, 54 000) urokinase separated by gel filtration. 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 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 organ 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 kinogen (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 Edgington3 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 al13 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 al9 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 al17 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.

This investigation was supported by grants from the Swedish Medical Research Council (17X-04523, 19X-04997, 19X-00087).

References

Immunological characterisation of plasminogen activators in the human vessel wall


Requests for reprints to: Dr H Ljungnér, Department for Coagulation Disorders, University Hospital, S Forstadsrgatan 101, S-214 01 Malmö, Sweden.

The August 1983 issue

THE AUGUST 1983 ISSUE CONTAINS THE FOLLOWING PAPERS

Review article

Formaldehyde in pathology departments RP CLARK

A data processing system adapted to the special needs of the emergency laboratory D NEUMEIER, H SATOR, GE RINDFLEISCH, M KNEDEL

Computer programs in cytology reporting and record keeping KV SWETTENHAM, CD NICKOLS, CL BERRY

The synovium and synovial fluid in multicentric reticulohistiocytosis—a light microscopic, electron microscopic and cytotoxic analysis of one case AJ FREEMONT, CJP JONES, J DENTON

Changes in the Paneth cell population of human small intestine assessed by image analysis of the secretory granule area MARGARET E ELMES, J GWYN JONES, MR STANTON

Blood group antigens in the normal and neoplastic bladder epithelium SUSAN J THORPE, P ABEL, G SLAVIN, TEN FEIZI

Demonstration of light chain monotypia in B cell non-Hodgkin's lymphomas using unfixed freeze-dried and formalin-fixed tryptsinised paraffin sections Z NEMES, V THOMÁZY, G SZEIFFERT

Calcium pyrophosphate dihydrate (CPPD) deposition in ochronotic arthropathy J MCCLURE, PS SMITH, AMANDA A GRAMP

Use of the cryostat section in electron microscopy P NORRIS, DWR GRIFFITHS

Increased transfer of iron to the fetus after total dose infusion of iron dextran during pregnancy D BINGHAM, MM KHALAF, G WALTERS, JT WHICHER

Differential effect of detergents on the alkaline denaturation of haemoglobin in maternal and fetal blood, with particular reference to Triton X-100 CG DUCK-CHONG

Analysis of the complexity of the multimeric structure of factor VIII related antigen/von Willebrand protein using a modified electrophoretic technique MS ENAYAT, FGH HILL

Faecal carriage rate of *Aeromonas hydrophila* SALLY E MILLERSHIP, SR CURNOW, B CHATTOPADHYAY

The value of screening blood donors for antibody to hepatitis B core antigen AC ARcher, BJ COHEN, PP MORTIMER

A miniaturised and simplified technique for typing and subtyping herpes simplex virus JM DARVILLE

Growth hormone and malignancy GS ANDREWS

Angiotensin-converting enzyme and its clinical significance — a review PR STUDDY, RUTH LAPWORTH, R BIRD

Quality assessment of blood glucose monitors in use outside the hospital laboratory RF DRUCKER, DRR WILLIAMS, CP PRICE

Technical method

A new bone marrow aspiration needle to overcome the sampling errors inherent in the technique of bone marrow aspiration A ISLAM

Letters to the Editor

Book reviews

Some new titles

Notice

Copies are still available and may be obtained from the PUBLISHING MANAGER, BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR. Price £5.00, including postage.