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Tissue-type Plasminogen Activator Concentrations in Plasma from Patients with Psoriasis

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An ELISA for tissue-type plasminogen activator (t-PA) was developed and used to measure the concentration of t-PA in plasma from 15 healthy donors and 23 psoriasis patients. The mean value for the healthy donors was 2.4 ± 1.3 ng/ml (SD) of t-PA in plasma. The mean value for the psoriasis patients was 4.4 ± 3.2 ng/ml of t-PA. The psoriasis patients were divided into two groups: patients with mild psoriasis, i.e. less than 20 % of the skin affected; and patients with severe psoriasis, i.e. more than 20 % of the skin affected. Patients with mild psoriasis had t-PA concentrations of 2.8 ± 2.3 ng/ml, while patients with severe psoriasis had 5.4 ± 3.3 ng/ml. t-PA concentrations were higher in the psoriasis patients than in the normal controls (at the 5 % level). The patients with severe psoriasis had t-PA concentrations significantly higher than the normal controls (at the 1 % level).

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Proteolysis caused by activation of plasminogen to plasmin plays an important role in many biological processes. In mammals there are two types of plasminogen activators: the tissue-type (t-PA) and the urokinase-type (u-PA). Both are serine proteases, but they differ in M_r (70 000 and 50 000, respectively), immunological reactivity, and amino acid sequence. The two activators are produced by different cell types in the organism and seem to be involved in different functions. u-PA is believed to be a key enzyme in breakdown of extracellular matrix proteins during tissue destruction in a variety of normal and pathological conditions, including the invasive growth of cancer cells, while t-PA, among other functions, is involved in thrombolysis (for review, see ref. 1). u-PA and t-PA are both found in blood (2–4).

Plasminogen activator activity has been demonstrated in the scales and epidermis of psoriatic skin (5–8). In a previous immunochemical study on psoriatic scale extracts (6), we found that t-PA, but not u-PA, could be detected. In a later immunohistochemical study (7) we found that the epidermis from psoriatic lesions showed focal staining for u-PA in and between the basal keratinocytes in the suprapapillary epidermal areas, while t-PA was found in the superficial keratinizing cells, including both stratum spinosum and the parakeratotic layer. No staining of keratinocytes was observed in uninvolved and normal skin.

In the present study we describe the use of an ELISA for t-PA to determine the concentration of t-PA in plasma from healthy donors and psoriasis patients.

MATERIALS AND METHODS

Patients and healthy controls

Included in the study were 15 healthy controls and 23 patients who had stable, chronic plaque psoriasis. The patients with psoriasis were divided into those with mild psoriasis ($n = 9$) with less than 20 % of the skin affected, and those with severe psoriasis ($n = 14$) with more than 20 % of the skin affected (5).

Human plasma

For preparation of human plasma, blood was obtained by venepuncture without stasis in tubes containing EDTA. The tubes were centrifuged at 2 000 *g* for 10 min. The plasma was stored at −20°C until analysis. A pool of plasma was prepared of equal volumes of plasma from 10 different healthy donors. For some experiments, samples of plasma were passed through a Sepharose column coupled with monoclonal anti-t-PA clone 1 antibodies. Recovery of plasma was monitored by measuring the absorbance at 280 nm.

Materials

Avidin–peroxidase complex was obtained from Kem-En-Tec, Hellerup, Denmark; biotin-*N*-hydroxysuccinimide from Sigma Chemical Co., St. Louis, Mo, USA. All other materials were those described previously (9, 10), or of the best grade commercially available.

Tissue-type plasminogen activator

Human t-PA was prepared as described (6) from culture medium from the Bowes melanoma cell line by affinity chro-

matography on a Sepharose column coupled with a monoclonal antibody against t-PA (anti-t-PA clone 1). The protein concentration of this preparation was determined by Lowry's method. When comparing our t-PA standard with the international t-PA standard (Lot 83/517 obtained from the National Institute for Biological Standards and Control, Holly Hill, London) in the t-PA ELISA 1 ng corresponds to 0.44 IU. Complexes between t-PA and PAI-1 were formed by incubation of two-chain t-PA (25 ng/ml) for 1 h at 25°C with highly purified SDS-treated PAI-1 (500 ng/ml) as described (11, 12). The complexes were removed from this mixture by incubation with anti-PAI-1 IgG (12) followed by adsorption on protein A Sepharose.

Antibodies

Anti-t-PA clones 1, 2, and 3 and anti-u-PA clone 6 is described in our previous report (7). Anti-TNP (Hy 2.15) and anti-plasminogen is described by Shulman et al. (13) and us (7), respectively. The monoclonal antibodies were purified from culture fluids with the use of protein A Sepharose. Rabbit antibodies against human t-PA were raised against t-PA purified as described by affinity purification on a Sepharose column coupled with monoclonal anti-t-PA clone 1 (10). Rabbits were immunized intradermally with 10 μg of purified t-PA per injection, according to a previously reported scheme (14). IgG was purified from the rabbit serum by affinity chromatography with protein A Sepharose (10). Twenty-two mg of this IgG was affinity purified on a column coupled with t-PA, resulting in 3 mg of IgG. Biotinylation of the polyclonal antibodies was performed as described (7).

ELISA for t-PA in plasma

Three different monoclonal antibodies against t-PA (7) were tested and clone 3 was found to give the highest sensitivity. The t-PA ELISA is essentially performed as the u-PA ELISA described by Grøndahl-Hansen et al. (15) with the following modifications: Anti-u-PA clone 6 is replaced by anti-t-PA clone 3, the standard was t-PA, the sample layer was diluted in PBS–Tween, 10 mM EDTA, 5% newborn calf serum, the plasma was diluted to 10%, the biotinylated rabbit antibodies against u-PA were replaced by biotinylated rabbit antibodies against t-PA and used at a concentration of 0.5 μg/ml. The biotinylated antibody was followed by avidin–peroxidase and the peroxidase reaction was performed with *o*-phenylendiamine, as described (15, 16).

Characterization of the t-PA ELISA

Several control experiments were performed to characterize the ELISA. In the standard ELISA, a linear dose–reponse was obtained with t-PA concentrations up to at least 600 pg/well. The detection limit for the assay was about 10 pg t-PA in a volume of 100 μl. When the anti-t-PA IgG was replaced by monoclonal anti-u-PA, anti-plasminogen or anti-TNP, or when the biotinylated second rabbit antibody against t-PA was replaced by biotinylated nonimmune rabbit IgG, no reaction with t-PA was detected. Complexes of t-PA and plasminogen activator inhibitor type 1 (PAI-1) was detected with an efficiency of about 80%. In order to ensure that the mixture of t-PA and PAI-1 contained no free t-PA, a sample was depleted of PAI-1, by adding polyclonal anti-PAI-1 antibody followed by protein A Sepharose. This resulted in total elimination of the t-PA, as measured by the ELISA, verifying that all the t-PA in the mixture was bound to PAI-1. No reaction was seen with proteins structurally related to t-PA (see 17), including human u-PA and plasminogen, assayed in concentrations higher than those physiologically present in blood plasma.

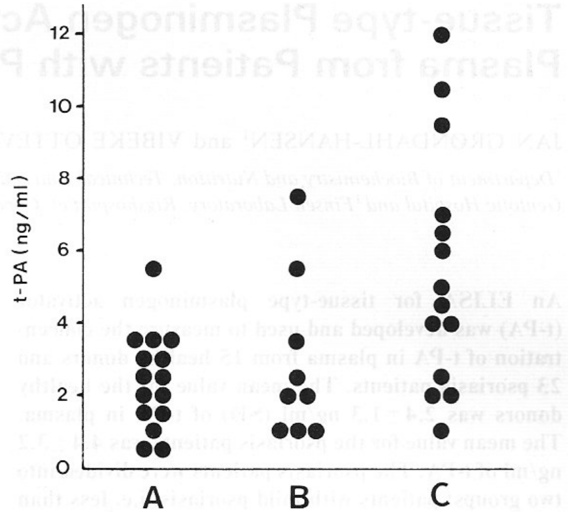

Fig. 1. t-PA concentration in plasma from normal donors (*A*) and patients with mild (*B*) and severe (*C*) psoriasis determined by ELISA. The normal donors had a mean concentration of 2.4±1.3 ng/ml ($n=15$), the patients with mild psoriasis had 2.8±2.3 ng/ml ($n=9$) and the patients with severe psoriasis had 5.4±3.3 ng/ml ($n=14$). All the psoriasis patients taken together had a concentration of 4.4±3.2 ng/ml ($n=23$) of t-PA in plasma.

Dilutions of a pool of human plasma from healthy donors had a dose–response in the standard t-PA-ELISA that was linear up to a concentration of at least 20% plasma. The regression coefficient was calculated to be 0.987. The intra-assay variation of the ELISA was determined by assaying 16 independent 1:10 dilutions of the plasma pool on the same ELISA plate; this gave a coefficient of variation of 6%. The inter-assay variation was determined by assaying the plasma pool in 7 ELISAs, which gave a coefficient of variation of 13%.

Plasma that had been passed twice through a Sepharose column coupled with monoclonal anti-t-PA clone 1, showed no reaction. The recovery in the plasma ELISA was tested by adding known amounts of t-PA to the human plasma pool and to the same pool depleted of t-PA immunoreactivity. The recovery of an internal standard of t-PA in a 10% dilution of plasma and in a 10% dilution of t-PA-depleted plasma was 100% and 114%, respectively.

When a 1:5 dilution of the human plasma pool was applied to wells coated with monoclonal anti-u-PA, anti-plasminogen or anti-TNP instead of monoclonal anti-t-PA as the first antibody, no ELISA-reaction above background was obtained. No reaction was seen with serum from rabbit, horse, swine and calf.

Other procedures

Glu-plasminogen was purified from fresh human male plasma, human u-PA and human PAI-1 from culture fluid from HT-1080 fibrosarcoma cells (6, 11, 18).

RESULTS

t-PA in plasma of healthy donors and psoriasis patients

The t-PA ELISA was used to determine the t-PA concentration in the plasma from 15 healthy donors and 23 patients with psoriasis. The results are shown in Fig. 1. The t-PA concentration was higher in the psoriasis patients than in the normal controls (at the 5% level), the patients with severe psoriasis having significantly higher concentrations than the normal controls (at the 1% level), as evaluated by the Wilcoxon rank sum test.

DISCUSSION

The t-PA concentration of 2.4 ± 1.3 ng/ml (corresponding to 1 IU/ml) in healthy donors is slightly lower than the concentration found by others using ELISAs for t-PA, 4.0 ± 1.8 ng/ml (19) and 3.4 ± 0.8 ng/ml (20).

In the present study we found that psoriasis patients had elevated concentrations of t-PA in the plasma, patients with severe psoriasis having the highest levels (while no differences in the u-PA concentrations were found, unpublished results). The origin of the increased amounts of t-PA found in the blood from the psoriasis patients remains to be determined, but our recent immunohistochemical finding of t-PA in the upper keratinocytes in psoriatic plaques does point to this site, though other places than the affected skin could also be responsible for the increased amounts of t-PA. The endothelial cells in the veins are a possible source.

t-PA has been correlated to thrombolysis and clinical trials indicate that intravenous t-PA is an effective coronary thrombolytic agent (21). The elevated concentrations of t-PA in patients with psoriasis do not seem to correlate with increased thrombolysis, since a study by McDonald & Calabresi (22) indicated that there is an increased incidence of occlusive vascular disease in the psoriatic patient.

From a diagnostic point of view, the plasma t-PA seems to be of limited value in psoriasis, but it might be of interest, when t-PA is measured in plasma from other patients, for other purposes, to know that psoriasis can give elevated levels of t-PA.

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