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RELEVANCE OF FREE t-PA ASSAY FOLLOWING VENOUS OCCLUSION IN PATIENTS WITH VENOUS THROMBOEMBOLIC DISEASE. M.C. Alessi (1), I. Juhan-Vague (1), J. Valadier (1), C. Philip-Joet (1), P. Holvoet (2) and D. Collen (2). Laboratory of Hematology, CHU Timone, Marseille, France (1) and Center for Thrombosis and Vascular Research, University of Leuven, Belgium (2).

Out of 100 consecutive patients with a history of venous thromboembolism, 80 were subjected to 10 min venous occlusion (VO). Blood samples were collected on citrate, immediately cooled on ice, centrifuged and frozen. Euglobulin fibrinolytic activity (EFA) was measured on fibrin plates, plasminogen activator inhibitor (PAI) activity according to Verheijen et al., and total t-PA antigen with an ELISA. In addition, free t-PA was assayed in plasma collected on the monoclonal antibody MA-2G6, which is directed against the active site of t-PA.

The assays allowed to differentiate three groups of patients. Good responders (GR) to VO with at least a doubling of the EFA after venous occlusion. Poor responders (PR) with less than a two-fold increase in EFA could be classified either as having a high PAI level resulting in rapid inhibition of t-PA (PR-I) or as having a deficient release of t-PA from the vessel wall (PR-II).

	GR	PR-I	PR-II
n	50	15	15
EFA before VO	8.7 ± 3.2	5.8 ± 1.9	7.9 ± 3.7
after VO	19 ± 4.4	8.9 ± 3.7	11 ± 4.9
t-PA Ag before VO	12 ± 6.9	16 ± 11	6.7 ± 3.8
after VO	43 ± 22	41 ± 22	9.3 ± 5.2
free t-PA Ag before VO	5.0 ± 2.4	4.7 ± 2.2	5.4 ± 1.6
after VO	20 ± 14	8.7 ± 3.4	7.0 ± 1.9
PAI before VO	4.8 ± 3.0	20 ± 4.0	4.0 ± 3.4

It is concluded that patients with a history of venous thromboembolism may have an abnormal fibrinolytic response to venous occlusion which is due either to an increased inhibitor level or to a deficient t-PA release. Both abnormalities are recognized by the free t-PA assay.

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DETERMINATION OF FREE CIRCULATING t PA AND MAXIMAL t PA CONTENT IN NORMAL HUMAN PLASMA USING A SPECTROPHOTOMETRIC METHOD. B. Boutière, D. Arnoux, J. Sampol, C. Masson*, F. Hamon*, and E. Anglés-Cano*. Laboratoire Central d'Hématologie, Hôpital de la Conception, Marseille, and *INSERM U. 143, Hôpital de Bicêtre, Paris, France.

Recent studies indicate that tPA released by endothelial cells circulates in plasma as a complex with its inhibitor (PAI). Quantitative data have been difficult to obtain however, due to the interference of plasmin inhibitors and other activators on liquid-phase tPA assays. In the present study an activity test (SOFIA-tPA ; Anal. Biochem. 153;201, 1986) on solid-phase fibrin has been used to determine the physiological forms of tPA in normal plasma. In this assay an affinity separation step allows the selective binding of tPA to fibrin and the elimination of plasmin inhibitors and other activators (pro UK, UK). Fibrin-bound tPA is subsequentely detected by adding plasminogen and a chromogenic substrate selective for plasmin. Under these conditions the reaction rate depends on the presence of tPA, tPA-PAI complexes and PAI present in plasma. Plasma from 38 normal human volunteers obtained before and after venous occlusion was tested. Free tPA was determined in undiluted plasma while the fibrinolytic potential (maximal tPA activity) of plasma was detected following dissociation of tPA-PAI complexes by dilution and acidification of plasma, i.e. euglobulin fractionation. A 1:2 to 1:5 dilution of euglobulins was necessary to obtain a maximal dissociation of precipitated complexes. A similar dilution induced dissociation of tPA-PAI complexes was obtained with plasma at pH 6.8. The amount of free detectable tPA increased as a function of the dilution of plasma until a plateau value (usually at dilution 1:20), which was similar to that observed with the euglobulins. Our results (see Table) indicate that most of the tPA in human plasma circulates as tPA-PAI complexes and that free tPA can be detected only in minute amounts. Plasma instead of euglobulins can be accurately used to detect both free (undiluted plasma) and total tPA (plasma diluted 1:20).

Venous occlusion	Free tPA Plasma	Total tPA Euglobulins	Total tPA Plasma
Before	0.04 ± 0.03*	0.88 ± 0.58	0.89 ± 0.55
After	0.82 ± 0.88	12.4 ± 13.4	11.4 ± 13.6

* Figures indicate IU/ml, mean ± SD, n = 38

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FIBRINOLYTIC ACTIVITY OF ARMS AND LEGS IN PERIPHERAL VENOUS HYPERTENSION IN MAN. M.R. Carriero, F. Annoni (1), L. Mussoni, C. Cerletti, G. de Gaetano. Istituto "Mario Negri "and Clinica Chirurgica III-Università (1), Milano, Italy

Spontaneous fibrinolytic activity of venous specimens is greater in the arms than in the legs of normal subjects. This difference might be caused by the different hydrostatic pressure in arms and legs. We tested, on standard fibrin plates, the fibrinolytic activity of euglobulins prepared from venous blood obtained from arms and legs of normal subjects and patients with chronic peripheral hypertension. Normal subjects (26-38 yrs old, n= 5) were tested both before and after 10 min venous occlusion (VO) of an arm and after 10 min occlusion of a leg. VO was obtained by applying the cuff of a sphigmomanometer at a pressure value intermediate between systolic and diastolic pressure. Patients (39-64 yrs old, n= 7) were tested both before and after VO of the arm and after 10 min orthostatic posture (mean 100 mmHg). For each individual the fibrinolytic activity in the arm before VO was considered as basal value of both the arm and the leg. In normal subjects fibrinolytic activity induced by VO was greater in the arm than in the leg (262.9±74.9 versus 165.5±52.9 mm^2). The average increase of fibrinolytic activity after VO was 3.4 (arms) and 2.1 (legs). In patients with peripheral venous hypertension fibrinolytic activity was 298.3±46.7 mm^2 in the arm and 131.1±19.2 mm^2 in the leg. The average increase induced by VO in the arm was 3.5 while the activity of the legs after orthostatic pressure was 1.6. In conclusion patients with peripheral venous hypertension did not show any reduced fibrinolytic response after VO of the arms. Fibrinolytic activity in patients' legs after orthostatic pressure was also similar to that in the legs of volunteers after venous occlusion.

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ASPIRIN DOES NOT INHIBIT FIBRINOLYTIC ACTIVITY IN TIA PATIENTS AFTER VENOUS OCCLUSION. M.R. Carriero (2), G. Pintucci (1), M.N. Castagnoli (1), R. Colombo (2), B. Lombardi (2), L. Mussoni (1), G. de Gaetano (1) Istituto "Mario Negri" (1) and Istituto Neurologico "Besta" (2), Milano, Italy.

We have recently shown that in normal subjects aspirin (1,300 mg) and indobufen (400 mg), a new cyclo-oxygenase inhibitor structurally unrelated to salycilate, lower the fibrinolytic activity, without modifying t-PA antigen levels, after venous occlusion (VO). The aim of this study was to investigate whether aspirin reduces fibrinolytic response to VO also in patients with TIA. These patients were selected in view of controlled clinical trials showing reduction of TIA recurrency and stroke by treatment with high dose aspirin. Six males (56-65 yrs old), with previous TIA (< 1 year) were selected; the presence of diffuse atherothrombotic lesions was demonstrated by doppler sonography and angiography. All patients were given, ten days apart, aspirin (600 mg daily x 2) or indobufen (200 mg daily x 2) following a randomized cross-over single blind scheme. In all patients 10 minutes VO applied before any drug administration, induced activation of the fibrinolytic system as assessed by euglobulin lysis area on fibrin plates (from 226±47 to 643±57 mm^2), t-PA antigen (from 13.8±1.0 to 40.9±3.1 ng/ml) and PA-I activity (from 39.5±5.0 to 14.8±1.6 AU/ml). Neither aspirin nor indobufen ingestion resulted in any inhibitory effect on fibrinolytic response to VO while both drugs suppressed serum thromboxane B_2 generation by more than 98%. In conclusion high dose aspirin and indobufen do not impair the fibrinolytic potential in TIA patients with atherothrombotic lesions. The reasons for the different behaviour of patients in respect to young healthy volunteers remain to be established.