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EXPRESSION OF HUMAN FIBRINOGEN Aα CHAIN cDNA MUTANTS, TO STUDY THE ACCELERATION OF TISSUE PLASMINOGEN ACTIVATOR (t-PA) INDUCED PLASMIN FORMATION. Jaap Koopman (1), Susan T. Lord (2) and F. Haverkate (1). Gaubius Institute TNO, Leiden, the Netherlands (1) and University of North Carolina, Chapel Hill, NC, U.S.A. (2).

The capacity to accelerate t-PA induced plasminogen activation, is located on the Aα chain of fibrinogen.
To determine more closely which parts of the Aα chain are involved in the accelerating activity, a number of deletion mutants were constructed using the cloned cDNA. The exact sequence of the mutated cDNA's was determined, using the dideoxy sequencing method according to Sanger et al. Plasmids containing the complete Aα cDNA or the constructed mutants were expressed in E. Coli cells. After treatment with thrombin, the cell lysates showed fibrinopeptide A levels ranging from 40 ng/ml to 200 ng/ml. Western Blotting of the cell lysates, using a monoclonal antibody which reacts specifically with the N-terminal part of the Aα chain of human fibrinogen (Koppert et al., Blood, 66, 503-507, 1985), showed protein bands of the molecular size as predicted by the DNA structure. The complete Aα chain expressed in E. Coli was equally effective in the acceleration of plasmin formation as Aα chain isolated from purified fibrinogen.
Using a number of deletion mutants we showed that the accelerating capacity is associated with amino acids within the Aα stretch 44-199, which is in agreement with previous studies (Nieuwenhuizen, Biochim. Biophys. Acta, 748, 86-92, 1983). This result shows that the approach using molecular biology seems promising for further defining the site(s) involved in the acceleration of t-PA induced plasminogen activation.

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CHARACTERIZATION OF THE 5'FLANKING REGION FOR THE HUMAN FIBRINOGEN β GENE. P. Huber, J. Dalmon, M. Laurent, G. Courtois, D. Thevenon and G. Marguerie. DRF/Laboratoire d'Hématologie/INSERM U217, CEN G 85X, F 38041 Grenoble

Fibrinogen is coded by three separate genes located in a 50kb region of chromosome 4 and organized in a γ - α - β orientation with an inversion of the gene β. A human genomic library was constructed using the λEMBL4 phage and screened with cDNA probes coding for human fibrinogen Aα, Bβ and γ chains. Clones, covering the fibrinogen locus, were identified, and their organization was analyzed by means of hybridization and restriction mapping. Among these clones one recombinant phage containing the β gene and large 5' and 3' -flanking sequences was isolated.

To identify the regulatory sequences upstream from the human β gene, a 1.5 kb fragment of the immediate 5'-flanking region was sequenced. The S1 mapping experiments revealed three transcription initiation sites. Potential TATA and CAAT sequences were identified upstream the initiation start points at the positions -21 and -58 from the first initiation start point.

Comparison of this sequence with that previously reported for the same region upstream from the human γ gene revealed no significant homology which suggests that the potential promoting sequences of these genes are different. In contrast, comparison of the 5'flanking regions of human and rat β genes showed more than 80% homology for 142 bp upstream from the gene. This highly conserved region is a potential candidate for a regulatory sequence of the human β gene.

To verify this activity, a β fibrinogen minigene was constructed by deletion of the internal part of the normal gene and including 3.4kb of the 5'flanking region and 1.4kb of the 3'flanking region. The minigene was transfected into HepG2, a human hepatoma cell line, to show whether the 5'flanking region of the human fibrinogen gene contains DNA sequences sufficient for efficient transcription in HepG2. Constructions of several parts of the sequenced 5'flanking region of the human β gene with the gene of the chloramphenical acetyltransferase have been also transfected in the HepG2 cells to determine the specificity of the gene expression and to localize the sequences controlling the transcription of the gene.

DETECTION OF DEEP VEIN THROMBOSIS AND PULMONARY EMBOLISM Monday

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SILENT PULMONARY EMBOLISM IN PATIENTS WITH DEEP VEIN THROMBOSIS. M.V. Huisman(1), H.R. Büller (1), J.W. ten Cate (1), E.A. van Royen (2), J. Vreeken (1). Center for Hemostasis, Thrombosis and Atherosclerosis (1), Dept. Nuclear Medicine (2) Academic Medical Center, Amsterdam, The Netherlands.

In patients presenting with clinically suspected deep vein thrombosis symptomatic pulmonary embolism is rarely apparent. To assess the prevalence of asymptomatic pulmonary embolism in outpatients with proven deep vein thrombosis, perfusion ventilation lungscans were performed in 101 consecutive patients at the first day of treatment and after one week of therapy. Fifty-one percent of these patients had a high probability lungscan at the start of treatment. In control patients (n=44) without deep venous thrombosis but referred through the same filter, the prevalence of high-probability scans was only 5%. After one week of anticoagulant treatment complete to partial improvement was observed in 55% of the patients while in another 24% of the patients the scan remained normal.
It is concluded that lungscan detected asymptomatic pulmonary embolism occurs frequently in patients presenting with symptomatic deep venous thrombosis and that the majority of these emboli resolve within one week of anticoagulant treatment.

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THROMBUS IMAGING WITH MURINE MONOCLONAL ANTIBODIES AGAINST FIBRIN FRAGMENT D-DIMER IN A RABBIT JUGULAR VEIN THROMBOSIS MODEL. P. Holvoet, J.M. Stassen, and D. Collen. Center for Thrombosis and Vascular Research, University of Leuven, Belgium.

Three murine monoclonal antibodies (MA-6C1, MA-8D3 and MA-15C5) reacting with fragment D-dimer from crosslinked fibrin but not with monomeric fragment D were obtained by immunization of Balb/c mice with the highly purified fragment, fusion of spleen cells with a myeloma cell line, production of ascites fluid in mice and purification of the antibodies on Affigel Blue. Fab fragments were isolated from papain digests. The IgG and Fab fragments were labeled with ^{125}I, ^{131}I or ^{123}I using lactoperoxidase. The disposition rates ($t_{½}$) and thrombus to blood ratios, measured in groups of 3 rabbits with a non-occlusive jugular vein thrombus composed of whole human plasma were :

	MA	$t_{½}$ (hr)	Thrombus/blood ratios 4 hr	8 hr	17 hr	24 hr
IgG	6C1	15	-	-	-	2.8±0.1
	8D3	16	-	-	-	2.3±0.2
	15C5	21	-	-	-	3.4±1.0
	8D3+C61	16	-	-	-	5.7±2.2
Fab	6C1+8D3	9	3.1±0.8	6.7±0.9	6.1±1.1	-
	6C1+15C5	7	3.3±0.8	5.3±1.3	3.2±1.2	-
	8D3+15C5	7	3.2±1.2	-	7.2±1.4	-
	6C1+8D3+15C5	7	4.1±0.6	-	7.1±13	-
	(6C1+8D3+15C5)*	6	3.4±0.4	-	4.9±0.1	-

*with simultaneous injection of 90 mg/kg human fibrinogen and 60 µg/kg human fibrinogen degradation products.

These results indicate that, after 1 to 3 half-lives of the IgG or Fab fragments, using combinations of 2 or 3 monoclonal antibodies, thrombus to blood ratios of isotope of 5 to 7 are obtained. Such signal/noise ratios are sufficient for in vivo detection by external gamma scintigraphy. This was preliminarily confirmed using ^{123}I-labeled Fab fragments of the three antibodies in rabbits.