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ISOLATION AND CHARACTERIZATION OF cDNA CLONES FOR HUMAN FACTOR V B. Dahlbäck and Å. Lundwall. Department of Clinical Chemistry, University of Lund, Malmö General Hospital, S-214 01 Malmö Sweden.

Coagulation factor V is a single chain, 330 kDa glycoprotein functioning as a cofactor to factor Xa in the activation of prothrombin. Thrombin cleaves factor V into four major fragments, out of which the N-terminal (105kDa) and the C-terminal (71-74kDa) fragments together constitute the active factor V species. To isolate cDNA clones a λ -gt 11 liver library was screened with a polyclonal, monospecific antiserum against human factor V. Four positive clones (two "weak", A1 and A2 and two "strong", A3 and A4) were identified and isolated. A1 (0.7kb), A2 (1.25kb) and A4 (0.85kb) reacted strongly with an antiserum against the 105 kDa, N-terminal fragment (heavy chain of factor Va), whereas A3 (1.25kb) gave the best signal with an antiserum against the 71-74 kDa, C-terminal fragment (light chain of factor Va). A1 hybridized with A2 and A4, whereas A2 only hybridized with A1. A3, which did not hybridize to any of the other clones, was used to rescreen the library and 9 positive clones (B1-9) were isolated. B9 (3kb) coded for the entire C-terminal factor V fragment and the 3' noncoding sequence. B8 (1.8kb) partially overlapped B9 but extended the 5' sequence with 0.8kb. In a third screening round A1 was used in combination with B8 and a 1.1kb clone (C10) was identified which hybridized to both. C10 did not hybridize with A2. The following overlapping cDNA clones can be ordered from the 5' end: A2-A1-C10-B8-B9 and together they cover 6 kb of coding sequence.

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THE COMPLETE AMINO ACID SEQUENCE OF HUMAN FACTOR V. Richard J. Jenny (1), Debra D. Pittman (2), John J. Toole (2), Ronald W. Kriz (2), Randal J. Kaufman (2) and Kenneth G. Mann (1). University of Vermont, Department of Biochemistry, Burlington, VT, U.S.A. 05405 (1) and Genetics Institute, Cambridge, MA, U.S.A. 02140 (2).

cDNA clones encoding human factor V have been isolated and sequenced. The cDNA sequence of factor V obtained from overlapping clones includes a 6672 bp coding region, a 90 bp 5'-untranslated region and a 163 bp 3'-untranslated region including a poly-A tail. The deduced amino acid sequence consists of 2224 amino acids including a 28 amino acid leader peptide. A direct comparison to human factor VIII reveals considerable homology between both proteins with respect to amino acid sequence and domain structure. A triplicated "A" domain and duplicated "C" domain show an approximate 40% identity to the corresponding domains in factor VIII. Factor V and Factor VIII both possess a heavily glycosylated B domain that separates the heavy and light chains of the activated cofactors, although no significant homology is observed in this region. The B domain of factor V contains 35 tandem and approximately 9 additional semi-conserved repeats of nine amino acids of the form (D-L-S-Q-T-T-L-S-P) and 2 additional semi-conserved repeats of 17 amino acids. Factor V contains 37 potential N-linked glycosylation sites, 25 of which are in the B domain, and a total of 19 cysteine residues. By direct comparison to amino acid sequence obtained from both human and bovine factor V, the thrombin (IIa) cleavage sites have been assigned as Arg-709/Ser-710, Arg-1018/Thr-1019, and Arg-1545/Ser-1546.

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rt-PA THROMBOLYSIS VERSUS SURGICAL THROMBECTOMY OF PERIPHERAL BYPASS GRAFTS: A COMPARATIVE TRIAL. R. Graor, J. Young, B. Beven, N. Hertzler, L. Krajewski, P. O'Hara, J. Olin, W. Ruschhaupt. The Cleveland Clinic Foundation, Cleveland, OH, U.S.A.

Twenty-nine of 33 patients (88%) with thrombosed lower extremity bypass grafts had angiographic and clinical successful lysis of graft thrombi with rt-PA. Of the successful group, 18 were saphenous vein grafts and 11 PTFE grafts in the femoropopliteal-tibial position. Following lysis, 76% required a secondary procedure (2 PTA, 20 surgical repair and 7 required anticoagulation) to maintain patency.

A matched cohort of patients with bypass grafts who had surgical thrombectomy were compared to the rt-PA successfully treated grafts and analyzed for duration of patency after opening and limb salvage. The Kaplan-Meier curve compared both treatment groups and demonstrated improved graft survival in the rt-PA treated group ($p=.01$) (median graft survival rt-PA 195 days, surgery 30 days). Limb salvage was marginally significant ($p=.064$) in favor of the rt-PA treatment group. Single and multi-variant risk factor analysis found smoking and age of the graft adversely affected patency ($p=.05$ and $p=.08$ respectively). Graft type, age of the patient, diabetes mellitus, and high blood pressure were not significant factors ($p>.15$).

Systemic fibrinolysis was identified to varying degrees. Mean decreases in the fibrinolytic constituents include: 59% decrease in clottable fibrinogen, 18% decrease in sulfite fibrinogen, 78% decrease in alpha-2 antiplasmin and varying degrees of increases in DDIMER, B-Beta₁₋₄₂ and B-Beta₁₅₋₄₂ coincident with the constituent changes.

Complications were unrelated to constituent changes. One patient had major bleeding secondary to graft anastomosis disruption.

Thrombolysis with rt-PA is an effective and more durable adjunct treatment option for thrombosed bypass grafts, especially when combined with PTA or surgical repair to maintain patency.

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IMMUNOELECTROPHORETIC CHARACTERIZATION OF SYSTEMIC FIBRINOGEN DURING THROMBOLYSIS OF PERIPHERAL ARTERIAL EMBOLI WITH tPA. John R. Shainoff, Youko Hishikawa-Itoh, Fred M. Lucas, Robert Graor, Bernadine Healy. Thrombosis Center of The Cleveland Clinic Foundation, Cleveland, Ohio 44106, U.S.A.

Prompted by uncertainties over the possibility of *ex-vivo* alterations of fibrinogen in plasma samples from patients receiving thrombolytic agents, we sought to 1) use sodium dodecyl sulfate (SDS) and mild acidification to pH 5 as a general means for inhibiting post-sampling proteolysis, and 2) assess the chemical state of fibrinogen by immunoelectrophoretically profiling the molecular weight distribution of the fibrinogen-related antigens in the sample. Blood samples taken into EDTA and PPACK were immediately centrifuged, and plasma diluted 5X in 2% SDS and 0.04% mono-chloroacetic acid. Such samples showed no changes in molecular weight distribution of fibrinogen-related antigens over 7 days storage at room temperature when analyzed by SDS-electrophoresis on 3% glyoxyl agarose followed by fixation with NaCNBH₃ and staining with fluorescent, affinity-purified anti-fibrinogen antibody. The method of study was applied to 16 patients with occlusive peripheral arterial emboli, all of which were successfully treated by catheter-directed administration of tissue plasminogen activator (tPA). All patients presented abnormally high levels of degraded (predominantly 170-300 kD) and dimeric forms of fibrinogen both prior and subsequent to treatment, and only 3 of the 16 patients underwent appreciable change in content or composition of fibrinogen derivatives in course of treatment. Concentrations of dimers ranged from 6 to 23 percent of the total fibrinogen. *De novo* elevations in degraded forms of fibrinogen observed to accompany treatment of 3 patients were reflected in prolongation of thrombin time while tests of sulfite precipitation underwent relatively minor change. The more frequent absence of change in content of derivatives, particularly the dimeric forms suggestive of a coagulopathic process, indicated that they were derived systemically rather than from the occluding thrombus that was removed by treatment. The findings raise a prospect that the clinically significant thrombosis in these patients may be symptomatic of a much more generalized vascular coagulopathic process. The absence of measurable change in molecular composition of fibrinogen-related antigens in course of successful treatment of these patients attests to the efficacy of tPA as a thrombolytic agent. Support: NIH Grants HL-19361 and HL-19767.