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SYNTHESIS, PROCESSING AND SECRETION OF HUMAN FACTOR VIII IN MAMMALIAN CELLS: REQUIREMENT FOR VON WILLEBRAND FACTOR. Louise C. Wasley, Andrew J. Dorner, and Randal J. Kaufman. Genetics Institute, Cambridge, MA

In the plasma factor VIII exists as a complex with von Willebrand factor (vWF). The cloning of the cDNA for factor VIII has provided the ability to develop mammalian cell lines which express high levels of factor VIII by using appropriatate expression plasmids and DNA cotransformation with selectable markers. We have studied the synthesis, processing, and secretion of factor VIII expressed in baby hamster kidney cells and in Chinese hamster ovary cells by ^{35}S-methionine pulse and chase labeling and analysis by immunoprecipitation with specific antibodies which recognize the light and heavy chains of factor VIII. In both mammalian cell lines, factor VIII is synthesized as a primary translation product of 230 kDa. A significant amount remains within the endoplasmic reticulum in a stable complex with a glucose regulated protein of 78 kDa. The remainder traverses into the Golgi compartment where it is cleaved to the heavy and light chain forms. Very shortly thereafter the mature factor VIII appears in the conditioned media as the mature heavy and light chain species. Very little single chain factor VIII is secreted into the conditioned media. The accumulation of factor VIII in the conditioned media requires the presence of vWF factor. In the absence of vWF, the factor VIII appears as unassociated heavy and light chains which are rapidly degraded. Bovine, porcine, or human 3WF all effectively stabilize human factor VIII expressed in these rodent cell lines. These results suggest the presence of vWF promotes factor VIII chain association which stabilizes the factor VIII to proteolysis.

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SUSTAINED EXPRESSION OF FULL LENGTH AND VARIANT RECOMBINANT FACTOR VIII IN GENETICALLY ENGINEERED CELLS. Nava Sarver and George A. Ricca, Meloy Laboratories, Inc., Springfield, Virginia, U.S.A.

A major effort is presently underway to provide factor VIII (FVIII) in a form free of viral pathogens via a recombinant DNA approach. We have constructed two chimeric FVIII cDNA vectors based on the bovine papillomavirus mammalian expression system. The first vector (FVIII) contained a full length FVIII cDNA; the second vector (ΔFVIII) contained a cDNA insert with an extensive deletion, corresponding to amino acid residues 747 to 1560 in the region encoding the "B" domain. This internal region is removed during activation of the parental FVIII molecule and is believed not to be required for coagulant activity. We have found that recombinant FVIII produced by stable cell lines harboring either the full length or the variant FVIII was capable of restoring coagulant activity to FVIII deficient plasma in vitro. This expressed activity was neutralized by anti-FVIII antibodies. Similar to observations with FVIII derived from human plasma, the two recombinant FVIII forms were (i) inactivated by the chelating agent EDTA, (ii) demonstrated a biphasic response of an initial activation followed by a decay in activity when treated with thrombin, and (iii) presented the expected peptide banding pattern by western blot analyses. A higher percentage of ΔFVIII transformants were isolated expressing coagulant activity compared to transformants harboring the complete FVIII cDNA. Among the positive transformants isolated, those harboring ΔFVIII produced higher levels of coagulant activity than their full length counterparts. Comparable steady state levels of FVIII specific transcripts were detected in FVIII and ΔFVIII transformants indicating that the difference in expression levels is due to a post transcriptional event(s). Our study demonstrates the efficacy of a full length and an abridged recombinant FVIII produced by stably transformed cells in correcting coagulation deficiency in vitro. It further suggests the potential usefulness of other molecular variants for efficient expression in genetically engineered cells.

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EFFECTS OF HEPARIN AND COUMARIN ON DEPOSITION OF FIBRIN, PLATELETS AND PLATELET THROMBI ON RABBIT AORTA SUBENDOTHELIUM EXPOSED TO FLOWING HUMAN BLOOD.
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The effect of heparin and phenprocoumon on thrombogenesis induced by rabbit aorta subendothelium (SE) was investigated in 20 volunteers using an ex vivo perfusion system. Blood was drawn directly from an antecubital vein through an annular chamber with exposed SE at 10ml/min flow rate (650sec^{-1} shear rate) for 5min. Following buffer perfusion for 15sec, the middle portion of SE was removed for plasmin digestion and adjacent segments were fixed and embedded for morphometric analysis. Perfusions were performed 20 min after i.v. injection of heparin 1000, 2500 and 5000 IU, respectively; and during the decline and steady-state of prothrombin activity during a 2 weeks treatment with phenprocoumon to target INR of 5.0.

The amount of fibrin attached to SE, as measured by fragment E RIA in plasmin digests, correlated negatively with the dose of heparin (r=-0.83, P<0.001, n=48) and with INR during coumarin intake (r=-0.58, P<0.01, n=40). After high doses of either heparin or coumarin fibrin deposition on SE was virtually abolished (table). Platelet adhesion was increased. Platelet thrombus volumes and heights were reduced by heparin and coumarin.

		control	heparin 5000 IU	coumarin INR 5.0
fibrin on SE	ng/mm^2	102±25	1.1±0.4**	0.4±0.4**
fibrin coverage of SE	%	34 ± 5	0**	0.9±0.7**
platelet adhesion on SE	%	35 ± 2	52 ± 4*	45 ± 3
platelet thrombus volume	$\mu m^3/\mu m^2$	4.4±0.6	2.4±0.4**	1.3±0.8**
maximum thrombus height	μm	44 ± 3	34 ± 3*	25 ± 7*

mean±SEM. n=12 for heparin, n=8 for coumarin.
*P<0.01, **P<0.001 vs control (paired t-test).

We conclude that both heparin and coumarin dose-dependently inhibit fibrin formation induced by SE. In addition, both drugs impair platelet thrombus growth and/or stability indicating that these processes may also depend on the coagulation mechanism.

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RESPONSE OF PROTEIN C AND PROTEIN S INHIBITORS IN LONG-TERM ORAL ANTICOAGULANT THERAPY. M. Mukhlova Montiel and H. Bussey, The University of Texas Health Science Center, San Antonio, TX, USA.

Protein C (PC) and its coenzyme Protein S (PS) are physiologic inhibitors of activated factors Va and VIIIa. Deficiency of either one of these inhibitors has been associated with venous thrombosis. Their activity is dependent on vitamin K for hepatic gamma carboxylation and it is depressed during oral anticoagulant therapy. Because rebound thrombosis complicates cessation of anticoagulant therapy, we investigated the response of PC and PS during long term oral anticoagulation. The study encompassed 30 patients ranging between 26 and 76 years of age, who have received therapeutic doses of coumadin from 15 days to more than 8 years. The conditions for which treatment was initiated were deep vein thrombosis, cerebral vascular accidents and cardiac valve replacements.

Factor VII and X activity was assayed by one step routine clotting assays. PC antigen (ag), total PSag and free PSag were assayed by Laurell Rocket electroimmunodiffusion method. The measurement of the free PS was carried out after precipitation of C4b-binding protein with polyethylene glycol. PC activity was measured by clotting assay using PC deficient plasma to which was added patient plasma as a source of PC. Control group of 30 individuals in similar age group were assayed parallel with the patient samples. Compared with the control group the coumadin-treated patients showed substantial decrease of all factors studied. Statistical regression analysis of the coumadin group showed a significant increase in PS free (p = 0.014) during long term anticoagulation, while all of the other variables did not change significantly.

PCag and total PSag were decreased and their activities, as expected, were more severely affected. The ratio of PC activity to PCag averages 0.39 (normal >0.80) and free PS represented only 27% of the total PSag (normal about 40%). The inhibitors' persistent activity parallels that of the depression of Factors VII and X and there appears to be a balanced coagulation-inhibition system. If PC and PS play a role in rebound thrombosis after a prolonged anticoagulation therapy, the changes may occur after discontinuation of medication.

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