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VARIABLE EXPRESSION OF ALLOANTIGENS IN PLATELET COHORTS OF DIFFERENT MEAN DENSITY: AN EFFECT OF AGING IN VIVO? J. Pereira, C. Cretney, R.H. Aster. The Blood Center of Southeastern Wisconsin, Milwaukee, WI, U.S.A.

Platelets differ widely in size and density, but the relationship of this heterogeneity to platelet age and function is not established. Published evidence suggests that platelet alloantigens of the HLA and $P1^A$ systems may be acquired by or released from platelets in the circulation. We therefore studied expression of HLA, $P1^{A1}$, and other markers in platelet cohorts of high density (HD) and low density (LD) separated on a linear, isoosmotic arabinogalactan gradient. HD and LD cohorts contained 11-14% of total platelets and did not differ significantly in mean cell volume. Alloantibodies reactive with antigens $P1^{A1}$, Bak^a, and HLA-A2 were used to saturate alloantigen sites. Surface markers were quantified (Human Immunol. 15:251, 1986) with radiolabeled monoclonal probes specific for HLA A,B,C antigens (W6/32), the Fc fragment of IgG (Hb-43) and the glycoprotein IIb/IIIa complex (AP-2).

Platelet Cohort	$P1^{A1}$ (Mol/Plt)	Bak^a (Mol/Plt)	HLA-A2 (Mol/Plt)	HLA-ABC (Mol/Plt)	GPIIb-IIIa (Mol/Plt)
HD	46939	22714	4571	11007	42067
LD	42410*	22709	5757*	17371*	42635

*p 0.05 LD vs HD platelets (Mean values: N = 5-12)

As shown in the Table, HD platelets carry significantly more $P1^{A1}$ (located on GPIIIa) and significantly less HLA than LD platelets. However, HD and LD cohorts express the same number of GPIIb/IIIa and Bak^a (located on GPIIb) molecules. These findings are consistent with preferential loss of HLA molecules from HD platelets in the circulation or acquisition by LD platelets. The variable expression of $P1^{A1}$ in HD and LD cohorts is apparently due to a conformational change in GPIIIa, rather than acquisition or loss of the GPIIIa molecule, because total GPIIb/IIIa was the same in the two platelet populations. Whether antigen differences in HD and LD platelets are determined at the time of platelet production or result from aging of platelets in the circulation is under investigation.

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HEMOSTATIC PARAMETERS DURING PREPARATION OF LEUKOCYTE-POOR BLOOD. R. Losito, M. Sternbach, A. Masson, M. Paquin, M. Boyer and A. Trépanier. Blood Transfusion Service, Canadian Red Cross, Montreal, P.Q., CANADA, H1W 1B2.

Non-hemolytic febrile transfusion reactions are believed to be due to sensitization to granulocytes. Leukocyte removal can diminish or prevent the reaction. Use of the Pall filter (Pall Bio Medical Prod. Corp.) has resulted in some patient reactions. The purpose was to re-investigate this filter in vitro and compare it to the Imugard (Terumo) and the Erypur (Organon Teknika) filters and study their influence on hemostatic activation pathways. Method: RBC concs., aged 5 or 10 days, were centrifuged (5,200 R.P.M., 4° C, 15 m.). Cells were left undisturbed for 6-24 hours before filtration. Hematological parameters analysed included: leukocyte, red cell and platelet counts, differential and scanning electron microscopy. Activation studies related to clotting, fibrinolysis, kinin and complement pathways including both biological and immuno-assays; they consisted of the recal time, PTT, pro time, Factors I, VIII, XII, HMW kininogen, kallikrein, F.D.P., AT-III, alpha 2-macroglobulin, alpha 1-antitrypsin, plasminogen, C1 inactivator, C_3, C_4 and C_5. Over 75 filtrations have been performed. Results of cell recovery are illustrated in table below. Changes in tests related to activation were minimal with the Pall filter while a wide spectrum of derangements were common with the other two filters.

FILTER	CELL AGE (Days)	LEUKOCYTES (% recovery)	RED CELLS (% recovery)	PLATELETS (% recovery)
PALL	5	59 ± 26	92 ± 8	39
	10	29 ± 14	95 ± 5	
ERYPUR	5	2.5 ± 1.3	85 ± 11	2
	10	1.6 ± 1.1	89 ± 8	
IMUGARD	5	6.3 ± 5.4	96 ± 6	18
	10	3.0 ± 3.0	93 ± 9	

Conclusion: it appears the Erypur filter gives optimal results in the removal of leukocytes and platelets, whereas there is good recovery of red cells by the Pall and Imugard filters. Secondly, the Pall filter (nylon) does not appear to be involved with the contact pathways whereas Erypur (cellulose acetate) and Imugard (cotton) have an influence on these pathways.

HEPARINS

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ANTITHROMBOTIC ACTIONS OF A SULFOMUCOPOLYSACCHARIDE MIXTURE (ATERIOD) IN ANIMAL MODELS. *U. Cornelli, J.M. Walenga, J. Fareed, X. Huan and D. Hoppensteadt. *Crinos SPA, Como, Italy, and Loyola University Medical Center, Maywood, IL 60153.

Ateriod obtained from beef mucosal lining is a sulfomucopolysaccharide mixture of various glycosaminoglycans which contains dermatans, heparatans and traces of heparin. It has been used in the treatment of atherosclerosis and related vaso-occlusive disorders. Ateriod is standardized in terms of its lipoprotein lipase activation actions. Ateriod contains signficant in vitro anticoagulant and antiprotease (anti-factor Xa and anti-factor IIa) activities as measured by clot-based and chromogenic substrate methods. However, this in vitro activity is 7-10 times lesser than heparin. In order to study the antithrombotic actions of this agent in subcutaneous, intravenous and oral routes, we utilized a rabbit stasis thrombosis model with a prothrombin complex concentrate/Russell's viper venom thrombogenic challenge and prolonged stasis. The apparent ED_{50} for the antithrombotic action were found to be: IV (75-100 ug/kg), SC (0.8-1.3 mg/kg) and oral (20-30 mg/kg). In both the IV and SC studies, sustained anticoagulant and antiprotease actions were evident. The observed antithrombotic actions did not relate to the anti-factor IIa or anti-factor Xa actions. Pretreatment of Ateriod with equigravimetric amounts of protamine and platelet factor 4 did not neutralize the antithrombotic actions of this agent in the rabbit model. In a primate (Macaca mulatta) model of pharmacokinetics, ex vivo analysis following subcutaneously administered Ateriod showed sustained anticoagulant and antiprotease effects. The time course of the subcutaneously administered Ateriod was markedly different than heparin and a low molecular weight heparin. Treated animals were shown to resist induced hypercoagulability following injection of homologous serum as measured by FPA generation for extended periods. These studies suggest that Ateriod produces a strong antithrombotic action and that it has highly sustained pharmacokinetics. The antithrombotic activity appears to be primarily mediated via non-antithrombin - III dependent events which may be related to heparin cofactor II and vascular/cellular modifications.

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ANTITHROMBOTIC ACTIVITY OF A SYNTHETIC HEPARIN PENTASACCHARIDE IN A RABBIT STASIS THROMBOSIS MODEL USING DIFFERENT THROMBOGENIC CHALLENGES. J.M. Walenga, J. Fareed, M. Petitou+, J.C. Lormeau+, M. Samama*, J. Choay+. Department of Pathology, Loyola University Medical Center, Maywood, Illinois 60153, USA. +Institut Choay 46 Ave. Th. Gautier, 75782 Paris, France. *Laboratoire Central Hematologie, Hotel Dieu, 75181 Paris, France.

The synthetic pentasccharide, representing the critical sequence required in heparin for binding to antithrombin III, provides a unique tool to study the question of whether an agent solely capable of inhibiting factor Xa but devoid of anti-factor IIa activity in vitro, has the capacity to produce an antithrombotic effect in vivo. We have previously demonstrated in a rabbit stasis thrombosis model using a human serum challenge, a significant antithrombotic effect of the pentasaccharide (Walenga et al., Thromb Res 43:243, 1986). To extend and confirm these studies, four modifications of the stasis thrombosis model were developed using more specified induction sites of thrombosis. The following thrombogenic challenges were selected: monkey brain thromboplastin, an activated prothrombin complex concentrate, a non-activated prothrombin complex concentrate administered simultaneously with Russell's viper venom, and factor Xa. Dose-dependent antithrombotic responses were obtained in all four systems with ED_{50} values between 25-43 ug/kg for pentasaccharide as compared to 16-47 ug/kg for heparin. Complete inhibition of induced thrombosis was obtained in all four systems for pentasaccharide. Ex vivo analysis revealed expected anti-factor Xa levels but no anti-factor IIa activity. It was also shown that pentasaccharide in the rabbit was capable of inhibiting the generation of thrombin without directly inhibiting formed thrombin. It is concluded that an oligosaccharide with high anti-factor Xa activity, devoid of anti-factor IIa activity, is capable of inhibiting thrombosis induced in rabbit stasis models, but that higher dosages than heparin are required for this effect in terms of anti-factor Xa activity.