0404

THROMBOTECT: AN OPTIMIZED SAMPLE COLLECTION SYSTEM FOR THE INHIBITION OF IN VITRO PLATELET RELEASE. E.F.Workman, Jr., and D. M. Clark, Abbott Laboratories, N. Chicago, IL 60064

Platelet activation occurs in a variety of thrombotic disease states and may be monitored via measurement of Platelet Factor 4 (PF4) levels in plasma. In order for PF4 levels to reflect in vivo activation, care must be taken to eliminate in vitro α-granule release during the collection and preparation of blood specimens. Thrombotect is an evacuated blood collection tube containing an anticoagulant (EDTA) and two platelet inhibitors (2-chloroadenosine and procaine-HCl). This system is effective in preventing in vitro release under a variety of centrifugation conditions. Subsequent to a 30 minute icing step, whole blood samples may be centrifuged at room temperature and one of three centrifugation speeds - 2500 x g for 20 minutes, 1500 x g for 30 minutes, and 1000 x g for 40 minutes. Plasma specimens from normal individuals were assayed after being prepared under the above conditions. PF4 levels were as follows: 2500 x g, *2.79± 1.00 (73); 1500 x g, 3.84± 1.78 (20); 1000 x g, 3.35± 1.65 (20). Variations in the temperature of centrifugation had little effect on the ability of Thrombotect to inhibit PF4 release, whereas other commonly used blood collection cocktails became progressively less effective with increasing temperature (See Table).

Cent./Temp.	*EDTA	EDTA-Theophylline	Thrombotect
2-4°C	4.4±3.0(173)	3.9±1.1 (4)	2.16±.4(4)
10-12°C	19.6±21.3(4)	12.9±12.8(4)	2.63±1.2(4)
25-30°C	176.0±139.0(11)	115.0±26.0(4)	2.79±1.0(75)

*(Mean PF4 in ng/ml ± 1 S.D., "n" values in parenthesis). Results similar to those obtained for EDTA/Theophylline were obtained when EDTA was used in conjunction with adenosine and/or aspirin. The Thrombotect system has been employed in an examination of PF4 levels in a variety of thrombotic disease states. It also provides an excellent collection system for use with other "platelet-release" assays.

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ISOLATION OF PLASMA MEMBRANE VESICLES OF HUMAN PLATELET BY AFFINITY CHROMATOGRAPHY. T. Kobayashi, M. Sakon, H. Ohno, J. Kambayshi and G. Kōsaki. The Second Department of Surgery, Osaka University Medical School, Osaka, Japan.

Platelets undergo a unique morphological changes leading to the formation of hemostatic plug. In recent years, its intermediaty metabolism has been extensively studied and the important function of plasma membrane in the platelet reaction has been recognized. The method of Barber and Jamieson has been employed in order to prepare plasma membrane vesicles of platelet of excellent quality but it is rather time consuming and the yield is relatively low. In this study, an attempt was made to isolate plasma membrane vesicles of human platelets by wheat germ agglutinin affinity chromatography.

Freshly collected human citrated blood was subjected to glycerol loading and hypotonic lysis to obtain lysed platelet suspension. Then, it was applied to the affinity chromatography and the fraction of plasma membrane vesicles was eluted by 0.2 M N-acetyl glucosamine. Electron micrograph of the fraction showed round membrane vesicles with some scattered intracellular organelles. Several marker enzymes were assayed in the fraction. No appreciable amount of β-glucuronidase or cytochrome c oxidase was detected in the fraction, indicating no contamination of mitochondria or α-granules. Relatively high activity of G-6-Pase was detected, suggesting possible contamination of endoplasmic reticulum. The yield was 11.6% in dry weight and 7.9% in protein.

By this method, the isolation was much faster than the centrifugal method and as low as 20 ml of human citrated whole blood may be used as starting material. Upon characterization of the plasma membrane fraction by electron microscopy and marker enzyme assays, the quality of the fraction was found comparable with the centrifugal method. The yield by this method was approximately two times higher than by the conventional method.

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ISOLATION OF DENSE BODIES FROM HUMAN BLOOD PLATELETS ON METRIZAMIDE GRADIENT. F. Rendu, M. Lebret and J.P. Caen. U. 150 INSERM, Hôpital Lariboisière, 6, rue Guy Patin, Paris 10, France.

In view of the prominent role of dense bodies in platelet activation suggested by the platelet dysfunctions observed in storage pool diseases, we have developed a method for the isolation of human platelet dense bodies, using mepacrine to follow the purification.

Each step of the purification (washing procedures, lysis and subcellular separation) has been controlled in order to obtain the minimum release of these granules. Platelet lysates were centrifuged on a short two step discontinuous metrizamide gradient which allowed the attainment of a high density pellet. This pellet consisted of isolated mepacrine fluorescent granules which showed the typical appearance of dense bodies by electron microscopy. The granule pellet was relatively free from plasma membranes as estimated by the remaining (^{3}H) -concanavalin A or ^{125}I after labelling the whole platelets before the fractionation. The low contamination by other granule populations was estimated by the different assayed markers, β-glucuronidase, monoamine oxidase and platelet factor 4. The method is simple, reproducible and allows the highest enrichment in dense bodies obtained until now with human platelets(x 170 enrichment in calcium and x 110 enrichment in (^{14}C) 5-HT after labelling the whole platelets as compared to the homogenate). Functional studies performed with the isolated granules showed a rapid accumulation of (^{14}C)-5-HT, and the initial uptake was inhibited by reserpine but remained insensitive to imipramine.

The technique can be applied to the study of inherited disorders where the serotonin uptake and release mechanism has to be clarified.

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HISTAMINE IN PIG PLATELET DENSE GRANULES. M.H. Fukami, K. Ugurbil, H. Holmsen, G.L. Pakstis and C.A. Dangelmaier. Thrombosis Research Center and Department of Pharmacology, Temple University Medical School, Philadelphia, PA and Department of Biochemistry, Columbia University Medical School, New York, N.Y.

In the course of high resolution nuclear magnetic resonance (n.m.r.) studies of dense granule storage complexes, an amine with two aromatic protons was observed in a proton n.m.r. spectrum of dense granules isolated from pig platelets. This amine was identified as histamine by the exact coincidence of the n.m.r. peaks of added histamine with the unknown peaks in the extract. The pH dependence of chemical shifts, paper chromatography and flurometric analysis after coupling with o-phthalaldehyde confirmed the identification. The concentration of histamine in isolated dense granules was about 700 nmol/mg of protein (n=3) or 1.6 times that of serotonin. In intact platelets, the histamine content was 11 nmol/mg compared to 7 nmol/mg of serotonin. The addition of 1 unit/ml of thrombin to suspensions of washed pig platelets resulted in the secretion of more than 90% of the histamine under conditions in which only 3.8% of thelactate dehydrogenase appeared extracellularly. These findings indicate that histamine is a major constituent of dense granules in pig platelets which can be released during hemostasis and thrombosis and may exert its pharmacological effects under these conditions.