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LIPID COMPOSITION OF PLATELETS FROM PATIENTS AFFECTED BY IIa HYPERLIPOPROTEINEMIA. D. Prisco, P.G. Rogasi, R. Paniccia, A. Panetta, M. Coppo and G.F. Gensini. Clinica Medica I, University of Florence, Italy.

Platelets from patients with familial hypercholesterolemia (IIa HLP), a condition associated with high prevalence of atherosclerosis and of its thrombotic complications, are known to be hyperresponsive to aggregating stimuli and to synthesize increased amounts of thromboxane A2. In order to search if these functional alterations can depend on a different platelet lipid composition, we studied 12 young patients (aged 20 to 34 years) affected by IIa HLP and 12 suitable controls with similar dietary habits. Lipids were extracted from washed platelets with a chloroform/methanol (2/1) mixture. After silicic acid chromatography and thin-layer chromatography different lipid fractions were eluted and fatty acid methylesters were prepared by acid transmethanolysis. The esters were extracted with hexane and analyzed by gas-liquid chromatography. Different fatty acids were identified on the basis of retention time with respect to standard methylester mixtures and silver nitrate thin-layer chromatography. Cholesterol and lipid phosphorus were assayed by colorimetric methods. Both cholesterol and phospholipid content of platelets were higher in patients than in controls with a significant increase of cholesterol/ phospholipid molar ratio ($p < 0.05$). The percentage content of the phospholipid fractions was not different from that of controls. On the contrary the proportion of saturated fatty acids esterified in the different phospholipid fractions was significantly increased (minimum $p < 0.05$). In addition thromboxane A2 production by platelets from patients with IIa HLP was higher than in controls ($p < 0.001$). Our results indicate that lipid composition of platelets from patients with IIa HLP is altered and may be responsible for the enhanced platelet activity described in these patients.

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VARIATION OF FUNCTIONAL PLATELET PARAMETERS DURING STORAGE AND DURING VENOUS OCCLUSSION MEASURED BY FLOW CYTOMETRY. M. Spannagl (1), G. Valet (2), W. Schramm (1). Medizinische Klinik Innenstadt, 8000 München 2, Germany (1), Mildred-Scheel-Labor für Krebszellforschung, Max-Planck-Institut für Biochemie, 8033 Martinsried, Germany (2).

Information on platelet function would be of great importance for many clinical situations in addition to platelet count and bleeding time. It was the purpose of this study to test DiOc6 (3,3-dihexyl-oxacarbocyanine: transmembrane potential), AO (acridine orange: granular content), and ADB (1,4-di-acetoxy-2,3-dicyanobenzene: intracellular esterase-activity and pH) stained platelets after 1 and 5 hours storage (anticoagulated with EDTA, Heparin and Sodium-Citrate) and 2, 6, 12 and 20 minutes after venous occlussion (immediately diluted in HEPES-Buffer (1:50)). A fresh whole blood sample diluted in Buffer served as control. All blood samples were gained from normal persons. Platelets were finally diluted 1:200 in HEPES buffered saline and stained. Cell volume, green and blue fluorescence were then measured in a Fluovo-Metricell-II flow cytometer.

- The mean platelet volume increased to 111% (1 h) and 117% (5 h) of control during storage. The volume remained stable during venous occlussion.
- The transmembrane potential (DiOc6) decreased to 52% of control after 5 hours storage. We saw an increase to 141% after 20 minutes venous occlussion.
- The granular content (AO) decreased to 81% of control during storage. There was no variation during VOT.
- Esterase activity (ADB) remained constant during storage and had the lowest coefficient of variation (CV = 51%). There was an increase to 132% after 20 minutes venous occlussion.
- We saw most increase in volume and decrease in DiOc6 and AO dye content after storage in EDTA compared to Citrate and Heparin.

The present results show that the dyes of functional platelet parameters are sensitively picked up by flow cytometry. The methodology seems attractive for clinical purposes because measurements can be performed in diluted blood samples within less than five minutes after venipuncture.

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AGONIST INDUCED CALCIUM MOBILISATION IN PLATELETS OF PATIENTS WITH "ASPIRIN-LIKE" DEFECTS. P.R. Kingston (1) K.R. Bruckdorfer (1) and R.A. Hutton (2). Department of Biochemistry (1), Department of Haemophilia (2), Royal Free Hospital School of Medicine, London, U.K.

A group of patients have been described with a condition often referred to as an 'Aspirin-like" defect, which is characterised by easy bruising and prolonged bleeding following dental extraction or surgery. Initial studies eliminated a deficiency in coagulation factors, plasma factors, platelet glycoproteins or platelet storage granules as being the cause of this condition and demonstrated a diminished aggregatory and/or secretory response in platelet-rich-plasma to ADP and collagen. Aggregation responses in isolated platelets to thrombin, arachidonic acid and ionomycin are within the normal range, however the response to ADP is dimished. Recent studies have concentrated on the various mechanisms involved in platelet aggregation amongst which is the change in intracellular calcium concentration with accompanied secretion.

Using the fluorescent indicator quin2 we have monitored the intracellular calcium changes induced by various agonists in the presence and absence of extracellular calcium in five patients with an "Aspirin-like" defect. In the presence of 1mM extracellular calcium, thrombin and ionomycin caused a rapid elevation in intracellular calcium to greater than 1µM within 30 seconds of stimulation. In the absence of extracellular calcium, thrombin and ionomycin caused rises in intracellular calcium of 200nM and 300nM respectively. All the responses observed were within the normal range and indicate that both influx of extracellular calcium and mobilisation of calcium from internal stores has occurred. Therefore the defect that causes this "Aspirin-like" condition is not commonly associated with a defect in calcium mobilisation.

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PLATELET IONIZED CALCIUM MOBILIZATION (AEQUORIN METHOD) IN PATIENTS WITH PRIMARY PLATELET DYSFUNCTION. R. Nagayama, A. Hattori, J. Fuse, T. Takeshige, S. Takizawa and A. Shibata. The First Department of Internal Medicine, Niigata University School of Medicine, Niigata 951, Japan.

Intracellular calcium level of platelets of the patients with primary platelet dysfunction (thrombasthenia, platelet cyclooxygenase deficiency, familial defect of A23187 induced platelet aggregation, Hermansky-Pudlak syndrome, Bernard-Soulier syndrome, each 1 case, and other 3 cases of platelet release mechanism defect of unknown etiology) were measured with the photoprotein Aequorin according to the method by Johnson et al. The peak level and the lag time to the peak were evaluated. Activation was done by 4 or more different concentrations of either thrombin (0.125-1.0u/ml), A23187 (0.25-2.0µM), ATA_2 (0.05-0.4µM) or occasionally arachidonate (0.25-100µM).

In case of stimulation by thrombin, the maximum [Ca^{2+}] level in thrombasthenia was much lower than those in normal. The lag time was prolonged in Bernard-Soulier syndrome. In case of stimulation by STA_2 the maximum [Ca^{2+}] level was very much lower in thrombasthenia and was lower in a familial defect of A23187-induced platelet aggregation and a case of platelet release mechanism defect than normals. In case of stimulation by A23187, the maximum [Ca^{2+}] level was much lower in thrombasthenia and PCO deficiency and platelet release mechanism defect (2 cases) in which the lag time of A23187 induced platelet aggregation was also prolonged. In PCO deficiency, arachodonate less than 1µM produced a dose-dependent rise in intracellular calcium level and that (1-25µM) caused a rise of a consistent level although it didn't induce aggregation. Arachidonate (25-100µM) caused both higher rise and aggregation. Mobilization was normal in response to STA_2 and thrombin but decreased to A23187 in this deficiency.

These findings suggest that [Ca^{2+}] mobilization was deteriorated by many mechanisms such as defect of PGG_2H_2-and Tx-formation, membranous abnormality (lack of glycoproteins) and storage pool deficiency, and further that atachidonate even at low concentration may cause mobilization without conversion to PGG_2H_2 or Tx.

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