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PLATELET SIZE, NOT PLATELET MASS, DETERMINES INTRINSIC KINETIC DIFFERENCES IN PLATELET RECRUITMENT INTO AGGREGATES FOR ADP, U46619, AND PAF, BUT NOT FOR RISTOCETIN. Truman Wong and Mony M. Frojmovic. Department of Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

Previous studies of platelet aggregation using resistive counting methods (PA) have suggested a dependence on platelet size ($\bar{v}$), but have not been evaluated for varying platelet number (N_0) and associated total platelet mass. Here, the relationship between $\bar{v}$, N_0 and function was examined in size dependent human subpopulations fractionated by counterflow centrifugation. The original platelet population and three size dependent platelet fractions were concentrated and resuspended into autologous citrated platelet poor plasma at varying N_0 for 5 donors. The initial rate and sensitivity of PA were determined generally at 3-5 seconds following ADP/ristocetin addition. Extent of PA was determined at 10 seconds. At similar N_0 ($180 \pm 50 \times 10^3$ μl^{-1}), large platelets (L; $\bar{v} = 7.4 \pm 0.3$ fl; $16 \pm 4\%$ of total population) were two-fold more sensitive and more rapidly recruited into both PA and turbidometrically measured macroaggregates (TA) in response to ADP than the smallest platelets (S; $\bar{v} = 4.6 \pm 0.4$; $16 \pm 5\%$). Aggregation kinetics and sensitivity for the mid-sized platelets ($\bar{v} = 5.9 \pm 0.3$; $31 \pm 7\%$) were intermediate between the large (L) and small (S) platelet fractions. When platelet counts were adjusted to yield similar total platelet mass ($N_0 \times \bar{v}$), these differences persisted for PA, but not for TA. Subsequent studies were all made for platelet suspensions at similar mass. Maximal rates of ADP-induced shape change were comparable for L vs. S platelets. Significant differences in the initial rate and maximal extent of PA between the size-dependent fractions were also seen for a stable PGH_2 analogue (U46619) and platelet activating factor (PAF). Most platelets were maximally recruited into micro-aggregates (60-80% PA) for all sized fractions. Kinetics and sensitivity for ristocetin-induced agglutination were comparable between the different sized fractions. The above size-dependent differences in aggregation for physiological activators appear to arise from intrinsic membrane/cell biochemical differences, not observed for ristocetin-von Willebrand (Factor VIII)-induced agglutination.

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AGGREGATION RESPONSE OF PLATELETS DURING INHIBITION OF PHOSPHOLIPASE A_2. **R.S. Labow (1), E. Meek (1), G.A. Adams (1,2), G. Rock (1,2,3).** **Ottawa Centre, Canadian Red Cross, Blood Transfusion Service (1), Depts. of Biochemistry (2) and Medicine (3), University of Ottawa, Ottawa, Ontario, Canada**

Arachidonic acid (AA) is liberated from platelet membrane phospholipids during stimulation and promotes cellular aggregation through the synthesis of thromboxane A_2. Two pathways; phospholipase A_2 (PLA_2) or phospholipase C (PLC) followed by the action of acylglycerolipases, are thought to be activated during platelet stimulation and supply the necessary AA. We have reported that mono (2-ethylhexyl)phthalate (MEHP), a physiological metabolite of the plasticizer di(2-ethylhexyl)-phthalate (DEHP), commonly used in a variety of medical devices and storage containers, inhibits PLA_2, but not PLC in platelet lysates. The effects of MEHP on intact platelets were studied. PLA_2 activity in intact platelets or lysates was assayed by incubating them with 2-^{14}C-arachidonyl-phosphatidylcholine and measuring formation of free ^{14}C-arachidonic acid in 10 min. Platelet lysates hydrolyzed 10% of the substrate while 2.6% was hydrolyzed by intact platelets. The amount of MEHP needed to inhibit ^{14}C-AA liberation was 0.35 mM for platelet lysates and 0.7 mM for intact platelets. Platelet aggregation induced by collagen was inhibited by MEHP (1 mM), although responses to adenosine diphosphate, AA and ionophore were unaffected. Identical effects on platelet aggregation were found when indomethacin (0.1 mM) was added. Higher concentrations of MEHP blocked platelet aggregation induced by adenosine diphosphate or AA but not ionophore or synergistic pairs of these stimuli, indicating a more generalized membrane disruption at higher MEHP concentrations. These results suggest that MEHP acts in a similar manner to indomethacin to block PLA_2-mediated liberation of arachidonate during platelet aggregation.

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CHANGES IN PLATELET HALF-LIFE, SENSITIVITY TO PROSTANOIDS AND AGGREGATION INDUCED IN THE DOG BY BODY HYPOTHERMIA. L.M. Cunha-Ribeiro, S. Cunha, T. Brandão, F. S. Gonçalves, A. Almeida-Dias and J.M. Pina-Cabral. Haemostasis Center (INIC) and Dept. of Physiology, Porto Medical School, Porto, PORTUGAL.

During body cooling, there is a progressive thrombocytopenia, which is reversible after rewarming and is not prevented by previous treatement with aspirin, ticlopidine or prostacyclin. In this work, in order to evaluate if hypothermia induces alterations of platelet function we studied, in the dog, the platelet aggregation (PA) and the inhibitory action of PGE1 and of a stable prostacyclin analog, iloprost, before hypothermia(37°C.) and after rewarming(37°C.). Platelet half-life was also studied in another group of dogs before induction of hypothermia and after rewarming and recuperation of the animals. PA has been evaluated by platelet counting in whole blood. Platelet half-life was estimated by serial determinations of MDA following administration of aspirin. PA induced by ADP (30µM) decreased 40% after rewarming(n=8). Platelet sensitivity to PGE1 (35 nM -1.4 µM f.c.) and iloprost (7nM-172nM f.c.) was also decreased after rewarming: inhibition index 2.08±1.082 versus 1.19±0.362 (n=8; $p<0.01$) and 2.48±1.250 versus 1.10±0.227 (n=8; $p<0.005$) respectively. Platelet half-life increased after hypothermia from 3.99±0.730 days to 4.48±0.846 days(n=8 $p<0.05$). In the control group(n=6) platelet half-life determined twice with one week interval, did not change significantly.

We conclude that body hypothermia decreases platelet reactivity to ADP; renders platelets less sensitive to the inibitory effect of prostanoids and increases platelet half-life. These results are probably due to alterations in platelet membrane induced by cooling and rewarming the animals.

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ABNORMALITIES OF PLATELET AGGREGATION AND ENHANCED FACTOR X ACTIVATOR ACTIVITY OF WASHED PLATELETS IN SICKLE CELL DISEASE. D.A.F. Chamone (1), A.Y. Hoshikawa-Fujimura (2), C. Massumoto (1), G. Bellotti (3), F. Arashiro (2) and M. Jamra (1). Department of Internal Medicine, Division of Hematology (1), Faculty of Pharmaceutical Sciences (2) and Institute of Heart Disease (3), São Paulo, Brazil

The occurence of microvascular occlusion is one of the most prominent pathologic features of sickle cell anemia. The mechanism of vaso occlusion has generally been attributed to the abnormal shape and reduced deformability of the sickled erithrocytes. However, the involvement of vascular endothelium, platelets and their interactions with coagulation factors may also be of pathogenic significance in microvascular occlusive crises.

We investigated the interaction between vascular endothelium, platelets and blood coagulation factors in 23 patients with Sickle Cell Disease (SCD) and in normal volunteers.

Factor X activator activity in washed platelets was performed according to Semeraro and Vermylen (1977), thromboxane B_2 (TXB_2) and 6-keto-$PGF_{1\alpha}$ were determined using specific radioimmunoassays. PAF-acether from platelets was determined according to Chignard et al (Nature, 1979, 279:799). Platelet aggregation was performed with a Chrono-Log Aggregometer (Model 440) on platelet rich plasma (PRP) using the Born method. Prostacyclin release from endothelium was performed according to Moncada et al (Lancet i:18, 1977).

Our results showed that platelets from patients with SCD have enhanced factor X activator activity ($p < 0.0001$), produce more PAF-acether than controls ($p < 0.02$) and showed hyperaggregability in these patients as compared to normal volunteers ($p < 0.00001$).

We concluded that platelets from homozygous sicklers have enhanced factor X activator activity as well as increased capacity for PAF-acether production. These abnormalities may contribute to the incidence of vaso occlusive crises in these patients.