1948

ABNORMAL CALCIUM TRANSPORT INTO MICROSOMES OF GRAY PLATELET SYNDROME. S. Levy-Toledano, J. Enouf, M. Lebret, R. Bredoux and J.P. Caen. U-150 INSERM, UA 334 CNRS, Hôpital Lariboisière, Paris, France.

Gray platelets initially described as lacking α granules also show thrombin-induced aggregation and release lower than normal. One of the possible explanation is a modified intracellular Ca^{2+} concentration which is involved in platelet activation. We then decided to investigate the relationship between the morphological abnormality and a possible regulation of platelet Ca^{2+} concentration.

We isolated a platelet membrane fraction (100,000 g) enriched in intracellular membranes which actively sequesters Ca^{2+}. This Ca^{2+} uptake was mediated by a characterized (Ca^{2+} + Mg^{2+}) ATPase.

The isolated membrane vesicles from two patients show an increase in the calcium uptake. The stimulation reaches a factor 2 to 3 and the Ca^{2+} uptake appears greatly increased whatever the Ca^{2+} concentration used. This led us to investigate the Ca^{2+} + Mg^{2+} ATPase activity. The enzymatic activity appears increased in the first 10 minutes which correlates with the increased rate in calcium uptake. The specific activity of the enzyme is increased by a factor 2.4 to 2.7 which again agrees with the calcium uptake results. Therefore we suggest that the severe impairment in secretion found in the Gray platelets is probably related to the low cytoplasmic mobilization as it is found by Hardisty et al, 1985 ; this would be the consequence of an increased Ca^{2+} uptake rather than a decrease in the Ca^{2+} liberation.

The absence of α granules in Gray platelets together with the described abnormality in internal membranes and the recently described modification of external membrane fluidity (Rendu et al 1985) would suggest that these platelets have a general membrane disorder.

1949

ABNORMALITIES OF CYTOPLASMIC [Ca^{++}] IN PLATELETS FROM UREMIC PATIENTS STUDIED WITH AEQUORIN AND INDO-1. J.A. Ware, B.A. Clark, M. Smith, E.W. Salzman. Beth Israel Hospital and Harvard Medical School, Boston, MA., U.S.A.

Uremic patients have a hemorrhagic tendency, often with prolonged bleeding times and abnormalities of platelet function in vitro. Whether these defects result from plasma factors, abnormalities in platelet surface receptors, or intracellular mediators is unknown. Accordingly, blood was obtained from 16 patients with severe uremia (BUN >90), and platelets were washed, loaded with aequorin or indo-1, gel-filtered, and resuspended in either plasma or buffer. Of the 16 patients, 4 had template bleeding times greater than 12 minutes, but platelet aggregation in plasma was not consistently impaired. However, the rise in cytoplasmic [Ca^{++}] in response to the Ca^{++}-ionophore A23187 or ADP in aequorin-loaded platelets from the 4 patients with long bleeding times was much lower than in uremic patients with normal bleeding times or in normal volunteers. The reduced [Ca^{++}] response was associated with decreased aggregation of gel-filtered platelets in buffer. Prolonged bleeding time was less consistently correlated with decreased responses to epinephrine or arachidonate. Suspending washed aequorin-loaded uremic platelets in normal plasma for 10-20 min did not reverse the decreased agonist-induced rise in [Ca^{++}]; platelets from a normal donor resuspended in uremic plasma responded normally. The agonist-induced rise in [Ca^{++}] shown by indo-1 was not abnormal in patients with prolonged bleeding times; however, uremic patients generally had higher indo-1-indicated basal platelet cytoplasmic [Ca^{++}] than normal. We conclude that the hemorrhagic tendency in some patients with uremia is associated with abnormal intracellular platelet [Ca^{++}] regulation marked by elevated resting [Ca^{++}] and a decreased rise in cytoplasmic [Ca^{++}] in response to certain agonists; this latter abnormality appears to be correlated with prolonged bleeding times.

1950

HUMAN RECOMBINANT ERYTHROPOIETIN (rHuEpo) CORRECTS ANEMIA AND SHORTENS THE BLEEDING TIME (BT) IN UREMIC PATIENTS. M. Moia (1), S. Casati (2), P. Della Valle (1), P. Passerini (2), C. Ponticelli (2) and P.M. Mannucci (1). A. Bianchi Bonomi Hemophilia and Thrombosis Centre (1) and Nephrology Div. (2), Univ. Milano, Italy.

A hemorrhagic tendency and anemia are two major complications of uremia. There is a significant negative correlation between BT and hematocrit (Ht) in uremic patients. We studied the efficacy of rHuEpo in correcting both these abnormalities in 10 patients on chronic hemodialysis with severe anemia (basal Hb levels 6.2±0.8 g/dL). Seven patients had BT longer than 10 min., 5 had BT longer than 30 min. rHuEpo was given to the patients 3 times a week at increasing doses, from 24 U/Kg to a maximum of 432 U/Kg, aiming to reach Hb levels ⩾12 g/dL. BT was measured (with the template-like disposable device Simplate II) before starting rHuEpo therapy and after reaching the target Hb values. One patient (n° 6) was excluded from the study after 5 weeks of rHuEpo treatment because of development of an arterio-venous fistula thrombosis. The data are shown in the table.

	BT (min.)		Hematocrit (%)	
PATIENT	BEFORE	AFTER	BEFORE	AFTER
1	6	4	20	36
2	8	7.5	19	39
3	20	6	21	41
4	>30	9	19	40
5	25	4.5	18	35
6	7.5	—	17	—
7	>30	8	20	36
8	>30	9	22	40
9	>30	7.5	19	34
10	>30	19	14	33

Eight of the 9 patients who concluded the study had final BT shorter than 10 min. This demonstrates the efficacy of rHuEpo in correcting both anemia and prolonged BT in uremic patients.

1951

ANALYSIS OF PATIENTS WITH A PROLONGED BLEEDING TIME. H.K. Nieuwenhuis , J.J. Zwaginga and J.J. Sixma. Department of Haematology, University Hospital Utrecht, P.O.Box 16250, 3500 CG Utrecht, The Netherlands.

The bleeding time (BT) is one of the most important screening tests in patients with a bleeding tendency. Investigations of Von Willebrand Factor and aggregation tests form routine diagnostic tools to analyse a prolonged BT. In many patients, however, this approach fails to explain the bleeding tendency because no abnormality is found. In order to deal with this problem , we chose three methods: I) determination of platelet nucleotides and serotonin in all patients with a prolonged BT, independent of the results of aggregation tests, II) measurement of platelet adherence to purified collagen and the matrix of endothelial cells in a perfusion system, III) measurement of adherence of normal blood to matrix of patients' fibroblasts.

I) In a group of 145 patients with a prolonged BT and a normal platelet count the diagnosis von Willebrand's disease was made in 52 patients (36%), congenital Storage Pool Disease (SPD) in 27 pts (18%), defect of thromboxane synthesis in 4 pts (3%) and platelet function disorders with miscellaneous aggregation abnormalities in 23 pts (16%). No abnormalities were found in 39 pts (27%). Analysis of aggregation tests, disclosed normal aggregation tracings in many patients with SPD: 23% of 106 patients with congenital or acquired SPD had normal aggregation tests.

II) Adhesion studies were performed with the blood of 7 patients with an unexplained prolonged bleeding time. Two patients had a severe defect and one patient a mild defect of adherence. Further studies revealed the presence of an autoantibody on the platelets in one of these patients.

III) In order to diagnose vessel wall defects which may cause a prolonged BT, we studied the adherence of normal blood to the matrix of the fibroblasts of one patient. We found a decreased adherence, which suggests the presence of a vessel wall defect causing a prolonged BT.

We conclude that in many patients with a prolonged BT and normal VWF parameters and normal aggregation tests, SPD or adhesion defects may be responsible for a bleeding tendency.