

STUDIES OF SUPEROXIDE PRODUCTION IN HUMAN PLATELETS. A.J. Marcus, S.T. Silk, L.B. Safier, H.L. Ullman and M.J. Broekman. NYVA Hospital and Cornell Medical College, N.Y., N.Y., U.S.A.

Superoxide radicals are measurable in platelet suspensions by two different assay systems (SOD-inhibitable cytochrome c and nitroblue tetrazolium reduction). The O_2^- -producing mechanism is detectable in platelet supernatants and the rate of O_2^- generation is proportional to platelet concentration. Prior ingestion of aspirin does not inhibit O_2^- production by platelets. Following stimulation by collagen and thrombin platelets develop a marked increase in their capacity for cytochrome c and NBT reduction, but in contrast to leukocytes stimulation does not result in an increase in superoxide production. Sodium fluoride, a powerful stimulus for O_2^- production in leukocytes, and also a platelet aggregating agent, is under study. A concentration of 20 mM sodium fluoride increases platelet superoxide production approximately two-fold over baseline values. This level of fluoride induces 88% 5-HT release in the absence of LDH leakage. The increase in superoxide generation does not correlate directly with 5-HT release, and the fluoride effect on O_2^- may not be related to its influence on secretion. Electrophoretic studies indicated that fluoride had no effect on platelet superoxide dismutase. Experiments with ionophore A23187 indicated that the action of this agent was not comparable to fluoride but was similar to collagen and thrombin in that O_2^- production did not exceed baseline levels. In some instances the ionophore appeared to inhibit O_2^- production. Superoxide radicals may play an important role in platelet-platelet or platelet-blood vessel interactions.

REDUCED GLUTATHIONE AND PLATELET FUNCTIONS. S. Matsuda, Y. Ikeda, M. Kikuchi, Y. Ando, M. Aoki, T. Ogawa and M. Hasegawa. School of Medicine, Keio University, Tokyo, Japan.

The role of reduced glutathione (GSH) on platelet functions was investigated utilizing diamide $[(CH_3)_2NCON=NCON(CH_3)_2]$, which was shown to be a rather specific agent for the intracellular oxidation of GSH to oxidized glutathione (GSSG). Human normal citrated PRP was incubated with various concentration of diamide for 30 min at 22°C and platelet aggregation studies were performed using ADP, epinephrine, or collagen as aggregating agents. Clot retraction was examined in PRP treated with diamide by adding 100mM $CaCl_2$ and 1.0u/ml thrombin. Simultaneously, GSH in platelets were measured by Beutler's method. The maximum aggregability of platelets treated with 25μM diamide was significantly decreased with 2.5μM ADP, 5μM epinephrine, or 64μg/ml collagen as aggregating agent. When platelets were incubated with 50μM diamide, no secondary aggregation with 2.5μM ADP or 5μM epinephrine was observed. At a concentration of 100μM, diamide completely abolished primary aggregation with 2.5μM ADP. Clot retraction was grossly inhibited by diamide at concentrations above 50μM. GSH levels of platelets which were incubated with 100μM and 1mM diamide at 22°C for 30 min was 11.4×10^{-9} and 1.16×10^{-9} moles/ 10^9 plts, respectively, while that without diamide treatment was 15.28×10^{-9} moles/ 10^9 plts. Decrease of GSH levels seemed to be dependent on diamide concentration. Membranes were isolated from diamide treated platelets according to the method of Jamieson et al, and were electrophoresed on SDS polyacrylamide gels. There was no difference in Coomassie blue or PAS stained bands between control and diamide treated platelets. Marked inhibition of platelet aggregation and clot retraction accompanied by intracellular oxidation of GSH to GSSG may imply an important role of GSH on platelet aggregation and clot retraction.

THROMBIN-INDUCED CHANGES IN PHOSPHATIDIC ACID (PA) AND PHOSPHOINOSITIDES. N.L. Leung, R.L. Kinlough-Rathbone and J.F. Mustard. McMaster University, Hamilton, Ontario, Canada.

Thrombin-induced changes in PA, monophosphoinositide (MPI), diphosphoinositide (DPI) and triphosphoinositide (TPI) of washed rabbit platelets have been examined. Platelets prelabeled by incubation with ^{32}P -orthophosphate, 3H -glycerol, 3H -inositol or ^{14}C -arachidonate were exposed to 0.33 u/ml thrombin for one minute, and the phospholipids extracted and separated by thin layer chromatography. Measurement of absolute amounts of PA and MPI by phosphorus assay showed that PA increased by 180% while MPI decreased by 15%.

	Mean % change in labeling in 1 minute vs control					
	PA	MPI	DPI	TPI	DG	AA
^{32}P	+1267	+4	+20	+12	--	--
3H -glycerol (3H -G)	+100	-22	0	0	+70	--
3H -inositol (3H -I)	--	-20	+8	+29	--	--
^{14}C -arachidonate (^{14}C -AA)	+1149	-27	+10	+20	+337	+78

The changes in MPI with 3H -G, 3H -I and ^{14}C -AA and in 1,2-diacyl glycerol (DG) indicate that some MPI may be converted to PA via DG. Changes in the 3H -G and 3H -I labeling of DPI and TPI suggest that the changes observed with ^{32}P -labeled platelets are a result of the turnover of the phosphorylinositol moiety. The increase in AA with ^{14}C -AA indicates that some of the decrease in MPI may be due to the formation of lyso MPI and free fatty acid. These results indicate that thrombin stimulation of platelets may affect inositol phospholipid metabolism through three pathways: (1) involving PA, DG and MPI; (2) the cleavage of free fatty acids from MPI; (3) turnover of the ester phosphates on DPI and TPI.