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10:00 h

STUDIES ON THROMBOTIC THROMBOCYTOPENIC PURPURA: PURIFICATION AND PROPERTIES OF PLATELET AGGREGATION FACTOR INHIBITOR A. E.C.-Y. Lian, P. Mui, and L. Chiu. Department of Medicine, Veterans Administration Medical Center and Center for Blood Disease, University of Miami School of Medicine, Miami, Florida, U.S.A.

Patients with thrombotic thrombocytopenic purpura (TTP) can be treated with plasma infusions, suggesting that deficiency of certain plasma factor(s) may be responsible for the syndrome. We demonstrated that TTP plasma induced the aggregation of autologous as well as homologous platelets, an activity not affected by hirudin, diisopropylfluorophosphate, aspirin, or prostacyclin, but inactivated when preincubated with normal plasma. One of the plasma factors designated TTP-PAFI A which inhibits the platelet aggregation caused by TTP plasma has been purified to homogeneity. The preparation procedures include ammonium sulfate fractionation, ion exchange chromatography, ultracentrifugation, gel filtration and preparation gel electrophoresis. The purified protein showed a single line in analytical gel electrophoresis and immunodiffusion. The amino acid composition of the inhibitor has been characterized, and its molecular weight was estimated to be 180,000 daltons. This inhibitor did not reduce the platelet aggregation induced by ADP, collagen, ristocetin, or thrombin, suggesting a specific interaction with an as yet undefined platelet aggregating factor in TTP plasma. This inhibitor could be attached to, and then eluted from, protein A. Preliminary studies indicate that this inhibitor as isolated from TTP plasma was partially defective. It appears that functional deficiency of this plasma inhibitor may contribute to the development of TTP.

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ROLES OF THROMBOXANE A₂, RELEASABLE ADP, AND cAMP IN FLUID SHEAR-INDUCED AGGREGATION OF HUMAN PLATELETS. M.W. Moritz, S.P. Suter and J.H. Joist. Departments of Surgery and Mechanical Engineering, Washington University, St. Louis, Missouri, and Departments of Medicine and Pathology, St. Louis University School of Medicine, St. Louis, Missouri, U.S.A.

Fluid shear-induced platelet alterations may be responsible for both thromboembolic complications and platelet dysfunction associated with extracorporeal circulation. Previous work in our laboratory using a Couette rotational viscometer with controlled laminar flow conditions demonstrated that shear-induced platelet aggregation (SIPA) in citrated human platelet-rich plasma at 24°C is associated with the release of substances from both the dense and α -granules of platelets and is not inhibited by aspirin (ASA) induced complete suppression of thromboxane A₂-synthesis. We studied SIPA in suspensions of washed human platelets in Tyrode albumin solution at 37°C to determine the importance of releasable ADP, thromboxane A₂ synthesis and the level of intracellular cAMP in SIPA. SIPA in this system was found to require the presence of added Ca⁺⁺ (but not Mg⁺⁺ or fibrinogen) in the medium. ASA (100 μ M) did not cause appreciable inhibition of SIPA. Creatine phosphate-creatine phosphokinase (CP/CPK), an enzyme system that converts ADP to ATP, caused 40% inhibition of SIPA. With both ASA and CP/CPK present in the suspension, SIPA was identical to that obtained with CP/CPK alone. However, preincubation of the suspension with PGE₁ (1 μ M) and theophylline (100 μ M) caused complete inhibition of SIPA. These findings indicate that SIPA in this system can occur by thromboxane A₂- and ADP-independent pathway(s), which may be modulated by intracellular cAMP.

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THE TIME RELATION BETWEEN THE HAEMOSTATIC AGGREGATION OF PLATELETS AND THEIR RELEASE REACTION IN VIVO. P. Grg and G.V.R. Born. Department of Pharmacology, King's College, Strand, London WC2R 2LS, U.K.

The release reaction of platelets has been assumed to subserve a positive feedback mechanism responsible for their aggregation in haemostasis and thrombosis. This assumption is based mainly on *in vitro* experiments. Considerable uncertainty remains about the contribution of the release reaction to the initiation of haemostasis *in vivo*. The rapidity of the process and the presence of other tissues makes it impossible to follow the reaction quantitatively *in vivo* by methods which permit this *in vitro*. We have therefore applied quantitative electron microscopy to find out how quickly the concentration of dense bodies decreases in platelets during their haemostatic aggregation. In mice, platelets were enriched in dense bodies by pretreatment with serotonin.

Mesenteric arteries were incised with a sharp blade. Bleeding was stopped by a micromanipulator-operated device about 15 sec and 60 sec after the cut. The cut segments were immediately fixed *in situ* with glutaraldehyde and postfixed. Serial sections were made for electron microscopy. Platelets isolated from peripheral blood of the same animal were prepared similarly. Electron micrographs were projected on to a television screen and numbers of dense bodies and total platelet areas were determined by an image analysing computer. After 15 sec there were no significant differences in numbers of dense bodies in platelets from different parts of the haemostatic plugs ($8.31 \pm 0.57/100 \mu^2$ mean \pm s.e.m.) and in platelets from the blood (8.93 ± 0.38). On the other hand, after 60 sec the parts furthest from the cut contained fewer dense bodies than the nearer parts and the overall dense body number (5.86 ± 0.05) was considerably smaller ($p < 0.001$) than that of platelets from the blood (14.45 ± 0.09).

The results suggest that haemostatic aggregation of platelets does not initially depend on their release reaction.

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INCREASED AGGREGATION OF HUMAN PLATELETS IN A VORTEX IN CORONARY ARTERY DISEASE. T. Karino and H.L. Goldsmith. McGill University Medical Clinic, Montreal General Hospital, Montreal, Quebec, CANADA.

Previous studies of normal human platelets circulating in an annular vortex distal to a sudden expansion of a 150 μ m into a 500 μ m tube showed that collisions between cells led to spontaneous formation of aggregates without addition of any aggregating agent (Karino and Goldsmith, *Microvasc. Res.* 17: 217-237, 1979). Using the same technique, we have compared the reactivity of platelets from 27 patients with suspected coronary artery disease (CAD) at time of coronary angiography with that from 20 healthy volunteers. Platelet-rich plasma (PRP) was prepared from 40 ml of citrated blood and subjected to steady flow at a mean velocity of 7.5 cm/s in the 150 μ m tube at which aggregation in the vortex was known to be maximal. The rate (time to form) and extent of aggregation (size of the largest aggregate) in the vortex were measured by observations through a microscope. Platelet aggregation was normal compared to volunteers in 5 of 6 patients found without CAD (largest aggregate $< 20 \mu$ m formed in > 90 s). By contrast, platelets in 12 of 21 patients with diagnosed CAD were markedly more reactive, forming aggregates $> 50 \mu$ m in < 60 s. The effect of propranolol therapy on aggregation was also investigated since we had found that both the active and inactive forms of the drug at 10^{-5} - 10^{-4} M partially or totally inhibited aggregation of normal platelets in the vortex. Thus, it became apparent that both rate and extent of aggregation in PRP from CAD patients not taking the drug were appreciably higher (4 of 5 having hyper-reactive cells) than that of patients on the drug (8 of 16 with hyper-reactive cells). Finally, it was noted that platelets from patients with a previous record of myocardial infarction (MI) were more reactive than those of non-MI patients. The above technique may serve as a sensitive tool for measuring platelet hyperaggregability in certain disease states.