

A MORPHOLOGICAL STUDY OF CLOT CONTRACTION DURING THE DEVELOPMENT OF ISOMETRIC TENSION

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Under isometric conditions, and in the presence of micromolar Ca^{2+} , clots of normal platelet-rich plasma develop tension at an initial rate of 0.1 to $0.2 \text{ g min}^{-1} \text{ cm}^{-2}$ (initial cross-sectional area). When fixed under tension, after attaining a force of 1.6 g cm^{-2} , electron microscopy reveals that platelets extend long pseudopods along the fibrin strands which are oriented in cable-like fashion in the direction of the isometric tension. Cross-sections of the pseudopods show microtubules in most. The extension of pseudopods paralleled the development of tension and was dependent on the quality of the platelets as well as on Factor XIIIa, catalyzed cross-linking of fibrin fibers. Normal platelets suspended in factor XIII deficient plasma failed to develop tension or long pseudopods but did so on addition of pure factor XIII. Pseudopod extension and development of tension were inhibited by either 10^{-4} M vincristine which disrupted platelet microtubules or by 20 ug/ml cytochalasin B which interfered with platelet microfilament function. When platelet numbers were decreased, tension development decreased approximately linearly. The decrease in platelet number was not compensated for by an increased length of platelet pseudopods suggesting that platelet-fibrin interaction is essential for tension, while platelet-platelet contact is not. We suggest that clot contraction requires the extension of pseudopods along cross-linked fibrin strands, followed by their contraction orienting the fibrin fibers in the direction of tension and compacting the fibrin network into platelet-fibrin clumps.

HUMAN BLOOD PLATELETS CAUSE COLLAGEN GEL RETRACTION. M. Kopeć and M. Jeleńska. Department of Radiobiology and Health Protection, Institute of Nuclear Research, Warsaw, Poland.

Contraction of collagen gels under the influence of human blood platelets was studied and compared with platelet-mediated fibrin clot retraction. Soluble type I collagen from the rabbit skin was allowed to polymerize in the presence of washed platelet suspensions. Collagen gels formed in the presence of intact platelets retract with concomitant exclusion of the fluid. Collagen gel retraction is positively related to platelet numbers/ $5-60 \times 10^6$ platelets/ml/ and negatively to collagen concentrations/ 0.5 to 2.0 mg/ml /. Under optimal conditions collagen gels shrink to about 12% while fibrin clots to 8% of initial volumes. Similarly to fibrin retraction collagen shrinkage is abolished by disruption of platelets or chelation of Ca^{++} ions. Collagen retraction is inhibited by cytochalasin B $/5 \mu\text{g/ml}/$ and N-ethylmaleimide $/25 \mu\text{g/ml}/$. Colchicine, indomethacin, PGE_1 , dipyridamol and serpasil did not influence collagen gel retraction. Thrombin, Defibrase, Arvin and hirudin remain also without effect. Platelets from patients with severe deficiencies of f.VIII or of vWillebrand factor exhibit normal collagen retracting potency. Platelets from patients with Glanzmann's thrombasthenia, defective in fibrin retracting capacity, provoke collagen shrinkage of nearly the same degree as platelets from healthy people. Certain tumor cell lines inactive in fibrin retraction have the potency to contract collagen. The presented data can be relevant to wound healing and tumor progression.

EFFECTS OF HEMOSTATIC AGENTS ON PLATELET AGGREGATION

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Several new collagen derived hemostatic agents have been introduced into the surgical armamentarium. The presumption is they produce platelet aggregation, ADP release and blood clotting through the intrinsic system.

Studies of interreaction of these new hemostatic agents with both blood and platelets suggests that the type of reaction with whole blood is not known or due to primary platelet activation. Avitene interreacts in part by platelet aggregation but also by interreacting with other components in blood including fibrinogen.

The available evidence with a new dissolving collagenous hemostat, "Superstat"TM indicates probable activation of the extrinsic coagulation system, directly converting prothrombin to thrombin, and fibrinogen to fibrin. Thrombin time ratios (experimental vs. control) are Superstat 1% - $0.81 \pm 0.16/0.93 \pm 0.06$, Superstat 2% - $0.84 \pm 0.11/0.93 \pm 0.06$, Superstat 3% - $0.61 \pm 0.15/0.71 \pm 0.02$. Comparative studies have been completed on several other agents, among them, Kollagen Haemo.Vlies - $0.66 \pm 0.1/0.71 \pm 0.02$, Gelfoam - $0.75 \pm 0.15/0.71 \pm 0.02$, Surgicel - $0.73 \pm 0.4/0.71 \pm 0.02$, Avifene - $0.75 \pm 0.3/0.71 \pm 0.02$, Collatamp - $0.73 \pm 0.4/0.71 \pm 0.02$. Derived data from thrombin times, clotting times and, when possible, platelet aggregation displays widely different rates of thrombus formation. We believe the enzymatic mechanisms for these reactions are currently only partially known.

The fact that clinically useful hemostatic agents each appear to produce hemostasis by several routes, and do not all use the same route, is of extreme interest in basic thrombosis studies as well as in clinical surgery.