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• HEPARIN, PLATELETS, AND VASCULAR CELLS

FUNDING

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Located at the Veterans Administration Puget Sound Health Care System, the Vascular Research Laboratories are led by Michael Sobel, M.D., Errol Wijelath, Ph.D., and supported by other Ph.D.'s and postdoctoral trainees. The principal focus of Dr. Sobel's research group is understanding the structure-function relations of heparin's interactions with vascular proteins and cells. Heparins are a family of structurally heterogeneous sulfated polysaccharides. Heparin is best known for its anticoagulant properties, which are exerted by heparin binding to the plasma protein antithrombin-III. But beyond their conventional anticoagulant actions, heparins have a wide range of other biological effects — antiproliferative, anti-inflammatory, as well as stimulatory actions on some vascular cells. And while the interaction between heparin and antithrombin-III is known to depend on a well defined structural domain — the heparin pentasaccharide — heparin interactions with other proteins

laboratories have found that heparin directly influences platelet function by at least two separate mechanisms.

Heparin Interactions with von Willebrand Factor

Using biophysical methods, binding assays, and molecular modeling, they demonstrated that heparin binds to a specific domain of von Willebrand factor (vWf) (1;2). This plasma protein is essential for normal platelet hemostatic function, and mediates the adhesion of platelets at sites of vascular injury (especially under high shear, arterial conditions). When heparin binds vWf it interferes with the platelet hemostatic properties of the protein. Specific sub-species of heparin were purified that bound vWf with especially high affinity. Through scientific collaborations with Dr. Yasuo Suda, a carbohydrate polymer chemist in Japan, a structurally defined disaccharide motif was identified that was responsible for heparin's binding to vWf. A refined heparin with high affinity for vWf (and low affinity for antithrombin-

Beyond their conventional anticoagulant actions, heparins have a wide range of other biological effects — antiproliferative, anti-inflammatory, as well as stimulatory actions on some vascular cells.

and cells have not been as well characterized. In part, the structural complexity of carbohydrates and heparin in particular has hindered efforts to better understand its structure-function relations. Also, the biological effects of heparins have often been contradictory or confusing, due to the complexity of the biological models used.

The interactions between platelets and heparin have been especially confusing. The autoimmune-mediated phenomenon of heparin-induced thrombocytopenia is one aspect of heparin-platelet interactions. But apart from this unusual immune reaction, Dr. Sobel's

III) was effective at preventing arterial occlusion in an animal model of platelet-vWf dependent arterial thrombosis (3;4). This work holds future promise for developing novel antithrombotic heparins that interfere with vWf-mediated platelet adhesion, rather than retarding plasma coagulation.

Heparin Binds Directly to the Platelet Integrin

Heparin also has a contradictory, direct stimulatory effect on platelet function. In related work, it was shown that heparin binds directly to the platelet surface, and

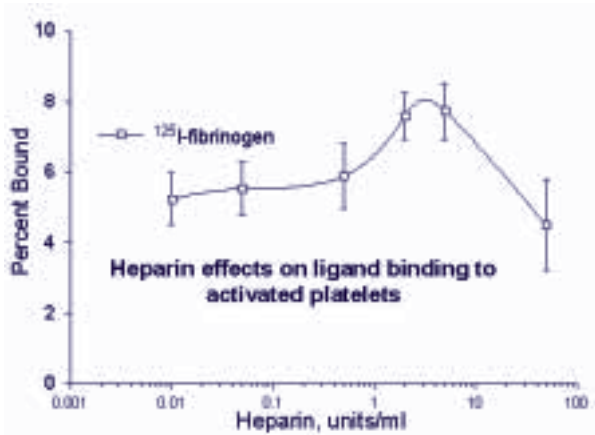


FIGURE 1: 125I-Fibrinogen binding to thrombin-activated platelets was measured over a range of heparin concentrations. At concentrations of 2 and 5 units/ml heparin, fibrinogen binding was significantly increased.

that one of the important binding sites may be the platelet fibrinogen receptor, GpIIb/IIIa (integrin $\alpha_{IIb}\beta_3$). Unlike vWf, which mediates platelet adhesion at high shear rates, the fibrinogen receptor is responsible for platelet aggregation and clumping at lower shear rates. Through physiological studies of platelet aggregation, photoaffinity crosslinking, and cell-signaling work, heparin was found to bind to this platelet integrin, and enhance its binding of fibrinogen (5).

Heparin Modulates α_3 Integrins

How does heparin activate or enhance integrin function in the platelet? To see whether these effects were unique to the platelet integrin ($\alpha_{IIb}\beta_3$), the K562 cell line was transfected with different integrins, and the effects of heparin on integrin-mediated cell adhesion were studied. Surprisingly, the effect of heparin on integrin function depended on the integrin subunit. A stimulatory effect was observed in all α_3 containing integrins ($\alpha_{IIb}\beta_3$, $\alpha_V\beta_3$) but the type of β subunit did not seem to be as important. The effect of heparin was structure specific, as other glycosaminoglycans and low molecular weight heparins showed no enhancement of adhesion (6). Because integrins are such ubiquitous receptors in vascular cells, a detailed understanding of precisely how heparin modulates these receptors may lead to novel drugs to modulate thrombosis and vascular healing.

Heparin Modulation of Endothelial Cell Migration and Proliferation

Matrix proteins and growth factors (and their respective cellular receptors — integrins and receptor tyrosine kinases) are key actors in angiogenesis and vascular

healing. Integrins and growth factor receptors work together to enhance the extracellular signals from each pathway, leading to increased endothelial cell proliferation and migration. Vascular Endothelial Growth Factor (VEGF) and fibronectin appear to have a unique complementary relationship. In a recent publication, VEGF was shown to preferentially bind to fibronectin over other matrix proteins (7). Platelets actually release pre-formed VEGF/fibronectin complexes, and these complexes have significantly more potent mitogenic effects than VEGF or fibronectin alone on endothelial cells. Heparin further supports the synergistic biological effects of VEGF/fibronectin. Once again, heparin (and cell-surface heparan sulfate proteoglycans) may be playing a key role in modulating the extracellular assembly of specific ligands on their cellular receptors.

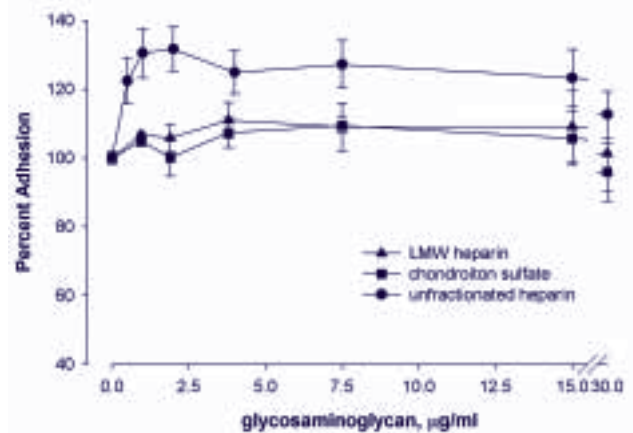


FIGURE 2: Thrombin activated platelets.

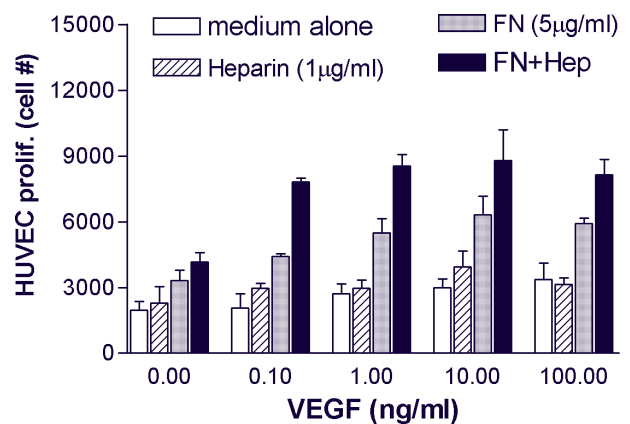


FIGURE 3: Adhesion of K562 $\alpha_V\beta_3$ cells to vitronectin. Unfractionated heparin enhances integrin-mediated adhesion, but other glycosaminoglycans do not.

RELATED PUBLICATIONS

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