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LOW MOLECULAR WEIGHT HEPARIN (Enoxaparin) COMPARED WITH UNFRACTIONATED HEPARIN THRICE DAILY IN PREVENTION OF POSTOPERATIVE THROMBOSIS. A RANDOMIZED MULTICENTRE TRIAL. M. Samama (1), P. Bernard (2), J.P. Bonnardot (3), E. Tissot (4), Y. Lanson (5), S. Combe-Tamzali (1), on behalf of the participants of the "Groupe d'Etude de l'Enoxaparine (GENOX)" multicentric trial. (1) Laboratoire Hématologie, Hôtel-Dieu, Paris; (2) C.H.U. Grenoble; (3) Hôpital Tenon, Paris; (4) C.H.U. Lyon; (5) C.H.U. Tours, France.

Three consecutive randomized open studies have been carried out in 892 patients undergoing abdominal, gynecological, thoracic or urological surgery. They were over 40 years old and presented at least one of the following risk factors for thrombosis: previous thromboembolism, obesity, varicose veins, malignancy (30% in these studies), pre-operative hospitalization over 5 days, estrogen therapy, chronic cardiac disease or bronchitis. The two groups of each trial were well matched with regard to population characteristics. The third trial included higher rate of patients undergoing urologic surgery. Isotopic venous thromboses and bleeding complications were assessed after subcutaneous administration of a low molecular weight (LMW) heparin fragment (Enoxaparin, 1 mg = 100 Anti-Xa I.U.) or unfractionated heparin (UH). The 3 studies compared 3 x 5,000 IU UH daily with 1 x 60 mg, 1 x 40 mg, or 1 x 20 mg LMW heparin daily. Thromboembolic event rates were not significantly different among the groups (UH : 3.6, 2.8, 7.6% respectively compared to LMWH : 3, 2.8, 3.7%). Significant decrease of hematocrit and hemoglobin were only observed in patients receiving 60 mg Enoxaparin (as compared to UH) whilst in the 2 other trials no difference could be evidenced between the 2 populations. The metaanalysis of the three studies on the "intention to treat" patients gave results consistent with those observed in good compliers. The three consecutive studies showed homogeneous results ($p = 0.20$), the Mantel Haenszel test did not evidence a global difference between Enoxaparin and unfractionated Heparin ($p = 0.54$). These results suggest that an optimal dosage of 20 mg per day of Enoxaparin is safe and as efficient as UH 5,000 IU x 3 in the prevention of post-operative thrombosis in this population.

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EFFECT OF COLLAGEN AND CITRATE ON HEPARIN-MEDIATED PLATELET ANTIAGGREGATORY ACTIVITY. S.R. Saba (1), H.I. Saba and G.A. Morelli (2). Division of Hematology, Departments of Internal Medicine (2) and Pathology, University of South Florida and J. A. Haley Veterans Hospital, Tampa, FL, U.S.A.

Heparin has been reported to inhibit platelet aggregation. Our studies show that this activity is easily demonstrable in washed platelet systems, but fails to occur in citrated platelet-rich plasma (PRP) in the presence of a variety of agonists, except collagen. Studies were performed to answer the following questions: (1) Why does heparin inhibit the aggregation of washed platelets but not of citrated PRP, which is the system commonly used for platelet aggregation studies? (2) What is the effect of heparin on platelet aggregation occurring in whole blood, where it can be examined both with and without the presence of sodium citrate? (3) Why does heparin consistently inhibit the collagen-induced aggregation even in citrated PRP, while it fails to inhibit aggregation caused by other agonists? Results of the studies clearly demonstrated that heparin has the ability to directly react with sodium citrate, causing loss of its inhibitory activity on platelets. The antiaggregatory activity of heparin in the presence of collagen as the agonists appears to be directly related to the blocking of collagen's agonist activity by heparin. Small concentrations of heparin which were unable to inhibit aggregation per se, effectively blocked the collagen agonist activity on platelet aggregation when heparin was directly added to collagen. Further studies showed that heparin, in a native whole blood platelet aggregation system (in the absence of any anticoagulant), exhibited significant inhibitory activity. This activity was lost when citrate was present in the whole blood preparation. These studies, therefore, indicate that failure of heparin to inhibit platelet aggregation in citrated PRP does not negate the importance of this inhibitory activity. Reactivity of heparin with sodium citrate renders citrated systems unsuitable for studying heparin's effect upon platelets. The whole blood platelet aggregation system without the presence of anticoagulants appears to be a more suitable system for the study of heparin and platelet aggregation, and is closer to the physiological system. Heparin exhibits marked inhibitory activity on platelet aggregation in this system, and this suggests it may be an important activity which deserves further attention.

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HEPARIN-INDUCED PLATELET AGGREGATION (H-IPA): DOSE/RESPONSE RELATIONSHIPS FOR TWO LOW MOLECULAR WEIGHT (LMW)HEPARIN PREPARATIONS (CY 216 and CY 222). L. D. Brace, *J. Fareed and D. Hoppensteadt. Univ. of IL. at Chicago, Chicago, IL., USA and*Loyola Univ. Med. Center, Maywood, IL, USA.

We have previously demonstrated that unfractionated heparin causes platelet aggregation (>50%) in about 40% of normal healthy donors tested. H-IPA occurs in a dose-dependent manner and can be inhibited by antogonists of the thromboxane pathway. Using a LMW heparin preparation (PK 10169) and fractions of this agent separated on the basis of molecular weight (MW) by gel permeation chromatography, we showed that H-IPA was dependent upon the MW of the agents tested. In order to further examine this MW dependence, we tested two other LMW heparin preparations, CY 216 (Mol. wt: 5600) and CY 222 (mol. wt: 3800), and 9 subfractions of each of these agents separated on the basis of MW. Blood was drawn from the same donors whose platelets aggregated when heparin was added to their platelet-rich plasma (PRP), and placed into citrate anticoagulant. PRP was prepared, various concentrations of the agents or their fractions were added and aggregation was monitored for 40 minutes at 37°C. Dose/response curves were constructed from the data obtained with each agent. Compared to unmodified heparin with M_r = 15,000 daltons (D), the dose/response curves for CY 216 (M_r = 5000 D) and CY 222 (M_r = 3,500 D) were shifted progressively down and to the right. Dose/response curves for each of the fractions of CY 216 and CY 222 demonstrated that as the molecular weight of the fractions decreased, the dose/response curves were also shifted progressively down and to the right. These results indicate that as MW decreases, higher concentrations of the fractions are required to cause aggregation, and the maximum aggregation obtained decreases. Fractions with MW less than 2,500 daltons caused aggregation only at concentrations exceeding supra therapeutic range. Since heparin and LMW fractions have inhibitory activity to the activated clotting factors IIa and Xa and LMW fractions have higher anti-Xa than anti-IIa activity, we measured these activities and attempted to correlate them with the ability to cause H-IPA. No correlation between AXa and H-IPA was found. We conclude that the ability to cause H-IPA is an inherent property of heparin and is molecular weight dependent.

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STUDIES ON THE INTERACTIONS BETWEEN THE HEPARIN-LIKE SUBSTANCES AND HUMAN PLATELETS USING THE MONOCLONAL ANTIBODIES TO PLATELET GLYCOPROTEINS. C. Ruan, H. Wan and W. Zhang. Thrombosis and Haemostasis Research Unit, Suzhou Medical College, Suzhou, China.

We reported the new sulphated polysaccharides, SJAMP and HLAMP that have been isolated from the sea cucumbers and compared their biological effects with those of standard heparin. (Med. J. Australia, 1986; 144: HS 17-21). SJAMP and HLAMP have an action that is independent of the antithrombin III and do not inhibit factor Xa, although they have potent antithrombin effect. SJAMP has been used as an antithrombin agent. As does heparin, SJAMP and HLAMP cause platelet aggregation of platelet-rich plasma. At the final concentration of SJAMP or HLAMP of 25μg/ml, a single wave of aggregation was observed. A second wave of aggregation can be induced at a concentration of SJAMP or HLAMP of 50ug/ml. In washed platelets, the platelet aggregation that was induced by SJAMP or HLAMP was fibrinogen-dependent. Furthermore, platelet aggregation and the secretion of serotonin that is induced by ADP or arachidonic acid were enhanced by the prior addition of SJAMP or HLAMP. This effect may explain the cases of thrombocytopenia that may be observed in patients who are undergoing treatment with SJAMP. Using the monoclonal antibodies (MoAb) to human platelet glycoproteins (GP), we studied the SJAMP-platelet and heparin-platelet interactions. SZ-21 is a MoAb directed against GPIIIa and the epitope for SZ-21 is essential for the binding sites of fibrinogen on GPIIb/IIIa complex. SZ-2 is a MoAb to GPIb and recognize an epitope located on the peptide tail (Mr=35K) of the α-chain of GPIb. The platelet aggregation and the secretion of serotonin induced by SJAMP were inhibited by SZ-21, but not by SZ-2. However the preincubation of platelets with SJAMP at the final concentration of 25μg/ml or with heparin at 5u/ml prevented the binding of radioiodinated-SZ-2 to human platelets. But SJAMP or heparin did not compete for the binding of radioiodinated-SZ-21 to human platelets. These results suggest that the platelet aggregation induced by SJAMP is dependent of the binding sites of fibrinogen on GPIIb/IIIa complex, and that the binding sites of SJAMP and heparin on human platelets are related with GPIb, but not with GPIIb/IIIa.