

**RAPID FORMATION OF LARGE MOLECULAR WEIGHT  $\alpha$ -POLYMERS IN CROSSLINKED FIBRIN INDUCED BY HIGH FACTOR XIII CONCENTRATIONS: ROLE OF PLATELET FACTOR XIII.** C.W. Francis and V.J. Marder, Hematology Unit, Department of Medicine, University of Rochester School of Medicine, Rochester, NY, USA.

Following fibrin polymerization, activated factor XIII stabilizes the clot by catalyzing the formation of specific intermolecular covalent crosslinks between pairs of  $\gamma$  chains to form dimers and also among two or more  $\alpha$  chains to form polymers. We have identified a series of previously uncharacterized  $\alpha$  chain polymers with a wide range of sizes, including some with apparent  $M_r$  in excess of several million. Additionally, we establish the role of high concentrations of factor XIII in the extent and rate of  $\alpha$ -polymer formation and provide evidence that the factor XIII required can be provided by platelets. Using SDS gel electrophoresis, we find that fibrin prepared from purified fibrinogen or from platelet-deficient plasma contains a series of 21 factor XIII  $\alpha$  crosslinked  $\alpha$  chain polymers with  $M_r$  from 140,000 to 770,000. The mean  $M_r$  difference between individual polymers of 32,000 is consistent with a staggered, overlapping sequential addition of monomers to the growing  $\alpha$ -polymer chain. In plasma containing no platelets,  $\alpha$ -polymer formation was incomplete with residual  $\alpha$ -monomer remaining. Progressively higher platelet counts facilitated more rapid crosslinking of  $\alpha$  chains into larger polymers. Intact platelets were not required to promote crosslinking, since platelets lysed by freezing and thawing were also effective. Enrichment of plasma with placental factor XIII in an amount equal to that contained in platelets was as effective as platelets in accelerating the rate of formation and increasing the size of  $\alpha$ -chain polymers. We conclude that platelets are a principal source of factor XIII for maximal fibrin stabilization, providing a larger quantity than is available from plasma alone and regulating both the rate and extent of  $\alpha$ -polymer formation in thrombi or hemostatic plugs at sites of vascular injury.

FIBRINOGEN - FIBRIN

**ANTI-THROMBOTIC PROPHYLAXIS THROUGH MODIFICATION OF FIBRIN NETWORK STRUCTURE: A NEW APPROACH.**

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Morphometric analysis of electron micrographs have demonstrated that diameters of fibrin strands follow a bimodal distribution comprising a major network of thicker fibres with a minor network interspersed and made up of very fine fibres. The relationship between the two networks is not fixed and invariant. It will be shown that the two networks are highly responsive to changes in the physiological conditions of clotting. Such a system has biological adaptability and tends to result in fibrin which is more suited to serve its varying roles in haemostasis, in limiting sepsis, in promoting neovascularization and in acting as a scaffolding for wound repair.

The response of the two network system to dextran, an agent widely used and well known for its antithrombotic properties, has been examined. It will be shown that *in vitro* dextran increases fibrin fibre thickness, reduces permeability and tensile strength of networks developed in plasma. Similar changes were found to follow when dextran is infused in clinical dosage in man. It was found that the increase in the thickness of major network fibre is at the expense of protein in the minor network. Such means of modulating distribution of fibrin fibre diameter within fibrin networks provide a new approach to antithrombotic prophylaxis.

**FACTOR XIII CONCENTRATE FOR PROPHYLAXIS OF REBLEEDING IN SUBARACHNOID HEMORRHAGE (SAH) - RESULTS OF A PROSPECTIVE MULTICENTER PILOT STUDY.** Thie A, Henge Th, Degger D, Oberling M, Clemens R, Klein HJ, Lombard GF, Muchnik, Neurolog. Univ.-Klinik Hamburg-Eppendorf, Neurol. Univ.-Klinik Göttingen, Ev. Stift St. Martin Koblenz, Behringwerke Marburg, Bezirkskrankenhaus Günzburg, Neurochir. Univ.-Klinik Turin, Spanish Hospital Buenos Aires.

Rebleeding occurs in subarachnoid hemorrhage (SAH) in 20 - 25 % of patients, with a mortality rate being above 50 %. The cause of rebleeding is considered to be premature fibrinolysis of the fibrin clot surrounding the site of rupture. Since the stability of the fibrin clot is influenced by the activity of coagulation factor XIII, and moreover, a factor XIII deficiency has been reported in SAH patients, the question arises as to whether the incidence of rebleeding can be influenced by the administration of F XIII concentrate.

During a period of 6 months, 69 patients with acute SAH were enlisted in an open, prospective, multicenter study. On admission, 5 patients were classified as stage I (7.2%) according to the Hunt and Hess scale, 22 as stage III (31.9%), 11 as stage IV (16%) and 9 as stage V (13%). Aneurysm was confirmed by angiography in 52 patients (75%). All the patients received 10 x 1250 U F XIII concentrate during the first 15 days after the initial hemorrhage. Surgery on the aneurysm was performed between day 3 and 32 (median: day 13) in 35 patients. A total of 7 rebleedings occurred in 6 patients (8.7%), of whom 2 were stage I - II and 4 were stage III - V cases. Cerebral infarction was observed in 10 patients (14.5%), and hydrocephalus requiring shunting occurred in 1 patient. There were no cases of peripheral thromboses or embolisms. After 4 weeks, the overall mortality rate was 26%. (stage I - II: 11.1%, stage III - V: 37.5%).

The conventional approach in the prophylaxis of rebleeding in SAH is an early operation or intravenous administration of antifibrinolytics. However, as none of these measures significantly reduce overall mortality, the present pilot study investigated a new, therapeutic approach in which F XIII concentrates were administered in order to stabilize the fibrin clots and prevent premature fibrinolysis. The data so far show that Fibrogammin P<sup>®</sup> is an effective and well tolerated agent for the prophylaxis of post-SAH rebleeding. In order to statistically confirm the results of the pilot study, we have, in the meantime, started a prospective, randomized, placebo-controlled, multicenter double-blind study, which will involve 750 patients over a period of 2 years.

**ON THE DETERMINANTS OF FIBRE THICKNESS AND FIBRIN NETWORK CHARACTERISTICS.**

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Spectacular differences are found between characteristics of networks developed in plasma and those developed in pure fibrinogen solution. Networks in plasma have thicker fibres, are more permeable and have lower tensile strength. In this investigation determinants of network structure under physiological conditions of clotting have been examined in an attempt to account for differences in network structure in plasma and in fibrinogen solution.

Two independent variations of mass-length ratio ( $\mu_p$  and  $\mu_r$ ) from permeability and turbidity respectively were used. Effect of varying fibrinogen and thrombin concentrations and the effect of physiological concentrations of Antithrombin III, fibronectin, albumin,  $\gamma$ -globulin and platelet extract on fibrin network structure was examined.

Fibrinogen and thrombin alter network characteristics through the modification of kinetics of network development. In these and several other studies it has been found that the kinetics of fibrin formation ultimately determine the final network structure through events preceding the appearance of visible fibrin. In separate experiments it was found that spectacular differences in network structure developed in plasma and fibrinogen were not entirely accounted for by alterations induced in network properties by albumin,  $\gamma$ -globulin, fibronectin, ATIII and platelet extract.

It is concluded that the final network structure is determined by kinetics of fibrin fibre growth and is highly responsive to the presence of plasma proteins and platelets. The findings may have fundamental applications to haemostasis and thrombosis.