ASSESSMENT OF PLATELET HYPERREACTIVITY
R.E. Scharf

Clinical and pathological studies indicate that platelet hyperreactivity occurs in a number of vascular disorders, including unstable angina, stroke, peripheral vascular disease, and following angioplasty or coronary thrombosis. To detect activated platelets in vivo, sensitive and specific methods are required that may permit the identification of a prethrombotic or thrombotic state. Demonstration of circulating platelet aggregates has been used for this purpose but this test lacks both specificity and sensitivity. At the present time, the most reliable markers of platelet activation in vivo are constituents, such as B-thromboglobulin and platelet factor 4 that are released from activated platelets and measured in the plasma or urine, and metabolites of thromboxane A2. However, widespread clinical application of these markers is limited by a number of technical requirements including sample collection, processing, and analysis. Another little used test for activated platelets is the determination of platelet density distribution since activated degranulated platelets are lighter than normal. Recent advances in flow cytometry have made it possible to study platelets rapidly with respect to size and qualitative or quantitative changes of membrane glycoproteins. Using murine monoclonal antibodies that bind specifically to "activation-dependent" epitopes at the platelet surface, it is now feasible to identify individual activated circulating platelets. This review will summarize recent developments in this area and discuss advantages and limitations of different techniques that are being used for the detection of activated platelets in clinical disorders.

Institut für Exp. Hämatologie und Transfusionsmedizin, Univ. Bonn, Sigmund-Freud-Str. 25, D-5300 Bonn 1

PLATELET HYPERREACTIVITY IN CORONARY HEART DISEASE (CHD) AND MYOCARDIAL INFARCTION (MI)
J. Klumpp

Several lines of experimental and clinical evidence indicate that platelets are involved in the initiation of coronary atherosclerosis (platelet-derived atheroma) and contribute to the precipitation of acute coronary events such as unstable angina, acute MI and sudden cardiac death. Acute coronary syndromes may be triggered by platelet-released vascular mediators (serotonin, thromboxane A2) leading to transient flow reductions in diseased coronary arteries and/or by aggregate formation ultimately resulting in thrombotic vessel obstruction. Various indices of platelet activation and hyperreactivity have been detected in patients with manifest CHD. These include augmented platelet aggregability, circulating platelet aggregates, reduced platelet survival, elevated plasma levels of beta-thromboglobulin and platelet factor 4, and increased thromboxane formation. Further evidence may be awaited from studies using monoclonal antibodies to detect activation-dependent membrane epitopes and platelet-derived microparticles.

A key question with respect to both the sequence of pathophysiological events and the rationale for therapeutic intervention is: does platelet activation in CHD merely result from the interaction of functionally normal platelets with the atherosclerotic vessel wall or do abnormally reactive platelets predispose to acute coronary events? Recent longitudinal studies suggest that elevated platelet counts, changes in platelet volume and density and finally enhanced platelet aggregability predict an increased risk of first occurrence or recurrence of acute coronary events. Hormonal and dietary factors but also intrinsic platelet abnormalities such as abnormal calcium handling or serotoninergic dysfunction could account for platelet hyperreactivity. ATP and thromboxane-dependent but thrombin-induced mechanisms of platelet activation appear to be involved. These findings open the perspective for new strategies in antiplatelet therapy in the various settings of CHD.

Med. Klinik und Poliklinik der Westf. Wilhems-Universität, Albert-Schweitzer-Str. 35, 4400 Münster

PLATELET FUNCTION IN PATIENTS WITH TRANSIENT ISCHEMIC ATTACKS AND STROKE

TIA and stroke are frequently due to thromboembolic mechanisms arising either from cardiac sources or as arterial embolism from atherosclerotic plaques of the vascular system. Among other mechanisms, platelet aggregation plays an important role in the formation of structural vascular lesions as well as of its embolic capacity. As a result of this concept in the pathogenesis of cerebral vascular events, the prevention of TIA and stroke is based on drugs interfering with platelet function. In contrast to the considerable amount of clinical trials to be reviewed, which supports the reduction of stroke risk in patients with generalized atherosclerosis, only limited data exists on the incidence of cerebrovascular events in patients with the defined atherosclerotic lesions of the brain's supplying arteries and the role of platelets involved. Among these is a five year prospective follow-up study investigating patients with carotid plaque lesions for progression and regression by means of ultrasound in parallel with platelet function analysis (Cerebrovasc Dis 1991; 1:142-148): This study suggests that intervals of progression of atherosclerosis are closely associated with hyperactivity of platelet function whereas stable phases and in particular regressive periods are more likely to be linked to normal status of platelet function.

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PLATELET HYPERREACTIVITY IN DIABETES MELLITUS
D.Taechoepe

Vascular accidents are the main cause of death in patients with diabetes of both types (I, IDDM and II, NIDDM). So far, improved management of the metabolic derangement was not paralleled by a satisfactory reduction of the cardiovascular morbidity and mortality. Platelet hyperreactivity is well known in diabetes and is considered to be a key signature of the prethrombotic state in these patients contributing to the vascular excess mortality. We showed recently that the increased functional properties of diabetic platelets result from the primary release of larger platelets with enhanced thromboxane formation capacity and increased numbers of functional glycoprotein receptors GPIIb and GPIIIa which are synthesized in the megakaryocyte.

"The megakaryocyte-platelet system is turned on in diabetes mellitus". It was however a matter of controversy whether the increased functional potential of diabetic platelets becomes operative in vivo: "Circulate platelets in an activated state in diabetes mellitus"? The Duesseldorf III method of single platelet flowcytometry (SPFC) for the activation dependent molecular markers CD62 and CD63 allowed the direct answer: "Large platelets circulate in an activated state in diabetes mellitus." At present, metabolic normalization is the main therapeutic option in the treatment of patients with diabetes. It is however questionable whether this normalizes the underlying primary platelet hyperreactivity. Antiplatelet therapy, e.g. ASA, calcium-channel-blockers or ticlopidin, has not yet become established in diabetics as a beneficial therapeutic adjunct despite even more rational indication compared to the general population. For the first time the platelet membrane activation marker test will be able to identify single patients with a discrete activated platelet system as present at prethrombotic states and this allows an active selection of patients with diabetes who will possibly benefit from additional antiplatelet therapy.

"Cellular Haemostasis Group", Diabetes Research Institute at the Heinrich-Heine University, Duesseldorf, Auf'm Hennelamp 66, D-4000 Düsseldorf 1.
NEW ASPECTS OF THROMBOLYTIC THERAPY IN ACUTE MYOCARDIAL INFARCTION

C. Bode and W. Kübler

Whereas the beneficial effects of thrombolytic therapy in acute myocardial infarction have been shown beyond doubt, the results of the GISSI-2 and ISIS-3 studies keep the question open which therapeutic agent should be preferred. New application schemes of known plasminogen activators, new possibilities of adjunctive therapy and the development of new plasminogen activators have been undertaken in an effort to overcome present limitations of this form of therapy. Among the limitations are a primary failure rate to achieve reperfusion of 20-40%, an early reocclusion rate of 5-20% and an increased risk of bleeding that makes many patients ineligible for thrombolytic therapy.

"Front-loading", the rapid infusion of large amounts of activator has been successfully tested for rt-PA. A number of combinations of plasminogen activators have been evaluated. A combination of prourokinase and rt-PA appears more promising in terms of efficacy and specificity as combinations of urokinase and prourokinase, rt-PA and urokinase or rt-PA and streptokinase. Early, preclinical application of thrombolytic agents is also being evaluated in an effort to reduce the ischemic period.

In an effort to improve lysis of platelet-rich, arterial clots and to prevent early reocclusion, a number of agents beyond aspirin and heparin have been evaluated as adjunctive therapy among them hirudin, argatroban, activated protein C and GPIIb/IIIa inhibitors on the basis of peptides or antibodies. Among the new plasminogen activators, a t-PA mutant with altered pharmacokinetics has yielded encouraging results in clinical trials. Experimental in vivo data make bat-PA and antibody-activator fusion proteins promising candidates for the future.

Medical Clinic III (Cardiology), University of Heidelberg, Bergheimerstraße 58, 6900 Heidelberg

THE PROBLEM OF REOCCLUSION FOLLOWING THROMBOLYSIS AND PTCA

H.J. Rupprecht, J. Meyer

Reocclusion is seen in 6-18% of the patients with acute myocardial infarction within 24 hours following successful thrombolysis and in 11 to 24% of the patients within the next days. Reocclusion is usually associated with further deterioration of left ventricular function and increased mortality. Post-mortem studies have illlucidated that ruptured atherosclerotic plaques with a high grade residual lesion and thrombi are found in most patients with reocclusion. As there is ongoing thrombosis inspite of thrombolysis, antithrombotic treatment with heparin should be performed simultaneously with the initiation of thrombolysis. Concomitant treatment with vasodilating agents should be considered to avoid coronary artery spasm. Moreover aspirin may be valuable in the prevention of reocclusion in the long-term run. Patients with postinfarction angina or incipient left heart failure should undergo urgent coronary angiography, and in case of need PTCA or coronary artery bypass grafting. Within the first days following successful PTCA about 4-5% of the patients develop reocclusion with the clinical picture of recurrent angina or acute myocardial infarction. In most of these patients, a major dissection or intracoronary thrombi can be seen immediately following the procedure. Thus, a thrombotic process in combination with elastic recoil and early restenosis can lead to early reocclusion following PTCA.

EVIDENCE FOR SYNERGISTIC EFFECTS OF CATHEPSIN G AND PAF IN THE ACTIVATION OF PLATELETS BY STIMULATED NEUTROPHILS

A. Maras, A. Ruf, R. Schlenk and H. Patscheke

Cathespain G seems to be the major stimulus involved in neutrophil (PMNL) induced platelet aggregation (Evangelista V., 1991, Blood 77:2379). An additional role of platelet activating factor (PAF) and other mediators is still controversially discussed. We used eglin C (a serine protease inhibitor) and Web 2086 (a PAF receptor antagonist) in order to investigate whether cathespain G and PAF are effective in mixed suspensions of platelets and PMNL. Aggregation and luminol-enhanced chemiluminescence (CL) were simultaneously measured with a lumiaaggregometer as parameters for platelet and PMNL activation, respectively. The chemotactic peptide FMLP induced both CL and platelet aggregation in the presence of cytochalasin D. The prostacyclin mimetic iloprost and EDTA inhibited the aggregation but did not affect the CL of PMNL. When cytochalasin D was omitted, CL decreased and no platelet aggregation occurred. Both eglin C and Web 2086 inhibited the aggregation induced by FMLP in the presence of cytochalasin D. At a high concentration of FMLP 2x10^{-5} M, eglin C 800μg/ml and Web 2086 2x10^{-5} M produced a maximal inhibition of no more than 54% and 36%, respectively. Both inhibitors used in combination showed an inhibition of 91%. At a lower FMLP concentration 2x10^{-5} M the inhibition of platelet aggregation by eglin C was 75% and by Web 2086 74%. Both inhibitors added together produced an inhibition of 92%. In the controls, neither eglin C nor Web 2086 inhibited the FMLP-induced CL of PMNL. These results suggest that neutrophil derived cathespain G and PAF may act synergistically in platelet activation in mixed suspensions of platelets and PMNL. (Supported by the DFG, Pe-263)

Medizinisch-Diagnostisches Institut, Klinikum Karlsruhe, 7500 Karlsruhe

Hirudin in the treatment of acute coronary events: a new perspective?

H.R. Bülter, M.T. Nurmohamed, J.W. ten Cate.

The first description of the anticoagulant action of a water-soluble, heatresistant substance derived from the salivary glands of leeches (Hirudo Medicinales) dates back to 1894. This substance was later called hirudin in 1904. Almost no research was done with this compound until the 1960's. More recently, after hirudin or fractions of it have been produced by recombinant techniques as well as by synthetic methods, there has been a fast growing interest in the clinical potential of this specific thrombin inhibitor. Hirudin is in particular effective in the inhibition of the feedback action of the coagulation system by thrombin. In addition Hirudin is a potent inhibitor of the thrombin mediated platelet aggregation. At present this compound is investigated both in the treatment and prevention of venous and arterial thromboembolic diseases in experimental models as well as in man. With respect to acute coronary events Hirudin is evaluated in models and patients with unstable angina, or with acute myocardial infarction treated with thrombolytic therapy, and also in patients undergoing PTCA in order to prevent restenosis. The evidence of the potential effectiveness and the preliminary clinical data in these indications will be reviewed.

Centre for Hemostasis, Thrombosis, Atherosclerosis and Inflammation Research, Academic Medical Centre, Amsterdam, The Netherlands.
THE INFLUENCE OF NEUTROPHIL PREPARATION ON THEIR ACTIVATION INDUCED BY PLATELETS

R. Schienk, A.Ruf, A.Maras and H.Patscheke

The activation of neutrophils (PMNL) by platelets requires mutual contacts and fibrinogen as a cofactor (Ruf et al. 1991 Thromb. Haemostas. 65:169). We examined how the procedure of PMNL preparation influenced platelet induced activation of PMNL. Luminal-enhanced chemiluminescence (CL) of PMNL was measured in platelet PMNL suspensions or human whole blood with a lumiaaggregometer. Preparation 1 (PMNL1): PMNL were isolated by dextran sedimentation from blood anticoagulated with ACD, subsequent centrifugation through Ficoll-Hypaque and hypotone red cell lysis. All steps were carried out at room temperature. Preparation 2 (PMNL2) comprised sedimentation with hydroxyethyl starch, hypotone lysis and centrifugation through Percoll without eluents. Preparation 3 (PMNL3) was used for PMNL isolation at room temperature in preparation 3 (PMNL3). Unstimulated PMNL showed a bipolar shape with surface ruffling and a basal CL, whereas PMNL2 and PMNL3 were spherical with a smooth surface and did not show CL. The extent of the CL induced by the chemotactic peptide (FMLP) was: CLPMNL1 > CLPMNL2 = CLPMNL3. Addition of unstimulated platelets to the PMNL induced a CL of PMNL1 and PMNL2 but not of PMNL3. The extent of CL induced by platelets stimulated with U 46619 was also different in the three PMNL preparations: CLPMNL1 > CLPMNL2 > CLPMNL3. These observations suggest different degrees of preactivation and priming in PMNL1 > PMNL2. Both PMNL3 and whole blood required stimulation by FMLP or U 46619 to show CL. This indicates the absence of preactivated or primed PMNL. We conclude that response of PMNL1 and PMNL2 to unstimulated platelets are due to preactivation and priming of neutrophils during their preparation. (Supported by the DFG, Pa=263)

Medizinisch-Diagnostisches Institut, Klinikum Karlsruhe, 7500 Karlsruhe 1

BILIRUBIN-INDUCED PLATELET AGGREGATION EX VIVO

A.Evangelou, S.Karkabounas, K.Liveris, G.Sofis, K.Charalambopoulos.

Technical Assistance, A.Beca.

Activation of platelet aggregation is induced by a variety of endogenous agonists. Bilirubin, a metabolic product of heme degradation, is not known as a platelet aggregating agonist so far. On rabbit washed platelets, action of non-conjugated bilirubin was tested by a photoaggregometer. Bilirubin induced a potent irreversible aggregation at conc. of 10-5M, which became maximal at concentration of 10-4M. Bilirubin failed to induce aggregation of platelets suspended in rabbit plasma, even at conc. of 10-5M, in contrast to platelets suspended in deferoxamined serum where bilirubin proked aggregation at conc. of 10-5M. Platelets treated by CP/EPK, aspirin, and ginkgo biloba extract, in order to prevent aggregation by activating ADP, Arachidonic Acid and platelet activating Factor biochemical process, exhibited a strong aggregatory effect to bilirubin at conc. of 10-5M. Hematoporphyrin, a relative to bilirubin substance, induced as well platelet aggregation at similar to bilirubin conc., while hemine, cyanocobalamine and biliverdin had no effect. Data indicate that bilirubin and other substances, induce an irreversible platelet aggregation ex vivo, which seems to be unrelated to ADP, AA and Paf biochemical process. This aggregation is probably inhibited in vivo by plasma protein.

Laboratory of Exp. Physiology, Faculty of Medicine, University of Ioannaia, 45110-Ioannina, Greece
COMPARISON OF THE EFFECT OF ALPHA-TOCOPHEROL (VITAMIN E) ON THE ADOPTION AND AGGREGATION OF PLATELETS IN PATIENTS WITH THROMBOCYTOSIS AND IN HEALTHY VOLUNTEERS

J. Pieper, M. Sosada and H. Pollwoda

10 healthy volunteers (m/f=6/4, age=24.9±3.8) and 16 patients (m/f=10/6, age=44.3±14.8) with thrombocytosis (>500 000/μl blood) suffering of myeloproliferative diseases were treated during 14 days with alpha-tocopherol (3 x 300 mg per day orally). Measurement of platelet aggregation (Born test) did not show any significant (student t-test) changes in the collagen and ADP-induced aggregation before and after 14 days of alpha-tocopherol treatment in either group. The thrombometer time (time in which a collagen channel is blocked by platelet adhesion and aggregation) was not affected by alpha-tocopherol in the healthy volunteers (28±12s = normal range of the used charge, n=10). Pathological decreased thrombometer times were obtained before alpha-tocopherol treatment in the patient group indicating increased platelet adhesion and aggregation (25±5±1.4s, n=16, p<0.05). A 14 day alpha-tocopherol treatment induced a significant increase of the thrombometer time not exceeding the normal range (29±12±1.1s, n=16, p<0.05).

In this study alpha-tocopherol is inducing a normalization of platelet function in patients suffering of myeloproliferative diseases accompanied by thrombocytosis as indicated by thrombometer time.

Abteilung Hämatologie und Onkologie der Medizinischen Hochschule Hannover, Konstanty-Gutschow-Straße 8, D-3000 Hannover 61
FISH OIL PREPARATIONS CONTAINING DIFFERENT DOSES OF EPA AND DHA MODIFY PLATELET RESPONSIVENESS TO U 46619

Jaschonek K., M. Schuerfen, M. Kirchner, M.R. Clemens
Medizinische Klinik und Pharmazeutisches Institut der Universität Tübingen, Germany

Changes in the receptor microenvironment and membrane microviscosity regulate the accessibility of ligands for its corresponding binding sites. In previous experiments (Jaschonek et al., Thromb Res 1989/1991) we demonstrated that platelet-PGI2 receptors behave as syndromic proteins; an increase in membrane microviscosity induced by cholesterol decreased whereas fluidizing fatty acids increased platelet surface PGI2-receptor expression. There is some evidence from in-vitro studies that TXA2/PGH2-reeoptors are regulated in a opposite fashion. In this study, we investigated the effects of supplementation with either 4.5 g eicosapentaenoic (EPA) and 3.35 g docosahexaenoic (DHA) acid (group I, n=6; EPA/DHA = 1.43) or 3.5 g EPA and 6.4 g DHA (group II, n=6; EPA/DHA = 0.544) on platelet thromboxane (TXA2) synthesis and platelet responsiveness to the stable endoperoxide-analogue U 46619. Dose-response-curves (DRC) of U 46619-induced aggregation were analyzed by computerized nonlinear curve-fitting. The synthesis of TXB2, TXB1, and PGE1, by platelets was measured after incubation with thrombin (5 U/ml) by HPLC. In group I, the dose of U 46619 required for half-maximal platelet aggregation (K) remained unchanged, whereas the Hill-coefficient decreased from 6.2 to 3.3 (p<0.02). In group II, characterized by a high intake of DHA, a considerable increase of K from 0.3 to 1.4 µM was found (p=0.02). These results suggest different effects of EPA and DHA on the platelet thromboxane/endoperoxide-amplifying system. After the EPA-rich preparation, the reduced cooperative effect found presumably is simply the result of the reduced endogenous thromboxane synthesis. However, the considerable shift of the DRC in group II suggests an effect of DHA on the presentation of endoperoxide receptor and/or postreceptoral events.

LOVASTATIN IMPROVES PGI PLATELET SENSITIVITY IN PATIENTS WITH HYPERCHOLESTEROLEMIA - A PROSPECTIVE DOUBLEBLIND PLACEBO-CONTROLLED TRIAL

D. Kaczmarek, Th. Hohlfeld, G. Wambach, K. Schröer

Hypercholesterolemic patients (n = 18) were randomized and treated with Lovastatin (LOV, 20 mg/day) or placebo for 3 months. Platelet parameters (aggregation, PGI-receptors, cAMP) and plasma lipids were determined before and at the end of the study. Total serum cholesterol was reduced from 267 ± 5 to 213 ± 11 mg/dl (p < 0.05) in LOV-treated patients but remained unchanged in the placebo-group: 277 ± 6 vs. 255 ± 5 mg/dl. There was a significantly enhanced inhibition of ADP-induced platelet aggregation by iloprost which was accompanied by a 40-70% increase in platelet cAMP in the LOV-group. In this group the number of PGI-receptors was enhanced from 1623 ± 252 to 2564 ± 379 fmol/mg protein with a tendency for reduced affinity (K0). No such changes were obtained with placebo.

These data confirm our previous retrospective trial with simvastatin (Krocobonid 3: 67, 1990) and are the first to demonstrate a HMG-CoA-reductase-induced increase in PGI-receptors of hypercholesterolemic patients in a placebo-controlled prospective trial.

Institut für Pharmakologie, Heinrich-Heine-Universität, Moorenstr. 5, D-4000 Düsseldorf, FRG.

CORRELATION OF BLEEDING COMPLICATIONS WITH THE NUMBER OF PLATELET GP IIB-IIIa RECEPTORS IN PATIENTS WITH THROMBASTHENIA GLANZMANN

C.M. Kirchmaier, A. Schirmer, B. Jablonka, M. Meyer, M. Just, H.K. Brüdgen

It remains still unclear when and to what extent bleeding complications have to be expected in patients with Thrombasthemia Glanzmann. We investigated 20 patients with a reduced platelet GP Iib-IIIa content to establish a correlation of the clinical disease with the molecular platelet abnormalities. We compared bleeding history and bleeding time with the following laboratory parameters: induced platelet aggregation, platelet adhesion, clot retraction, GP Iib-IIIa content in the EIA and I-125 fibrinogen binding.

ADP-induced aggregation was performed with gel filtrated platelets in a dose dependent manner (from 10 µM up to 100 µM ADP/ml) before these preparations of gel filtrated platelets were used in the J-125 fibrinogen binding assay. With the exception of one family we found a good correlation between the number of the fibrinogen receptors and the GP Iib-IIIa level in the EIA. Two patients of the family showed GP Iib-IIIa levels of 50 % but a significantly reduced fibrinogen binding (7 % and 10 % of normal) and the bleeding time was prolonged up to 20 min. In general aggregation response was normal if more than 50 % of intact fibrinogen receptors were present on the platelets. Bleeding time was slightly prolonged if ADP-induced platelet aggregation was reduced to 40 % of normal and markedly prolonged at reductions under 20 % (correlation ADP-induced aggregation - bleeding time r = 0.8763, p < 0.001).

The bleeding time was significantly correlated with the fibrinogen receptor on the platelet membrane (r = 0.8198, p < 0.001). From 30 to 20 % intact receptors bleeding time was slightly and at less than 20 % intact receptors markedly prolonged. These results demonstrate that in patients with Glanzmann's Thrombasthemia bleeding complications have to be expected if the fibrinogen receptors on the platelet membranes are reduced under 20 %.

J.W. Goethe-Universität, Zentrum der Inneren Medizin, Abt. Angiologie, Theodor Stern Kai 7, D-6000 Frankfurt/Main
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INCREASED PLATELET ACTIVITY IN POSTSURGICAL PATIENTS
C.B. Reiningier, A.J. Reiningier, B. Steckmeier, A. Hörmann, W. Schramm, L. Schwilbeier
Background: In spite of present advances in anticoagulant and antiplatelet therapy, an increased incidence of thrombosis - usually within the first week - is still observed following surgery. Hence, a quantitative assessment of platelet function could provide information valuable for improvement of antithrombotic therapy.
Methods: In our model - the Stagnation Point Flow Adhesio-Aggregometer (SPAA) - citrated PRP is perfused perpendicular to a glass stagnation plate, which is observed by means of dark field microscopy. The intensity increase of light scattered by deposited platelets is recorded as a function of time. A biomathematical evaluation of the obtained growth curve, based on respective reaction kinetics, permits a separation and subsequent quantitative assessment of the platelet adhesion and aggregation functions. Using the SPAA, the PRP of 20 vascular surgical and 20 general surgical patients was examined before and after surgery. The experimental results were compared to those obtained with the PRP of 30 healthy volunteers. Documentation and statistical evaluation of all data were performed using the Statistical Analysis System Software (SAS).
Results: All surgical patients (n=50) exhibited an increased in platelet adhesion postoperatively as compared to their respective preoperative values and as compared to those of the control group (n=30). Maximal values were attained 2 to 5 days following surgery. This increase in platelet activity also coincided with an increase in plasma fibrinogen levels. In the vascular surgical subgroup (n=30), the adhesion was increased up to threefold as compared to that of the controls and was regularly combined with a pathological spontaneous aggregation of the platelets. This increase in platelet function occurred in spite of antithrombotic treatment.
Conclusions: An increase in platelet adhesion and aggregation could be detected using the SPAA in all patients after surgery although under routine antithrombotic medication. This increase in activity was observed within the time period generally associated with a high risk for thrombosis. We therefore conclude that the highly sensitive registration and quantitative assessment of platelet function by means of this method can be of benefit in improving postoperative antithrombotic therapy.

Theoretische Chirurgie, Chir. Klinik und Poliklinik Innenstadt der Ludwig-Maximilians-Universität München, Müßbaumstr. 20, D-W-8000 München 2

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SEROTONINE UPTAKE AND RELEASE OF THROMBOCYTES IN CHRONIC URAEMIC PATIENTS BEFORE AND AFTER DIALYSES
U. Frick, K.-U. Möritz, I. Wiedenhöft, G. Kreutz und G. Frick
Control of platelet function by induction of aggregation include raw and in part unphysiological test procedures. In contradiction the uptake of serotonin is an active membrane process and based on a metabolic performance of living thrombocytes. So it is more sensitive. In 15 patients with chronic uraemia before and after dialysis 4C-serotonin uptake and release of platelets 1, 2 and 4 minutes after addition of collagen were measured. The same parameters were checked in an age-matched healthy control group of 20 persons.

While the ability of thrombocytes to take up serotonin in contrast to the control group is significantly reduced, the results of serotonin release of thrombocytes of uraemic patients against values of healthy persons are increased. These observations speak in favour of an fundamental irritation of platelet membrane for their life time by the uraemic process and partial depletion of their granular storages. So clinically we can see as well thromboses as haemorrhages.

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PLASMIN (PL) MODIFIES PLATELET RESPONSIVENESS TO THROMBIN AND PROSTACYCLIN (PGI2)
Jaschonek K., C. Faul, B. Wannke
The question as to whether PL modulates platelet function has been addressed in several in-vitro studies with conflicting results. In our hands, at low temperatures (20°C) PL caused time- and dose-dependent platelet activation (EC50 0.04 CU/mL). PL-induced platelet activation could be inhibited by SQ 29548 and iloprost but not by the thrombin inhibitor MCI 905. After incubation of washed platelets with PL at 20°C, platelet responsiveness to thrombin was considerably impaired. Flow cytometry studies using Mab ANS1 and P2 excluded that this might simply result from proteolytic degradation of platelet glycoproteins (GP IIb/IIIa, Ib). In addition, binding studies with Hi-Si 29548 demonstrated that neither PL, t-PA or streptokinase interfere with the TXA2-endoperoxide-mediated signal amplification at the receptor level. Thus, PL-mediated activation of protein kinase C and the subsequent modification of postreceptor responses (PLC-actin/v) might provide a more likely explanation. This mechanism depends largely on platelet activation that particularly occurs at low temperatures and is not longer demonstrable when incubation is carried out at 37°C. In further experiments PL was shown to potentiate the antiaggregatory effectiveness of PGI2 without any modification of platelet PGI2-receptor surface expression. The finding that plasmin causes an increase of basal cyclic AMP in platelets prelabelled platelets points towards a modification of postreceptor responses (e.g. GJ-cyclase-interaction) and deserves further investigation. In the light of these observations we conclude that PL under "physiological conditions" (37°C, 3mM Ca2+) does not modify the platelet responsiveness to thrombin. However, PL acts synergistically with PGI2 to inhibit platelet activation. Thus, PL may efficiently operate together with EDRF and PGI2 to localize platelet activation in vivo.

Medizinische Klinik & Poliklinik der Universität Tübingen, Gerinnungslabor, W-7400 Tübingen

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CALCIUM ANTAGONIST (NIFEDIPIN) AND PLATELET FUNCTION IN HEALTHY VOLUNTEERS
J. Pieper, M. Hosada and H. Poliwoda
Platelet adhesion and aggregation was measured by our thrombometer method (time in which a collagen channel is blocked by platelet adhesion and aggregation) and in the Born test before and after 60, 90, 120 and 180 min following a sublingual application of 20 mg nifedipin in 20 healthy volunteers (n/f=12/8; age=30.5±12.2 years). No significant changes were observed in the collagen and ADP-induced aggregation during the whole observation period (student t-test).

Starting with a normal thrombomter time of 22.4±1.3s (normal range of the used charge, n=20) an increase to 27.2±1.5s (p<0.05) was measured 60 min after nifedipin application, reaching a maximum of 30.3±1.7s (p<0.05) after 90 min. 120 min after nifedipin application a normalisation of the thrombometer time occurred (23.7±2.1s, p<0.05) and was maintained until the end of the experiment after 180 min (21.4±1.5s, p<0.05).

In this study a reversible inhibition of platelet function was induced by sublingual application of nifedipin correlating with its biological kinetic as determined by the sensitive thrombometer method.

Abteilung Hämatologie und Onkologie der Medizinischen Hochschule Hannover, Konstanty-Gutschow-Straße 8, D-W-3000 Hannover 61
Numerous studies have demonstrated that low-dose ASA inhibits platelet-derived thromboxane (TX) formation, which lacks its known above the consequences for platelet secretion. This study investigates the effects of daily oral low-dose ASA (aspirin; 40 mg for 8 days) on collagen-induced thromboxane (TX) formation and serotonin (5-HT) secretion in citrated PRP. Blood was taken by venipuncture from 15 healthy male non-smokers before treatment and 12 h after the last intake of ASA. TXB2 was measured by RIA; 1C5-HT release by radioimmunoassay. Aspirin resulted in a significant inhibition of urinary TX-excretion: 9815 vs 27525 ng/g creatinine (p < 0.05).

Effects of ASA on collagen-induced TX2-forma- tion and 1C5-HT release (% inhibition of control):

| Parameter | COLLAGEN [µg/ml] |
|-----------|------------------|
| TXB2      | 94±4            |
| 1C5-HT    | 77±4            |

In conclusion, the inhibition amounted only to 22%. Further studies on this issue are necessary.

Institut für Pharmakologie, Heinrich-Heine-Univer- sität, Moorstr. 5, D-4000 Düsseldorf, FRG.

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THE STIMULATION OF NEUTROPHILS BY ACTIVATED PLATELETS IS MITIATED BY THE FIBRINOGEN RECEPTOR CD11c/CD18

A. Ruf and H. Patscheke

The stimulation of neutrophils (PMNL) by activated platelets requires mutual contacts and platelet-expressed fibrinogen as a cofactor (Ruf et al. 1991, Thromb. Haemostas. 65:169). Neutrophils have two types of fibrinogen receptors, called Mac-1 and p150/95. Besides LFA-1 they belong to the glycoprotein family of the B2-integrins. Monoclonal antibodies (MoAb) directed against the subunits of these integrins and GPRP-peptide were used to investigate the role of these inte- grins in the platelet-induced neutrophil activation. The luminometry (CL) as a parameter of PMNL activation was measured simultaneously to the platelet aggregation in mixed sus- pensions of PMNL and platelets. The thromboxane A2 mimetic U 46619 induced both CL as well as platelet aggregation in mixed cell suspensions. In contrast, U 46619 neither stimulated CL or aggrega- tion in pure neutrophil suspensions nor CL in platelet suspensions. MoAb directed against CD11a (a-subunit of Mac-1) which blocks fibrinogen binding to Mac-1 (common 8-subunit of the neutrophil integrins) did not inhibit the CL induced by U 46619 stimulated platelets. The neutrophil activation was also not affected by MoAb against CD11b (c-subunit of Mac-1) and CL formation was completely inhibited by MoAb against CD11c (a-subunit of p150/95) strongly reduced the CL. Furthermore, a peptide with the sequence GPRP which is recognized by p150/95 on the Aa-chain of fibrinogen also inhibited the CL of PMNL. Control peptides with the sequences GHRP or GPGS were ineffective. In contrast to the platelet-induced CL, neither MoAb against CD11c nor GPRP-peptides inhibited the CL induced by N-formyl-methionyl- leucyl-phenylalanine (FMLP). They did also not affect U 46619-induced platelet aggregation. Therefore, the platelet-induced neutrophil activation is mediated by CD11c/CD18 interacting with the Aa-chain of platelet-associated fibrinogen. (Supported by the DFG, Pa-263).

Medizinisch-Diagnostisches Institut, Klinikum Karlsruhe, 7500 Karlsruhe 1.

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REGULATION OF THE EICOSANOID BIOSYNTHESIS IN LYSATES OF HUMAN PLATELETS: ROLE OF THE REACTIFYING ENZYMES

I. Freus and H. Patscheke

Liberation of esterified arachidonic acid (AA) from membrane phospholipids (PL) is the rate-limiting step in the biosynthesis of prostaglandins, thromboxanes A2 and 12-hydroxyeicosatetraenoic acid (12-HETE) upon platelet activation. The concentration of free AA depends on the activities of the deactivating and reacting enzymes of the LANDS cycle; phospholipase A2 (PLA2) arachidonoyl-CoA synthetase (AS), and lysophosphatidyl acyltrans- ferase (LAT). The aim of our study was to investigate the role of the reactiv- ing enzymes in the regulation of eicosanoid biosynthesis. For this purpose, platelet lysates served as a model system. Aspirin-treated washed human platelets were sonicated and incubated at 37°C in the presence of all cofac- tors required for the enzymes mentioned. Analysis of extracted [14C]AA, [14C]1AA, and [14C]12-HETE was performed by radio TLC. Lysates of [14C]AA-prelabelled platelets continuously released [14C]AA and [14C]12- HETE. Eicosanoid formation reached 4.1 % of the total radioactivity during 40 min after platelet sonication at pH 7.0. Inhibition of the ATP-de- pending AS by apyrase 4 U/ml significantly increased the [14C]eicosanoids to 8.0 % (p < 0.004). Even at the extensive [14C]eicosanoid production of 20.6 % mediated by added PLA2 from bee venom 0.02 U/ml, apyrase still caused an increase to 30.6 %. Lysates of non-labelled platelets formed [14C]eicosanoids from exogenous [14C]AA and [14C]1AA-CoA, respectively, through the actions of AS and LAT. Moreover, [14C]eicosanoids were released from previously esterified [14C]1AA-CoA. If the deactivating activity was prevented by EGTA 0.5 mM, [14C]AA 0.1 μM was almost completely incorporated into PL within 20 min, but [14C]12-HETE was simultaneously formed at 0.5 μM of [14C]AA. Nevertheless, AS activity had to be blocked by apyrase 4 U/ml to produce an exclusive formation of [14C]12-HETE. Conclusions: 1) Platelet lysates show an AA turnover within the LANS cycle. 2) Inhibition of AA re- esterification by apyrase increases the net amount of eicosanoids released from exogenous PL. 3) Blockades of the reactivating activity also enhances the availability of AA for metabolism into 12-HETE and other eicosanoids AA. (Supported by the Deutsche Forschungsgemeinschaft, Pa 263).

Institute for Clinical Chemistry, Klinikum Mannheim of the University of Heidelberg, 6800 Mannheim, and Institute of Medical Diagnostics, Klinikum Karlsruhe, 7500 Karlsruhe.

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DETECTION OF ACTIVATED PLATELETS: MICROSCOPY OF THE SHAPE CHANGE IS MORE SENSITIVE THAN FLOW CYTOMETRY OF FIBRINOGEN BINDING AND GMP140 EXPRESSION

A. Ruf and H. Patscheke

Flow cytometry has been used for the measurement of activated platelets at a single cell level. Surface bound fibrinogen, expression of GMP140 and other neoantigens were immunologically detected on activated platelets. We compared these methods with a morphological approach by evaluating the platelet shape change. There was only a partial inhibition of 5-HT secretion which was inversely related to the collagen concentra- tion. At 10 μg/ml collagen the inhibition amounted only to 22%. Further studies on this issue are necessary.

Institut für Pharmakologie, Heinrich-Heine-Uni- versität, Moorstr. 5, D-4000 Düsseldorf, FRG.

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INCOMPLETE INHIBITION OF COLLAGEN-INDUCED PLATELET SECRETION EX VIVO BY LOW-DOSE ACETYLSALICYLIC ACID (ASA)

M. Braun, J. Kramann, H. Strobach, M. Palmér, K. Schrör

Acetylsalicylic acid (ASA) is commonly used in the treatment of rheumatic diseases, due to its anti-inflammatory and anti-thrombotic properties. In vitro studies have shown that ASA inhibits platelet aggregation and secretion, but the mechanism of this inhibition is not fully understood. The aim of this study was to investigate the incomplete inhibition of collagen-induced platelet secretion ex vivo by low-dose ASA.

Numerous studies have demonstrated that low-dose ASA inhibits platelet-derived thromboxane (TX) formation, which lacks its known above the consequences for platelet secretion. This study investigates the effects of daily oral low-dose ASA (aspirin; 40 mg for 8 days) on collagen-induced thromboxane (TX) formation and serotonin (5-HT) secretion in citrated PRP. Blood was taken by venipuncture from 15 healthy male non-smokers before treatment and 12 h after the last intake of ASA. TXB2 was measured by RIA; 1C5-HT release by radioimmunoassay. Aspirin resulted in a significant inhibition of urinary TX-excretion: 9815 vs 27525 ng/g creatinine (p < 0.05).

Effects of ASA on collagen-induced TX2-forma- tion and 1C5-HT release (% inhibition of control):

| Parameter | COLLAGEN [µg/ml] |
|-----------|------------------|
| TXB2      | 94±4            |
| 1C5-HT    | 77±4            |

In conclusion, the inhibition amounted only to 22%. Further studies on this issue are necessary.

Institut für Pharmakologie, Heinrich-Heine-Uni- versität, Moorstr. 5, D-4000 Düsseldorf, FRG.
in pathophysiological events e.g. Tangier disease a change of C-P ratio in total human blood platelets correlates with an increase of platelet aggregability and a reduced phosphatidyl inositol (IP3) content in platelet lipid extracts (Shastri et al., J Lipid Res 21: 467-472, 1980). In density dependent human blood platelet subpopulations (SP I: = 1.04-1.05 g/ml; SP II: = 1.065-1.07 g/ml; SP III: = 1.07-1.08 g/ml) (Rahel et al., Br J Haematol 72: 387-401, 1990) we found differences in membrane properties such as C-P ratio, phospholipid composition, and membrane anisotropy. Furthermore the second messenger systems - CAMP levels and phospholipid metabolism - differed significantly among the three subpopulations. SP I, the subpopulation with the highest C-P ratio and the highest membrane anisotropy showed a significantly lower basal level of IP3 whereas after thrombin stimulation the percentage increase of [PH2] inositol monophosphate was highest in SP I (624 ± 62% compared to the unstimulated SP I) as compared to SP II (540 ± 51%) and SP III (323 ± 48%) (n=9). After stimulation of the three subpopulations with ADP and thrombin the TX A2 release showed the highest percentage increase again in SP I. The results ascertain that the varying lipid composition in platelet plasma membranes is correlated with the observed differences in signal transduction as well as with the release of TX A2, an extracellular messenger in platelet function.

Institut Physiol. Chem., Abt. Neurochemie, Philippus-Universität Marburg, Hans-Meiserinstr., 3550 Marburg

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NITROGLYCERINE MODIFIES THE BLOOD PLATELETS MEMBRANE STRUCTURE
J. Gorksi, J. Nowak, A. Slonecka, A. Winnicka

In patients with unstable angina and myocardial infarction treated with i.v. nitroglycerine/NTG/ blood platelets disaggregation and prolongation of bleeding time have been observed.

The aim of our study was to estimate the influence of NTG on platelet membrane structure utilizing spin markers/methyl 5-doxylspaltoxyme and EPR spectrometor analysis of labeled platelets in platelet rich plasma /PRP/.

The analysis of PRP spectrum demonstrated:
1. In vitro incubation of platelets from healthy donors with NTG alters the platelet membrane phospholipids layer and spin marker easily penetrates into the membrane.
2. Ex vivo investigation on platelets from patients treated with i.v. NTG shows the effect mentioned above; additionally the spin marker remains firmly immobilized within the membrane.

CONCLUSIONS:
1. NTG markedly alters the structure of platelet membrane.
2. It is not excluded, that this alteration disintegrates membrane receptors and modifies platelet functions.

CLINICAL and LABORATORY DEPARTMENT
Institute of Maritime and Tropical Medicine
Gdynia, Poland

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Flexibility of fibres from biomaterial influences the action of the platelet cytoskeleton
U. Hubertus, E. Morgenstern

In this study the ultrastructural rearrangement of the cytoskeleton and the shifting of the membrane contact sites in human platelets during interaction with flexible or rigid fibres from biomaterial (BM) was investigated. Fibrillar networks of BM were produced from textured (polyurethane PU) or porous (polysulfone PS) source material by cryo-ultramicrotomy. SEM investigation showed that the networks consist of flexible i.e. thin or incompletely tied fibres and immobile i.e. thick or texture-interwoven fibres. These BM-networks were incubated for 7 - 45 min at 37°C with:
- citrated PRP and ADP (0.03 mg/ml) or
- washed platelets and thrombin (0.2 - 0.5 IU/ml) or
- washed platelets, thrombin and 10% homologous plasma.

For TEM and SEM the samples were fixed with glutaraldehyde or impact frozen and substitute substituted. Platelets adhering to the fibres developed multifocal contacts. With flexible fibres the cytoskeleton of platelets formed a constricting sphere. Membrane-bound fibres, situated in membrane invaginations, were found to be associated with this sphere. In contrast, if platelets adhered to immobile fibres the associated cytoskeleton formed cable-like bundles of microfilaments proceeding from the multifocal adhesion contacts of the plasmalemma running through the cell. The findings suggest that the action of the constricting sphere which is associated with the membrane contact areas - retracts fibre-networks as long as the fibres are mobile. Under isometric conditions (immobile fibres), cytoskeletal microfilaments form cable-like bundles.

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Medizinische Biologie, Universität des Saarlandes, D-6650 Homburg/Saar
COMPUTER ASSISTED THREE-DIMENSIONAL RECONSTRUCTION OF REARRANGEMENT OF THE CONTRACTILE CYTSKELETON IN PLATELETS DURING RETRACTION

D. Bastian, U. Hubertus, R. Dierichs* and E. Morgenstern

Activated platelets form a contractile gel. Under isometric conditions cable-like filamentosus bundles are visible. We studied the formation of different types of cytoskeletal arrangement depending on the platelet-ligand interaction. Fibres from collagen type I (CF) with a final concentration of 40 µg/ml and fibres of textured polyurethane (PUF) were incubated (7-25 min at 37°C) with a suspension of washed platelets resp. citrated PRP fixed and embedded for TEM. Ultrathin serial sections with an average thickness of 65 nm were made in both attempts. TEM pictures of sections were digitalized on a Kurta tablet and reconstructions were drawn on a PC-Apple Macintosh Ilcx using the software BioModel by Dierichs & Strömpel (Münster).

With CF the platelets formed multifocal contacts associated with the constraining sphere. The reconstructions show that membrane-bound CF adhere to the contractile sphere. CF were found to be internalized due to the contracting action of this sphere (retraction). Fibres in the platelet periphery were surrounded by (pseudopodial?) membranes without visible connections to the contractile sphere. The role of this type of contact is not yet clear.

With the immobile fibres in PU-textures the platelets formed contacts associated with cable-like microfilamentosus bundles. The reconstructions show bundles running across the cell. They do not connect only platelet-PII-contact but also interplatelet contacts. Thick filaments were found within those bundles as observed in stress fibres of other cells and they were identified as myosin filaments in an other study.

MEDIZINISCHE BILOGIE, UNIVERSITÄTS DES SAARLANDS
D-W-6650 Homburg/Saar

*ANATOMISCHES INSTITUT DER UNIVERSITÄT, D-W-4400 MÜNSTER

CO-LOCALIZATION OF VITRONECTIN AND PLASMINOGEN ACTIVATOR INHIBITOR-1 IN RESTING HUMAN PLATELETS K.T. Preissner, *E. Morgenstern

The adhesive glycoprotein vitronectin (VN) is present in the circulation as soluble protein, stored within resting platelets as well as correlated with extracellular matrix sites in the vessel wall. Besides its adhesive properties relevant for endothelial cell adhesion and spreading, VN acts as major stabilizing protein for plasminogen activator inhibitor-1 (PAI-1) in the subendothelium, thereby contributing to the initial phase of thrombus formation at the site of vessel wall injury. Although the concomitant release of VN and PAI-1 from activated platelets is well known (Preissner et al., 1989), the fine structural localization of both components or their pathway of release has not been demonstrated by immunolabeling. In the present study, we used rabbit antibodies against human VN and antibodies against PAI-1 for primary labeling and 10 nm gold-marked goat anti-rabbit IgG (100) as secondary labeling on ultrathin sections of paraformaldehyde (4%), glutaraldehyde (0.1%) fixed platelets from citrated platelet rich plasma subsequently cytofixed in the presence of 2M sucrose, 10% polyvinylpyrrolidone and 6% gelatin in liquid propane. VN and PAI-1 indicating gold markers were found to be located within α-granules and within the surface-connected system. Here, the distribution of PAI-1 appeared to be less dense as compared to VN. Strong labeling was also seen on the platelet plasma membrane surface. These characteristics of co-localization of VN and PAI-1 in α-granules are in agreement with the described concomitant release of both proteins; yet, their fate during platelet release reactions and during haemostatic plug formation remains to be determined.

Haemostasis Research Unit, Korkhoff-Klinik, MPG, Bad Nauheim and
*Medizinische Biologie, Universität des Saarlandes, Homburg/Saar

BIOTINYLLATION OF INTEGRATED PLATELETS FOR THE ANALYSIS OF RECEPTOR PROTEIN DEFECTS IN THROMBOCYTOPATHIES M. Meyer, I. Schellenberg, Ch. Ströhl

Surface labeling of intact platelets by radioactive isotopes (125-I, 3-H) has been introduced for the analysis of membrane glycoprotein defects. In order to circumvent potential hazards and limitations we explored the possibility to use nonradioactive labeling of (membrane) proteins in intact platelets. Washed platelets were labeled by protein- and carbohydrate-specific biotinylating reagents. After solubilization biotinylated proteins were separated by one- and two-dimensional electrophoresis and detected by avidin-peroxidase after Western blotting. Sensitivity and specificity of the labeling methods were investigated by comparison of the patterns of biotinylated components to conventionally stained protein patterns and to radioactively labeled samples.

The biotinylation procedure was applied to platelets from patients with Glanzmann's thrombasthenia and a patient with a structurally abnormal GP Ib. Both quantitative and qualitative disorders could be detected by analysis of biotinylated proteins. Another successful application of the labeling procedure has been the characterization of the antigens of a platelet autoantibody by immune-precipitation.

Abteilung Medizinische Genetik, Medizinische Akademie Erfurt, Arnstädtener Straße 34, D-0-5010 Erfurt
PLATELETS FROM PATIENTS WITH LIVER CIRRHOSIS EXHIBIT A DEFECT OF THE VON WILLEBRAND-FACTOR BINDING DOMAIN
Jaschonek K., C. Paul, R. Klein

In this study, we examined the platelet von Willebrand-factor (vWF) binding domain in patients with liver cirrhosis. Binding studies were performed with monoclonal antibody (moab) AN51 with specificity for the glycoprotein Ibα. In immuno-blotted studies with Triton X-100 solubilized platelet membranes, the moab was shown to interact with a 120 kDa glycoprotein (glycocalcin). The binding of AN51 to intact washed platelets was monitored by an enzyme-linked immunosorbent assay. Half-maximum binding to intact washed platelets occurred at concentrations as low as 94 ng/ml. Binding saturation analysis with AN51 revealed a more than fifty percent reduction of AN51-binding capacity (p<0.001) in cirrhosis (N=13) as compared to sex- and age-matched healthy controls (N=12). These data demonstrate an impairment of the platelet surface vWF-binding domain in patients with liver cirrhosis. Thus, a defect in primary haemostasis, i.e. the vWF-mediated interaction between platelets and the vessel wall, can be expected to contribute to the increased risk of haemorrhagic complications in these patients.

Medizinische Klinik & Poliklinik der Universität Tübingen, Gerinnungs labor, W-7400 Tübingen

BINDING OF PLASMODIUM FALCIPARUM INFECTED ERYTHROCYTES TO CD 36 DEFICIENT PLATELETS
A. Kronenberg, M. Schreiber, C. Hemmer, and B. Kehrel

The adherence of trophozoite-infected erythrocytes to vascular endothelium, platelets, and monocytes plays an important role in the pathogenesis of Plasmodium falciparum malaria. Purified CD 36 binds with receptor-like specificity to infected red blood cells (IRBC) and inhibits cytoadherence of IRBC providing direct evidence in support of the hypothesis that CD 36 is a sequestration receptor. We found two persons with CD 36 deficient platelets by dot-blot assay with the anti CD 36 monoclonal antibody OKM 5 and IOP 36. In crossed immunoelectrophoresis, flow cytometry analysis showed only background silver-staining, and staining of the glycoproteins on nitrocellulose blots with a polyclonal anti CD 36 antibody and lectin avidin biotin alkaline phosphatase staining. Flow cytometry analysis showed only background binding of OKM 5 and IOP 36. In crossed immunoelectrophoresis the CD 36 precipitation arc was missed in the analysis of the CD 36 deficient platelets. Knob-positive (K+) IRBC infected with P. falciparum strain Uganda Palo Alto were cultured and shown to bind to C32 melanoma cells as control. K+ IRBC bound in the presence of Ca2+ to the CD 36 deficient platelets. Our findings strengthen the hypothesis that CD 36 is not the only receptor for IRBC in P. falciparum malaria.

Medizinische Klinik A der Westfälischen Wilhelms-Universität Münster, Exp. Hämostaseoforschung, Domagkstr. 5, D-4400 Münster/Westf.

DETECTION OF VITRONECTIN mRNA IN TISSUE AND CELLS OF THE MOUSE
D. Seiffert*, M. Keeton, Y. Eguchi, M. Sawdey and D.J. Loskutoff

Mouse vitronectin (Vn) was isolated from serum by heparin affinity chromatography. The purified protein (M, 71,000) supported adhesion of mouse and human cells in an Arg-Gly-Asp-dependent manner and bound to type I plasminogen activator inhibitor with kinetics similar to those observed using human and bovine Vn. To further characterize murine Vn and its biosynthesis in vivo, a mouse Vn cDNA was isolated from a liver cDNA library. The amino acid sequence of mouse Vn was deduced from the cDNA and was aligned with that of human Vn. Based on this alignment mouse Vn was inferred to be 457 amino acids long and to have extensive (82%) homology with human Vn. Northern blot hybridization analysis of RNA from mouse tissues, using the mouse Vn cDNA as a hybridization probe, revealed the presence of a single transcript of 1.7 kilobases in mouse liver. Vn mRNA was not detectable in heart, lung, kidney, spleen, muscle, brain, thymus, testes, uterus, skin, adipose tissue, and aorta. The cellular localization of liver Vn mRNA was studied by in situ hybridization. Strong staining was observed only in hepatocytes, suggesting that these cells are the primary source of Vn in vivo.

The Scripps Research Institute, La Jolla, CA, USA. *Present address: Clinical Experimental Physiology, University of Vienna, Austria

INFLUENCE OF CONFORMATION AND MULTIMERIZATION OF VITRONECTIN ON ITS INTERACTION WITH ADHESIVE CELLS
A. Stockmann, S. Hess, G. Müller-Berghaus, K.T. Preisner

Vitronectin (Vn) is a multifunctional adhesive glycoprotein (Mr=75,000), present in the circulation predominantly in its native conformation (VN=) and associated with different tissues. The RGD-sequence of VN, which is responsible for cell-attachment activity and a polycationic cluster of basic amino acids, which functions as a heparin-binding site, are located at distal ends of the molecule. The latter epitope becomes exposed by conformational changes from the closed native form (VN=) via the open denatured form (VN<=>) of VN. Induced by surface coating, complex-formation with other proteins (e.g. thrombin-antithrombin III-complex) or treatment with denaturing agents (e.g. urea), the contribution of both forms with regard to structure-function-relationship was studied. While high protein concentrations of VN already represent populations of oligomeric forms, treatment of VN<=> with urea in a concentration-dependent manner lead to multimerization into high Mr self-association products. Human umbilical venous endothelial cells (HUVEC) and other adhesive cells attached in a concentration- and time-dependent manner to both forms of VN, but only adhesion to VN<=> was inhibited by heparin and by heparin sulfates up to 40%. Moreover, VN<=> preferentially associated with monolayers of HUVEC in a non-dissociable manner at 37°C, as evidenced by direct binding studies as well as by immunofluorescence analysis. These results indicate that multimerization of VN may determine the ability of the adhesive protein to interact with adhesive cells in a different fashion compared to the form of VN present in plasma.

Haemostasis Research Unit, Kerckhoff-Klinik, MPG, Sprudelhof 11, 6350 Bad Nauheim
PAF INDUCES PAF SYNTHESIS IN HUMAN ENDOTHELIAL CELLS.

R. Heller, F. Buissinolino, G. D'Ghio, G. P. Pescarmona, A. Bosia, and U. Tüll

Endothelial cells (EC) are targets for platelet-activating factor (PAF), a phospholipid mediator that leads to increased EC permeability and adhesivity. EC also synthesize PAF upon agonist-induced stimulation. We examined the synthesis of PAF in human umbilical cord vein EC after stimulation of PAF itself. PAF (1-100 nM) induced a time- and dose-dependent activation of the remodeling pathway of PAF synthesis. This was demonstrated by 1) increase of 45Ca influx; 2) activation of (45)Cl-achidonic acid release indicating a stimulation of phospholipase A2; 3) enhancement of the lyso-PAF acetyltransferase activity; 4) increase of PAF synthesis as detected by [3H]acetate incorporation into PAF. The non-metabolizable analog of PAF 1-0-alkyl-2-N-methyl-carbamoyl-syn-glycero-3-phosphocholine (C-PAF) was able to mimic the effect of authentic PAF on EC permeability and adhesivity. EC also synthesize PAF upon agonist-induced stimulation.

COMPARISON OF DIFFERENT METHODS FOR THE PRODUCTION OF HUMAN ECM AND THEIR INFLUENCE ON THE ADHESION OF HUMAN PLATELETS

C.M. Kirchmair, J. Altarjov, K. Kruglinski, A. Schliermer, H.K. Bredin

Extracellular matrix (ECM) produced by cultured endothelial cells (ECs) closely resembles the vascular subendothelial lamina in its organization and chemical composition. The incubation of platelets with ECM results in platelet adherence, aggregation, thromboxane formation and release of ADP and serotonin. We suggest that PAF-induced PAF synthesis in EC may be responsible for increased platelet adhesion. PAF-induced PAF synthesis might be an important mechanism for amplifying original PAF signals thereby potentiating adhesive interactions of circulating cells with EC and altering the thromboresistance of EC.

PAF INDUCES PAF SYNTHESIS IN HUMAN ENDOTHELIAL CELLS.

F. Buissinolino, G. D'Ghio, G. P. Pescarmona, A. Bosia, and U. Tüll

We examined the synthesis of PAF in human umbilical cord vein EC after stimulation of PAF itself. PAF (1-100 nM) induced a time- and dose-dependent activation of the remodeling pathway of PAF synthesis. This was demonstrated by 1) increase of 45Ca influx; 2) activation of (45)Cl-achidonic acid release indicating a stimulation of phospholipase A2; 3) enhancement of the lyso-PAF acetyltransferase activity; 4) increase of PAF synthesis as detected by [3H]acetate incorporation into PAF. The non-metabolizable analog of PAF 1-0-alkyl-2-N-methyl-carbamoyl-syn-glycero-3-phosphocholine (C-PAF) was able to mimic the effect of authentic PAF on EC permeability and adhesivity. EC also synthesize PAF upon agonist-induced stimulation.

REGULATION OF THE PAI-1 GENE BY TNFalpha

Ch. Kunz, G. Müller-Berghaus, D. von der Ahe

Plasminogen activator inhibitor-1 (PAI-1) is a rapid and specific inhibitor of both tissue-type and urokinase-type plasminogen activator (t-PA and u-PA, respectively) and may be the primary regulator of plasminogen activation in vivo. Synthesis and secretion of PAI-1 can be modulated by a number of growth factors, hormones and cytokines. The regulation of the PAI-1 gene by the cytokine TNFalpha was studied in early passage human umbilical vein endothelial cells (HUVEC) and human lung carcinoma cells (A549). Exposure of the cells to TNFalpha was found to cause a 10-fold increase of PAI-1 transcription within 4 h in HUVEC resp. a 16-fold increase within 24 h in A549. This effect involves de novo protein synthesis and proceeds to be independent of an activation of protein kinases A and C. In contrast, dephosphorylation by phosphatases 1 and 2A appeared to be essential for signal transduction. Transient transfection experiments using PAI-1 promoter deletion plasmids gave evidence for a positive TNFalpha-inducible element located between positions -1500 and -1500 of the PAI-1 gene. Deletion of this regulatory region revealed a repressing effect of TNFalpha on PAI-1 promoter activity which was maintained down to about 100 bp promoter length. This finding suggests the existence of an additional negative TNFalpha-responsive element quite close to the transcriptional start site. Constitutive promoter activity seems to be determined by at least two positive and one negative element located below position -216 in HUVEC and in A549 cells only positive elements between positions -142 and -53 exert an influence on the rate of basal PAI-1 transcription.

Haemostasis Research Unit, Korkhoffklinik, NPG, Sprudelhof 11, D-6350 Bad Nauheim
FALCIPARUM MALARIA: PATIENT SERUM CONTAINING TNF-Alpha UP-REGULATES TRANSCRIPTION OF PAI-1 AND PAI-2 (BUT NOT tPA) IN CULTURED ENDOTHELIAL CELLS.

Christoph J. Hemmer, Angelika Bierhaus, Thomas W. Stief, Peter P. Nawroth, Manfred Dietrich

Procoagulant alterations in falciparum malaria can be associated with excessively high plasma activity levels of Plasminogen Activator Inhibitor (PAI). tPA was found to significantly up-regulate PAI-1 expression in circulating endothelial cells (EC), and since the vascular endothelium is activated in human malaria, we examined whether patient serum containing TNFs can upregulate PAI-1 and PAI-2 transcription, and whether this effect is suppressed by a TNF-antibody.

Cultured human umbilical EC were incubated with serum (diluted 1:10 with DMEM) from two different patients with severe falciparum malaria and high TNF-α serum levels (732 and 140 pg/mL respectively, ELISA) or from a healthy control. Patient serum was used with or without addition of a neutralizing anti-TNF-α-antibody. Next, the EC nuclei were isolated and allowed to synthesize mRNA using RNA-UTP (Run-on transcription). The labeled mRNA was slot-blotted against cloned DNA probes for PAI-1, PAI-2 and tissue Plasminogen Activator (tPA). Bound mRNA was then visualized by autoradiography.

Incubation of EC with either of the two patient sera induced a strong signal for PAI-1 and a weak signal for PAI-2, as compared to control sera. Incubation of EC with TNF-α, on the other hand, was not induced. Addition of an anti-TNF-α-antibody reduced the induction of PAI-1 and PAI-2 transcription, which indicates that this effect depends upon TNF-α.

Thus, TNF-α in patient serum seems to be responsible for the up-regulation of PAI-1 and PAI-2 transcription in cultured EC. Since intravascular fibrin strands have been described in fatal malaria, this mechanism might be relevant for the pathogenesis of this disease.

Bernhard-Nocht-Institute for Tropical Medicine, Department of Medicine, 2000 Hamburg 36, Germany

TNF INCREASES THROMBOMODULIN PLASMA LEVELS

A. Böhme, J. Lin, B. Kemkes-Matthes, A. Bierhaus, G. Leidig, H. Minne, B. Langer, Ch. Hemmer, M. Dietrich, B. Komorrell, R. Ziegler, H.O. Lasch, H. Ishii, P.P. Nawroth

Incubation of cultured human umbilical vein endothelial cells (HUVEC) with TNF decreases Thrombomodulin (TM) mRNA levels and protein secretion into the supernatant in a time dependent manner. The steady state levels of TM in an ELISA in patients treated with TNF. In contrast to the in vitro results we observed an increase in plasma TM levels after patients were treated with TNF. The time course showed a lag period of 72 h, followed by a rapid increase from 7 to 15 ng/ml within the next 24 h. Plasma TM levels continued to rise over a period of about 24 h. The mean plasma TM levels of the studied patients was increased by about 50% within 6 h. Plasma TM appeared on Western Blots with several bands, but the relation of the bands was not altered by TNF treatment. TNF did not increase TM secretion into the cultured supernatant when HUVEC were treated with TNF in combination with HUVEC. In contrast TNF led to an increase in plasma TM levels when patients with carcinomas were treated. Consistent with the activation of coagulation noted in these patients, we found with positive ELISA for tPA a 10-fold mediated damage of cultured endothelial cells or HUVEC, mediated damage of cultured endothelial cells led to TM release into the supernatant. We conclude, that i.e. TNF treatment might not directly release TM from the native endothelial cells, but might induce TM release by thrombosis mediated endothelial cell damage.

Univ. Heidelberg, Bernhard-Nocht Institut Hamburg, Univ. Giessen

GENE REGULATION OF THROMBOMODULIN

K. Meyer, C. Nischan, G. Müller-Berghaus and D. v. d. Ahe

Thrombomodulin (TM) is an anticoagulant protein present on the surface of vascular endothelial cells. By modifying the substrate specificity of thrombin, TM functions as a cofactor for the activation of protein C. Binding to TM reduces the procoagulant properties of thrombin, and increases the rate of protein C activation at least 1000-fold. TM activity decreases when endothelial cells in culture are exposed to cytokines like Tumor Necrosis Factor (TNF-alpha), Interleukin (IL-1 alpha) or Endotoxins. The modulation of endothelial cell TM activity by TNF occurs primarily at the level of TM gene transcription. Our work focuses on the elucidation of the TM gene regulatory elements modulated by TNF. We cloned the TM genomic sequences including the 5′ flanking and putative regulatory region. In order to test these promoter fragments we cloned them in front of the bacterial CAT gene and transfected these reporter constructs into HUVEC and an EC Hybrid cell line. After treatment of the cells with TNF-CAT there is a significant decrease in CAT activity. This decreased Reporter activity depend on the TM promoter sequence within the boundaries −111 to −39. By Nase I footprinting we identified a protein-binding site in this region homologous to the DNA binding sequence of the transcription factor c-ets.

Haemostasis Research Unit, Kerckhoff-Klinik, MPG, Sprudelhof 11, D-6350 Bad Nauheim

CULTURED HUMAN MESOTHELIAL CELLS ARE A CELL MODEL FOR THROMBOMODULIN EXPRESSION IN NON-ENDOTHELIAL CELLS

H. Kawamura*, B. Petzet, K.T. Preissner, H. Ishii**, G. Müller-Berghaus*

The glycoprotein thrombomodulin(TM) serves as a potent cofactor for thrombin-induced protein C (PC)-activation. Immunohistochemical analysis demonstrated in non-endothelial cells, such as mesothelial cells and squamous epithelial cells of the epidermis. To study the role of the TM, affinity thrombin receptors on cells which are not in close contact to the flowing blood, we used cultured human mesothelial cells(HMNC). In a first series of experiments, we studied whether functionally active TM antigen was synthesized and expressed by HMNC. In all experiments, human umbilical vein endothelial cells(HUVEC) were used as positive control cells. TM activity was tested by a PC activation assay. Briefly, confluent monolayers of cultured cells were incubated with thrombin, purified PC was added, and the amount of activated PC generated was measured using a chromogenic substrate. The PC activation tested on ten cell lines isolated from ten different individuals was 75% of that observed on HUVEC. Indirect immunofluorescence staining using polyclonal anti-human TM antibodies revealed a strong and diffuse staining of the cell membranes in nearly 80% of the cultured HMNC. 25% of HMNC failed to stain for TM. A similar staining pattern was seen in HUVEC. Metabolic labelling with [35S]-methionine and subsequent immunoprecipitation with monoclonal anti-TM antibodies revealed a band in the position of molecular weight over 200.000 under reduced conditions. Although the molecular weight was different from that observed for isolated TM, this HMC derived protein was identified with a polyclonal TM antibody. These data demonstrate that functionally active TM is expressed by cultured HMNC. Although the physiological role of TM on these non-endothelial cells is not clarified yet. TM on HMNC may have a role to localize and neutralize thrombin released into the peritoneum during inflammation. By this way, TM would prevent adhesion between surfaces of the peritoneum in vivo.
IS THERE ANY ASSOCIATION BETWEEN PLasma THRombomODulin AND DEEP VEIN THROMBOSIS?
M. Steiner, O. Anders, Chr. Burstein and B. Ernst

Currently, there are few accepted pathogenic factors of venous thrombogenesis including stasis, hypercoagulability, inhibitory deficiency and hypofibrinolysis. Nevertheless, many patients suffering from deep vein thrombosis (DVT) remain with no detectable biochemical abnormality associated with thrombogenesis. Thrombomodulin (TM), a membrane-bound endothelial cell thrombin receptor, plays a key role in the regulation of haemostasis and fibrinolysis. Alterations of the TM molecule may theoretically contribute to venous thrombogenesis. In an attempt to verify this hypothesis we measured plasma TM in 15 patients younger than 60 years who had developed DVT. An immunoassay was employed. Venous occlusion (VOC) was applied for stimulation of vascular endothelium. Normal values were obtained from 29 healthy controls. Compared to healthy subjects, significantly increased plasma TM values were found before and after VOC (14.8 ± 3.7 ng/ml vs 0.05 before VOC and 10.9 ± 4.4 ng/ml, p<0.01 after VOC vs 14.2 ± 3.6 ng/ml). 11 out of 15 patients had higher plasma TM values after VOC whereas in 4 patients decreased values were observed. No differences could be found between patients with one or recurrent thrombotic events. Increased plasma TM is probably the result of damage to vascular endothelium. Therefore, it should be investigated in order to evaluate a possible addition to the list of factors associated with thrombogenesis and thrombophilia.

Institut für Klinische Chemie und Laboratoriumsdiagnostik und Klinik für Innere Medizin der Universität Rostock, Heydemann-Str. 6, 0-2500 Rostock

ENDOTHELIN - A POTENT STIMULUS OF VASOCONSTRICTION AND COAGULATION
E. Schulz, F. Ruschitzka, E. Lüders, J. Coenen, C. Gronau, J. Schrader

The interaction of the endogenous vasoconstrictors Endothelin (ET), Angiotensin II (ANG II) and catecholamines with the kallikreine-kinine-, prostaglandine- and renin-angiotensin-system is still to be defined. In 16 minipigs the effect of i.v.-infused ET (200-800 pmol/kg), ANG II (5-15 ng/kg) and Norepinephrine (NE; 5-30 ng/kg) on haemodynamics and coagulation-parameters (anti-FXa, aPTT, AT III, prekallikreine, fibrin monomers and F VIII) was investigated. ET-infusion caused an initial blood-pressure (BP)-reduction with heart-frequency (HF)-elevation followed by a transient BP-increase and reduced HF, elevated PGI2-, PGB2-levels, whereas PGF2α-values remained unchanged. ET caused a hypercoagulability featuring a shortened aPTT, a reduced anti-FXa-activity and elevated fibrin monomers. ANG II and NE-infusion induced only a short-lasting BP-elevation and lower prostaglandine-levels without any alteration of the coagulation-parameters. These studies reveal Endothelins property as a potent stimulus both of vasoconstriction and coagulation which may be of pathophysiologial importance when counteractive endothelial release of EDRF and prostacyclin is impaired.

Dept. of Nephrology/Rheumatology, University of Goettingen, Federal Republic of Germany.

ENDOTHELIAL CELL ACTIVATION BY JUN AND FOS
A. Bierhaus, P. Pitronico, R. Ziegler, P.P. Nawroth

Tissue Factor (TF) and Endothelin-1 (ET) are involved in the regulation of free blood flow. Phorbol esters can induce transcription of both genes. Phorbol ester as well as TNF induce jun and fos. The complex of Jun and Fos (AP-1) can bind to regulatory elements. Both genes contain in their 5' untranslated regions elements related to AP-1 binding elements. Therefore we transfected cultured endothelial cells (EC) with jun and fos cloned into vectors containing SV 40 promoters by the calcium phosphate method. Transfection of endothelial cells with jun or fos alone (or a control gene) did not markedly induce procoagulant activity, however transfection of EC with jun and fos resulted in induction of coagulant activity. Transfection led to an increase of TF from 25 to 78 pg/ml. Gel mobility shift assays confirmed induction of proteins recognizing AP-1 homologous regions in tissue factor.

Univ. Heidelberg

STIMULATION OF ENDOTHELIAL CELLS BY AGE-PROTEINS: A MODEL OF DIABETIC COMPLICATIONS

AGE-proteins (non enzymatic glycosilated proteins) will accumulate with increasing age and at an accelerated rate in diabetics. Since specific binding sites have been demonstrated on cultured endothelial cells, studies of the receptor mediated effect of AGE-proteins on cultured endothelial cells have been possible. AGE-albumin was incubated with cultured endothelial cells at varying doses and times. To better understand the molecular mechanism of diabetic vasculopathy we looked at the expression of genes possibly involved in thrombosis, vessel wall leakiness and vasoconstriction. We found that the genes coding for von Willebrand Factor, Tissue Factor and High Molecular Weight Kininogen are induced by AGE-albumin. In addition we observed an induction of endothelin-1. In contrast to the relatively "acute" mediator TNF is the AGE-response present over several hours. AGE-albumin did not only induce genes related to procoagulant mechanisms, but also cellular protooncogenes, such as jun and fos. Tissue factor and endothelin-1 contain AP-1 binding sites. Band shift assays were used to study binding of the AGE-albumin induced AP-1 to the known AP-1 consensus sequence related structures in tissue factor and endothelin-1.

Univ. Heidelberg, Columbia University New York
53 EFFECTS ON EICOSANOID-METABOLISM IN HUMAN ENDOTHELIAL CELLS EXPOSED TO REACTIVE OXYGEN SPECIES GENERATING AGENTS

A. Griesmacher, G. Weigl, I. Schimke and M. M. Müller

Reactive oxygen species (ROS), which are generated during reperfusion after oxygen deficiency and inflammatory situations, are suggested as promoter of artherosclerotic process. Confluent endothelial cells (ECs) from umbilical veins were incubated with increasing concentrations (0.001-1 μmol/l) of H2O2 or cumene hydroperoxide (CHP). Released prostacyclin (PGI2) and thromboxane A2 (TXA2) were measured. Simultaneously the content of intracellular ATP and the peak level of cytosolic free Ca2+ were measured. The maximum PGI2-release was 13.9 +/- 0.76 pmol/million and the highest TXA2-release was 1.16 +/- 0.2 pmol/million using 0.1 μmol/l H2O2. On stimulating ECs with 0.1 μmol/l CHP, the PGI2-release increased to 18.6 +/- 4.76 pmol/million. Observing the time course of the eicosanoid release the release of PGI2 started after 60 seconds. In contrast, a measurable increase in TXA2-release was observed not before 10 minutes after onset of the stimulant. Ca2+ increased within 180 seconds after starting the stimulation. Stimulating ECs with physiological stimuli (ATP, thrombin) increased Ca2+-levels (EC) after 20-30 seconds followed by the release of PGI2 and TXA2 after approximately 60 seconds. In conclusion, our results indicate, that ROS generating agents stimulate PGI2- and TXA2-release in ECs. The observed delayed increase of Ca2+-levels gives hint that eicosanoid-synthesis is directly started by ROS used. The subsequent elevation of Ca2+ is probably induced by the interaction of ROS with membranes of cellular Ca2+-reservoirs.

2nd Dept. of Surgery, Div. Clin. Biochemistry, Univ. of Vienna, Spitalgasse 23, 1090 Vienna, Austria

54 IL-4 REGULATES PYROGEN-INDUCED EXPRESSION OF VCAM-1, ELAM-1 AND ICAM-1 IN HUMAN VASCULAR ENDOTHELIAL CELLS

S.Kaptolis, P. Quehenberger, C. Pflaum, R. Enter, H. Strolz, D. Bevec, I. Schwarzinger, C. Mannhalter, K. Lechner, W. Speiser

Interleukin 1 (IL-1), tumor necrosis factor (TNF), and bacterial lipopolysaccharides (LPS) are known to increase endothelial cell adhesiveness for leukocytes by stimulating surface expression of various cell adhesion molecules (CAM). IL-4, a product of activated T-cells is known to modulate upregulation of EC-CAM surface expression; this effect is enhanced by the presence of IL-4, ICAM-1 upregulation was reduced to about 40 % (n=4; p<0.05) of stimulated cells not treated with IL-4; ELAM-1 upregulation was not significantly influenced by IL-4; induction of VCAM-1 was enhanced by IL-4 about 2-fold (n=4; p<0.05) of cells stimulated in the absence of IL-4. IL-4 alone had no significant effect on the adhesion molecules investigated. Northern blot analysis showed that the changes in ICAM-1, ELAM-1 and VCAM-1 mRNA content of HUVEC during incubation with pyrogens and IL-4 resembled the phenomenon observed on the cell surface. Determination of ICAM-1 antigen in HUVEC conditioned medium revealed corresponding changes in surface expression and antigen concentration in the conditioned medium. We conclude from our results that IL-4 differentially regulates pyrogen-induced upregulation of adhesion molecules in EC and that these phenomena are mainly regulated at a transcriptional level.

55 EFFECT OF PYROGENS AND INTERLEUKIN 4 ON PECAM (CD31) SURFACE EXPRESSION OF CULTURED HUMAN VASCULAR ENDOTHELIAL CELLS

P. Quehenberger, S. Kaptolis, Ch. Pflaum, I. Schwarzinger and W. Speiser

Endothelial cells (EC) express a variety of adhesion molecules (e.g. Intercellular Adhesion Molecule 1 ICAM-1, Vascular Cell Adhesion Molecule 1 VCAM-1 and Platelet Endothelial Cell Adhesion Molecule PECAM). The pyrogens interleukin 1 (IL-1), tumor necrosis factor (TNF) and bacterial lipopolysaccharides (LPS) upregulate expression of ICAM-1 and VCAM-1. IL-4 was shown to have modulating effects on these phenomena. In the present study, the effects of IL-1, TNF and LPS and a potential regulating effect of IL-4 on pyrogen induced changes in EC - PECAM surface expression were investigated. We used a human umbilical vein endothelial cell (HUVEC) - culture system; PECAM surface expression was quantified by flow cytometry. PECAM was found to be constitutively expressed on the surface of unstimulated HUVEC. During an incubation period of 48 hours with the pyrogens IL-1(100 U/ml), TNF(500 U/ml) or LPS(10 μg/ml) PECAM surface expression was not altered. Incubation of HUVEC with IL-4 decreased PECAM surface expression to about 80 +/- 13 % of controls. Coincubation of the pyrogens IL-1, TNF or LPS with IL-4 for 48 hours caused a further decrease in EC - PECAM surface expression to about 65 +/- 13 % of unstimulated cells. Our data suggest that IL-4 expresses a downregulating effect on HUVEC - PECAM surface expression; this effect is enhanced by the pyrogens IL-1, TNF and LPS.

Klin. Inst. Med. Chem. Lab. Diagn., Univ. of Vienna; Währinger Gürtel 18-20, A-1090 Vienna, Austria

56 PLATELETS ACTIVATE NEUTROPHILS VIA CELL-CELL CONTACT.

W. Lösch, H. Redlich, S. Krause, I. Bergmann and F. Spangenberg.

The aim of this study was to prove whether adhesion of platelets to neutrophils ("rosette formation") causes an activation of the neutrophils. To avoid effects of any material released from platelets, non-activated or thrombin-activated platelets were fixed with formaldehyde and washed before they were added to neutrophils (ratio 50:1).

Adhesion of platelets to neutrophils increased their capacity to generate reactive oxygen species as revealed by measuring luminol-enhanced chemiluminescence (CL). After a 10 min incubation of the cells the CL signals that were measured in presence of non-activated or activated platelets exceeded that obtained in absence of platelets by about 200 % and 550 %, respectively. About the same % increases were obtained when CL was additionally stimulated by zymosan. Incubating neutrophils and platelets in presence of zymosan allowed us to estimate the effect of adherent platelets on the phagocytic activity (PA) of neutrophils. We found that platelet adhesion increased the PA by about 500 %.

The results obtained indicate that adhesion of platelets to neutrophils activates the neutrophils and enhances their reactivity to respond to other stimuli.

Institut of Pathological Biochemistry, Medical Academy of Erfurt, Nordhäuser Straße 74, 0-5010 Erfurt, Germany
LIPOPROTEIN (A) IS A RISK FACTOR FOR RETINAL VASCULAR OCCLUSION. H.M. Müller, F.F. Diekstall, E. Schmidt, W. März, H. Canzler, and U. Demeler

Starting from a study project by Diekstall et al. (Fortschr Ophthalmol 1987, 84: 369) we have studied atherogenic risk factors in 84 patients (60 men and 24 women) with retinal vascular occlusion. As controls we used two reference groups of 40 and 46 healthy persons studied at the Clinic of Ophthalmology in Bremen and at the University Hospital Frankfurt, respectively. Lp(a) was determined by radial immunodiffusion.

29 % of the patients had Lp(a) levels above 300 mg/L whereas in the two reference groups only 10 and 9 % of the subjects exceeded this threshold. On ch2 testing the association between Lp(a) levels and retinal vascular occlusion was significant. Several studies have shown that Lp(a) concentrations above 300 mg/L indicate a 2- to 5-fold increase in coronary risk (Kostner GM et al., Atherosclerosis 1981, 38: 51). This may also hold for the occlusion of retinal vessels.

There were no differences in total cholesterol, triglycerides, and HDL-cholesterol between patients and controls. Lp(a) was not different between patients with arterial and those with venous occlusion. Furthermore, Lp(a) neither correlated with age, cholesterol, triglycerides, LDL-cholesterol, nor with pre-β-lipoproteins. This suggests that Lp(a) represents an independent risk factor for retinal occlusion.

Agglopiprotein (a) is structurally homologous to plasminogen (McLean JW et al., Nature 1987, 330: 139). Therefore, interferences of Lp(a) with fibrinolytic mechanisms at the endothelium are being discussed (Miles LA, Plow EF, Thromb Haemost 1990, 63: 331).

Zentrum der Augenheilkunde, J. W. Goethe-Universität, Theodor Stern Kai 7, W-6000 Frankfurt/Main, FRG

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NATURAL RUBBER DRAINAGE TUBE WITH ANTITHROMBOTIC LUMEN SURFACE

H.-P. Klöcking1, W. Schunk2, G. Merkmann3, C. Gießmann3, H. Knott4 and S. Bergmann4

Previous investigations have shown that antithrombotics such as streptokinase, heparin or pentosan polysulfate sodium (NaPPS) bound to synthetic zeolites and incorporated in elastomers (crude rubber, rubber or latex) may generally be released by diffusion in hydrous solution (W. Schunk et al., Folia Haematol 115:218, 1988).

The present study reports on the antithrombogenic properties of drainage tubes made from natural rubber into which a zeolite NaPPS adduct was incorporated during manufacturing. The tubes were tested for their anticoagulant properties during perfusion with Tris buffer solution, citrated plasma and native blood, resp. The amount of NaPPS released from the tube walls during perfusion in an open circulatory system was sufficient to exert an anticoagulant effect on the streaming plasma. The antithrombogenicity test according to Chandler in a closed circulatory system revealed thrombus formation times (TBZ) of more than 24 h (control: TBZ = 1-3 min in native blood).

Institute of Pharmacology and Toxicology1, Medical Academy Erfurt, Institute of Occupational Medicine2, Medical Academy Erfurt, Phoenix AG Thuringia Waltershausen3 and Chemistry AG, Bitterfeld-Wolfen4

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EFFECT OF LDL, oxy-LDL, and Lp(a) ON PROSTAGLANDIN BIOSYNTHESIS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS. A. Beckmann, W. März, G.M. Kostner, R. Siekmeier, W. Schneider, W. Groth, and M. Kaltenbach

In human skin fibroblasts, LDL have been shown to provide arachidonic acid (AA) as the precursor for prostaglandin biosynthesis via the LDL-receptor pathway (Habenicht AJR et al. Nature 1990, 345, 634). However, the contribution of lipoproteins to endothelial prostacyclin (PGI2) production is controversial (Specter MD et al., J. Lipid Res 1985, 26, 268). Therefore, we have studied the effects of apoB containing lipoproteins on (LDL, oxyLDL, and Lp(a)) on the PGI2 synthesis in cultured human umbilical vein endothelial cells. PGI2 release was quantified in terms of its stable metabolite 6-keto-PGF1α.

All lipoprotein fractions studied stimulated the production of PGI2. Neither LPDS nor FCS alone brought about a measurable release of PGI2. Lp(a) and oxyLDL were approximately half as efficient as LDL in stimulating PGI2 production. When AA was supplemented in incubations with LDL, PGI2 synthesis further increased by no more than 30 %, and AA addition abolished the differences between the PGI2 stimulating capacity of LDL on the one hand and oxyLDL/Lp(a) on the other.

The experiments show that PGI2 production in endothelial cells largely depends on lipoproteins. OxyLDL and Lp(a) are less efficient in promoting PGI2 production than LDL. The diminished capacity of oxyLDL to stimulate PGI2 production may be related to the oxidation of AA. In the case of Lp(a), reduced PGI2 liberation may be due to the lower affinity of this particle for LDL-receptors. However, at present no specific effect of apo(a) cannot entirely be ruled out.

Centre of Internal Medicine and Centre of Biological Chemistry, J. W. Goethe-University, Theodor Stern-Kai 7, W-6000 Frankfurt/Main, FRG

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REDUCED FIBRINOLYTIC CAPACITY AND PROLONGED DISTURBANCE OF HEMOSTATIC BALANCE IN PATIENTS WITH CHRONIC RESTENOSIS FOLLOWING PERCUTANEOUS TRANSLUMINAL CORONARY ANGIOPLASTY (PTCA).

K.-H. Zurborn, E. Hohenstein, S. Meyer, H.O. Brunn

The coagulation and fibrinolytic system was analyzed in 59 patients with coronary heart disease allocated for PTCA and compared to 19 control patients who showed normal coronary vessels by angiography. It was demonstrated that patients with arteriosclerotic lesions of coronary artery had significantly elevated thrombin-antithrombin III complexes (TAT; X = 2.3 vs. 1.4 ng/ml) and an impaired fibrinolytic capacity determined by venous occlusion compared to patients without coronary arteriosclerotic lesions. These results demonstrated a procoagulant state of the blood of patients with coronary arteriosclerosis. In a prospective part of the study, blood analyses were done before PTCA, 24 h, 48 h and 3 months after PTCA. All patients underwent a control angiography after 3 months and restenosis was documented in 24 patients. PTCA induced further activation of coagulation system assessed by increased TAT (p<0.01) to the same extent in both patient groups with and without restenosis. The increase of D-Dimer/TAT significantly increased 24 h and particularly 48 h after PTCA indicating prolonged disturbed balance between coagulation activation and fibrinolysis in the group with restenosis. Baseline t-PA-antigen and t-PA-1 concomitantly increased after PTCA. Fibrinolytic capacity (venous occlusion) was markedly lower only in the restenosis group before PTCA compared to controls (X = 5.7 vs 13.3 IU/ml). The present findings suggest that disturbed balance between coagulation activation and fibrinolysis as well as low fibrinolytic capacity may be a risk factor for developing restenosis after primarily successful PTCA.

I. Medizinische Klinik, Christian-Albrechts-Universität Schloßdorferstr. 12, 2300 Kiel 1, FRG

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IMPROVEMENT OF WHOLE BLOOD VISCOSITY IN HYPERLIPIDEMIC PATIENTS FOLLOWING HELP THERAPY
A.J. Reinsinger, M. Pfeffer, D. Seidel

Background. Heparin-induced Extracorporeal LDL Precipitation (HELP) is an established therapy to lower plasma low-density lipoprotein (LDL) levels in patients with otherwise only ineffectively treatable familial hypercholesterolemia. As such therapy also has been shown to reduce plasma viscosity as well as to improve muscle oxygen tension we investigated its influence on whole blood viscosity at low to medium shear rates.

Methods. In seven patients who underwent HELP therapy at weekly or biweekly intervals continuous viscosity profiles of whole blood and plasma were measured at 37°C and at shear rates ranging from 1:5 to 1500 sec/min before and after treatment. The hematocrit in patient blood was normalized to 35%. Hematocrit calibration of the viscosity was performed with blood from healthy subjects which was diluted using autologous plasma to hematocrit values ranging from 30% to 49% (1% steps). Viscosity curves were then obtained for each hematocrit. In addition, plasma fibrinogen, total cholesterol, LDL and HDL levels were measured from each blood sample.

Results. HELP therapy markedly decreased plasma cholesterol, LDL and fibrinogen levels - but not HDL levels. Whole blood viscosity could also be lowered within the entire shear rate range measured (e.g. decrease of 0.6 mPa sec at 1500 sec/min). This obtained reduction was equal to a decrease in hematocrit from 37% to 31% when compared to the calibration curves and was calculated to be equivalent to an isoviscous blood of approximately 0.8 liter. HELP therapy also resulted in a reduction in plasma viscosity which was, however, less marked than that observed for whole blood.

Conclusions. Our results indicate that in patients with familial hyperlipoproteinemia, a risk factor for atherosclerosis and thrombosis, HELP therapy can not only lower plasma LDL and fibrinogen levels without affecting the HDL levels but can also effectively decrease whole blood viscosity at low to medium shear rates. This suggests that such patients profit from the improved hemorheology produced by HELP therapy.

Abt für Physiologie, GSF - Forschungszentrum für Umwelt und Gesundheit, Ingolstädt
Landstr. 1, D-W-8042 Neuherberg

THE INFLUENCE OF FIBRINOGEN ON HEMORHEOLOGICAL PARAMETERS INVESTIGATED BY FACTOR ANALYSIS
U. Staedt, W. Kirschstein and D.L. Heene

Previous studies have shown that high levels of fibrinogen (FI) are associated with an increased risk for coronary artery disease and stroke. In order to establish the influence of plasma fibrinogen (FI) on hemorheology we measured FI, hematocrit, hemoglobin, red cell aggregation, plasma (PV) and blood viscosity (BV) at different shear rates in 100 healthy subjects (20-80 years).

A factor analysis was calculated (principal component method, varimax rotation) to assess covariations between these parameters. Results: VARIABLE FACTOR 1 FACTOR 2 MEAN (+/- SD)

Fibrinogen 0.82 2.85 0.96 (g/l) Hematocrit 0.83 41.5 3.44 (%) Hemoglobin 0.79 13.8 1.13 (g/dl) Red Cell Agg. 0.53 13.7 2.84 (-) PV 48.8/s 0.55 1.60 0.16 (cp) BV 48.8/s 0.82 5.64 0.04 (cp) BV 14.5/s 0.81 7.52 1.23 (cp) BV 2.3/s 0.68 13.3 3.63 (cp) BV 0.2/s 0.81 38.1 15.9 (cp) Age 0.81 51.4 17.1 (y)

Two factors result, compromising correlated variables; the individual factors being independent from one another. Beside hematocrit FI seems to be the most important single variable, which determines BV at low shear rate, PV red cell aggregation and increases with age. Apart from the enhancement of thrombus formation elevated FI has unfavorable effects on several hemorheological factors. This may play an important part in the development of ischemic diseases, especially in aged patients.

I. Med. Klinik, Klinikum Mannheim, Univ. Heidelberg, Theodor-Kutzer-Ufer, W-6800 Mannheim, FRG

VARIABLES OF COAGULATION AND FIBRINOLYSIS IN PATIENTS AFTER MYOCARDIAL INFARCTION WITH AND WITHOUT CHD, DETECTABLE BY CORONARY ANGIOGRAPHY
J. Heinrich, H. Schulte, R. Schönfeld, E. Köhler, G. Assmann

The fact that coronary artery thrombosis plays a pivotal role in the pathogenesis of acute myocardial infarction (MI) is widely accepted. However, MI also occurs in the absence of coronary atherosclerosis. In order to elucidate the effect of atherosclerotic vessel walls on haemostasis, we measured variables of coagulation and fibrinolysis in MI patients (234 men), admitted to a convalescent hospital and undergoing coronary angiography.

Compared to an age matched group of healthy individuals, the mean whole blood concentration of D-dimer was lower (39.4±9.5 vs. 44.7±10.8 mg/dl) and the mean triglycerides were higher (174.7±124.5 vs. 145.6±94.1 mg/dl). We ascertained differences in patients without and with CHD as to fibrinogen (298.7±73.8 vs. 310.8±81.1 mg/dl), factor VIII vWF (141.0±66.8 vs. 156.0±64.1%), prothrombin activation peptide F1+2 (0.9±0.48 vs. 1.02±0.55 nmol/l), PAI-1 (2.1±2.1 vs. 2.3±2.4 U/ml), t-PA-Antigen (7.5±3.5 vs. 8.1±4.3 ng/ml) and d-dimer (3.0±22.6 versus 403.6±262.4 mg/dl). Especially F1+2 and d-dimer exhibited a distinct increase with the severity of CHD. F VIIc, AT III, protein C, plasminogen and euglobulin fibrinolysis activity were nearly identical in both subgroups.

In summary, we could demonstrate a stronger activation of coagulation and fibrinolysis in MI patients with CHD, compared to those free of vessel wall alterations.

Institut für Klin. Chemie und Laboratoriumsmedizin, Westfälische Wilhelmsuniversität Münster, A.-Schweitzer-Str. 33, W-4400 Münster

EFFECT OF INTERMITTENT INTRAARTERIAL INFUSION THERAPY WITH PROSTAGLANDIN E1 ON THE FIBRINOGENIC POTENTIAL AND THE PLATELET FUNCTION IN PATIENTS WITH SEVERE CLAUDICATION
M. Sosada1, J. Pieper1, M. Barthels1, A. Creutzig1, K. Alexander1, H. Poliwo1

19 patients (m/f=14/5, age=61, range=33-77 years) with severe claudication received during a period of 21 days 36 infusions with 5 mg prostaglandin E1 each (50 min infusion) intraarterial into the femoral artery. The therapy success was defined and measured by a statistical and clinical significant increase of the painfree and maximal walking distances under standardized conditions. Before as well as 7, 14, and 21 days after initiation of therapy the following parameters were determined before and directly after the infusion: t-PA, PAI-1, F2-antiplasmin, plasminogen, D-dimers, fibrinogen (Clauss) and thrombometer test (time in which a collagen channel is blocked by platelet adhesion and aggregation). Significant elevated D-dimer and fibrinogen levels were measured before therapy. During the whole observation period (7, 14 and 21 days) similar levels were measured. All other parameters were obtained in the respective normal range before and during the study. The comparison of the laboratory parameters measured directly before and after each intraarterial infusion did not show any significant changes.

The improvement of severe claudication by prostaglandin E1 is not related to the fibrinolytic potential or platelet function with respect to the measured parameters in this study.

Abteilung Hämatologie und Onkologie und Abteilung Angiologie1 der Medizinischen Hochschule Hannover, Konstanty-Gutschow-Straße 8, D-W-3000 Hannover 61
**CAPILLARY MICROSCOPIC FINDINGS IN PATIENTS WITH VON WILLEBRAND-JÜRGENS SYNDROME**

Koscielny, J.; Groß, J.; Jung, F.; Kiesel, I.; Seyfert, U.T.; Mrowietz, C.; Wenzel, E.

**Abstract**

In a cross-sectional study 25 patients with von Willebrand-Jürgens syndrome, predominantly of type 1 showing low to medium intensity, were studied up to now in all patients vWF-concentration and activity (reduced to between 20% to 45% of the normal value). Factor VIII-activity, factor VIII-antigen, aPTT and bleeding time were determined, and a microscopical study of nailfold capillaries was carried out. Compared to a group of 100 healthy volunteers (Jung et al., Biomed. Tech. 28, 1987) our patients showed morphological and dynamic changes in cutaneous microcirculation. Capillary density was within physiological range. In 25 of 25 patients continuously and discordant capillary perfusion at rest, which progressed to temporary stasis in 5 patients. 13 patients showed a mean capillary erythrocyte velocity below the normal range. Thus, in future differential diagnosis could profit from capillary microscopy. Prospective studies are planned.

**Abteilung für klinische Hämostaseologie und Transfusionsmedizin**

(Direktor: Prof. Dr. E. Wenzel) der Universitätskliniken Homburg/Saar

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**66a DECREASED EXPRESSION OF TISSUE FACTOR ON STIMULATED MONOCYTES FROM HIV-INFECTED PATIENTS ANALYSED BY FLOW CYTOMETRY**

Th. Luther, S. Albracht, V. Menzel, B. Pinzet, M. Müller

Whereas the apoprotein of tissue factor (TF), a 47 kDa transmembrane glycoprotein, is constitutively present in certain tis- sues such as epithelial tissue, brain and placenta, normally it is not expressed by cells within the vasculature. However, the stim- ulation of monocytes and macrophages, effector cells of the inflam- matory component of the cellular immune response, by a va- riety of inflammatory and immunological settings including end- toxin, phorbol esters, lectins, immune complexes, and cytokines results in the induction of cell surface procoagulant activity (PCA), i.e. TF expression. There is described, that in response to endotoxin the induced level of TF mRNA is decreased in mo- nocyes isolated from AIDS patients. In our present study, we are detecting TF expression on monocytes from HIV-Positive pa- tients by flow cytometry using a FITC-conjugated anti-TF mAb (generated in our laboratory) in comparison to noninfected do- nors. We observed a significant depression of TF expression on LPS-stimulated monocytes in relation to the stage of HIV in- fection. Flow cytometry seems to be a simple method for moni- toring molecular TF expression on monocytes in HIV infected pa- tients eventually developing AIDS.

Institut für Pathologie und Hautklinik der Medizinischen Akademie Dresden, Fetscherstr. 74, 0-8019 Dresden

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**66 IN VITRO EXPERIMENTS ON THE PREANALYTICAL CONDITIONS FOR F.2 DETERMINATIONS**

H.-U. Kolde, R. Simmel, J. Ruiz, U. Pelzer, B. Pinzet, M. Müller

The determination of the prothrombin activation peptide F.2 is now possible using radio immuno assay or enzyme linked immuno sorbent assay (ELISA). A critical variable for assay results is certainly the quality of blood sampling. A contamination of the sample with tissue factor may happen during the venepuncture or the sample may contain with blood cells which express tissue factor activity. In e.g. sepsis and also in tumor patients this activity seems to be very common and may result in prothrombin activation during storage of the sample before the assay. The recommended anticoagulant solution for F.2 is either heparin (Bauer et al) or citrate (Pelzer et al). In a new monoclonal based ELISA for F.2 on microtiter modules (Ruiz et al) we simulated the effect of tissue factor contamina- tion by adding highly diluted rabbit brain thromboplastin (Thromboplastin IS) into blood that was either drawn into heparin or citrate. After defined incubation times the samples were stabilized by a protease inhibitor cocktail to prevent further prothrombin activation. The de- termination of F.2 with the method of Ruiz et al or Pelzer et al showed that in the heparin anticoagulated blood F.2 was more suppressed than in the samples with citrate. The amount of F.2 generated in the samples was dependent on the dilution of the added thrombo- plastin and showed also some minor variances in the blood of different individuals. The results with the two ELISAs showed similar trends but were quantitatively not identical. According to these results the determination of F.2 seems to be possible both in citrate or heparin anti- coagulated blood samples but heparin seems to protect the sample better against in vitro pro- thrombin activation than citrate.

Baxter Deutschland GmbH, Edisonstraße 3, D-M-8044 Unterschleißheim, *Baxter Diagnostics Incorporated, Miami, USA*
A. Hubbuch, K. Schmitt

We have evaluated a new transparent reagent (Neoplastin® Plus) for the measurement of prothrombin time in 7 laboratories. Source of the thromboplastin is rabbit brain.

Results: Within and between run coefficients of variations of the times measured were below 2 %, recoveries of assigned values in PreciClot®II and PreciClot®III were excellent as well: relative mean deviations were 0.5 % and 3.6 %.

The new reagent showed a high sensitivity to factor VII (ratio 1.15 at 60 % factor VII), while heparin up to 1.0 U/ml did not interfere. Good agreement with the comparison methods' INR values (ISI of the evaluated reagent: 1.85) was yielded with patient plasma: for example: y = 0.03 + 1.02 x, n = 100, r = 0.979. The therapeutic range (20 to 35 %) was confirmed by comparisons with other commonly used methods.

In conclusion, Neoplastin® Plus is well suited for the measurement of prothrombin times in routine laboratories, especially on automated instruments.

Boehringer Mannheim GmbH, Sandhofer Str. 116, D-6800 Mannheim
COMPARISON OF TWO AUTOMATED APTT METHODS AND HEPARIN ASSAYS IN SELECTED PATIENT GROUPS
R.M. Loreth, R. Simmoteit, H.-J. Kolde

The performance of the aPTT on an automated instrument (Electra 1000c) using two ellagic acid activated aPTT reagents (Actin FS, soy bean phospholipids, and Actin FSL, soy bean plus rabbit brain phospholipids) was compared in selected groups of patients and in normals. In heparin treated patients an anti-Xa based heparin assay was performed on the same instrument and, additionally, on a manual photometric instrument (Chromotimer®). The aPTT results in normals (n = 60) agreed very well with the assay results. The median values were 29.15 and 28.15 sec for FS and FSL respectively. The corresponding upper normal ranges (mean + 2SD) were 35.8 and 36.8 sec. In patients with cumarin treatment and after operations clotting times with FS were slightly longer than with FSL (47.8 vs 41.3 and 43.4 vs36.4 sec). These differences may be explained by a different factor sensitivity of Actin FS versus Actin FSL. In 69 patients on heparin treatment (unfractionated heparin from Braun/Melsungen) Actin FS showed a trend towards longer clotting times than Actin FSL (median 62.6 vs 51.3 sec) although the in vitro heparin sensitivity of both reagents is very similar. In patients on heparin treatment (unfractionated heparin from Braun/Melsungen) Actin FS showed a trend towards longer clotting times than Actin FSL (median 62.6 vs 51.3 sec) though the in vitro heparin sensitivity of both reagents is very similar. The correlation of the two reagents was very good up to clotting times of around 70 sec with Actin FS or 60 sec with Actin FSL respectively whereas at higher heparin doses the results showed a higher degree of scatter. The other hand the two chromogenic heparin assays showed a slightly better correlation coefficient than the two aPTT reagents (r 0.889 vs 0.843) but a considerable degree of scatter, in particular in the low and high concentration range. The two aPTT methods correlated with the two specific heparin assays but for individual patients the results did not agree very well in several cases. This may reflect methodistical difficulties both in the low and in the high heparin measuring range. The results of the two chromogenic assays showed a rather good regression line (y = 1.015 x + 0.16) but with major individual deviations at all concentration ranges. These data show that the monitoring of heparin treatment using two different aPTT reagents does not cause more difficulties in interpretation than using two different chromogenic heparin assays. A standardization of both aPTT and heparin assay is needed to improve heparin monitoring.

Klinikum Kaiserslautern, Medizinische Klinik III, Abteilung für Klinische Hämostaseologie, Friedrich-Engels-Str. 25, D-W-6750 Kaiserslautern
Baxter Deutschland GmbH, Scientific Department, Edisonstr. 3-4, D-W-8044 Unterschleißheim

TECHNICAL PROBLEMS IN THROMBIN CLOTTING TIME ANALYSIS
E.M. Solleder, J. Mayer, B. Schwenk, H.-J. Kolde*, R. Simmoteit* and F. Keller

Thrombin clotting time is a widely used parameter in monitoring heparin therapy. However, this parameter is standardized only to a certain degree. Concentrations of thrombin, incubation times and pipetting schedules are subject of variability between laboratories as well as reagent manufacturers and this method is influenced by the different types of endpoint detection more than other routine methods. Therefore great differences(5,120),(989,897) between various techniques can be found, mainly in patients with prolonged clotting times under heparin therapy. In plasma pool supplemented with standard heparin (0.1 IU/ml) e.g. thrombin times in an intra-assay with three techniques but with identical thrombin concentrations in the reagent differed for more than 10 seconds. With the KC 40 a mean of 25.39 ± 2.19 sec was found, with the “wire loop” technique a mean of 31.06 ± 0.88 sec and with the ELECTRA 1000C a mean of 20.3 ± 0.21 sec. The intra-assay precision of the three methods were cv’s: 8.6%, 2.8% and 1.0% respectively. Out of the reasons mentioned above the development of new, well-standardizable methods seems to be urgent for a reliable monitoring of heparin therapy.

Zentrallabor der Med. Universitätsklinik Würzburg, Josef-Schneider-Str. 2, D-8700 Würzburg, Germany.
* Baxter Deutschland GmbH, Edisonstr. 3-4, D-8044 Unterschleißheim, Germany.
DEVELOPMENT OF A CHROMOGENIC THROMBIN GENERATION INHIBITION ASSAY FOR ANTICOAGULATORY DRUGS

H.-J. Kolde, A. Stecher-Schilling, J. Epple, H. Straub

Laboratory monitoring of non coumarin type anticoagulant drugs like heparin or hirudin is necessary to avoid overdosage and so to prevent bleedings. The most important and commonly used tests until now are the aPTT and the TCT. These methods are influenced by individual coagulation factors and FDPs. Other methods to measure the anticoagulant activity of heparins are anti Xa- and anti IIa-assays. They have the disadvantage that they do not measure the biological activity of heparin like substances because they do only measure the activity against one specific enzyme. Furthermore they are not sensitive for all kinds of anticoagulants and difficult to standardize. According to newer results the main activity of heparin is the inhibition of thrombin generation. Therefore, a well standardized highly sensitive chromogenic assay was developed which measures the true biological activity by measuring the thrombin generation inhibition.

The test is insensitive for platelet factor 4. The anticoagulant activity of different drugs can be determined, for example UFH, LMWH, hirudin, a2-APAP, heparin sulphate, pentosan polysulphate (SP 54). Our method is a further development of a method described recently by R.J. Wagenvoord, H.H. Hendrix, H.-J. Kolde and H.C. Hemker, Thromb. Haemostas., 1991, 65, 930. The Procedure can be adapted on automated instruments and shows very low c.v. values.

The reagents contain a mixture of purified or recombinant coagulation factors of the intrinsic pathway, phospholipide and antithrombin III. To this mixture of coag factors diluted plasma containing heparin or other coagulation inhibitors is added and the coagulation cascade is started by addition of factor IXa. After a certain incubation time generated thrombin is measured with a chromogenic substrate. Heparin or several other anticoagulatory substances produce a dose dependent inhibition of thrombin generation. The assay is not influenced by alterations of coagulation factors in the sample, the fibrinogen concentration or split products. This assay may be useful to determine the degree of thrombin generation inhibition in patient plasma when patients are treated with anticoagulatory drugs. In addition it may be possible to compare the effect of different drugs in a well standardized system.

Baxter Deutschland GmbH, Scientific Department, Edisonstraße 3, D-W-8044 Unterschleißheim

DETERMINATION OF ANTITHROMBIN ACTIVITY WITH AN AUTOMATED SYSTEM, COMPARISON BETWEEN THROMBIN AND FACTOR Xa BASED METHODS

K.v.Pape, M.Blaurock, F.Rabe, N.E.Andersson, J.Bohner

We have analyzed more than 500 patient plasma samples on a Hitachi 717 instrument utilizing the two chromogenic antithrombin methods Coamate AT 400 from Kabi Diagnostica and Boehringer Mannheim Antithrombin non dilution. The results obtained show a very strong correlation between the two methods (r=0,98). However, when plasma samples from patients treated with Heparin(final levels about 1 IU/ml) are analyzed, the results obtained with the Thrombin based method are consistently higher than those obtained with the Factor Xa based method. In vitro addition of Heparin to achieve the same levels in number of plasmas did not result in any difference between the two methods. It is suggested that the difference obtained with patient plasmas might be due to the influence of the slowly acting Heparin - Co-factor II.

Klinikum Fulda, Institut f. Laboratoriumsmedizin Pacelliallee 4, 6400 Fulda

WHOLE BLOOD CLOTTING TEST TO MONITOR HEPARINS IN CHRONIC HEMODIALYSIS

B. Haaf, M. Schäfer, C.E. Dempfle and J. Harenberg

Whole blood clotting methods are effectively used to control the anticoagulant effect on unfractionated heparin (UFH) during chronic intermittent hemodialysis. These methods remain ineffective with fractionated heparins (FH). Anti-factor Xa (aXa) chromogenic substrate assays are sensitive and useful to control anticoagulation of the fractionated heparins during hemodialysis. The present study was designed to establish the correlation of a chromogenic and a clotting aXa-method from whole blood and to determine the therapeutic range of this aXa specific whole blood clotting method.

20 patients were randomized to receive UFH or FH for anticoagulation during 5 subsequent hemodialyses. The aXa-activity in the chromogenic assay ranged from 0.2 - 0.5 U/ml with UFH and from 0.4 - 0.8 U/ml with FH. The aXa-activity from whole blood samples ranged from 0.4 - 0.8 U/ml with UFH and from 0.8 - 1.4 U/ml with FH. The coefficient of correlations of the aXa-activities with the chromogenic assay, heptest assay from plasma and heptest assay from whole blood were between r = 0.5 and r = 0.6. The data demonstrate that the aXa-specific coagulation assay from whole blood is sensitive, reproducible, valid and rapid to control the anticoagulant effect of unfractionated as well as of fractionated heparin during chronic intermittent hemodialysis.

1. Medizinische Klinik der Fakultät Klinische Medizin Mannheim der Universität Heidelberg, Theodor-Kutzer-Ufer, D-6800 Mannheim
EVALUATION OF AN AMIDOLYTIC TEST FOR COMPARATIVE CALIBRATION OF HMW- AND LMW-HEPARINS

K. Müntstedt (1), H. Bleye (2), L. Röka (3)

Amidolytic chromogenic substrate assays are frequently used to determine the anticoagulant activities of various commercial heparins. With the help of a combined assay method heparin characterization is made possible using the TAT/XAT quotient under consideration of the simultaneous inhibition of the two serine proteases thrombin and factor Xa by antithrombin III. This is made possible by choosing a buffer with a compromise between range of best serine protease activity and best serine protease inhibition. The test is primarily designed for qualitative characterization where a numerical value can be assigned to every heparin and shows the heterogeneity of low molecular weight heparins as well as the homogeneity of high molecular weight heparins. It shows that heparins obtained by the same or similar production techniques show similar results in the test system, whereas different LMW-heparins express different AT III modulating effects. The TAT/XAT quotient correlates with anti-IIa and anti-Xa plasma activities after subcutaneous application of various heparins to rabbits. The test is very sensitive and distinguishes between different heparins, heparins of same or similar production techniques, and different lots of the same heparin. How the effect on the TAT/XAT ratio may be used for dosage and selection of the heparin preparation for their therapeutic use has to be established.

(1) Frauenklinik, Klinikstr.32, 6300 Gießen
(2) Städt. Kliniken, Sterkenburgring, Offenbach
(3) Klinische Chemie, Klinikstr.36, 6300 Gießen

DERIVED FIBRINOGEN - A RAPID, SIMPLE AND RELIABLE METHOD FOR ROUTINE SCREENING OF FIBRINOGEN.

E.M. Solleder, J. Mayer, B. Schwenk, M.J. Kraus, H.-J. Kolde*), R. Simmoneit*, P. Brauer and P. Keller

Despite its striking importance in hemostaseological diagnostics all common fibrinogen assays reveal technical problems. With activity tests comparable and reproducible results can be expected only when activity and concentration of fibrinogen are close to or within the normal range. Immunological assays supply reliable results but they give no information how much of the fibrinogen content is active in coagulation processes. A rapid and cost saving method is represented by the "derived fibrinogen" assay. In this test fibrinogen content is determined via the prothrombin time on optical hemostasis analyzers. In the assay the coagulation system in patient plasma is activated and the thrombin generated converts fibrinogen into fibrin. This process increases the turbidity in proportion to the clottable fibrinogen concentration of the plasma.

In more than 100 patients subjected the routine screening in our hemostaseological laboratory the fibrinogen content in plasma was determined according to the method of CLAUS and performed on the RC 10 as well as with a "derived fibrinogen" assay via a prothrombin time method applied to ELECTRA 1000 C. Comparison of the results revealed a very good correlation between both methods (Passing / Bablok equation: y = 0.03 + 1.15 x). The results demonstrate that derived fibrinogen" as an additional result of the prothrombin time test is a parameter that can be analyzed easily, cost-savingly and reproducibly.

Zentrallabor der Med. Universitätsklinik Würzburg, D - 8700 Würzburg, Germany.
*) Baxter Deutschland GmbH, Edisonstr. 3-4, D - 8044 Unterschleißheim, Germany.

CLOTTING FACTOR ANALYSIS WITH PLASMA SAMPLES CONTAINING INCREASING CONCENTRATIONS OF HMW- AND LMW-HEPARIN AS WELL AS OF AT III J. Stödt, B. Fickenscher, T. Berndt, R. Klingelhöfer

The influence of increasing plasma concentrations (p.c.) of HMW-, an LMW-Heparin (LH) and of AT III on the PTT, the Thrombin-, and the Prothrombin Time (TT, Quick), and on several clotting factor assays (II, V, VII, X, VIII, IX, XI, XII, Fibrinogen) has been examined with conventional clotting factor assays (II, V, VII, X, VIII, IX, XI, XII, Fibrinogen). This is made possible by choosing a buffer with a compromise between range of best serine protease activity and best serine protease inhibition. The test is primarily designed for qualitative characterization where a numerical value can be assigned to every heparin and shows the heterogeneity of low molecular weight heparins as well as the homogeneity of high molecular weight heparins. It shows that heparins obtained by the same or similar production techniques show similar results in the test system, whereas different LMW-heparins express different AT III modulating effects. The TAT/XAT quotient correlates with anti-IIa and anti-Xa plasma activities after subcutaneous application of various heparins to rabbits. The test is very sensitive and distinguishes between different heparins, heparins of same or similar production techniques, and different lots of the same heparin. How the effect on the TAT/XAT ratio may be used for dosage and selection of the heparin preparation for their therapeutic use has to be established.

(1) Frauenklinik, Klinikstr.32, 6300 Gießen
(2) Städt. Kliniken, Sterkenburgring, Offenbach
(3) Klinische Chemie, Klinikstr.36, 6300 Gießen

A CLOTTING ASSAY FOR FIBRINOGEN WITHOUT SAMPLE PREDILUTION

K. Fickenscher, T. Berndt, R. Klingelhöfer

Fibrinogen is the most frequent quantitatively assayed protein in coagulation and increasing attention is paid to it due to its importance as a risk factor for cardiovascular disease. The method of Clauss is widely used but is hampered by the need of sample predilution. We therefore developed a new assay for fibrinogen based on the method of Clauss, but without the need of a predilution of the plasma sample. This allows the assay to be performed more easily and convenient with a standard pipetting scheme. The assay can be done on all types of instrumentation used in the coagulation laboratory like coagulometers, photometers and others. To 100 ~l of undiluted plasma 200 ~l of the reagent are added. A high surplus of thrombin immediately converts all fibrinogen into fibrin monomers. Coagulation of fibrin monomers is slowed down by the use of a special fibrin aggregation inhibitory peptide (Gly-Pro-Arg-Pro-Ala-amid). This specific inhibitor allows adjustment for coagulation speed dependent on the fibrinogen concentration over a wide range. We adjusted the coagulation time to about 10 sec for 6 g/l and 70 sec for 1 g/l.

Since the same basic principle as in the Clauss method is employed, the assay correlates well with other common methods from pre diluted samples.

Behringwerke AG, Postfach 11 40, D-W-3550 Marburg
EVALUATION OF FIBRINOGEN ASSAY METHODS AND BATROXOBIN TIME ON ELECTRA 1000C AND KC10 FOR PATIENTS WITH LIVER DISEASE AND THROMBOLYSIS THERAPY

Y. Schmitt, I.F. Ramirez, H.-J. Kolde

With the introduction of optical hemostasis analyzers the quantitative determination of PT and fibrinogen became possible. In the present study the PT derived fibrinogen (DF) value on the Electra 1000, using a turbidimetric endpoint detection, was compared with the Clauss (CF) assay on the KC10. In addition the application of the batroxobin time with Fibrinoclotin® on the Electra and the KC10 was investigated in the same samples. The patient group consisted of 31 patients with liver disease (Pseudo-cholin-esterase < 2000 u/l) or clinical symptoms of liver cirrhosis. Another group consisted of patients with fibrinolytic therapy. For comparison also 31 normals were analyzed with the different fibrinogen assays. The DF assay was calibrated with patient plasmas at different concentration ranges assuming that the CF values of those plasmas were accurate. The results showed a very close relationship between DF and CF in the group of normals (r = 0.927, y = 0.1x + 0.93x). In the group of patients with liver disease DF showed higher values than CF in several cases but still lower values than the fibrinogen antigen determination on a laser nephelometric analyzer using polyclonal antibodies against fibrinogen. The correlation between CF and DF in the liver patients was r = 0.982, y = 0.1 + 0.9x. In the group of patients with fibrinolytic therapy, however, CF values were lower than DF values, in some cases with extremely low CF values where there was still a considerable amount of DF and even higher concentrations of fibrinogen antigen. The overall correlation of 31 normals, 31 patients with liver disease and 11 samples of patients during thrombolysis therapy gave a correlation coefficient of r = 0.563, y = 0.4 + 1.1x. The batroxobin time in the groups of liver patients showed a surprisingly good correlation (r = 0.95, y = 1.7 + 1.0x). Only in one patient who had a fibrinogen value of almost 10 g/l, the Electra value was only slightly prolonged whereas the K10 value was almost twofold prolonged in comparison to the normal range.

In conclusion these data show a good correlation of the derived fibrinogen and the Clauss fibrinogen assay in normals and an acceptable correlation of these parameters in liver disease. In thrombolytic therapy sometimes the Clauss assay gives very low values in contrast to the derived fibrinogen or the fibrinogen antigen.

Institut für Klinische Chemie und Laboratoriumsmedizin, Katherinenhospital, Kriegsbergstr. 60, D-W-7000 Stuttgart 1

Baxter Deutschland GmbH, Scientific Department, Edisonstr. 3-4, D-W-8044 Unterschleißheim

A NEW CLOT LYSIS ASSAY FOR THE EVALUATION OF HYPOFIBRINOLOGIN

Mondorf, W., Robbins, I., Scharer, I.

Impairment of the fibrinolytic system may be a major factor in thromboembolic diseases. Although methods are available for clot lysis analysis (euglobin lysis time, fibrin plate method), these are not practical for routine laboratory screening of the fibrinolytic system. We developed a simple and reliable assay for investigation of fibrinolysis. The assay is initiated by adding citrated plasma to a microtitre plate containing buffer, thrombin and streptokinase at various dilutions. Clotting is followed by lysis. The degree of lysis can be expressed as a percentage of that found in wells with no streptokinase.

With streptokinase 70 IU/ml, lysis in 30 randomly selected fresh frozen plasma samples was normal (80-145 %) in 24 (80 %) and slightly diminished (55-79 %) in 6 (20 %) samples. Fibrinolytic activity as low as 19 % was found in a female patient with a history of thromboembolic disease. The plasma of one daughter with a history of thromboembolic disease showed 44 % and that of another daughter, who had no history of thromboembolic disease, showed 58 % lysis. Plasminogen function and antigen was diminished in all three. The father, who had no history of thromboembolic disease and normal plasminogen levels showed 137 % lysis.

We suggest that the preliminary data provide the basis for the development of a simple and reliable method to screen for hypofibrinolysis.

Department of Internal Medicine, University Hospital, Frankfurt/M, Germany

The influence of blood taking conditions on some fibrinolysis and thrombophilia parameters

R. Stahl, A. Feinemann and B. Kempter

Klinikum Großhadern, Institut für Klinische Chemie (Dir. Prof. D. Seidel), Marchioniinstr.15, 8000 München 70

Blood collecting with the aid of venous occlusion is daily clinical routin. We tried to mimic different conditions under which a certain sample may be taken, to see the influence on the fibrinolysis parameters thrombin-antithrombin (TAT), fibrin- and fibrinogen degradation products (FDP, PDG), PAI, ATIII and factor II in seven healthy subjects. A manometer cuff was inflated above diastolic pressure and butterflyneedles (21 G) were used for venipuncture. Thereafter blood was taken every minute. In the fourth minute the pressure was released and blood taken for another five minutes. Under these experimental conditions factor II, ATIII and PAI showed a steady level; FbDP, FgDP and especially TAT, however, showed a marked increase up to highly pathological values. This was the same in one person under coumarin therapy. To see if this effect is due to the occlusion we took blood samples in the same manner but without any occlusion and yielded more or less the same time course. By only applying the occlusion and releasing it and taking blood once after the eight minutes, normal values were found. So the time how long after venipuncture a certain blood sample is taken is crucial for these parameters. That means also that care should be taken if these parameters are to be determined out of samples from daily routin and not taken under controlled conditions.

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Quantitative Measurement of Factor XIII Activity in Plasma using a Novel Microtiter Plate Assay

C.E. Dempfle, K. Hochreuter, J. Harenberg, D.L. Heene

A novel activity assay for measurement of factor XIII activity in plasma and other biological fluids is introduced. The assay is based on the incorporation of soluble DNP-cadaverine into solid phase bound casein by activated factor XIII, and detection of incorporated DNP-cadaverine by a monoclonal antibody-enzyme conjugate. A range between 2% and 200% of standard plasma factor XIII activity can be measured accurately, using a sample dilution of 1:40. The correlation of the procedure with F XIIIa-values measured with Laurell electroimmunoassay is 0.94, correlation with the dansylcadaverine incorporation method using gel filtration for separation of casein-bound and free dansylcadaverine is 0.88. The assay procedure consists of three simple steps: 1) Casein-coated microwells are filled with diluted sample, DNP-cadaverine substrate solution, and thrombin reagent, and incubated for 10 minutes. 2) Plates are washed with TTBS, and incubated for 60 minutes with an antibody-enzyme conjugate solution. 3) Plates are washed, and wells filled with enzyme substrate solution. Plates are read at a wavelength of 405 nm using standard microtiter plate photometers. By use of the microwell format, the test allows parallel quantitative determination of factor XIII activity in 96 individual samples per plate, or smaller numbers of samples, using microtiter strips. In contrast to other tests available, the assay is not influenced by heparin, or by turbidity (e.g. lipidaemic plasma), colored or fluorescent compounds in plasma samples. The test allows diagnosis of inherited or acquired factor XIII deficiency, as well as monitoring of factor XIII substitution therapy.

Universität Heidelberg, Klinikum Mannheim, I. Medizinische Klinik, Theodor-Kutzer-Ufer, D-6800 Mannheim

Clinical Evaluation of a New Photometric Test for Factor XIII Activity in Plasma

E.M. Solleder, J. Mayer, D. Demuth, M. Bombhard, Th. Eller, P. Brauer and F. Keller

Due to the permanently increasing number of F XIII analyses there is a strong demand for new F XIII assays. Besides a high precision one expects from these assays that - compared with common tests - they are faster, easier to handle and that they can be carried out on automated analyzers. The clinical evaluation of a new photometric assay developed by Fickenscher et al. (1991) was performed in our laboratory. This test distinguishes itself from all other photometric assays by the admixture of a newly developed fibrin aggregation inhibitor to prevent increasing turbidity caused by fibrin polymerisation. Thus, with this method one has not to pretreat the plasma with bentonite to precipitate the fibrinogen of the samples. This deffibration leads to a coprecipitation of parts of the fibrinogen-linked F XIII as demonstrated by simultaneous F XIII analyses both in an untreated and a bentonite-pretreated sample of identical plasma.

The assay studied here, which enables the direct determination of the F XIII activity at 340 nm via a NADH-consuming reaction allowing quantification of the ammonia liberated in course of the F XIII catalyzed reaction, can be performed with a common photometric equipment as well as with automated analyzers like Cobas FarA. With the automated version of the cv's for intra-assay precision varied from 1.8% to 2.4%, the cv's for inter-assay precision from 2.4% to 4.8%. Due to the linearity of this rapid new test that allows a determination range between 0% and 150% and the good accordance to the clot lysis test, the Berichrom® F XIII assay promises to become a reliable simplification in the analyses of F XIII activity.

Zentrallabor der Med. Univ.-Klinik, Josef-Schneider-Str. 2, D-8700 Würzburg, Germany
Using the Bethesda method, factor VIII:C inhibitors are found in 5-15% of patients with hemophilia A. There is no way of confirming these figures, as there has been no alternative to the in-vivo recovery of factor VIII:C. The Bethesda method is not suitable for screening purposes, and is time-consuming. We have developed an enzyme-linked immunosorbent assay (ELISA) for the routine screening for inhibitors. Preliminary results show good correlation with the Bethesda method. In 54 hemophiliacs, all (n=9) those found to have more than one Bethesda unit showed highly positive ELISA results. In those whom Bethesda results were less than 1.0, more than half (n=13) had positive ELISA results (slight or moderate). Screening blood from 470 non-hemophilia patients showed similar correlations with the Bethesda results. Weakly positive results were found in 5.7% (n=27), indicating low-titer inhibitors. Further studies are needed to determine the role of this ELISA method in screening for factor VIII:C inhibitors.

**EVALUATION OF IN VITRO BLEEDING TIME AS AN INDICATOR OF PRIMARY HEMOSTASIS IN VARIOUS HEMORRHAGIC STATES**

C. Salat, B. Reinhardt, E. Hiler

Despite great clinical relevance few feasible laboratory methods for investigating primary hemostasis are available. We investigated the in vitro bleeding time according to the method of Kratzer and Born (Thrombostat 4000, VDG von der Goltz, Secon, Germany). Materials and methods: 1 ml of citrated whole blood was forced by a constant negative pressure to flow through a collagen covered aperture which was soaked with ADP imitating the injured vessel. Corresponding to the degree of platelet adhesion and aggregation the blood flow was diminished and normally stopped after some time. The initial blood flow (IF), bleeding time (t) and volume (V) were recorded.

Results (+/-SEM): Normal controls (n=10): IF=118.6 (+/-13.7) ul/min; t=66.6 (+/-11.1) sec; V=96.6 (+/-18.0) ul. Patients with leukemia (n=32): IF=156.1 (+/-31.8) ul/min; t=353.1 (+/-111.7) sec; V=552.3 (+/-216.5) ul. Patients with thrombocytopenia after chemotherapy (n=15): IF=142.6 (+/-26.3) ul/min; t=294.3 (+/-99.1) sec; V=412.7 (+/-187.9) ul.

In all of the patients investigated a prolonged in vitro bleeding time was found. In spite of very low platelet counts in all of these patients (<10,000/ul) remarkable interindividual differences were seen indicating that there is no close correlation between platelet count and effectiveness of primary hemostasis. In a patient with CML in acceleration a prolonged in vitro bleeding time (t=470 sec) was seen despite 500,000 platelets/ul corresponding with his bleeding tendency. A shortening of initially increased bleeding time (t=470 sec) occurred after DDAP infusion (t=58 sec) in a patient with von Willebrand disease showing a beneficial effect. We conclude that the measurement of the in vitro bleeding time is a valuable tool in the evaluation of primary hemostasis in thrombocytopenic and -pathic states.
INDUCTION OF CELLULAR ADHESION MOLECULES ON THE ENDOTHELIUM
W. Speiser, S. Kapoitiis, P. Quehenberger

Leukocyte adherence to endothelial cells (EC) and migration of blood cells through the endothelial layer are central steps in the development of inflammatory events. These phenomena are mediated on the one hand by ligands located on the leukocyte surface such as LFA-1, Mac-1, VLA-4, sialyl Lewis X and on the other hand by cell adhesion molecules (CAMs) expressed by EC. Endothelial CAMs belong to two main protein families. Intercellular cell adhesion molecule 1 and 2 (ICAM-1, ICAM-2), vascular cell adhesion molecule 1 (VCAM-1) and platelet endothelial cell adhesion molecule (PECAM) belong to the immunoglobulin superfamily, whereas endothelial leukocyte adhesion molecule 1 (ELAM-1) and granule membrane protein 140 (GMP-140) also called platelet activation dependent granule-external membrane protein (PADGEM) belong to the selectin family. In vivo and in vitro studies showed that the pyrogens interleukin 1 (IL-1), tumor necrosis factor (TNF) and bacterial lipopolysaccharides (LPS) are capable of inducing expression of several CAMs (ICAM-1, VCAM-1 and ELAM-1) on the EC surface. Increased CAM expression is followed by enhanced leukocyte adherence to the endothelial layer.

Recently, IL-4 a product of activated T-cells was found to exert modulating effects on pyrogen induced activation of cultured EC. The induction of the various CAMs is, however, modulated differentially. Pyrogen induced ICAM upregulation is counteracted by IL-4, whereas VCAM upregulation is enhanced by this cytokine. This regulatory phenomena are mainly mediated at a transcriptional level. ELAM induction is not affected by IL-4. PECAM surface expression, which is not affected by pyrogens was found to be reduced under the influence of a combination of pyrogens and IL-4.

Institute of Medical and Chemical Laboratory Diagnosis, University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria

ANTIGEN OF ENDOTHELIAL CELL DYSFUNCTION

P.F. Nawroth, A. Bierhaus, Y.M. Zhang, J. Lin, R. Waldherr, R. Ziegler

Endothelial cell dysfunction can be induced by different mediators, such as cytokines, tumor products or advanced glycosylation end products. It is of high importance to close the gap between in vitro knowledge about endothelial cell dysfunction and clinical use. Therefore we studied endothelial cell dysfunction with histologic methods in biopsies of patients with autoimmune vasculitis, allograft rejection and plasma of patients treated with TNF. Thrombomodulin, ELAM-1, ICAM-1 and vascular expression of tissue factor were useful markers of altered functional properties of endothelial cells. In vitro it is possible to mimic the clinical situation by studying the effect of cytokines, tumor products or NOS-proteins on endothelial cell properties, such as expression of thrombomodulin, tissue factor or endothelin-1. Transfection of endothelial cells with jun and fos allows understanding of the role of AP-1 in inducing markers of altered endothelial cell properties.

In conclusion: Elucidating the molecular mechanism of endothelial cell dysfunction may be useful in understanding the mechanism of disease involving endothelial cell activation.

Univ. Heidelberg

BLOOD-LOSS AND INCIDENCE OF DVT IN ORTHOPAEDIC SURGERY

F.U. Niethard, R. Pauschert

Still today, thrombo-embolic complications and postoperative blood-loss are one of the most common complications in operative medicine. In orthopaedic surgery, the highest incidence of DVT is found in hip and knee joint surgery (incidence of DVT 40-60 %).

The reasons for developing DVT can be found in the patient collective, which creates a great range of risk factors like age, DVT in past history, varicose veins or cardiac insufficiency.

Furthermore the kind of surgical procedure, i.e. the localisation, dimension or duration implies a considerable risk of DVT. During surgery, the veins of the lower extremities can be directly damaged or twisted through operative manipulations as well.

The above mentioned risk factors will be discussed and their significance in relation to blood-loss and DVT will be demonstrated.

Orthopädische Universitätsklinik Heidelberg, Schillerbacher landstr. 200 a, D-6900 Heidelberg

ENDOTHELIAL CELL DYSFUNCTION

P.F. Nawroth, A. Bierhaus, Y.M. Zhang, J. Lin, R. Waldherr, R. Ziegler

Orthopädische Universitätsklinik Heidelberg, Schillerbacher landstr. 200 a, D-6900 Heidelberg
THE SENSITIVITY AND SPECIFICITY OF DIAGNOSTIC PROCEDURES FOR THE DETECTION OF DEEP VEIN THROMBOSIS IN ORTHOPEDIC SURGERY. M.N. Levine

Deep vein thrombosis (DVT) is a common complication in patients undergoing orthopedic surgery. Despite the demonstrated benefit of prophylactic anticoagulant therapy, approximately 25% of patients undergoing hip surgery will develop post-operative DVT diagnosed by venography. However, most of these thrombi are asymptomatic and the clinical significance of asymptomatic DVT is unclear. Post-operative screening tests to detect DVT have been used for patient management and as outcome measures for clinical trials. Three types of tests have been used to screen for DVT and each has limitations. The combination of impedance plethysmography and 111th fibrinogen leg scanning is insensitive for proximal DVT (sensitivity < 50%). The sensitivity of compression ultrasonography for proximal DVT is only approximately 60%. Although venography will detect DVT, it is invasive, unsuccessful in approximately 20% of patients, and may induce DVT. The choice of post-operative screening test for DVT is problematic. The consequences of not diagnosing and thus not treating DVT must be balanced against the risk of anticoagulant-induced bleeding from treating asymptomatic thrombi and the cost of performing the tests. A model comparing three post-operative screening methods; 1. venography, 2. compression ultrasonography, and 3. no screening will be discussed.

OCTRF Hamilton Centre, 711 Concession St, Hamilton, ON, Canada L8V 1C3

LOW-DOSE ORAL ANTICOAGULATION IN ORTHOPEDIC SURGERY

F. Buchmann

The usefulness of very low doses of warfarin to prevent thrombosis in central venous catheters and postoperative deep venous thrombosis (DVT) after general, particularly gynecological, surgery has been well established. In one study, warfarin, 1 mg per day, started 7 days prior to surgery, resulted in minimal changes of thromboplastin time, APTT and vitamin K dependent clotting factors, but the postoperative increase of FI-1 was blunted and there was an increase of fibrin degradation products in the postoperative period compared to the group of patients receiving placebo. This suggests stimulation of the fibrinolytic system.

In hip surgery (elective and hip fractures) 10 prospective randomized clinical trials comparing placebo treatment with oral anticoagulation for the prophylaxis of DVT and/or pulmonary embolism have been undertaken. Oral anticoagulation was commenced preoperatively in 4 of these studies and postoperatively in 6, and in the majority warfarin was given to result in INRs of 1.5 to 2.0. The outcome measures were 111th fibrinogen scanning of the legs, impedance plethysmography, venography, autogamy, and, more recently, duplex ultrasonography or a combination of these methods. Each of these studies reported a significant reduction of the incidence of thromboembolic events and overall reduction of DVT was about 50%. In the 2 studies in knee surgery, risk reduction was over 80%. In the majority of studies there was an increase incidence of bleeding. Several other studies comparing oral anticoagulation with dextran, intermittent compression of the legs, fixed doses of unfractionated heparin yielded superior results in the oral anticoagulation group but a lesser efficacy then is obtained with LMW-heparin.

Hematologisches Zentrallaboratorium, CHUV, CH-1011 Lausanne

PROPHYLACTIC TREATMENT WITH DIFFERENT LMWH: INCIDENCE OF THROMBOSIS AND RISK OF BLEEDING COMPLICATIONS

D. Bergqvist

Prophylaaxis with LMWH and heparin analogues is the most recent branch of the prophylactic tree. Apart from having some practical advantages with a better s.c. bioavailability, a longer biological half-life and less interaction with platelet there are several studies showing a benefit in orthopaedic surgery. Five studies have been placebo controlled with a significant reduction of deep vein thrombosis in the LMWH group. At present there are 12 studies in hip surgery (3 fracture) comparing LMWH with standard heparin with altogether around 2200 patients. The relative risk reduction is 17% in favour of LMWH. Including only studies with mandatory bilateral phlebography there are 1535 patients with a relative risk reduction of 19% in favour of LMWH. LMWH is significantly better than dextran, three studies having been performed. Different LMWHs have been used but there are no data so far indicating much of a clinically relevant difference. The frequency of fatal pulmonary embolism using LMWH seems very low, based on 55.545 patients only 0.04%. In the comparative studies between standard heparin and LMWH on orthopaedic patients no fatal pulmonary embolism has been reported. Org 10172, a mixture of sulfated glycosaminoglycans, seems very effective in both elective hip and hip fracture surgery. DERMATAN sulphate, so far insufficiently documented, has at least a small effect. It is possible today to obtain good prophylactic effect with minimal risk for bleeding complications and other adverse events.

Department of Surgery, Lund University, Malmö General Hospital, S-214 01 Malmö, Sweden

PLATELET DYSFUNCTION IN UREMIC CAUSES AND THERAPY

G. Viganò and G. Remuzzi

An acquired platelet dysfunction plays a major role in the pathogenesis of the bleeding tendency of uremia. Several biochemical abnormalities have been reported, including reduction in platelet serotonin and adenosine-5'-diphosphate, higher cyclic AMP levels and a reduced ability to generate thromboxane. The latter defect can be attributed to a functional abnormality in one of the prostaglandin-forming enzymes, cyclooxygenase. A new finding is an intrinsic platelet defect that manifests itself by a smaller than normal rise in platelet Ca++ after stimulation. The synthesis of vascular prostacyclin is also increased in uremia and quantitatively as well as qualitatively defects of plasma and platelet von Willebrand factor have been consistently described. The bleeding tendency of uremia is known to be influenced by low hematocrit since anemia may have a negative effect in the rheological component of the platelet- vessel wall interaction. Recently, it was also found that nitric oxide (NO), a table humoral agent formed by vascular endothelial cells from L-arginine, is a mediator of the bleeding tendency of uremia. A specific inhibitor of NO formation from L-arginine, completely normalized the prolonged BT in experimental uremia. The impairment of primary hemostasis in uremia has two constant abnormalities: reduced adhesiveness of platelet and prolonged bleeding time (BT). The latter is considered the most sensitive marker of such hemorrhagic diathesis.

The current management of uremic bleeding includes an adequate dialysis schedule, and red-cell transfusion or recombiant human erythropoietin to patients with severe anemia. A partial correction of renal anemia (hematocrit between 27 and 32%) by erythropoietin treatment is enough to normalize BT. Acute bleeding episodes may be treated with desmopressin, a synthetic analog of the decapeptide hormone vasopressin, which in most patients is rapidly effective, at least on BT.

Patients undergoing major surgery and with gastrointestinal or intracranial bleeding need longer-lasting control of hemostasis and seem to be the most likely candidates for conjugated exogenous therapy, which can restore BT to normal for as long as 3-14 days.

Mario Negri Institute for Pharmacological Research, via Gavazzeni 11 - 24100 Bergamo
Abstract not submitted

**Hirudin and Renal Function**

Götz Nowak

The 65 amino acid anticoagulant miniprotein hirudin is a potent tight binding inhibitor of thrombin. Now recombinant pure hirudin is available. Apart from its anticoagulant effect, hirudin is pharmacodynamically inert and is well tolerated in vivo. Hirudin is distributed within the extracellular space of the body and the elimination half-life was about 1 hour. Hirudin is exclusively eliminated via the kidneys and is partly recovered in unchanged form in the urine (after an intravenous dose of 0.1 mg/kg about 38%). Therefore, the interaction of hirudin with the kidneys was studied in more detail. In studies with nephrectomized dogs and patients were found that hirudin is not extrarenally metabolized or excreted. Moreover the pharmacokinetics of hirudin was studied in patients with different degrees of renal function impairment. The renal clearance of hirudin was significantly and linearly correlated with the creatinine clearance. In these patients, the elimination half-life is 20 to 41 hours in some of these patients with interstitial nephropathies the cumulative urinary hirudin excretion is increased to 70 to 80%. It is possible that hirudin was reabsorbed and metabolized in proximal tubulus cells to a certain extent. The results allow the conclusion that hirudin is suitable for anticoagulation in renal failure with thrombotic risk and in clinical hemodialysis. Hirudin is also a potential diagnostic agent to discriminate between glomerular and interstitial renal dysfunction.

Institut für Pharmakologie und Toxikologie
Medizinische Akademie Erfurt
Nordhäuser Str. 74, 0-6010 Erfurt

**Haemostatic Effect of Recombinant Human Erythropoietin**

J. Vermylen and C. Van Geet

We have performed several studies on the effects of recombinant human erythropoietin administration on the haemostatic system of chronic haemodialysis patients. A first study was performed in adults. From the third week of administration onwards, not only haemoglobin and haematocrit but also the platelet count rose significantly, the peak of the latter coinciding with the peak dosage of erythropoietin. The bleeding time normalized in all nine patients, at least temporarily. Subnormal platelet aggregation before therapy also improved transiently and in parallel with the erythropoietin dosage. A second study was performed in ten children on chronic haemodialysis. Platelet aggregations, subnormal before therapy, again improved during treatment. The intracellular free calcium concentration in platelets after thrombin stimulation also increased significantly during erythropoietin administration. The patients showed an increase in blood pressure.

We hypothesize that the effect of erythropoietin on platelet aggregability and on blood pressure may be due to an increase in the intracellular free calcium mobilization in platelets and possibly in smooth muscle cells respectively.

Centre for Thrombosis and Vascular Research and Department of Paediatrics, University of Leuven, Herestraat 49, B-3000 Leuven

**Mechanisms of Pathophysiology and Diagnostic Strategies in Patients with Cerebral Ischaemia**

H. G. Rennerici, Klinikum Mannheim. University of Heidelberg, FRG

Studies from data banks evaluating the sources for cerebral ischaemia in large community series of patients have consistently demonstrated that the majority of transient ischaemic attacks and stroke are due to a cerebral embolism from extracranial sources. In contrast, haemodynamic compromise of the cerebral circulation is rare even in the presence of severe destructive lesions within the brain supplying extra- or intracranial arteries, and microangiopathy represents a well known but also less common entity in patients with hypertension and diabetes mellitus, which affect the intracranial small vessel system more severely than the extracranial large arteries. Identification of these different pathogenic mechanisms in the individual patients, however, is essential for the rational planning of an effective therapy and, in particular, for the design of clinical trials investigating the efficacy of antithrombotic drugs.

The patient’s history and clinical findings are useful for the determination of the time, course and topography of the affected brain area; however, they only occasionally contribute to the definition of the individual pathogenesis. Vascular and brain imaging methods (e.g. ultrasound, CT and MRI) are more useful for this purpose, which will be described in detail. Even with an adequate use of these modern facilities and extensive additional diagnostic studies (e.g. laboratory investigations, cardiac ultrasound, monitoring of cardiovascular function) about one third of the sources of cerebral vascular ischaemic events may not be detected. Strategies reducing this proportion are to be discussed.
Acute Anticoagulation. Selection of patients, results, complications.
D. Busse (Minden)

So far, no effective treatment of ischemic stroke is known, which avoids or limits the extension of ischemic tissue damage. Therefore therapeutic consideration focuses on treatment of the underlying vascular disease. Besides lysis of thromboembolic occlusion of cerebral arteries, acute anticoagulation with heparin is a therapeutic strategy. But the efficacy of heparin in acute ischemic stroke is still controversial. The risk is a secondary hemorrhage with clinical deterioration. In our opinion anticoagulants have their major role in the prevention of recurrent cardiogenic and intraarterial embolism.

101 pat. were anticoagulated with heparin for prevention of recurrent cerebral embolism. The treatment started in mean 1.4 days after stroke onset. PTT should be prolonged to 2 - 2.5 fold of the normal value. The patient selection: 76 atherothrombotic strokes, 20 extrapolranal dissections, 56 cardiogenic embolism and 39 embolism of unknown etiology. The average duration of heparin therapy was 11.6 days.

Results: 2 pat. (3.5%) with cardiogenic embolism had a recurrent stroke on the first day after onset of therapy. Of the patients with atherothrombotic stroke, 4% got an infarction, 15% a minor stroke with a reversible deficit. 7 pat. had haemorrhagic complications, 2 died after suffering an intracerebral haemotoma.

Conclusions: Probably the efficacy of heparin therapy is better in cardiogenic than in atherothrombotic strokes. In general it's a low risk therapy. Absolute contraindications are immediate hemorrhagic infarctions in CT, therapy-resistant hypertonus and coagulopathies, relative contraindications are large infarction and a septic embolism.

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PREVENTION OF STROKE WITH ANTIPLATELET DRUGS, ANTICOAGULATION AND CAROTID SURGERY
H.C. Diener, Essen

Antiplatelet drugs cannot be recommended for the primary prevention of stroke (Physicians Health Study). Four large controlled trials indicate, that anticoagulation is effective in stroke prevention in patients with atrial fibrillation. The risk of stroke following a transient ischemic attack (TIA) or a second stroke following a primary stroke is reduced by 20 to 30% with prophylactic treatment with acetylsalicylic acid (ASA), a combination of ASA and dipyridamole and ticlopidine. The optimal dosage of ASA is still under debate. Positive preventive results have been obtained with daily doses between 30mg and 1500mg. The side effects are clearly dose-dependent. Anticoagulants are effective in patients with cardiac emboli, probably effective in carotid dissection and are under investigation in rapid progressing asymptomatic carotid stenosis. Recent trials in patients with asymptomatic carotid stenosis <60% indicate, that carotid surgery is not recommended in these patients. Two large prospective trials in patients with carotid stenosis >70% and TIA or minor stroke showed, that carotid surgery may reduce the risk of stroke by 10 to 17% (NASCET, ECST). Whether surgery is useful in patients with stenosis <70% is not yet known.

Neurologische Universitätsklinik, Stadlassestr. 15, W-4300 Essen

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THE PREVALENCE OF FACTOR XII DEFICIENCY IN 103 ORALLY ANTICOAGULATED OUTPATIENTS SUFFERING FROM RECURRENT VENOUS AND/OR ARTERIAL THROMBOEMBOLISM
W.-M. Halmayer, Ch. Mannhalter, Ch. Feichtinger, K. Rubi and M. Fischer.

One hundred and three patients suffering from recurrent venous thrombosis (DVT), recurrent arterial thromboembolism (ATE) and/or recurrent myocardial infarction (MI) and 50 healthy subjects were tested for Hageman factor (F XII) activity and antigen. Among the 103 patients we identified 17 subjects with F XII deficiency (16%), 3 with protein C deficiency (3%) and 3 with protein S deficiency (3%). The 103 patients were divided into subgroups according to the kind of thrombotic complication. Of the DVT-group 8% of the patients were deficient in F XII. Among patients suffering from recurrent ATE and/or MI, the incidence of F XII deficiency was significantly higher (25%, p < 0.001). In 59% of the patients with F XII deficiency a positive family history of thrombosis could be established. In contrast, only 32% of all DVT and 28% of all ATE/MI patients had a positive family history. We believe that reduced levels of F XII should be considered as a risk factor in the development of thromboembolism. Consequently, more attention should be payed to the measurement of F XII when evaluating thromboembolic risk factors especially in cases of recurrent ATE and/or myocardial infarction.

Central Laboratory, Municipal Hospital, Wolkersbergenstraße 1, A-1130 Vienna, Austria.

1st Department of Medicine, University Vienna, Cent. Lab., Kaiserin Elisabeth Hospital, Vienna
LUPUS INHIBITOR AND CIRCULATING ANTIBODIES AGAINST F VIII, F V, AND PLATELETS LEADING TO RECURRENT VENOUS THROMBEMBOLISM AND MISSED ABORTIONS

S. Simianer, W. Kirschstein, J. Harenberg and D.L. Heene

We report on a female patient, who presented 1985 at the age of 16 with deep venous thrombosis after 14 days of oral contraceptives. Initial platelet count, aPTT and PT were reported normal. Sedimentation rate was moderately elevated. She had urinary infection and was treated successfully with antibiotics.

Subsequently she developed autoimmune thrombocytopenia, reflected by high loading of autologous thrombocytes with IgM, IgG and C 3d and a platelet count of 24,000/ul. Circulating anticoagulants against F VIII, quenching F VII:C to <0.5 %, and F V as well as a lupus anticoagulant were detected by dilution assays with normal plasma. Laboratory signs for DIC or a fibrinolytic defect were absent. Within the next months she experienced recurrent deep venous thrombosis and pulmonary embolism.

Steroids were administered for 4 months, leading to remission of the autoimmune thrombopathy. Titers of F VIII circulating anticoagulant decreased, F V circulating anticoagulants and lupus anticoagulant persisted.

In 1988 and 1999, she had two missed abortions within the second trimester. Then, circulating antibodies against F VIII had disappeared, lupus anticoagulant and F V circulating anticoagulants were still present.

In 1991, she presented with symptomatic urinary infection. Coagulation status was unchanged, platelet count and platelet immunostaining remained normal. Her family history was uneventful supporting thrombophilia or autoimmune disorders.

The clinical course of the patient is unique with thrombosis and missed abortions, probably caused by a lupus inhibitor, in spite of severe coagulation defects and autoimmune thrombocytopenia.

I. Medizinische Klinik, Klinikum Mannheim, Theodor-Kutzer-Ufer, W 6800 Mannheim

ERYTHROCYTE SUPEROXIDE DISMUTASE ACTIVITY DURING AND AFTER DEEP VEIN THROMBOSIS

R. Müller, R. Jaekel, O. Anders, Chr. Burstein, M. Steiner and B. Ernst

Impaired enzymatic and non-enzymatic antioxidant systems are believed to take part in the pathogenesis of a variety of diseases. There is accumulating evidence supporting the view that a dysbalance between prooxidant and antioxidant systems could contribute to thrombogenesis.

We measured erythrocyte superoxide dismutase (SOD) activities in 9 patients undergoing deep vein thrombosis (DVT) and in 32 patients at least 6 months after they had developed DVT. SOD activities were determined using xanthine, xanthine oxidase and nitroblue tetrazolium.

Normal values recorded from 75 healthy subjects were 334 ± 59 mg/1 erythrocytes. At the time of thrombosis manifestation, significantly reduced SOD activities were found (118 ± 65 mg/1 erythrocytes). Patients with thrombosis history demonstrated higher SOD activities (194 ± 41 mg/1 erythrocytes) which are still significantly decreased when compared to healthy controls.

Since erythrocyte SOD represents the major part of blood antioxidant system the observed reduced SOD activities could play a pathogenetic role in thrombogenesis which needs further evaluation.

Institut für Klinische Chemie und Laboratoriumsdiagnostik und Klinik für Innere Medizin der Universität Rostock, Heydemann-Str. 6, 0-2500 Rostock

ANTITHROMBIN III DEFICIENCY AND PREGNANCY - SUCCESSFUL MANAGEMENT WITH LMW-HEPARIN

E. Miller, C. Salat and B. Kempfer

The management of pregnancies in women with congenital antithrombin III (AT III) deficiency poses special problems. Affected persons who have not experienced thrombotic events should probably be given antithrombotic prophylaxis with heparin throughout pregnancy. Patients with a history of previous thrombotic episodes should definitely receive treatment. We report a 26 year old patient whose diagnosis of hereditary AT III deficiency was established 4 years prior to pregnancy following acute bilateral pulmonary embolism while on oral contraceptives. During continuous cumarin treatment values of AT III activity ranged between 60 and 65%. Since the patient wanted to become pregnant, cumarins were discontinued and, after confirmation of the pregnancy, low molecular weight (LMW)-heparin twice the recommended doses for prophylaxis (Fragmin P forte, 5000 anti Xa units) was started and given subcutaneously daily throughout the pregnancy. 1 week prior to term the patient was admitted to the hospital and AT III concentrate (Kybernin®), 1500 units, was infused daily intravenously. During pregnancy and on LMW-heparin AT III activity and antigen was in the range of 41 - 50%. Following infusions of concentrate the AT III activity levels rose to 90 - 100%. The patient delivered spontaneously a healthy girl 4 days following admission. The LMW-heparin prophylaxis was continued for 8 weeks. To our knowledge this is the first reported case of a pregnancy associated with AT III deficiency managed successfully with LMW-heparin prophylaxis.

Medizinische Klinik III und Institut für Klinische Chemie der Universität München, Klinikum Großhadern, D-8000 München 70

THROMBEMBOLIC RISK IN SURGICAL OUTPATIENTS WITH INJURIES OF THE LOWER LIMB

P. Kujath, B. Horst, U. Spannagel, W. Habscheid
Medizinische Universität Lübeck, Lübeck, Germany

In an open randomized clinical study the incidence of venous thrombembolism in outpatients with a plaster cast due to injuries of the lower limbs was examined. 251 patients with a plaster cast were randomized in two groups. Group I received a LMW-heparin (36 mg Fraxiparin once daily) for thromboprophylaxis, group II was left without prophylaxis. After removal of the cast, all patients were examined with compression real time ultrasonography. If thrombosis was suspected venography was performed.

6 patients with prophylaxis and 21 patient without prophylaxis developed thrombosis, this difference was statistically significant with p < 0.01. The thrombembolic risk was highly depending on the trauma. In group I, 10 of 89 patients with minor lesions such as ligamental ruptures developed DVT (11.2%), whereas DVT could be found in 11 of 38 patients with fractures (28.9%). The incidence of thrombosis was reduced in group I. 2 of 85 patients (1.5%) with minor lesions and 4 of 39 patients (10.2%) with fractures developed DVT.

There was no difference regarding risk factors in both groups. A general thromboprophylaxis for outpatients immobilized with a plaster cast should be performed.
HETEROGENEITY OF LIPOPROTEIN (A): STRUCTURAL AND IMMUNOLOGICAL CHARACTERIZATION OF LP(A) SIZE ISOMORPHS. W. März, R. Siekmeier, and W. Großer.

Lp(a) is an LDL-like particle to which apo(a) is attached by disulfide bonds to apoB. Apo(a) CDNA is homologous to plasminogen. The molecular weights of the major apo(a) isoforms are genetically determined, and the apo(a) size polymorphism is related to Lp(a) levels. Here we show that in plasma there is additional, intrapersonal heterogeneity of Lp(a).

Plasma was subjected to fast flow gel filtration. The elution profiles of Lp(a) were monitored with three non-competitive enzyme immunoassays (EIA). In all assays, a polyclonal anti-apo(a) was used on the solid phase. Plasminogen receptor antibodies (F. X. III-Antigen) and elevated PHN-Elastase but not to TAT levels it can malaria might not reflect intrinsic differences between clinical severity. Decreased Coagulation Factor XIII in falciparum malaria (as compared to vivax malaria).

These results show that reduced F.XIII levels in malaria are associated with high PHN-Elastase concentrations. Since low F.XIII levels correlate with high parasitemia and increased as the size of the Lp(a):B complexes decreased.

The data suggest that Lp(a) constitutes a complex system of particles differing in the size of the apo(a) moiety, the apo(a) to apoB mass ratio, and in the expression of apoB epitopes. An elution profile in the vicinity of the receptor binding domain of apoB-100 (MB47) is expressed high in number on smaller Lp(a):B particles. This raises the possibility that lower molecular weight Lp(a) particles are the preferential ligands for apoB receptors.

Gustav-Emden-Centre of Biological Chemistry, J. W. Goethe-University, Theodor Stern Kai 7, W-6000 Frankfurt/Main, FRG

ELEVATED FN-ELASTASE AND DECREASED COAGULATION FACTOR XIII LEVELS IN F. FALCIPARUM AND P. VIVAX MALARIA. P. Schuff-Werner, E. Schütz, S. Schulz, V.W. Armstrong and T. Eisenhauer

Procoagulant alterations in malaria correlate to clinical severity. Decreased Coagulation Factor XIII (F.XIII) in falciparum malaria might indicate an additional role for unspecific proteolysis. Therefore plasma Elastase levels from polymorphonuclear leucocytes (FN-Elastase) were performed in 45 patients with falciparum and in 45 patients with vivax malaria. Elastase was measured by sandwich-ELISA in its complex with alpha-1-proteinase-inhibitor. F.XIII-Antigen concentrations (subunit A and B) were determined by Laurell electrophoresis. F.XIII-Plasma activity was determined by measurement of ESR, blood cell counts and CRP which are unspecific. With anti-neutrophil cytoplasm antibodies (ANCA) laboratory assessment of DA of SNV has increased considerably. We investigated whether alteration of haemostasis allows an evaluation of DA as well. Prior to therapy (pt) and after clinical remission (cr) these investigations were performed in 22 pat. with Wegener's granulomatosis (WG), 3 pat. with microscopic polyarteritis (MP) in 6 pat. with M. Henoch-Schoenlein (HSP). Thrombin-Antithrombin III-complexes (TAT), Prothrombin fragments (F1+2) and Fibrin D-dimers were elevated pt and diminished or normalized with cr.

(median TAT pt: 23.2; cr: 3.2ug/l; F1+2 pt: 3.6; cr: 1.5umol/l; D-dimers pt: 3; cr: 0.5mg/l).

There was a significant correlation to ANCA-titers (ANCA pt: 160; cr:420). Initial F. VII- vWF-AG, Plasminogen inhibitor (PAI-I), a, anti-plasmin and antithrombin III were high and remained elevated after cr. F. X was diminished only in pt. with HSP. Preliminary investigations disclosed that cardiolipin-Ab detected only in NP, but not in WG or HSP. Conclusion: TAT, F1+2 and D-dimers are promising markers of DA, equivalent and additive to ANCA in SNV. They are superior to ESR and CRP because they reflect vascular endothelial damage and are not influenced by frequent concomitant infections in pt. with SNV. In contrast, F VII-vWF-AG as another marker of endothelial function is not acceptable for indicating DA.

Med.-Univ.-Klinikum Heidelberg und Marburg, FRG

Haemostaseological Effects of Different LDL-Apheresis Procedures. P. Schuff-Werner, E. Schütz, S. Schulz, V.W. Armstrong and T. Eisenhauer

Today LDL-spheres is established in the treatment of severe hypercholesterolemia refractory to conventional therapy. The most commonly used procedures are double filtration (DF), polyanion adsorption using dextran sulfate (DS), polyanion precipitation with heparin (HELP) and immunoadsorption (IA). Coagulation factors are eliminated to a various extend by DF, DS and HELP, whereas IA does not at all affect the coagulation system.

The complete elimination of fibrinogen and factor V and X in the extracorporeal circuit attests the thromboplastin time (TPT), whereas the partial thrombin time (PTT) is mainly influenced by the decrease in factor VIII and IX activity during HELP therapy. Significant changes in coagulation time can also be observed in patients treated by DS: the loss of factor V results in an abnormal TPT, whereas PTT is neither measurable in vitro nor in vivo. This is due to the extracorporal elimination of the factors VIII, IX, XI and XII.

DF has only a moderate influence on coagulation parameters: the prolongation of the PTT is mainly due to the loss of factor VIII.

Except immunoadsorption, LDL-spheres has a moderate to marked influence on haemostasis, thus leading from a hypercoagulatable to a hypocoagulatet state at least for a short time. This might be a positive effect in patients with coronary heart disease based on hypercholesterolemia.

Universitätsklinikum, Zentrum Innere Medizin, Robert-Koch-Str. 40, W-3400 Göttingen, FRG
SUCCESSFUL TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA IN EARLY PREGNANCY

E. Seifried, E. Rozdzinski, T. Schmeiser, E. Kurrie, H. Heimpel

Thrombotic thrombocytopenic purpura (TPP) is a hematologic disorder which is clinically characterized by thrombocytopenia, microangiopathic hemolytic anemia, fever, neurologic symptoms and cardiac and renal involvement. Up to now the pathogenetic mechanisms of this disease are poorly understood. It is well-known that TPP is associated with pregnancy and prognosis for the mother and child is poor. We present the first case of a severe TPP diagnosed in the first trimester of pregnancy (13th week of gestation) with maternal survival and birth of a healthy child which required continuous and intensive treatment with plasmapheresis until delivery. During 24 weeks several trials to discontinue plasma therapy failed because of continuous active disease, and it became evident that plasma infusions were not as effective as plasma exchanges. The fact that the patient came into remission soon after delivery of a healthy child by cesarean section in the 37th gestational week shows that in this case pregnancy induced an unknown factor which does not cross the placenta and which can be removed by plasmapheresis.

Medizinische Klinik und Poliklinik Ulm, Abt. Innere Medizin III, Robert-Koch-Str. 8, 7900 Ulm, FRG

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FATAL HEAT STROKE ASSOCIATED WITH DISSEMINATED INTRAVASCULAR COAGULATION

G. Stehle, C. E. Dempfle, J. Buss, J. Harenberg, D. L. Heene

A 29 year old healthy farm worker complained of severe headache after working for more than 12 hours in the field during a hot summer day without adequate fluid intake. He suddenly started running and screaming confusedly, and finally lost consciousness. A rescue team found a patient with generalized convulsions and high body temperature. Differential diagnoses included intracerebral hemorrhage or heat stroke. The patient was transferred to an intensive care unit. Upon admission temperature was 41.0°C. Cerebral hemorrhage was excluded by CT scan. Although cooling measures was started immediately and normal temperature was attained within a few hours, massive rhabdomyolysis occurred, causing serum creatinine kinase peak levels of 86000 U/l (normal: < 100 U/l) and serum myoglobin concentrations above 300000 mg/ml (normal: 8-80 mg/ml). Severe consumptive coagulopathy developed, which was treated with fresh frozen plasma, thrombocyte concentrates, and small intravenous doses of heparin. The further course of the disease was complicated by ARDS and renal failure. The patient died after 15 days. Autopsy revealed excessive hemorrhage was excluded by CT scan. Although cooling measures was started immediately and normal temperature was attained within a few hours, massive rhabdomyolysis occurred, causing serum creatinine kinase peak levels of 86000 U/l (normal: < 100 U/l) and serum myoglobin concentrations above 300000 mg/ml (normal: 8-80 mg/ml). Severe consumptive coagulopathy developed, which was treated with fresh frozen plasma, thrombocyte concentrates, and small intravenous doses of heparin. The further course of the disease was complicated by ARDS and renal failure. The patient died after 15 days. Autopsy revealed excessive hemorrhage was excluded by CT scan.

Universität Heidelberg, Klinikum Mannheim, I. Medizinische Klinik, Theodor Kutzer Ufer, W 6800 Mannheim

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PARAMETERS OF HYPERCOAGULABILITY IN ELECTIVE HIP SURGERY

H. J. Siemens, K. Gehmke, T. Wagner

Venous thromboembolic disease is the most frequent complication in patients undergoing total hip replacement. In 30 patients scheduled for elective hip surgery, the course of different coagulation parameters was investigated: a) preoperatively before induction of anesthesia, b) intraoperatively before the beginning of the traumatic surgical phase as well as c) directly afterwards, d) immediately after the end of the operation, e) 2 hours and f) 24 hours postoperatively; furthermore 2, 4 and 5 days (g, h, i) later. The inhibitors AT III, protein C and S showed a significant decrease of activity intraoperatively to marginally low normal values (d). After a moderate but significant initial decrease intraoperatively, fibrinogen increased to high values postoperatively as an acute phase reaction (i). Fibrinogen levels as well as D-dimers did not reach their highest concentrations until 2 hours after the end of the operation (e) as a sign of intraoperative tissue traumatization. Total plasminogen activators only dropped to significantly low values during the first days after surgery (i). TAT-activity reached maximum values on the first postoperative day (f) and TAT complexes did not reveal peak levels until 2 hours postoperatively (e). Thus according to our results, a maximum of hypercoagulable activity can be recognized 2 hours until 2 days postoperatively.

Deapt. of Hematology, Medical University of Lübeck, Ratzeburger Allee 160, D-2400 Lübeck.

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CHANGES IN HEMOSTASIS AFTER HEART TRANSPLANTATION

U. T. Seyfert, E. Helmling, H. G. Glunz, F. W. Albert, E. Wenzel

The changes of haemostasis were investigated in 9 patients undergoing heart transplantation. Methods: 2 Year Follow up. Rejection episodes were always confirmed by biopsy. Parameters studied: Thrombin generation, platelet activation, endothelial alteration, fibrinolytic system and elastase. Results: 1. No changes in thrombin generation rate compared to normals. Under rejection episodes vs. CMV infections there was a significant increase of TAM and Dimeres, but there was no predictivity. F1,2 fragments are only moderately increased (n.s. vs. normals) during a rejection episode (lack of specificity, sensitivity and predictivity). The TDP, PdDP seems to have a high specificity with regard to the discrimination of rejection episodes vs. PdDP, TDP during intercurrent viral infection. 2. Significant increases (p < 0.001) of thromboxane synthesis in the presence of a rejection episode. Prostacyclin values remain stable within the normal range. 3. Increased F1,2 liberation under combined cyclosporin/azathioprine/cortisone treatment. Conclusion: According to our findings the disturbances of arachidon acid metabolism in connection with thromboxane/fibrin turnover allow in connection with biopsy and non-invasive cardiac monitoring the differentiation between different clinical problems after heart transplantation.

Abt. f. Klin. Hämostaseologie u. Transfusionsmedizin, Universitätskliniken, D-6650 Homburg/Saar
The activation of haemostasis influenced by blood transfusions to children with a systemic or systemic heart disease who were undergoing open heart surgery has been investigated. The activation markers thrombin-antithrombin III complex (TAT), fibrinogen and fibrin degradation products TDP, FgDP, FbDP and D-Dimer complex (DD) have been analyzed. Immediately after the end of cardiopulmonary bypass (CPB) one group of children (n=14, median age 7.6 years, 20.65 kg body weight, b.w.) received whole blood (WB), another group (n=14, median age 4.75 years, 17.35 kg b.w.) packed red blood cells (RBC) and platelet-rich plasma (PRP) transfusions. All children received preoperative infusions of 7,500-10,000 KIU/kg aprotinin; during CPB 300,000-500,000 KIU/kg were administered. After CPB the infusions were continued with 5,000-7,500 KIU/kg until the first postoperative (postop) day. The pre- and intraoperative values of all parameters determined exhibited no significant differences between the two groups. In both groups the preop TAT were slightly elevated (5-8 µg/l) (the median of all data are presented). During CPB TAT increased from initially 2 µg/l to 10 µg/l. After the end of CPB the neutralisation of heparin by protamine chloride induced a sharp rise of TAT to 50-55 µg/l. In the WB group immediately after transfusion TAT declined to 31 µg/l, in the RBC/PRP group to 18 µg/l. In both groups TAT had nearly normalized at the second postop day. The pre-, intra- and postop TDP, FgDP, FbDP and DD levels were found to be within their lower normal ranges (150-250 µg/l). No significant differences between the two groups were evaluted. However, at the second postoperative day slight increases of TDP, FgDP, FbDP and DD towards the upper normal ranges (400-500 µg/l) were observed. The courses of TAT indicated a slight activation of the coagulation system during CPB, followed by markedly enhanced activation upon heparin neutralization. Compared with the WB group the more rapid decrease of TAT in the RBC/PRP group might be due to the absence of WB leucocyte-induced activation of the coagulation system by RBC/PRP. In both groups no transfusion-associated generation of fibrin(ogen) derivatives was measurable due to the protective α2-antiplasmin-like effect of aprotinin.

Institut f. Exp. Hämostaseologie und Transfusionsmedizin der Universität Bonn, Sigmund-Freud-Str. 25, W-5300 Bonn 1

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Influence of the Method of Anesthesia on Haemostasis During and After Open Chest Surgery

R. Kätzel, B. Wiedemann, H. Keuper

Two randomly assigned groups of 21 patients each who underwent open chest surgery received either general anesthesia (GA-Group) or epidural anesthesia (EA-group). Except for collagen-induced aggregation, there were no relevant differences between the two groups with respect to platelet morphology and aggregability. The intraoperative rise of factor VIII:C was less pronounced in the EA-group. In addition, the decrease of the anticoagulatory activity of the protein C system was less noticeable in this group. There were remarkable differences in the fibrinolytic system between the two groups. Whereas IPA-antigen was significantly higher perioperatively in the GA-group, after surgery the inhibitory activity of PAI-1 decreased faster in the EA-group. The decreased hypercoagulability during surgery and increased fibrinolytic activity after surgery in the EA-group is reflected in the D-dimer-values which increase only moderately during surgery and approach normal values more quickly postoperatively. The increased perioperative levels of vasopressin in the GA-group suggests that this stress hormone affects the release of FVIIa and IPA from the endothelium. Our results are in accordance with the well-known finding of a lower incidence of thrombotic episodes after surgery under epidural anesthesia.

Städt. Klinikum St. Georg, Leipzig, and Behringwerke AG, Marburg

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RISK-MARKER PROTEINS T-PA, PAI AND PROTEIN C DURING ECC AND IN THE PRIMARY POSTOPERATIVE PHASE

W. Heller

Aortic coronary venous bypass operations generally lead to the destruction of platelets and proteins active in the clotting process. At the same time, this phase, the fibrinolytic system is stimulated. Haemostatic abnormalities, along with changes in the endothelial cells which manifest themselves first, can lead to postoperative lung complications connected with infections of the respiratory system and subsequent ARDS. We examined this phenomenon within the framework of the present study with 90 patients who had to undergo bypass surgery. Blood was taken preoperatively, before thoracotomy during ECC at short intervals, at the end of the operation and on the 1st, 3rd, and 5th postoperative days. The following parameters were determined: the clotting and fibrinolytic systems, the stimulation of the platelets during ECC, the release of factors from the endothelium (tissue plasminogen activator, t-PA), and further plasminogen, plasminogen activator inhibitor (PAI), as well as protein C and the D-dimers. Upon the plasminogen activation immediately after the onset of ECC there is a significant release of t-PA (from 10.5 to 21.7 µg/l) while, during ECC, remains at a constant high level. With raised alpha2-antiplasmin levels this situation is maintained. The drop in fibrinolytic activity is dependent on the level of these parameters. Simultaneously there is a significant rise in plasmin- elastase (780 µg/l) complexes leading to the activation of Willebrand antigens. The latter is itself a marker for the stimulation of endothelial cells. In summary it can be said that during ECC a significant rise in t-PA and PAI (from 6.3 to 33.2 AU/ml) occurs. As a measurement of the platelet stimulation as a result of membrane damage a significant rise in platelet factor 4 is to be registered, signalling a consumption of heparin.

Abt. Thorax-, Herz- und Gefäßchir., Univ. Tübingen, Calwerstr. 7, D-7400 Tübingen

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POSSIBLE LUNG COMPLICATIONS DURING THE REPERFUSION PHASE (ECC) AND THEIR CAUSES

W. Heller, H. Engel, H. Hoffmeister

During extra-corporeal circulation (ECC) the circulation is for the most part removed from physiological regulation mechanisms. The contact of the blood with artificial surfaces causes the activation of the kallikrein-kinin system as well as the initiation of the endogenous coagulation pathway through various feedback mechanisms. In order to gain information on the activation of the kallikrein-kinin system, prekallikrein and kallikrein-like activity were determined as indicators of the endogenous clotting pathway factor XII. For both systems the total inhibitory activity as well as the C1-esterase inhibitor were recorded. Further the concentration of the PMN-elastase and its total inhibition as well as alpha1-antitrypsin were measured. Particular attention was dedicated to the reperfusion phase. In order to be able to carry out a comparison of haemofiltration and autotransfusion regards effect on the prekkins and their inhibitors two groups, each with 20 patients were formed. Besides the mechanisms of the release of elastase from the granulocytes (from 87 µg/l to 789 µg/l p.o.), such as in the case of the frustane phagoeytosis, in addition degranulation of these cells occurs through mechanical damage and through specific biochemical activation processes by means of the anaphylatoxin C3a, plasma kallikrein and beta-factor XIIa. The destruction of PMN-leucocytes and the release of elastase resulting from this show a linear dependence on the perfusion time. This fact allows us to conclude that the release rate of elastase is dependent on the length of the ischemia time and that there is inadequate perfusion of the lung could have an influence on the degranulation of the polymorphonuclear leucocytes. With the data at hand a time-dependent change in the behaviour of the elastase concentration, as a result of insufficient perfusion of the lung, is to be detected.

Abt. Thorax-, Herz- und Gefäßchir., Univ. Tübingen, Calwerstr. 7, D-7400 Tübingen
ALTERED ACTIVATION OF THE COAGULATION SYSTEM DUE TO ENDOTOXIN TOLERANCE IN A PORCINE SHOCK MODEL
B. Horst, P. Kujath, K.-H. Staubach, A. Kooistra, S. Jonas
Medizinische Universität Lübeck, Lübeck, Germany

In an animal model the influence of endotoxin tolerance in septic shock and related alterations of the coagulation system were investigated. Endotoxin from Salmonella abortis equi H1178 was administered i.m. to mixed-bred pigs (900 g on day 1, 90 g on day 2 and 40 g on day 3 and 4) inducing an immunological response to endotoxin. After a week, the animals were narcotized and mechanically ventilated after tracheostomy. An endotoxin shock was initiated by continuous infusion of 2.5 mg/kg/h endotoxin.

Cardiovascular monitoring was done by measuring mean arterial pressure, pulmonary capillary wedge pressure and extravascular lung water by intrathoracic and intravascular catheters. The following laboratory parameters were measured: haemoglobin, leukocytes, thrombocytes, fibrinogen, AT III, PTT, Quick, Lactat, GOT, GPT, Creatinine, C3a, C4a, a2-macroglobulin, a1-antiplasmin, TAT, TPA, D-DIMER, a1-antitrypsin.

Compared to animals without previous endotoxin-application, the progress of the septic shock was prolonged and haemodynamic compensation occurred about 6 hours later. Activation of the clotting and fibrinolytic system was delayed, in the state of compensation the alterations were moderate showing slightly increased normal ranges. Parallel to haemodynamic decompensation with low cardiac output and peripheral vasodilatation C4a, AT III, a2-antiplasmin, leukocytes and thrombocytes decreased whereas TAT levels increased from initially 3364 ng/ml to 108, 97 ng/ml and D-DIMER from 2786 ng/ml to 2214 ng/ml. The decreased a2-antiplasmin levels indicate a lack of reactive fibrinolysis during decompensation of septic shock, whereas increased ranges of D-DIMER and TAT are an indicator for thrombin formation in the capillary system. Endotoxin tolerance leads to an prolonged immunological activation of the haematological response.

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CIRCULATING FIBRINOGEN OLIGOMERS IN HEALTHY HUMAN PLASMA PROBES
J. Stopc, A. Badorrek-Iser and R.E. Zimmermann

The prethrombotic state needs a quantification of involved plasma factors or metabolites to find safe ground for therapeutic measures. Fibrin or fibrinogen oligomers are plasma factors which possibly reach significance as prethrombotic plasma markers. In the present study fibrin and fibrinogen oligomers were investigated in plasma probes of healthy volunteers by agarose gel electrophoresis. The oligomers were visualized by immunoblotting with specific antibodies and evaluated by scanning laser densitometry. Both fibrin and fibrinogen oligomers were found. The fibrin oligomers were very low or not detectable in contrast to the presence of fibrinogen oligomers could be demonstrated clearly in the plasma probes (table).

Table: Fibrinogen oligomers in plasma obtained from healthy volunteers. VN = volunteer number

| V1 | V2 | V3 | V4 | V5 | mean | SD |
|----|----|----|----|----|------|----|
| %  | %  | %  | %  | %  | %    | %  |
| monomers | 86.1 | 85.8 | 86.3 | 88.4 | 71.3 | 83.6 | 6.2 |
| dimers   | 10.0 | 10.1 | 8.2  | 5.5  | 17.4 | 10.0 | 4.0 |
| trimers  | 3.8  | 3.4  | 4.3  | 3.3  | 8.6  | 4.7  | 2.0 |
| tetramers| 0.8  | 0.5  | 0.9  | 1.6  | 1.9  | 1.1  | 0.5 |
| pentamers| 0.2  | 0.0  | 0.2  | 0.0  | 0.8  | 0.3  | 0.2 |

Exercise increased the amount of fibrinogen oligomers depending upon exercise intensity and metabolic rate.

Physiologisches Institut der Westfälischen Wilhelms-Universität Münster, Robert-Koch-Str. 28, D4400 Münster

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FENOLDOPAM LACKS EFFECTS ON THE HEMOSTATIC SYSTEM IN VITRO
R. Lorenz, A. Clemens, C. Paschke, N. Tornieporth

In vivo, catecholamines cause a slight elevation of coagulation factors and of antithrombin demonstrating an activation of the haemostatic system. We studied the influence of a new selective dopamine-agonist fenoldopam (SmithKline Beecham) on coagulating whole blood in vitro. Two different concentrations of fenoldopam (160 ng/ml; FI, and 320 ng/ml; F2) were added to native whole blood, and in sequential samples (1-minute-intervals) the concentrations of fibrinopeptide A (FPA) as well as of platelet factor 4 (PF4) were measured.

The controls (n=6) showed a parallel curve of both parameters with initially low concentrations ("slow phase") followed by high values ("rapid phase") after 3.7+/-.03 minutes (FPA: x+/-. SEM) and 3.7+/-.02 minutes (PF4: 4). In both concentrations (each n=6), fenoldopam did not show any effect in vitro compared to the controls and to each other. The start of the rapid phase was for FPA after 3.7+/-.02 minutes (F1) and after 3.5+/-.02 minutes (F2), for PF4 after 4+/-.05 minutes (F1) and after 3.5+/-.02 minutes (F2), resp. (n.s. vs. controls). The addition of dopamine (200 ng/ml) led only initially to increased values of PF4 (4.4+/-.0.3 ng/ml) compared to the controls (5.3+/-.0.1; p: 0.05). The following concentrations of PF4 and the values of FPA were unchanged, compared to the controls.

In sum, fenoldopam does not activate the coagulation system and shows no platelet activating effect. Therefore, fenoldopam seems to be suitable for the treatment of patients with thrombotic risks.

II. Med. Klinik und Poliklinik, TU München, Klinikum rechts der Isar, Ismaninger Str. 22, 8000 München
DO NITROPRUSSIDE AND NITROGLYCERINE INFLUENCE COAGULATION NATIVE WHOLE BLOOD?
K. Lorenz, C. Paschke, N. Tornloepoh, R. Cle-
COMPARISON OF POSTOPERATIVE PLASMA HEPARIN ACTIVITY IN PATIENTS WITH AND WITHOUT MALIGNANCIES
J. Kußmann, U. Naglik, R. Weinel, M. Rothmund

Perioperative low dose heparin (LDH) prophylaxis is less effective in patients with malignant disease than in patients with benign disease. A difference in plasma heparin activity in patients with and without malignancies given LDH could be part of an explanation for that observation.

As a marker for heparin activity pre- and postoperative Thrombin Inhibiting Capacity (TIC) of patients plasma was assessed in vitro using a chromogenic substrate assay. The data of 25 patients with large bowel malignancies were compared with those of 25 patients undergoing cholecystectomy. All patients were given low dose heparin prophylaxis (3x5000 IU Na-Heparinat (Thrombophob)) for at least seven days postoperatively. Blood samples were drawn preoperatively before heparin administration and on days 1, 2, 4 and 7 postoperatively.

Before heparin administration there was no difference in TIC in patients undergoing surgery for benign or malignant disease. When the areas under the curves of post op TIC were compared for both groups TIC in patients with cancer surgery was significantly lower than in patients undergoing cholecystectomy (t-Test p = .0162).

Both groups of patients were comparable with respect to sex distribution, average weight and height. Patients with malignant disease were older and duration of operation was longer but both items had no separate influence on TIC. Though APTT values were slightly higher in patients with benign disease the difference was not significant. This was also true for platelet count, Antiplasmin and cryoglobulin levels. Plasma activity of AT III was similar in both groups before operation but significantly lower in patients with malignancies on days 1 and 2 postoperatively.

Klinik für Allgemeinchirurgie der Philipps-Universität Marburg, Baldingerstraße, D-3550 Marburg

Determination of Glycoproteins of Platelets in Patients with Acute Leukemia and Chronic Myeloproliferative Syndromes
H. Müller, I. Wulle, E. Gunsilius, H. Wankmüller, G. Heil, E. Seifried

Patients with hematologic systemic diseases often present with hemorrhagic diathesis. Plasmatic clotting disorders and defects in platelet function are responsible for such complications. The platelet membrane glycoproteins Ib and IIb/IIIa and the laminin- and thrombospondin(TSP)-receptors were investigated in 26 patients with acute myelocytic (AML; n = 21) and lymphatic (ALL; n = 5) leukemia, and in 10 patients with chronic myeloproliferative syndrome (c-MPS) and myelodysplastic syndrome (MDS). 13 apparently healthy volunteers were taken as a control group. Fluorescence-labelled MAbs were used in a FACScan: AN 51 (Dakopatts) for GP Ib, P 2 for GP IIb/IIIa, Gi 9 for the laminin-, and OKM 5 for the TSP-receptors (all Dianova).

In conclusion, a significant decrease in the expression of platelet membrane glycoproteins in c-MPS and in the different forms of acute leukemia can be observed. Further investigations are necessary to find out whether there are any differences between the subtypes of these diseases and to assess the importance of our observations for the clinical course of these patients.

Erwinia-Asparaginase alters the coagulation system less than E.Coli-Asparaginase
A.H. Sutor, C. Niemeyer, S. Sauter, J. Witt, K. Kaufmann, M. Brandis

In the ALL/NHL-BFM 90 protocol for treatment of childhood acute lymphoblastic leukemia (ALL) E.Coli-Asparaginase (COLI-ASP) is given from day 12 to 30 in a dose of 10,000 IU/m² every 3 days (protocol I). After one application of COLI-ASP on day 12 the following changes were observed in 14 children with ALL (abnormal pre-ASP values were caused by the previous corticosteroid-therapy from day 1 to 12): AT III dropped from 130±8% (pre-COLI-ASP, mean±SE) to 87±5%, plasminogen from 102±5% to 59±6%, protein C from 183±17% to 91±8%. These alterations were reversed after discontinuation of COLI-ASP therapy on day 30.

From the following data we have evidence that ERWINIA-ASP acts differently from COLI-ASP: 1) 3 patients with ALL, who received ERWINIA-ASP in protocol I did not show the above mentioned alterations of coagulation observed in the 14 patients treated with COLI-ASP. 2) In one patient COLI-ASP was replaced by ERWINIA-ASP with prompt reversal of the alterations of coagulation factors. 3) During re-induction (protocol ID), when ERWINIA-ASP is applied instead of COLI-ASP, the above mentioned alterations of coagulation factors are not observed.

From these data we conclude that ERWINIA-ASP affects the coagulation system less severe than COLI-ASP.

Universitäts-Kinderklinik, Mathildenhöhe, D-7800 Freiburg

GLYCOPROTEINS AND PLATELET FUNCTION IN CRYOPRESERVED AUTOLOGOUS PLATELETS AFTER TRANSFUSION TO PATIENTS WITH ACUTE LEUKEMIA
E. Seifried, H. Müller, M. Wiesneth, K. Köhner, I. Funke, M. Cardoso, H. Heimpel, B. Kubanek

A major problem in patients with acute leukemia is refractoriness to platelet transfusions, because multitransfused patients get alloimmunized and for some of them no histocompatible donors are available. In these patients no curative antileukemic therapy is possible. 14 autologous platelet transfusions were performed in 5 patients with acute myeloid leukemia (AML). Platelets were taken in remission of AML, cryopreserved for 1 - 5 months and retransfused in the therapy-induced thrombocytopenic phase. Platelet function and glycoproteins were determined in vitro in the platelet concentrates before, 1 h and 24 h after transfusion of autologous platelets.

| Glycoproteins | Platelet conc. (n=14) | 1 h post Transf.| 24 h post Transf. (n=8) |
|--------------|-----------------------|-----------------|------------------------|
|               | In remission          | Pre Transf.     | n.d.                   |
| Adhesion     | H. Moiler, I. Wulle, E. Gunsilius, H. Wankmüller, G. Heil, E. Seifried | 6±1 | n.d. |
| Glycoproteins | n.d.                  | 62±10           | 62±10                  |
|              | 34±13                 | 34±8            | 34±8                   |
|               | 67±25                 | 67±25           | 67±25                  |
|               | 80±17                 | 80±17           | 80±17                  |

Bleeding complications could be prevented in all patients. In conclusion cryopreserved autologous platelets function hemostatically and can be used even for complete refractory patients to continue a curative antileukemic therapy.

Medizinische Klinik und Poliklinik der Universität Ulm, Robert-Koch-Str. 8 und DRK-Blutspendezentrale, Heimholzstr. 10, 7800 Ulm, FRG

ERWINIA-ASPARAGINASE ACTS DIFFERENTLY FROM COLI-ASPARAGINASE
A.H. Sutor, C. Niemeyer, S. Sauter, J. Witt, K. Kaufmann, M. Brandis

In the ALL/NHL-BFM 90 protocol for treatment of childhood acute lymphoblastic leukemia (ALL) E.Coli-Asparaginase (COLI-ASP) is given from day 12 to 30 in a dose of 10,000 IU/m² every 3 days (protocol I). After one application of COLI-ASP on day 12 the following changes were observed in 14 children with ALL (abnormal pre-ASP values were caused by the previous corticosteroid-therapy from day 1 to 12): AT III dropped from 130±8% (pre-COLI-ASP, mean±SE) to 87±5%, plasminogen from 102±5% to 59±6%, protein C from 183±17% to 91±8%. These alterations were reversed after discontinuation of COLI-ASP therapy on day 30.

From the following data we have evidence that ERWINIA-ASP acts differently from COLI-ASP: 1) 3 patients with ALL, who received ERWINIA-ASP in protocol I did not show the above mentioned alterations of coagulation observed in the 14 patients treated with COLI-ASP. 2) In one patient COLI-ASP was replaced by ERWINIA-ASP with prompt reversal of the alterations of coagulation factors. 3) During re-induction (protocol ID), when ERWINIA-ASP is applied instead of COLI-ASP, the above mentioned alterations of coagulation factors are not observed.

From these data we conclude that ERWINIA-ASP affects the coagulation system less severe than COLI-ASP.

Universitäts-Kinderklinik, Mathildenhöhe, D-7800 Freiburg

| Antibody | AN 51 | P 2 | Gi 9 | OKM 5 |
|----------|-------|-----|------|-------|
| Healthy volunteers | 99±1 | 98±2 | 41±18 | 92±3 |
| AML | 46±26 | 66±28 | 64±25 | 57±28 |
| ALL | 57±32 | 63±25 | 36±39 | 54±28 |
| MPS/MDS | 47±27 | 79±15 | 47±23 | 85±23 |

Data as % of positive platelets in mean ± SD

In conclusion, a significant decrease in the expression of platelet membrane glycoproteins in c-MPS and in the different forms of acute leukemia can be observed. Further investigations are necessary to find out whether there are any differences between the subtypes of these diseases and to assess the importance of our observations for the clinical course of these patients.

Abt. Innere Medizin III, Universität Ulm, Robert-Koch-Str. 8, 7800 Ulm, FRG
Elevation of thrombomodulin and F. VIII R:Ag in patients after allogeneic bone marrow transplantation (ABMT)

C. Salst, E. Hölzer, B. Reinhardt, J. Hahn, C. Hüttel, M. Kolb, E. Hölzer

Graft versus host disease (GVHD) is normally seen in patients after ABMT. Endothelial cell injuries play an important role in the development of complications. Therefore it would be helpful to establish "endothelial markers". An elevation of F. VIII R:Ag in patients after ABMT correlated with severe microangiopathy was described earlier. On the other hand thrombomodulin is located on the endothelial cell surface and can be cleaved. For that reason thrombomodulin is discussed as a marker of endothelial cell lesion. We studied thrombomodulin (TM) and F. VIII R:Ag levels in patients undergoing ABMT.

Materials and methods: Samples of citrated blood were taken from 5 patients on day -8, -5, -1, 0, 7, 14, 21, 28 and 35 before and after ABMT, respectively. Soluble TM was studied by an immunoassay based on monoclonal antibodies (Asserachrom Thrombomodulin, kind gift of J. Amrila, Serbio, Genneville, France). F. VIII R:Ag was measured by a commercially available assay (ELISA, Behring, Marburg).

Results: The TM levels were normal or slightly elevated before ABMT (61.3 +/- 20.4 ng/ml) and were increased between day 7 and 35 (105.6 +/- 45.4 ng/ml). The highest levels ranging from 75.6 ng/ml before to a mean of 145.4 (+/-18.6) ng/ml between day 7 and 35 were found in a patient with severe complications reaching a peak of 223.7 ng/ml one day before death. F. VIII R:Ag was slightly elevated before ABMT (139.4 +/-27.8%) and rose to a mean of 261.4 (+/-107.2%) between day 7 and 35. We conclude that elevated levels of TM are found in patients after ABMT. As well as F. VIII R:Ag seem to be connected with endothelial cell damage in ABMT. Studies in greater number of patients are under way.

Med. Klinik III des Klinikums Großhadern, Ludwig-Maximilians-Universität, Marchioninistr., 8 München 70

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Monitoring of platelet function in thrombocytopenic patients before and after transfusion of platelet concentrates

E. Stoczek, J. Groh (*) and M.A.A. Kratzer

There is no reliable test to monitor platelet function in thrombocytopenic patients. We developed the following techniques to improve the indication for and the monitoring of platelet transfusion in these cases.

1. The ex vivo bleeding time (BT) was determined with a patient sample of anticoagulated blood (Na-citrate 1:10) using the Thrombostat system (VDG von der Goltz, Seeon). To increase the sensitivity, standard pressure was reduced to < 40 mm Hg. 2. Measurement of the quality of platelet concentrates: platelet count was adjusted to 300 000/µl. 20 µl isotonic CacCl3 (Heilige) and 3.5 µl Fregmin (0.28 mg, Primaver, Kabl) was added to 200 µl of platelet suspension. ADP-induced aggregation was then determined with a platelet aggregometer (Biodata, Hatboro). 3. Prediction of the in vivo function of a platelet concentrate: Anticoagulated blood (Na-citrate 1:10) of the patient was added to the concentrate of the donor in the ratio (patients whole blood volume) / (expected transfusion volume) and the BT determined in the mixture. 4. Measurement of BT in a sample of anticoagulated blood drawn from the patient after transfusion of the platelet concentrate.

In 12 patients, who underwent bone marrow transplantation, we predicted BT correlated with the true one (R = 0.89).

Institut für Klinische Chemie und Anästhesiologie (*) der Universitäts-Klinikum Großhadern, Marchioninistr 15, D-8000 München 70

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ACTIVATION OF COAGULATION AND NEUTROPHILS IN LUNG CANCER PATIENTS R. Seitz, W.-H. Dürner, M. Zehrer, C. Görg, K. Havemann, R. Egbring

Background: Thromboembolic or haemorrhagic complications are known to occur frequently in cancer patients, though only rarely coagulation disturbances are found. Within tumor tissues, histochemical and experimental data show extensive fibrin formation, which is assumed to support neoangiogenesis and tumor growth.

Methods: The plasma levels of TAT, ATIII, fibrinogen (FBg), neutrophil elastase-α-antitrypsin complexes (ELP), as well as serum LDH as nonspecific tumor parameter were assessed in 74 lung cancer patients. The disease was localized to the thorax region (LD) in 9/37 patients with small cell (SCLC), and in 17/37 patients with non-small cell (NSCLC) lung cancer, the other patients had distant metastases (ED II).

Results: The TAT levels were elevated (>4.0 µg/l) in 15/37 SCLC and 13/37 NSCLC patients; neither the median TAT values 3.4 vs. 3.8 were significantly different between SCLC and NSCLC, nor AT III 96 vs. 96, nor FBG 433 vs. 520. ELP was elevated (>35 µg/l) in 32/37 SCLC and 35/37 NSCLC patients. There were no significant differences between localized and metastatic disease, with the exception of LDH (p = 0.021) in SCLC. In SCLC patients, correlations were found between ELP and LDH (r = -0.75, p = 0.002) and ELA and TAT (r = 0.38, p = 0.02), and in localized disease between TAT and AT III (r = -0.84, p = 0.008); in NSCLC there was a weak correlation between ELP and AT III (r = -0.38, p = 0.067).

Conclusions: An activation of coagulation is demonstrable in lung cancer patients; however, this study failed to demonstrate any relation to histologic type or tumor spreading. Enhanced ELP release from neutrophils in the majority of lung cancer patients points to inflammatory processes associated with the tumor or infectious complications. Systemic alterations of FBg and AT III appeared to be related rather to these inflammatory processes than to the activation of coagulation, suggesting that coagulation takes place predominantly within the tumor tissues.

Zentrum Innere Medizin der Universität, Baldinger Straße, 3550 Marburg

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QUANTITATIVE DETERMINATION OF SURFACE ANTIGENS, PROTEASES AND CELL CYCLE (S-PHASE AND PLOIDY) IN HUMAN BREAST CANCER CELLS IN COMPARISON TO NORMAL CELLS:

E. Schröder, M. Schmitt, N. Monlwa, N. Harbeck, H. Hölzer*, F. Jäncke 55, Raut and H. Graef.

Frauenklinik und *Pathologisches Institut der Technischen Universität München, und Frauenklinik der Universität Heidelberg, Germany.

We have developed a quantitative flow cytometric protocol which allows to measure simultaneously tumor-characteristic antigens, cellular proteases and the cell cycle (s-phase and ploidy status). Mechanical dissection of breast cancer tissues was preferred over enzymatic dissolution as we found that treatment of breast cancer tissue with proteases (collagenase II, trypsin, elastase from neutrophils in the majority of lung cancer patients) points to inflammatory processes associated with the tumor or infectious complications. Systemic alterations of FBg and AT III appeared to be related rather to these inflammatory processes than to the activation of coagulation, suggesting that coagulation takes place predominantly within the tumor tissues.

Zentrum Innere Medizin der Universität, Baldinger Straße, 3550 Marburg
DNA FLOW CYTOMETRY IN PRIMARY BREAST CANCER: CORRELATION OF PROGNOSTIC FACTORS TO S-PHASE-VALUES AND PLOIDY-STATUS.

N. Harbeck, M. Schmitt, N. Moniwa, E. Schüren, H. Höller*, F. Jäncke and H. Grooff.
Frauenklinik und *Pathologisches Institut der Technischen Universität München, Germany.

Due to differing techniques applied, the prognostic value of DNA-ploidy and S-phase value is still matter of debate in primary human breast cancer. We have developed an improved technique for the preparation of pure nuclei from formalin-fixed, paraffin-embedded breast cancer tissue sections (Harbeck et al., Gyn. Rundschau 31, in press, 1991) based on Hoechst's method (1993). DNA profiles were measured on a FACSscan and nucleus preparation controlled by confocal laser microscopy. Nuclear of peripheral blood lymphocytes and chicken erythrocytes served as internal controls.

Paraffin sections of 155 primary breast cancer patients were evaluated for ploidy and S-phase fraction. 40 tumors (26%) were diploid, 115 tumors aneuploid. In 36 (90%) of the diploid tumors the S-phase could be measured. A cutoff (median S-phase) of 5% was found: 21 (59%) had a low and 15 had a high S-phase. No correlation was found between established histomorphologic prognostic parameters and ploidy status or high S-phase except for an inverse relation between estrogen receptors status and S-phase in diploid tumors: 84% of tumors with a low S-phase were estrogen-receptor positive, but only 53% of tumors with a high S-phase. Of the patients with diploid tumors and a high S-phase, 4 patients have relapsed and 3 have died (median follow up of 24 months) compared to none of the patients with diploid tumors and low S-phase.

Currently, we are also correlating the S-phase fraction and ploidy status with new prognostic factors which are involved in tumor cell invasion and metastasis. These are the proteases cathepsin D and the urokinase-type plasminogen activator (uPA) and the urokinase-inhibitor PAI-1. These three proteins are independent factors for the prediction of the outcome of the breast cancer disease (Jäncke et al., Sem. Thromb. Haemost. 17, 303, 1991).

FLOW CYTOMETRIC ANALYSIS OF TUMOR-ASSOCIATED PROTEASES IN SINGLE BREAST CANCER CELLS DERIVED FROM TUMOR BIOSPSES.

N. Moniwa, M. Schmitt, N. Chucholowski, E. Schüren, S. Kauf*, F. Jäncke and H. Grooff.
Frauenkliniken der Technischen Universität München and der Universität Heidelberg, Germany.

Tumor-associated proteases such as the urokinase type plasminogen activator (uPA), plasmin and cathepsin B, D, L are of clinical relevance for tumor cell spread and metastasis. These are the proteases cathepsin D and the urokinase-type plasminogen activator (uPA) and the urokinase-inhibitor PAI-1. These three proteins are independent factors for the prediction of the outcome of the breast cancer disease (Jäncke et al., Sem. Thromb. Haemost. 17, 303, 1991).

We have devised techniques which allow, by flow cytometry or confocal laser scan microscopy, the rapid and quantitative single-cell analysis of living or fixed tumor cells in a heterogeneous cell suspension derived from fresh tumor biopsies. uPA, cathepsin B and D, as well as the inhibitor PAI-1 and the receptors for uPA and EGF were identified by antibodies directed to the respective antigens. These results were confirmed in parallel by immunohistochemistry on tissue sections or cytopsins.

Functional uPA-R on living cells was quantified by applying FITC-pro-uPA as the specific ligand (real-time analysis; Schmitt et al., Sem. Thromb. Haemost. 17, 291, 1991; Kobayashi et al., J. Biol. Chem. 266, 5147-5152, 1991). The antigen specificities of the respective antibodies were confirmed by assessing established tumor cell lines, fibroblasts and peripheral blood cells.

Our setup of experiments also allowed to determine simultaneously DNA-ploidy and S-phase as marker of cell proliferation. Both, measurement of the cell cycle and determination of proteases of prognostic relevance for the course of the breast cancer disease. The established experimental design thus should allow a precise analysis of markers of proliferation and cell invasion within hours after tumor resection. This should enable the clinician to select patients for individualized adjuvant hormone or chemotherapy.

INCREASED THROMBIN GENERATION AS A MARKER OF PROGRESSIVE DISEASE IN COLORECTAL CARCINOMA.

H. Ostermann, K. Malotki, A. Steinkamp, J. Kienast.

Systemic activation of the coagulation cascade is commonly found in colon cancer. This is probably mediated by a soluble procoagulant activity released by the tumor. We initiated a prospective study to investigate the association of changes of hemostatic parameters to disease progression in patients with colorectal cancer.

Patients were recruited at diagnosis, prior to initial therapy. If resection was not possible or if disease recurred, patients were treated by chemotherapy, Blood for hemostaseological studies was collected at diagnosis and during regular follow-up examinations.

39 patients had entered the study at the time of the present analysis. They have been investigated on a total of 45 occasions. Patients were stratified according to disease status at the time of blood sampling. Those with favourable response to therapy were either in complete (CR; n=4), or partial remission (PR; n=2) or no change (NC; n=4). Patients with a poor response to therapy had progressive disease (PD; n=25) or early death (ED; n=9).

We analysed citrated plasma samples for thrombin-antithrombin III complex (TAT, ELISA, Behringwerke) and prothrombin fragment 1+2 (F1+2, ELISA, Behringwerke).

Significantly (p<0.05) increased levels of TAT as well as F1+2 were found in patients with poor response compared to those with favourable outcome.

Mechanism of Pancreatic Carcinoma Cell Induced Platelet Aggregation

E. Heinmöller, R.J. Weinel, A. Bittinger*, D. Erb, M. Rothmund

The aggregation of host platelets by circulating tumor cells (TCIPA) is regarded to be an important step in the metastatic process. Studies with tumor cell lines from different origin revealed at least 4 pathways by which tumor cells might induce platelet aggregation, while whole mechanism is not fully understood as yet.

Furthermore, there is only little known about the interaction between pancreatic carcinoma cells and platelets. We studies TCIPA in two cell lines of ductal human pancreatic cancer (PC3, PC44). Both cell lines were able to induce the aggregation of human platelet rich plasma in vitro. The aggregation could not be inhibited by antibody, ASS or collagenase, suggesting that it was not ADP, thromboxane or collagen-dependent. Pretreatment of the tumor cells with trypsin or neuraminidase had no effect on TCIPA, showing that trypsin - or neuraminidase sensitive surface proteins were not involved in TCIPA. Hirudin, a thrombin-antagonist and treatment of tumor cells with phospholipase A2 and C completely abolished TCIPA, showing that TCIPA in pancreatic cancer is a thrombin-dependent process and that the thrombin generating activity might be a phospholipase-containing complex on the tumor cell surface. Furthermore pretreatment of tumor cells with RGD and antibodies (anti GPllb-IIIa anti-<VLA5 and anti-VLA6) blocking receptors for adhesive proteins on the cell surface had no effect on TCIPA. This indicates that pancreatic carcinoma cell induced platelet aggregation does not require direct tumor cell-platelet contact. These results were confirmed by electronmicroscopic studies. We conclude that pancreatic carcinoma cells induce platelet aggregation through thrombin activation without direct tumor cell-platelet contact.

Department of Internal Medicine, University of Münster, Albert-Schweitzer-Straße 33, D-4400 Münster
CHANGES IN HAEMOSTASIS ASSOCIATED WITH ISOLATED HYPERTERMIA OF EXTREMITIES IN MELANOMA PATIENTS

U.T. Seyfert, G. Omlor, P. Feindt, A. Jäger-Hamberger, E. Wenzel

Up to now, effects of hyperthermia on haemostasis have been studied only in a small number of cases where just global coagulation tests were performed.

Aim of the study: Effects of local hyperthermia on the coagulation system - possible interactions with additional local perfusion with high dosages of cytostatics (cis Platin).

Methods: n = 6 patients with melanoma located in the distal two thirds of the extremities. Blood samples were taken at different times (before hyperthermia, 20 min after onset of hyperthermia, end of hyperthermia - after therapy with cytostatics, 1. p.o. day) - venous and arterial line, systematically.

Results:
1. During hyperthermia there was an excessive activation of thrombin generation (TAT, Dimers, FbDP, TDP) despite controlled heparinization.
2. There was a significant increase of thromboxane/Prostacyclin ratio after onset of hyperthermia with a maximum after end of hyperthermia. These changes could be correlated with an increased pulmonary capillary resistance (p<0.001).

Conclusion: In connection with changes of the arachidon metabolism possible effects on occurrence of vasospasm and on the pharmokinetics of an cytostatic agent applied have to be considered.

Abt.f. Klin. Hämoustaseo. und Transfusionsmedizin, Universitätsklinik, D-6650 Homburg/Saar

EXPRESSION OF TISSUE FACTOR AND THROMBOMODULIN BY KAPOSI SARKOMAS

T.Y. Zhang, A. Bierhaus, P. Kern, R. Kaufmann, M. Bevilacqua, M. Dietrich, R. Ziegler, R. Waldherr, P.P. Nawroth

Some evidence points to the origin of Kaposis deromas (KS) from endothelial cells. To test this hypothesis we tested the endothelial cells markers thrombomodulin (TM), Tissue Factor (TF), ELAM-1, and INCAM-110. The expression of protein was evidenced by immunofluorescence, the expression of RNA by Northern blotting and in situ hybridisation, showing positive staining for KS cells, but not surrounding tissue. In addition we looked at the expression of TF and TM in the normal vessels surrounding KS or in normal skin of HIV infected patients. Vessels in the skin of HIV infected patients showed positive staining with TM and was indistinguishable from normal skin. However normal vessels surrounding KS were partly positive for TF, especially in areas of adhering leukocytes.

In conclusion: KS express TM, which gives further evidence to the close relation of KS to endothelial cells. Expression on ELAM-1 and INCAM-110 by KS suggests a possible interaction of KS with leukocytes. The TF expression of endothelial cells in normal vessels in proximity to KS indicates that endothelial cells can express TF in vivo and that KS cells can modulate surrounding endothelium of normal vessels.

Univ. Heidelberg, Univ. Ulm, BNI Hamburg

ELEVATED LEVELS OF PROTHROMBIN FRAGMENT 1+2 IN NEUTROPENIC PATIENTS WITH THE SEPSIS SYNDROME TREATED WITH GM-CSF

R. Kuphal, U. Riedel, M. Rotheburger, J. Kienast, H. Ostermann

Coagulation and fibrinolytic parameters were analysed in patients with acute myelogenous leukemia (AML; FAB M1-M6) undergoing treatment with intensive chemotherapy.

Patients were randomized for supportive application of human recombinant GM-CSF (Granulocyte-Monocyte Colony Stimulating Factor). 27 patients, receiving a total of 29 courses of chemotherapy, were recruited for the study. 11 Patients developed a sepsis-syndrome according to the definition of Bone et al (N Engl J Med 1989;317:653).

Blood collection was started before chemotherapy and repeated at 4 day intervals. If body temperature exceeded 38.5°C blood was obtained on days 1-2-4-7-11 of the febrile episode. Thromboplastin was collected at 4 day intervals until the WBC increased to >10000/ml.

Prothrombin fragment 1+2 (F1+2) levels were measured using an ELISA (Behring, Enzymuntest).

In 21 patients the development of febrile temperatures coincided with an increase in F1+2. No increase in F1+2 was found in patients without fever (n=4).

In patients treated with GM-CSF, F1+2 values were significantly elevated (2.7 nmol/l) as compared to untreated patients (2.0 nmol/l); p < 0.05.

There was no correlation between WBC and F1+2 values.

We conclude that GM-CSF treatment is associated with activation of coagulation as expressed by increased F1+2 levels.

Department of Internal Medicine, University of Münster, Albert-Schweitzer-Straße 33, D-4400 Münster

CORTICOSTEROIDS CAUSE MULTIPLE CHANGES OF COAGULATION FACTORS

A.H. Sutor, C. Niemeier, S. Sauter, J. Witt, K. Kaufmann, M. Brandis

In the ALL/NHL-BFM 90 induction therapy (protocol I) for treatment of childhood acute lymphoblastic leukemia (ALL) prednison (PRED 60 mg/m2/d) is given during induction therapy from day 1 to day 29 and then tapered, ASP (10,000 IU/m2 every 3 days) from day 12 to 30.

Prior to the initiation of ASP therapy the following changes were observed among 14 children newly diagnosed with ALL between day 1 and 12: fibrinogen dropped from 319±31(mean±SE) mg/dl to 146±21 mg/dl. AT III increased from 112±7% to 130±8%, protein C increased from 96±8% to 183±17%, plasminogen remained unchanged (102±5% to 102±5%). These changes were most likely caused by corticosteroids, although other factors, like decrease of leukemic blasts and treatment with vincristin and daunorubicin have to be considered. However, the same changes were observed during week 21, when corticosteroids (Dexamethason 10 mg/m2/d) were given during re-induction therapy (protocol II).

From these data and from our earlier results indicating that PRED reduces capillary resistance and bleeding intensity in vivo (Europ J Paediatr 129:67-72, 1978) we conclude that corticosteroids cause multiple and severe changes of coagulation factors which may increase thrombotic and/or hemorrhagic risks in patients.

Universitäts-Kinderklinik, Mathildenhstr. 1, D-7800 Freiburg
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QUANTITATIVE ASSESSMENT OF THE UROKINASE-TYPE PLASMINOGEN ACTIVATOR (UFA) AND ITS RECEPTOR (UFA-R) ON TUMOR CELLS.

M. Schmitt, N. Chucholowski, N. Moniwa, E. Schüren, F. Jörncke and H. Große.

Fraunhofer der Technischen Universität München, Germany.

The importance of elevated levels of the tumor-associated serine protease uPA (urokinase-type plasminogen activator) for tumor cell invasion and metastasis has been established for solid cancers of the breast, ovary, and colon. Determination of UFA antigen in breast cancer tissue is of predictive value for the course of the disease (independent prognostic factor) and UFA-R expression of those patients having a low or high risk of relapse or death, irrespective of the established risk factors (Jörncke et al., The Lancet, 1989). We have recently established techniques and devised protocols for the quantitative assessment of both, UFA antigen and UFA-R, in human tumor cells applying ELISA, immunohistochemistry, flow cytometry or confocal laser microscopy.

UFA-antigen:
ELISA based on mAbs #377 and 594 (American Diagnostica) allows very sensitive detection (< 1 pg / ml) of UFA in tumor tissue extracts. Immunohistochemistry based on mAbs #554 or 5559 (American Diagnostica) yields sensitive staining of formalin-fixed paraffin-embedded tissue sections (APAAP-Technique). Flow cytometry is used to assess receptor-bound or internal UFA in living or fixed tumor cells applying mAbs directed to the B-chain of UFA. Alternatively, UFA can be quantified and localized on tumor cells by confocal laser microscopy (CLSM, Leica).

UFA-receptor:
Flow cytometry is applied using a mAb to the second domain of the UFA-R which is not involved in UFA binding. This allows simultaneous binding of 1) the mAb to UFA-R and 2) FITC-labeled probes to UFA. This approach discriminates between free and ligand-occupied UFA-R. The mAb to UFA-R can also be used on breast or colon cancer tissue sections to detect UFA-R(+) tumor cells. In order to establish new reagents, which eventually may be used for nontoxic receptor-specific tumor therapy, these techniques are currently applied to demonstrate efficient inhibition of the UFA / UFA-R interaction by means of 1) mAbs directed to UFA or UFA-R, 2) competitive synthetic or recombinant UFA-analogues and 3) recombinant UFA-R.

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A SPECIAL VON WILLEBRAND CONCENTRATE FOR THE TREATMENT OF VON WILLEBRAND’S DISEASE.

E. Aygelen, L. Hatterbach, Zs. Vigh, I. Scherner

Univ. Hospital, Dept. of Angiology, B Frankfurt/Main, Germany.

Heamostasis HS, Cryoprecipitates and DDAVP are the drugs of first choice in the treatment of von Willebrand’s disease (VWD). Especially the posttransfusion antihaemophilic concentrate Hemostase HS has been shown to be effective in the correction of the bleeding defect in VWD.

A von Willebrand factor concentrate was developed by the Behring-Werke (Marburg, Germany). It is a posttransfusion F.VIII preparation of high purity. We investigated the in vitro properties in 2 lots. The product contained 3.1 U porcine factor Vlll:C, 36 K U F IX: Ag and 155 U ristocetin cofactor activity/ml. F. IX:Ag, F. VIII:C was 5.21, RCo/F.VIIa:C and RoC/F. Ag was 5.0 and 9.89. The quotient RoC/F.VIII:C with 50 was strikingly high. In the majority of preparations presently available we found this quotient much lower. The multimeric distribution of F IX in this product was similar to the pattern of normal plasma. The half-life of the new product will be demonstrated.

To 3 patients with VWD type I, IIa, III and III, the new concentrate was given in a dosage of 50 RoC/F. Ag bw. A complete and lasting correction of the bleeding time (according to Milkus) could only be seen in 2 type I and 1 type IIb-VWD. In the remaining type IIa - and III-VWD a correction of the bleeding time either was very short (30 min) or was not observed at all. In those 3 patients, who did not at all respond to the infusion of F IX concentrates in terms of bleeding time, a competition with Hemostase HS in the usual dosage (40 F.VIII:C/Ag/kg bw) was made. In 2 of those patients (type IIa-VWD) a correction of the bleeding time could be achieved by Hemostase HS in the usual dose, whereas a higher dosage (60 F.VIII:C/Ag/kg bw) was needed in a type III-VWD to get the same result.

Further investigations are necessary to improve this new product in order to become an effective alternative to DDAVP, cryoprecipitates or Hemostase HS.

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BIOCHEMICAL QUALITY PROFILE OF THE HIGH PURITY F IX CONCENTRATE IMMUNINE.

A. Weber, A. Schoppmann, M. Sazgary, F. Hondl, Y. Lignau

A new high purity factor IX concentrate (IMMUNE) in PEG: IMMUNE+ STIM plus) has been developed with a specific activity of 1000 IU/mg protein and is virtually free of factors II, VII and X. Purification includes chromatography by ion exchange and hydrophobic interaction (Weber et al., 1991). IMMUNE has been designed to be non-thrombogenic with an increased safety margin with respect to virus transmission.

Preclinical and clinical data suggest a very low thrombogenicity, if not absent altogether, while pharmacokinetic data were found to be within the range given in the literature (Anderle et al., 1992).

In this study, apart from the routine analytic test program being run in accordance with the Europ. Pharmacopoeia, additional tests were performed to study the composition and F IX integrity of IMMUNE lots containing low amounts of heparin (Sigma/100IU F IX) and of one lot free of heparin. No difference was seen in the properties of IMMUNE lots with and without heparin. Specific activity was >100 IU/mg in all of the lots tested. Factor IX antigen made up 68% of the total protein with a ratio of F IX Ag/F IX:C activity between 1.08 and 1.27. Using Superose gel filtration, 98.2% of F IX:Ag were eluted as a single peak. The major protein other than F IX was inter-alpha-trypsin inhibitor, an inert protein with no apparent physiological impact. Per 100IU of F IX:C, 0.15IU of F II, and 0.8IU of F X activities were found. Thrombinase activity in heparin-free thrombin was not found to be present in IMMUNE either enzymatically (highly sensitive clot formation test) or immunologically (modified TAT ELISA test). Prothrombin Fragments F1+2 were below plasma level (78±110IU/100IU F IX:C).

Comparative analysis including a number of other (purified) F IX preparations indicated large differences in some of the biochemical characteristics.

IMMUNE AG, Industriestr. 67, A-1220 Vienna, Austria.

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REDUCTION OF THE THROMBOGENIC RISK THROUGH SPECIFIC PURIFICATION IMMUNE STIM PLUS - A HIGHLY PURIFIED FACTOR IX CONCENTRATE.

K. Anderle, A. Schoppmann and G. Brodl

Clinical application of prothrombin complex concentrates has caused thromboclastic complications, above all in patients at high risk for thrombosis and in surgical procedures.

The following mechanisms seem to be involved:
1. Release of prothrombin activated clotting factors (Ila, VIIa, Xa and/or Xa) are formed during the manufacturing process.
2. Large quantities of synagogs the recipient already has in adequate amounts in his circulation are administered.
3. Through previous infections such as hepatitis or HIV endothelial cells or vessels are already damaged or prirised.

Despite the recommendations of the Task Force for Prothrombin Complex Concentrates in 1974 and 1976 to add heparin to the final product or to treat the recipient with heparin for the prophylaxis of thromboclastic complications the situation has not improved, according to a report by Lamb at the ISTH (SSC) meeting in Amsterdam in 1991. The risk for thrombotic complications seems to be associated with all brands of prothrombin complex concentrates, regardless of the addition of heparin.

The fact that up to now the thrombogenic risk could be reduced only surgically and that morbidity and mortality among the patients involved is quite high, necessitates further development in this field. Risk of thrombosis (as well as infection) must be significantly reduced in future preparations. To minimize these risks factors, higher purification of the clotting factor IX without its activation as well as removal of synagogs, which the patient does not need, is recommended.

IMMUNE has developed a new factor IX concentrate which contains only traces of factors II, VII, X and Xa and has a specific activity of 100-150 IU/mg of protein. The objective was to make available a product which is less thrombogenic than conventional prothrombin complex concentrates. Preclinical data obtained in the Wesler stasis model and first clinical applications were thus far those that the new preparation is highly efficacious and that the risk of thrombosis could indeed be minimized.

IMMUNE AG, Industriestr. 67, A-1220 Vienna, Austria.
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PURIFIED FIX AND FIX COMPLEX PRODUCTS: PARAMETERS INFLUENCING THROMBOGENIC POTENTIAL

K. Koltzscheke, G. Harbauck

Thrombotic complications of Factor IX complex concentrates (PCCs) and purified Factor IX concentrates (FIX) have been reported in 64 cases (ICTH FVIII and FIX Subcommittee, June 1991). No specific parameter responsible for the thrombogenic potential in these preparations has been identified up to now. The Subcommittee determined that the 1975 recommendation of the ICH that heparin be added to reconstituted PCCs shall not be extended to FIX. Commerciably available PCCs and FIX products do still contain heparin in concentrations ranging from 5 to 15 I.U./ml (Table 1). Factors possibly responsible for adverse side effects leading to DIC or thrombotic episodes after application of PCCs or FIX are those which give rise to fibrinopeptide A (a measure of thrombin activity) or prothrombin fragment F1+2 (a measure of Factor IX activity). Prothrombin fragments F1+2 in PCCs and FIX ranged from 1.65 to 42.60 nmol/1 (Tab.). Factors probably influencing the thrombotic potential of PCCs and FIX are Heparin, Protein C, Protein S and the enzymatic activity measured with the chromogenic substrate S-2238 (Table 2).

From the various animal models proposed for the detection of thrombogenic characteristics of FIX and NaPPS we used rats and rabbits.

Purified FIX (No. 1-5) and FIX complex (No. 6-7) products: parameters influencing thrombotic potential

| Product | FIX | Protein C | Protein S | S-2238 | Proth. F1+2 |
|---------|-----|-----------|-----------|--------|------------|
| 1       | 7   | 0         | 0.6       | 0      |
| 2       | 9   | 25.3      | 3.8       | 19     |
| 3       | 10  | 32.8      | 7.5       | 103    |
| 4       | 9   | 0.3       | 0.3       | 0      |
| 5       | 9   | 40.2      | 1.1       | 65     |
| 6       | 13  | 34.7      | 3.8       | 260    |
| 7       | 12  | 32.0      | 5.2       | 210    |
| 8       | 13  | 27.2      | 13.1      | 143    |
| 9       | 5   | 32.1      | 21.7      | 64     |

Biotest Pharma GmbH, Landeteinstr. 5, D-6072 Darmstadt

Abt. für Klin.-eXz. Chirurg., Chirurg. Uni-Klinik, 6650 Homburg/Saar

Biotest Pharma GmbH, Landeteinstr. 5, 6072 Darmstadt

Abt. für Klin.-eXz. Chirurg., Chirurg. Uni-Klinik, 6650 Homburg/Saar

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EFFECT OF SODIUM PENTOSAN POLYSULPHATE ON THE THROMBOGENICITY OF PROTHROMBIN COMPLEX CONCENTRATES

H.-P. Klöcking 1, G. Dornheim 2 and H. Schulze-Riewald 1

Earlier studies have shown that the thrombogenicity of prothrombin complex concentrates (PCC), which may be reduced by previous administration of pentosan polysulphate sodium (NaPPS) using the stability model according to Wessler in rats (H.-P. Klöcking et al, Folia Haematol 115:132,1988). The objective of this study was to compare the degree of thrombogenicity of PCC prepared in the presence of NaPPS (PCC I) to that where NaPPS was subsequently added to already fixed PCC (PCC II) in the stability model acc. to Wessler in rats.

No reduction of thrombogenicity was found during the in vivo thrombogenicity test with PCC I. The ED50 value effective dose at which thrombus formation was found in 50% of the experimental animals was 53 (30-93) U FIX/kg and thus below the tolerable ED50 of > 100 U FIX/kg. After administration of 200 U FIX/kg of PCC I the score of thrombus formation was 3.0.

PCC II led either to reduced size of thrombi or completely prevented thrombus formation, depending on NaPPS concentration in the stability model in rats. Complete prevention was yielded by addition of 1.5 mg NaPPS to PCC II.

The studies revealed that the thrombogenicity of PCCs may be prevented by adding small amounts of NaPPS after the manufacturing procedure of PCC had been finished.

Institute of Pharmacology and Toxicology 1, Medical Academy Erfurt, Nordhäuser Str. 74, 0-5010 Erfurt and Institute of Blood Transfusion 2, Albert-Schweitzer Str. 14, 0-6013 Suhl, FRG.

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PURIFICATION OF COAGULATION FACTOR PRODUCTS USING MONOCLONAL ANTIBODY TECHNOLOGY

F. Feldmann, S. Chandra, C.C. Huang, R.L. Weeks, M.S. Kielkamp, M.E. Hindia and A.B. Schreiber

Highly pure coagulation Factor VIII (Monoclate-P™) and Factor IX (Mononine™) have been prepared for the treatment of Hemophilia A and Hemophilia B, respectively. Monoclate-P (Purified) human Factor VIII:C, licensed in the USA and many European countries, is purified from plasma by monoclonal antibody-immunoaffinity chromatography. The product is puréized in full liquid form at 60°C for 10 hours, similar to human albumin, to inactivate viral contaminants potentially present in human source plasma. Purification results in a highly purified FACTOR VIII:C of specific activity 3000-4000 U/mg protein. Monoclate-P™ exhibits highly stable potency during processing as well as in final dosage form. Effective stabilization of the preparation results in retention of potency evidenced by identical assay results using one stage APTT, two stage TGT, or by synthetic substrate assay.

Monoclonal Factor IX (Mononine™) is also purified by immunoaffinity chromatography. A pure product is obtained with a specific activity of about 3000 U/mg. Only coagulation Factor IX is contained in the preparation with no contamination by Factors II, VII or X as found in prothrombin complex concentrates currently utilized in therapy. The concentrate is prepared in lyophilized form and reconstitutes to 100 Factor IX units per ml. No stabilizer albumin is utilized and the purity is confirmed by electrophoresis or HPLC. The product does not contain heparin. Sensitive bioassay and bioassay-assay demonstrated the absence of thrombogenic potential in the preparation. These include testing for Factor IXa and other Factor IX degradation components as well as evaluating the APTT and Wessler assay at high concentrations. Advances in methods for viral inactivation are used. HIV-1 is inactivated by a combination of steps and shows 8 log inactivation by sodium thiosulfate and 1 log removal by ultrafiltration. Model viruses are removed by 6-8 logs.

Armour Pharmaceutical Company, Kankakee, IL 60901, USA

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ACQUIRED FACTOR VIII:C INHIBITORS IN NON-HEMOPHILIC PATIENTS

D. Schönherr 1, B.M. Kuntz 2, C. Specker 3, A. Wehmeier 1, C. Aul 1, T. Südhoff 1 and W. Schneider 1

About 5-15% of hemophiliac patients develop antibodies to factor VIII:C and become refractory to factor VIII:C-infusions. In rare cases factor VIII:C inhibitors also develop in non-hemophiliac patients. These antibodies are of IgG class. For the time being, treatment strategies are still controversial. Although there are reports that these antibodies may disappear spontaneously, the clinical course is characterized by several hemorrhages in the majority of patients. Eight non-hemophiliac patients (three men and five women aged between 21 and 80) with acquired factor VIII:C inhibitors were studied at our clinic so far. In all cases bleeding tendency started suddenly after inadequate injury or surgery. Disorders that may be combined with the acquired inhibitor were found to be systemic lupus erythematosus, rheumatoid arthritis, asthma and infectious diseases caused by viruses or bacteria.

Initial clotting tests revealed a prolonged aPTT (> 80 sec) and a decreased F VIII:C level (< 5%). In seven patients other deficiencies of blood-clotting factors and acquired or hereditary von Willebrand's disease were excluded. In only one case an additional F I:C-deficiency was found. Measurement of F VIII:C-Inhibitor levels (Behring Units, BU) revealed values in the range between 9 and 108 BU. In all cases the correlation between aPTT, FVII:C and FVIII:C inhibitor levels was low and not predicable as in hemophiliac patients with inhibitors. Replacement therapy necessary to stop bleeding was done with FVIII:C concentrates and fibrin. The four patients subjects to long-term therapy with a combination of prednisone and azathioprin as well as the two patients treated with prednisone and cyclophosphamide responded positively. After starting immunosuppression the inhibitor vanished within 5 weeks. Two patients treated with prednisone or high-dose intravenous immunoglobulin alone did not improve.

Although the clinical course is not predicable and inhibitors may disappear spontaneously combined therapy with prednisone and azathioprin is recommended. In non-responsive disorders azathioprin should be replaced by cyclophosphamide. In pregnancy only prednisone should be given.

1 Dept. of Hematology, Oncology and Clinical Immunology 2 Dept. of Transfusion Medicine and Coagulation Physiology 3 Dept. of Endocrinology and Rheumatology, Heinrich-Heine-Universität Düsseldorf, FRG
ORTHOTOPIC LIVER TRANSPLANTATION IN HEMOPHILIA A: THE ROLE OF CAREFUL REGULATION OF HEMOSTASIS

P. Fischbach, I.O. Hutenbach, B. Martuza, H.C. Wenich, C. Alters*

Hemophilia A patients in the end stage of cirrhosis or hepatic carcinoma are cured of their bleeding tendency by liver transplantation. In Europe, 3 such patients are known; one had an emergency operation in Heidelberg. This paper describes the two Frankfurt cases.

Successful outcome of transplantation depends on very careful regulation of hemostasis, especially in hemophiliacs.

Systemic changes (acidosis, hyperkalemia, hypercalcemia and hyperphosphatemia), lack of factor VIII, thrombocytopenia and impaired liver function (reduction of all coagulation factors, AT III, protein C and S as well as qualitative changes of coagulation proteins) may cause severe bleeding. Besides that hyperfibrinolysis is a major factor; this is caused by the activation of plasminogen by t-PA.

In February 1989 a liver transplantation was performed in a 46-year-old HIV-seronegative patient with primary liver carcinoma, cirrhosis and severe hemophilia A. He needed 40,000 U virus-inactivated highly purified factor VIII concentrate and 10,000 U AT III before, during and 24 hours after surgery. He received 13 units of blood and 8 units of fresh frozen plasma. However he did not get aprotinin for inhibition of fibrinolysis.

Recombinant factor VIII was used for the first time in liver transplantation with combined splenectomy in April 1990. This was performed in a 47-year-old HIV-seronegative patient with end-stage cirrhosis, severe thrombocytopenia (18,000/µl) and severe hemophilia A. The T-PA-Ag, T-PA-activity and II-3-Dimers were measured hourly during the operation. He needed only 22,000 U recombinant VIII and 4000 U AT III perioperatively, in order to inhibit hyperfibrinolysis, in the preparation phase he was given 200,000 U aprotinin, 400,000 U/h during the anhepatic and reperfusion phase and then 100,000 U/h during the following 24 hours after surgery.

As hepatic artery thrombosis is such a serious postoperative complication, additional aprotinin was not given in spite of signs of hyperfibrinolysis in the anhepatic and reperfusion phase. He received a total of 12 units of blood, 11 units of fresh frozen plasma and 5 units of pooled thrombocyte concentrates.

Both patients are well. There are no signs of recurrence of either carcinoma, hepatitis or hemophilia A.

These clinical results show that the need for F VIII, blood, fresh frozen plasma and AT III can be markedly reduced during hepatic transplantation by the suppression of fibrinolysis.

Department of Internal Medicine and Surgery*, University of Frankfurt, Theodor-Stern-Kai 7, 60009 Frankfurt

Effect of Aprotinin on transfusion requirements and coagulation parameters in orthotopic liver transplantation (OLT)

S.C. Asad, J. Groh*, W. Welte*, E. Pratschke* and W.A.A. Kratzer

Aprotinin has been reported to reduce blood loss during OLT (Mallett et al. Lancet (1990) 336: 866). In order to clarify this observation we have performed a double blinded randomized study in 10 patients with Aprotinin (2 000 000 KIU loading dose, 500 000 KIU/h) and 9 controls with placebo. In all patients coagulation was monitored according to Kratzer et al. (Transpl. Proceed. (1991) 23:1905). Preoperative hemostatic parameters were comparable in both groups. AT III was administered at values lower than 80%. We found no significant differences between the intraoperative blood loss of the Aprotinin group (A); RBC=13+/+-7 and control group (C); RBC=14.7+/+-8.6. During the critical period 30 min after reperfusion no significant differences were observed for the prothrombin time (A: 53; C: 51%) and fibrinogen (A: 172; C: 172 mg/dl), however marked differences occurred for the aPTT (A: >98; C: 52 sec), PAI (A: 19; C: < 1 U/ml), the ratio of factor V/II (A: 0.64; C: 0.52) and TAT (A: 156; C: 208 µg/l). These results, which are in contrast to those cited above, can be explained by assuming that secondary fibrinolysis plays a induced role under the hemostatic condition of FFP, platelets and a high AT III level.

Institut für Klinische Chemie, Anästhesiologie (*) und Chirurgische Klinik ($) der Universität München, Marchioninistr. 15, 8000 München 70

TRANSFUSION OF WHOLE BLOOD VERSUS PACKED RED BLOOD CELLS AND PLATELET-RICH PLASMA IN OPEN HEART SURGERY OF CHILDREN

H.-J. Herrfelder, S. Popov-Cenie, A. Urban, M. Dösterwald, A.M. Brochter, G. Giers, P. Haftend

The supportive blood transfusion therapy is an important component in the acquisition of hemostasis in the postoperative (postop) management of children undergoing open heart surgery (OHS) with cardiopulmonary bypass (CPB). Commonly homologous whole blood (WB) is applied. However, the benefits of WB are questioned by the impairment of platelet activity due to storage of WB in the cold and to proposed side effects of the WB leukocyte content, e.g. alloimmunization and immunomodulation. In search of alternatives we examined the application of WB in comparison with packed red blood cells (RBC) and platelet-rich plasma (PRP), both leukocyte-depleted. The influence of the blood preparations on the hemostasis of the children are described here.

A total of 28 children with aSynoectic and cyanotic congenital heart disease underwent OHS with CPB. All children were peri- and intraoperatively treated with aprotinin infusions. Immediately after the end of CPB 14 children (median age 7.6 years, 20.63 kg body weight, b.w.) received WB and 14 children (median age 4.75 years, 17.35 kg b.w.) RBC/PRP. The median transfusion requirement was 15.9 ml/kg WB in the first group, 9.7 ml/kg RBC and 10 ml/kg PRP in the second group.

The platelet count, thrombelastogram (TEG), PT (Quick, %), aPTT, TT, TAT, fibrinogen, factors II, VII, VIII, X, antithrombin III, protein C (PC), protein S, plasminogen (PMG) and α2-antiplasmin have been analyzed.

The median preoperative (preop) parameters of the children were found to be within their normal ranges. Platelet count and the hemostatic proteins during CPB decreased 50-80% and recovered to 70-90% of preop levels within two postop days. During the observation period no significant differences between both groups were evaluated, but compared with the WB group the medians of F IX, PC, PMG in the RBC/PRP group showed a tendency towards 10-20% higher levels immediately after transfusions until second postop day. The median postop blood loss in both groups did not differ significantly.

Our results indicate that WB can effectively be replaced by RBC/PRP transfusions in OHS of children.

Institut f. Exp. Hämatologie und Transfusionsmedizin der Universität Bonn, Sigmund-Freud-Stiege 25, W-5300 Bonn 1

MONITORING POSTOPERATIVE F XIII SUBSTITUTION WITH A NEW PHOTOMETRIC ACTIVITY ASSAY

J. Mayer, E.M. Solleder, J. Grün*, C. Pfeiffer and F. Keller

Factor XIII (F XIII) activity is often reduced in patients after major operations. F XIII levels play an important role as prognostic markers for delayed or disturbed wound healing. Prophylactic administration of F XIII concentrate has been described to be effective in preventing postoperative wound healing impairments. Reliable and rapid screening can be performed with a recently introduced new photometric assay for the detection of F XIII activity in plasma (Bertichrom® F XIII). In order to investigate the time course of postoperative F XIII activity, levels were monitored in 10 patients (gastrectomy) supplemented with F XIII concentrate (Fibrogammin® HS). The new F XIII assay allowed the screening of F XIII activity in short intervals. As expected the reduction of F XIII activities to 50-80% and recovered to 70-90% of preop levels within two postop days. During the observation period no significant differences between both groups were evaluated, but compared with the WB group the medians of F IX, PC, PMG in the RBC/PRP group showed a tendency towards 10-20% higher levels immediately after transfusions until second postop day. The median postop blood loss in both groups did not differ significantly.

Our results indicate that WB can effectively be replaced by RBC/PRP transfusions in OHS of children.

Zentrallabor der Med. Universitätsklinik, Josef - Schneider - Str. 2, D - 8700 Würzburg, Germany.

Behringwerke AG, Postfach 1140, D - 3550 Marburg, Germany.
INDICATION AND USE OF CONTINUOUS DAILY THERAPY IN THE MANAGEMENT OF HEMOPHILIA

H. Pollmann, S. Kleine and H. Jürgen
University Children's Hospital, Munich

The administration of rt-PA (recombinant tissue-type plasminogen activator) has been demonstrated to be an effective treatment in retinal artery occlusion, if performed within the first hours after the onset of symptoms. The major problem in producing rapid clearance of an occluded retinal vessel is the hemorrhagic risk, and only a limited number of cases is suitable for fibrinolysis. We successfully treated a 42-year-old male and a 46-year-old female with central retinal artery occlusion in the left eye by a low dose of rt-PA and heparin. 50 mg of rt-PA were given intravenously over a period of 60 minutes. Heparin was infused subsequently for 8 days, at 1200 units/hour. In one patient, heparin therapy was started simultaneously with the administration of rt-PA. The patients also received pentoxifylline 1200 mg and aspirin 100 mg daily. In both cases, the visual acuity improved considerably, from 20/200 to 20/25 and from 20/200 to 20/20 after 8 days, by which time retinal edema had cleared and there were no abnormalities on ophthalmoscopy. Complete recanalization was observed in 6 infants. In an eight months old girl we had to stop rt-PA therapy. No severe side effects, for example cerebral hemorrhage, were observed. We conclude, that treatment with rt-PA at dosages of 0.25-0.75 mg/kg/day in combination with heparin leads to a high rate of recanalization in 57-67% of the treated cases in the groups I, II, III and IV. Complete recanalization was more pronounced in the higher dosage groups. Bleeding was observed in 46, 28, 24 and 6% (groups I, II, III and IV) of the patients. There was a strong correlation between the dosages used and the number of bleeding episodes (r = 0.95). We conclude, that treatment with rt-PA at dosages of 0.25-0.75 mg/kg/day in combination with heparin leads to a high rate of recanalization in 57-67% of the patients. The good effectiveness of the lowest dose (0.25 mg/kg/day) was accompanied by only a small rate of bleeding. In no case a serious or fatal pulmonary embolism was observed.

Departments of Internal Medicine, University of Heidelberg, Ulm, Munich, Rehabilitation hospital and thrombosis and hemophilia Center, Rehabilitation Foundation, Heidelberg, Dr. Karl Thomae GmbH, Biberach/R., FRG

* for the study group
IMPROVED THROMBOLYSIS IN ACUTE MYOCARDIAL INFARCTION WITH FRONT-LOADED ADMINISTRATION OF RT-PA:
RESULTS OF THE RT-PA - APSAC - PATENCY - STUDY (T.A.P.S.)
U.Tebbe, K.L.Neuhau, R.v.Essen for the Arbeitsgemeinschaft leitender kardiologischer Krankenhausärzte e.V.
The effects of a front-loaded dose regimen of rt-PA (Alteplase) and APSAC (Anistreplase) on early patency and reocclusion of infarct-related coronary arteries were investigated in a single-blind, randomized multicenter trial in 421 patients with acute myocardial infarction of < 6 h duration. 100 mg Alteplase were given i.v. in 209 patients with an initial bolus of 15 mg, followed by an infusion of 50 mg over 30 min, and 35 mg over 60 min. 30 mg APSAC were injected i.v. over 5 min in 212 pts. Angiography 90 min after onset of treatment revealed a patent infarct-related artery (TIMI grade 2 or 3) in 84.9% of 198 pts given rt-PA versus 70.0% of 203 pts given APSAC (p<0.001). Early reocclusion within 24-48 hours were documented by repeated angiography in 10.3% of 174 pts after rt-PA versus 2.5% of 163 pts after APSAC. Late reocclusion within 21 days was observed in 2.8% of 152 rt-PA versus 5.3% of 159 APSAC patients. There were 5 in-hospital deaths in the rt-PA group (3.8%) and 17 deaths (7.8%) in the APSAC group (p<0.001). The reinfarction rate was 3.8% and 4.8%, respectively. Peak serum creatine kinase and left ventricular ejection fraction were identical in both treatment groups. There were more bleeding complications after Anistreplase (45% vs. 31%, p<0.01). Two intracranial hemorrhages (0.9%) occurred in each group. It is concluded that front-loaded administration of 100 mg Alteplase (rt-PA) yields a significantly higher early patency rate of infarct-related arteries and causes less bleeding complications than Anistreplase (APSAC). The statistically significant difference in hospital mortality needs to be confirmed by a further trial with mortality as a primary endpoint.
Medizinische Klinik II, Kreiskrankenhaus Detmold, Möntgenstraße 18, W-4930 Detmold

SURVIVAL RATE AFTER INTRACORONARY (ICL) AND SYSTEMIC THROMBOLYSIS (SL) WITH AND WITHOUT ADDITIONAL AT III ADMINISTRATION IN 97 PATIENTS SUFFERING FROM MYOCARDIAL INFARCTION.
K. Rickert, M. Maasberg, R. Seitz, R. Egbring
Thrombolytic therapy of patients suffering from myocardial infarction lead to a significant reduction of mortality. But reocclusion still as reinfarction occur in 10-20% after successful recanalisation. To improve postthrombolytic anticoagulant treatment with heparin 42 of 97 patients had been additionally treated with Antithrombin III in a concentration of daily 1000 U. The results are demonstrated in table 1. While 9 of 45 patients not treated with AT III concentrate did not survive, all of 42 patients additionally treated with AT III no lethal complications occurred.

|    | ICL  | ICLL | SL | SLL |
|----|------|------|----|-----|
| without AT III | 14   | 5    | 41 | 4   |
| addit. AT III   | 38   | 0    | 4  | 0   |
|                 | 52   | 5    | 45 | 4   |

Table 1

ICL: Intracoronary thrombolysis
ICLL: ICL with lethal complications
SL: Systemic thrombolysis
SLL: SL with lethal complications

Department of Hematology, Philippa-University 3550 Marburg

COMPARISON AND COMBINED USE OF PA-I PLASMA LEVELS AND THALLIUM-SCINTIGRAPHY AS EARLY NON-INVAIVE MARKERS OF CORONARY RESTENOSIS AFTER SUCCESSFUL PTCA
K. Huber, M. Gottaauen-Wolf, M. Jörg, I. Roehl, I. Lang, H. Sochor, P. Probst, F. Kaindl and B.B. Binder
We could show recently (Thromb. Haemostas., in press) a strong association between a decrease of PA-I plasma levels and a lack of tendency to restenosis whereby PA-I was determined in plasma samples obtained immediately before, and 3 days and 3 months after PTCA. To characterize whether a determination of PA-I plasma levels after PTCA is clinically more useful than other methods to detect an early restenosis formation we compared the increase or decrease of PA-I plasma levels with the results of thallium-scintigraphy (TI) at the end of a follow-up period of 75 days (mean) by calculating sensitivity, specificity, or positive and negative predictive values for each method alone and for the combined use of both methods in 34 consecutive patients (M/F:27/7; mean age 55 years, range 38-70) after successful PTCA (LAD: 27; ACx: 2; RCA: 5). Within this group, 13 patients (38%) developed renewed angina due to angiographically proven coronary restenosis between 3 and 6 months thereafter. Sensitivity values were 46% for PA-I and 54% for TI (not significant), while specificities were 96% (PA-I) and 70% (TI), respectively (p<0.05). By combined use, sensitivity increased to 77%, while specificity decreased to 57%. The positive predictive value for PA-I was significantly higher as compared to TI (75 vs. 50%; p<0.05), while the negative predictive values were similar (PA-I: 73%; TI: 70%). From these data we conclude that determination of PA-I during the follow-up period after successful PTCA might be a clinically useful and effective non-invasive method to detect ongoing restenosis formation.
Department of Cardiology and Clinical Experimental Physiology, University of Vienna, Austria

PERMANENTLY DECREASED FIBRINOLYTIC POTENTIAL IN PATIENTS WITH RECURRENT RESTENOSIS AFTER PTCA
K. Huber, B. Beckmann, R. Pachor, S. Graf, P. Probst and B.B. Binder
We investigated the fibrinolytic potential in patients after percutaneous transluminal coronary angioplasty (PTCA) and compared in asymptomatic patients with a history of ≥2 coronary restenoses (n=36, group B) and a group of patients (n=71, group A) with no or only one event of restenosis. Patients of both groups were comparable with respect to age, gender, clinical characteristics, therapy and risk factors. We determined plasma levels of t-PA antigen and t-PA activity as well as PA-I activity and "active" antigen in blood samples obtained before and after venous occlusion (VO). Before VO, t-PA antigen (A: 5.7±5.5; B: 4.4±2.7), t-PA activity (A: 0.2±0.8; B: 0.1±0.0), and PA-I activity (A: 8.9±2.5; B: 6.1±2.1) did not show significant differences between the study groups. However, basal plasma levels of "active" PA-I antigen were significantly elevated in the group of patients with recurrent restenoses (A: 22.1±17.1; B: 61.8±15.1; p<0.01). VO had no influence on PA-I levels. We conclude that in those patients a defective fibrinolytic potential might be involved in the process of restenosis formation.
Department of Cardiology and Clin. Exp. Physiology, University of Vienna, Austria
RATE OF FIBRINOGEN BREAKDOWN IS RELATED TO CORONARY PATENCY AND BLEEDING COMPLICATIONS IN PATIENTS WITH THROMBOLYSIS IN ACUTE MYOCARDIAL INFARCTION: RESULTS FROM THE PRIMI TRIAL. H. Ostermann, U. Schmitt-Huebner, J. Winderer, F. Bar, H. Meyer, J. van De Loo.

401 patients with acute myocardial infarction of less than four hours duration were randomized to intravenous thrombolytic treatment with either 80 mg of ful length u-lycosylated single-chain-urokinase plasminogen activator (INN Saruplase) or 1.5 million IU of streptokinase delivered over a 60 minute period. Angiographic patency rates were higher at 60 minutes in saruplase treated patients (71.8% vs 48%; p<0.001), but did not differ significantly at 90 minutes (71.2% vs 63.9%; p=0.15).

Fibrinogen levels dropped markedly in both groups, the decrease being delayed and less pronounced with saruplase. Total fibrin and fibrinogen degradation products and D-dimer values rose earlier and to higher peak values in streptokinase treated patients. In both groups marked plasminogen and alpha-antiplasmin consumption was observed. Lower fibrinogen levels and in particular the faster rate of fibrinogen breakdown were associated with higher patency rates at 90 minutes (p<0.05). Patients with bleeding complications had lower nadir and a more rapid decrease in fibrinogen (p<0.05). These findings were not related to the drug used.

Increased heparin levels at 6 to 12 hours were correlated to bleeding complications in streptokinase treated patients.

It is concluded that the rate of fibrinogen breakdown during and following thrombolytic treatment for acute myocardial infarction is related to early vessel patency and bleeding complications.

Department of Internal Medicine, University of Münster, Albert-Schweitzer-Straße 33, D-4400 Münster

UROKINASE IN UNSTABLE ANGINA PECTORIS: EFFECTS ON THE SYSTEM OF COAGULATION AND FIBRINOLYSIS. M. Oehminger, H.-G. Schreiner, S. Ziesche, W. Haerer, W. König, V. Hombach and E. Seifried.

To determine the role of i.v. urokinase (urokinase-plasminogen activator, u-PA) in unstable angina pectoris, it was compared with heparin therapy in a prospective, randomized trial. 30 patients (pts.) were randomly allocated to treatment with either i.v. u-PA (1.5 Mio. l.U. bolus, followed by 2000 l.U./kg b.w./h over 12 h plus simultaneous heparin infusion) or to a continuous heparin infusion (1000 l.U./h adjusted to the aPTT) All pts. received aspirin, i.v. glyceryl trinitrate, calcium antagonists and/or beta blockers. At timed intervals blood samples were taken on the inhibitor GGACK to assess effects of u-PA on haemostatic parameters. Marked alterations of following parameters were seen in the u-PA group:

| Parameter | pre | 30' | 12 h | 48 h |
|-----------|-----|-----|------|------|
| \( \alpha_2 \)-antiplasmin (%) | 101 | 21 | 24 | 79 |
| plasminogen (%) | 102 | 45 | 33 | 65 |
| fibrinogen (g/l) | 3.1 | 1.5 | 1.1 | 2.0 |
| PAI-1 (AU/ml) | 20 | 0.6 | 3.3 | 12.5 |
| FDP (µg/ml) | 0.3 | 16.1 | 13.3 | 0.6 |
| FbDP (µg/ml) | 0.4 | 4.0 | 3.6 | 0.4 |
| TSP (µg/ml) | 1.1 | 18.4 | 19.9 | 1.9 |
| D-Dimer (µg/ml) | 0.4 | 15.5 | 12.0 | 2.6 |

1 Mean levels in n = 15, u-PA group

In contrast, no significant changes of haemostatic parameters were observed in the heparin group (n = 15). It is concluded that u-PA in this dose regime induces sustained plasminogen activation and results in ongoing in vivo fibrinolysis for more than 48 h. It is still a matter of debate whether these changes in the haemostatic system are of prognostic relevance. The results of correlation studies between the above reported haemostatic changes and clinical outcome will be reported in due course.

Haematologie, D-3550 Marburg, Department of Haematology, D-3550 Marburg.

THE INTERACTION OF VITRONECTIN WITH PLASMIN(OGEN) C. Kost, K.T. Preissner.

Vitronectin (VN) is a multifunctional adhesive protein present in the circulation as well as associated with the extracellular matrix of various tissues. VN interacts with components of the fibrinolytic system. In addition to its known function as major binding and stabilizing factor for plasminogen-activator-inhibitor (PAI-1) thereby expressing antifibrinolytic properties, we investigated the interaction of VN with plasminogen and plasmin. Although plasminogen-activators did not directly interact with VN, low affinity binding of plasminogen to immobilized VN (KD ~ 0.5 uM) was demonstrated. Compared to Glu-plasminogen, Lys-plasminogen and active site-blocked plasmin displayed an about 10-fold increase in affinity for immobilized VN. Binding of plasminogen could be inhibited by lysine-analogues but not by heparin. The binding site was localized adjacent to the heparin-binding site of VN, which is also recognized by PAI-1. Plasmin proteolyses VN within the heparin-binding site generating a stable intermediate fragment with Mr = 61,000, which has lost all heparin- and most PAI-1-binding ability, but displayed about 10-fold higher affinity to plasminogen. These results indicate that proteolytic processing during early fibrinolysis converts VN from an initially anti fibrinolytic into a pro-fibrinolytic component. The differential interaction with components of the plasminogen activation system might render VN an important link in the regulation and direction of pericellular proteolysis events.
FIBRINOLYTIC ANALYSIS IN LATE PREGNANCY AND AT THE ONSET OF LABOR

Shigenori Suzuki, Hitomi Matsuda

Rapid and profound alternations in the coagulation and fibrinolytic systems occur during late pregnancy and at the onset of labor in the kallikrein-kinin system as well. The relationship between the kallikrein-kinin system, the coagulation system and the fibrinolytic system were evaluated on 37 patients during pregnancy and onset of labor.

(Method) 37 cases of uncomplicated pregnancy were reported on by the examination of TAT, PIC, PC, ATIII and so on.

The profile of coagulation and fibrinolytic system was drawn by Thrombastat 4000.

(Results) TAT (ng/ml) PC (%) Prekallikrein

Non-Pregnant 1.5±0.8 76.1±8.9 73.4±9.8

Late Pregnancy 12.6±6.6 96.3±11.8 196.8±11.9

Onset of labor 14.6±9.1 98.8±10.8 90.6±10.9

The most prominent changes were those in the kallikrein-kinin system.

After the onset of labor, prekallikrein decreased rapidly (114.8% of 90.6%). This may trigger changes in the blood coagulation and fibrinolytic systems and it seems something to do with uterine contraction relating to onset of labor.

College of medical technology, Hokkaido university, K-12, W-5, Kita-ku, Sapporo, JAPAN

FIBRINOLYSIS IN PREMENOPAUSAL WOMEN BEFORE AND AFTER HYSTERECTOMY

S. Bauer, J. Grulich-Henn, C. Schubring and G. Müller-Berghaus

Reduced fibrinolytic activity in plasma has been associated with increased risk for thromboembolic diseases. Recent clinical studies indicate an increased risk for thrombosis after hysterectomy in premenopausal women. It is well known that the uterus is an organ with high fibrinolytic activity.

The present study investigated the fibrinolytic activity in plasma of premenopausal women before and after hysterectomy. The study included 23 women with a mean age of 41.5 years who underwent hysterectomy for nonmalignant diseases of the uterus. Blood was withdrawn in the morning between 7 a.m. and 9 a.m. after a 10 min venous occlusion (VO). Using this procedure the fibrinolytic activity of plasma was measured before and six weeks after hysterectomy. Euglobulin-lysis-time (ELT) and the difference of ELT before and after VO were not significantly different before and after hysterectomy. Furthermore, there was no significant difference in tissue-type plasminogen activator (t-PA) before (6.9 vs. 11.4 ng/ml after VO), and six weeks after hysterectomy (6.7 vs. 11.0 ng/ml after VO). Plasminogen activator inhibitor type 1 (PAI-1) was 9.2 ng/ml before and 9.0 ng/ml six weeks after hysterectomy (means, n.s.). These data indicate that hysterectomy in premenopausal women does not lead to measurable changes in fibrinolytic activity of plasma.

Forschungsgruppe Hämostaseologie, Kerckhoff-Klinik, Sprudelhof 11, Bad Nauheim

FIBRINOLYSIS IN PATIENTS WITH CROHN'S DISEASE

Scheuren M., A. Sauer, W. Daill, K. Jachounek

Epidemiological observations point towards an increased risk for thromboembolic complications in patients with inflammatory bowel disease. The reason for this observation remains to be established. In this study, we investigated fibrinolytic activity and markers indicating a prothrombotic state in 26 patients with active and inactive Crohn's disease (CD).

Twenty-six patients (21 men, 5 women) were included into the study. Group 1 (n=12) consisted of patients with high disease activity (Crohn's Disease Activity Index (CDAI) 52±18 pts.), group 2 (n=14) of patients with low activity (CDAI ≤18 pts.). Plasminogen-Activator-Inhibitor (PAI) and Tissue-Plasminogen-Activator (t-PA) were measured before and after 20 min. venous occlusion (VO). In addition, plasminogen, a2-antiplasmin (a2-AP), Protein C, soluble fibrin (SF), D-dimer and thrombin-antithrombin complexes (TAT) were determined. Basal t-PA a2-AP, SF, D-dimer and TAT did not differ significantly between the two groups. In patients with active disease, PAI decreased by 51% after VO, and remained constant in patients with inactive disease.

In patients with CD, the reduced secretion of t-PA and the relatively high residual activity of PAI might contribute to an increased risk of thromboembolic complications. However, this defect is not longer demonstrable during the active inflammation, presumably due to a reactive activation of fibrinolysis.

Medizinische Klinik & Poliklinik der Universität Tübingen, Gerinnungslabor, W-7400 Tübingen
THE FIBRINOLYTIC SYSTEM IN PATIENTS WITH CROHN'S DISEASE

W. Kirschstein, S. Simianer, U. Staedt, C.E. Dempflé, R. Gladisch, D.L. Heene

In Crohn's disease there is an increased tendency to thromboembolic complications and an increased procoagulant stimulation is considered as a pathogenetic factor (Lancet II, 1057, 1989). We studied pts. with acute disease (gr A: n = 12, Best Index 234) and during remission (gr B: n = 20, BI 72), control (gr C: n = 20, healthy individuals).

In the acute phase (gr A) there was a significant rise compared to gr C as well as to gr B of fibrinolytic capacity (A = 84±20, C = 50±6, PI-PA-release (A = 20.6±4.8, C = 12.7±1.1) and plasminogen (A = 136±25, C = 100±8) as well as Cl-inh. (A = 12±4±2, C = 100±8), a2-antitrypsin (A = 145±30, C = 100±8), and fibrinogen (A = 46±12, C = 102±12), no changes were observed regarding fibrinolytic activity, i.e., the main fibrinolytic inhibitors plasminogen activator inhibitor 1 and a2-antiplasmin, the contact factors prekallikrein, F XII, and a2 macroglobulin as well as protein C and antithrombin III.

During remission (gr B), no significant changes were measured except a significant rise of fibrinogen (B = 281±40, C = 100±16).

Thus, in plasma no changes of the fibrinolytic system could be detected, that induce an increased thrombotic risk in pts with Crohn's disease.

I. Medizinische Klinik, Klinikum Mannheim, Universität Heidelberg, Theodor-Kutzer-Ufer, D 6800 Mannheim.

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GLYCOSAMINOGLYCANS SPECIFICALLY STIMULATE THE INHIBITION OF 55kD BUT NOT 33kD UKINASE BY PROTEIN C INHIBITOR

S. Ecke, M. Geiger, B.R. Binder

Protein C inhibitor (PCI) is a rather non-specific glycosaminoglycan (GAG) binding serine protease inhibitor that inactivates its target proteases by forming stable 1:1 complexes. PCI seems to be an important inhibitor of uokinase (uPA) not only in urine but also in plasma, since uPA-PCI complexes have been shown in plasma samples from patients undergoing thrombolytic therapy with uPA (M. Geiger et al., Blood 1989). We have shown that the inhibition of uPA by PCI is stimulated by different GAGs (M. Geiger et al., J. Biol. Chem., 1991) and heparin is widely used simultaneously with thrombolytic agents. We were therefore interested in a possibly selective stimulation of the inhibition of different forms of uPA (55kD and 33kD) by PCI by GAGs. Inhibition of the 55kD uPA by PCI and complex formation of uPA with PCI was stimulated by heparin, heparan sulfate and dermatan sulfate at different optimal concentrations (0.3-3ug/ml, 1ug/ml, and 50ug/ml, respectively). Inhibition of the 33kD uPA by PCI was not stimulated by these GAGs. When GAG-binding of mixtures of 125I-labelled 55kD and 125I-labelled 33kD uPA was studied by affinity chromatography on heparin-Sepharose or dermatan sulfate-Sepharose, the two forms were always completely separated from each other, the 33kD form being present in the unbound fractions and the 55kD form eluted either at 0.1M NaCl (dermatan sulfate) or at 0.3M NaCl (heparin). These data suggest that the formation of a trimolecular complex of uPA, PCI and GAG is required for the stimulation of PCI inhibitory activity towards uPA.

Lab. Clin. Exp. Physiology, Univ. Vienna, Austria

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GLYCOSAMINOGLYCANS ALTER THE ENZYME SPECIFICITY OF PROTEIN C INHIBITOR (PCI)

S. Ecke, M. Geiger, B.R. Binder

PCI is a non-specific heparin-binding serine protease inhibi-
tor (serpin) present in plasma and urine (>200ng/ml). Although PCI inhibits several enzymes involved in coagulation and fibrinolysis by forming SDS-stable 1:1 complexes, the physiological role of PCI has still not been defined. We have shown that PCI is also an inhibitor of urinary (tissue) kallikrein (Ecke et al., J. Biol. Chem. 1992) and that it is possibly identical to the "kallikrein binding protein" described by Ticiano et al. (Blood, 1986). In the present study we analyzed the effect of glycosaminoglycans (GAGs) on the activity and enzyme specificity of PCI using its urinary target enzymes urokinase (uPA) and tissue kalli-
krein. Although all GAGs studied (i.e., unfractionated heparin, low M heparin, heparan sulfate and dermatan sulfate) stimulated the interaction of 55kD uPA with PCI, they interfered with the inhibition of tissue kallikrein by PCI and inhibited complex formation of 125I-kallikrein with PCI as judged from SDS-PAGE and autoradiography. The inhibitory effect of all GAGs was dose dependent. 30ug/ml unfractionated heparin or low M heparin, 160ng/ml heparan sulfate or 500ng/ml dermatan sulfate, respectively, completely abolished the interaction of tissue kallikrein (200ng/ml) with PCI (130pm). When equimolar concentrations of 125I-uPA and 125I-kallikrein were incubated together with PCI, PCI formed a complex mainly with kallikrein in the absence of GAGs and mainly with uPA in the presence of GAGs. We also studied GAGs isolated from epithelial kidney cells (TCL-988) that have been shown previously to stimulate the PCI-uPA interaction (Geiger et al., J. Biol. Chem. 1991). Similar results as with commercial GAGs (i.e., interference with kallikrein inhibition by PCI and with complex formation of 125I-kallikrein with PCI) were obtained with these kidney cell GAGs. Our data therefore indicate that GAGs present in a specific environment may not only modulate the activity, but also the enzyme specificity of PCI.

Clin. Exp. Physiology, University of Vienna, Austria

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A SIMPLE METHOD FOR THE ISOLATION OF LIPOPROTEIN(A): LP(A) ATTENUATES PLASMINOGEN ACTIVATION BY t-PA

E. Große, W. Mähr, R. Siekmann, J. Scharrer, and W. Große

LP(a) is an LDL-like particle that carries an additional protein constituent, apo(a). Apo(a) DNA is homologous to plasminogen. Therefore, Lp(a) is believed to interact with the fibrinolytic system. In order to study the influence of Lp(a) on fibrinolysis, Lp(a) has to be purified, preferably from a single blood donor. Conventional methods for the preparation of Lp(a) are tedious and cumbersome. Therefore, we have developed a simple method for the preparation of Lp(a) from the eluate of a dextran sulfate LDL-apheresis system.

Procedure. DS-Lp(a) was isolated from a 37-year-old male with heterozygous familial hypercholesterolemia and severe coronary artery disease. Treatment was carried out with automatically regenerated DS cellulose columns (Kanegafuchi MA 01 - Liposorb LA 15). The regenerate fluid was ultracentri-
fuged, first at 1.125 kg/L, then at 1.050 kg/L. DS-Lp(a) was ob-
tained from the infranate of the 1.050 kg/L spin by chromato-
ography on Biogel A-15M.

Results. As shown by intermediate gel electroimmunodiffusion DS-Lp(a) contained no LpB. On lipoprotein electrophoresis and two dimensional immunoelectrophoresis DS-Lp(a) could not be distinguished from Lp(a) isolated with conventional methods. Immunoblotting of DS-Lp(a) and of the patient's plasma obtained the same pattern of apo(a) isoforms. Immunoblots probed with polyclonal anti-apoB revealed apoB-100, and the thrombolytic fragments of apoB. The effect of Lp(a) on t-PA activity against glu-plasminogen was examined. In these experi-
ments, Lp(a) inhibited plasminogen activation in a concentration dependent fashion, irrespective whether fibrin was present or absent in the reaction.

Conclusions. We have developed a simple method for the purification of large quantities Lp(a) from DS absorbents. Physico-
chemically, DS-Lp(a) appears identical to Lp(a) obtained classical means. DS-Lp(a) is a suitable starting material for studies on the interaction of Lp(a) and fibrinolysis.

Centre of Internal Medicine and Centre of Biol. Chemistry, J.W. Goethe-University, Theodor Stern-Kai, W-6000 Frankfurt/Main,
INACTIVATION OF THE NOVEL RECOMBINANT PLASMINOGEN ACTIVATOR BM 06.022 IN HUMAN BLOOD AND PLASMA

U. Martin, G. Sponer, K. Strein

The novel recombinant plasminogen activator BM 06.022 is an unglycosylated deletion variant of human t-PA consisting of the kringle 2 and protease domains. Recent experiments indicated that the uptake of BM 06.022 in rat liver was significantly lower than that of wild type t-PA. The purpose of the present study was to evaluate whether blood or plasma contributed to the metabolism of BM 06.022. Alteplase (recombinant t-PA) and BM 06.022 were incubated each at a final concentration of 2000 U/ml buffer (n = 6), and in citrate-blood or citrate-plate from 5 human donors at 37°C for 6 h. At serial intervals, aliquots were taken for measurement of t-PA-like activity in buffer or plasma in an indirect spectroscopic assay. The concentrations of alteplase and BM 06.022 in buffer remained constant over time. The activity of BM 06.022 and of alteplase decreased over time in citrate-blood and citrate-plate. Compared with alteplase, the decrease of activity in plasma was more rapid (p<0.001) for BM 06.022 in citrate-blood (130 ± 3.3 min vs. 71.9 ± 3.1 min) and in citrate-plate (129.9 ± 5.3 min vs. 62.9 ± 1.2 min). The plot BM 06.022 and of alteplase at 1 min was higher by 51 ± 13 or 48 ± 0.8 %, respectively, in blood-incubation experiments than in plasma-incubation experiments, probably because of a concentration of plasminogen activator in the plasma-supernatant during centrifugation. This could indicate that BM 06.022 and alteplase did virtually not bind to blood cells. We conclude that blood components contribute to the inactivation of BM 06.022 more than for that of alteplase.

Dept. of Pharmacology, Boehringer Mannheim GmbH, Sandhofstr. 116, D-6900 Mannheim 51

D-PHE-PRO-ARG-CHLOROMETHYLKETONE PREVENTS IN VITRO FIBRINOLYSIS REDUCTION BY THE NOVEL RECOMBINANT PLASMINOGEN ACTIVATOR BM 06.022

U. Martin, D. Gärtner, H.J. Markl, R. Kanzig, G. Sponer

The novel recombinant plasminogen activator BM 06.022 which is an unglycosylated deletion variant of t-PA is currently under clinical investigation. The purpose of the present study was to investigate whether BM 06.022 reduced fibrinogen in vitro in human blood and whether the reduction could be prevented for use of D-Phe-Pro-Arg-Chloromethylketone (PCMK). Citrated human blood samples were incubated without or with 1000 U/ml BM 06.022 for 25 min at 25°C. Plasma-fibrinogen was measured coagulometrically. PCMK (2 μM final concentration) was available in lyophilized form in test-tubes. PCMK test-tubes (stored at 4°C) were used 1, 3, and 6 months (n) after lyophilization to incubate citrated human blood without or with 1000 U/ml BM 06.022 for 25 min at 25°C. Plasma-fibrinogen was reduced completely in lyophilized form in test-tubes. Fibrinogen reduction was prevented in PCMK test-tubes of various storage duration: 2.00 ± 0.13 vs. 1.94 ± 0.17 (1 m), 2.46 ± 0.13 vs. 2.27 ± 0.13 (3 m), and 2.22 ± 0.15 vs. 2.21 ± 0.21 g/l (6 m) (n = 4 each). Fibrinogen reduction was completely inhibited by 2 μM PCMK: 1656 ± 79 vs. 61 ± 2 (1 m), 1284 ± 122 vs. 11.1 ± 0 (3 m), and 1251 ± 134 vs. 1.1 ± 1.6 U/ml (6 m) (n = 4 each). We conclude that lyophilized PCMK test-tubes are at least 6 months stable and can be used to prevent in vitro fibrinogen reduction by BM 06.022 probably due to inhibition of BM 06.022-activity.

Dept. of Pharmacology, Boehringer Mannheim GmbH, Sandhofstr. 116, D-6900 Mannheim 51

THE PLASMINOGEN ACTIVATOR-PLASMIN SYSTEM IN HUMAN ARTERIOSCLEROTIC (AT) VESSELS.

T. Podz, D. Kötter, J. Kienast

The plasminogen activator-plasmin system besides its role in fibrinolysis is thought to have a regulatory function in tissue remodeling and cell migration. Since fibrin and fibrin degradation products are important components in the atheroma, and smooth muscle cell migration is a key process in the development of the arteriosclerotic lesion, we have studied whether the level of plasminogen activators (PAs) and their inhibitors (PAl's) in the different tunica of the arterial wall is modified in AT vessels, and whether this modification is related to the degree of lesion. PAI (t-PA, u-PA) and PAI levels (antigen activity) were analyzed in the different layers (intima, media, adventitia) of human artery samples (necropsy specimens). Adventitia has been found as the only tunica with a significantly higher t-PA and u-PA activity. In normal vessels, t-PA level in the adventitia was 5 times higher than in the intima and 10 times higher than in the media. In AT vessels, t-PA was by 120% increased in the media and by 60 % in the adventitia. It was, however, not affected in the intima. The u-PA level was similar in the three layers of normal aorta, but was highly increased in the intima (240 %) and media (934 %) of AT vessels. Plasmin was mainly present in the intima and media of the arterial wall, but only the latter showed a significant detectable activity. Plasmin levels in both tunica were more than 200 increased in AT vessels. The modification of t-PA, u-PA and PAI levels found in AT vessels, occurred in the area near the intima, but none or only minor changes were seen in the area near the adventitia, where arteriosclerotic plaques were not present.

Med. Klinik und Poliklinik der Westf. Wilhelms-Universität, Albert-Schweitzer-Str. 3, 4000 Münster

COMPARISON OF THE FIBRINOLYTIC ACTIVITY IN VITRO OF STAPHYLOKINASE AND STREPTOKINASE

J. Hauptmann, E. Glusa, D. Behnke*, B. Schott*

Staphylokinase (SAK), a non-enzyme plasminogen activator, was expressed in E. coli. SAK resembles streptokinase with respect to the mechanism of activation of plasminogen. The fibrinolytic activity of SAK was compared (on a molar basis) to that of SK in the following systems: Fibrin plate, plasma clot, and labelled fibrin clot in plasma. On plasminogen-containing fibrin plates SAK is more effective than SK. The dose-response curve of SAK is steeper and it shows a more pronounced time course of the effect. Incorporation of SAK or SK in plasma clots (human, rabbit, rat) leads to lysis of the clots dependent on the concentration and on the plasma origin. In human and rabbit plasma SAK and SK exert a fibrinolytic effect with a typical optimum dose-response curve. However, SAK is effective at higher concentrations only and shows smaller maximum effect. In rat plasma, addition of human plasminogen is needed for plasmin clot lysis with SAK and SK. Lysis of labelled fibrin clots in a human plasma system is achieved by SAK and SK in a manner which shows differences in the range of effective concentrations as well as in the time course.

The differences of the fibrinolytic efficacy of SAK and SK in various in vitro systems have to be taken into account in the overall evaluation of the activity of these fibrinolytic agents.

Institute of Pharmacology and Toxicology, Medical Academy Erfurt, 0-5010 Erfurt and *Institute of Microbiology and Experimental Therapy, 0-6900 Jena
DETECTION OF AN ATYPICAL FACTOR V INHIBITOR AND ITS THERAPY

U.Harbrecth and E.Obriga*

Factor V inhibitors are rare and their etiology is still unknown. A relationship to administration of antibiotics (streptomycin, penicillin or derivatives) has been discussed. The course of the inhibitors is short-lived, disappearing on average within 4 to 6 weeks in the majority of reported cases.

We report on a 76-year-old woman without former history of a bleeding disorder who developed an acquired factor V inhibitor which caused severe hematuria and cutaneous singulation requiring blood transfusion. Except of dihydropteroatoiminomethanesulfonate she received no other drugs at that time. Haemostatic analysis revealed F V activity of 35%, prolonged prothrombin time of 60.8 sec (control 13.6 sec) and APTT of 120 sec (control 32.4 sec). E-test was prolonged to 65 min in chrombelastogram. All other clotting factors were in the normal range except of enhanced fibrinogen and F VIII. The inhibitor capacity was determined by F V inhibitor assay via serial dilutions of patient plasma with either normal plasma or buffer. A very weak inhibitor activity of 0.7 Bethesda Units was evaluated. Initial therapy with PPSB-concentrate and fresh frozen plasma (8 U) did not result in any increase of F V activity; however mild symptomatic improvement with reduction of bleeding tendency was achieved. The inhibitor has been persisting over 14 months by now. Steroid therapy alone was unsuccessful, after introducing cyclophosphamide (100 mg/d) F V increased up to 29% within the first month, 25% after three and 64% after 14 months. In conclusion an unusually long-lived and very low-titer F V inhibitor could be observed in this patient. No trigger for its development could be recognized. Improvement of hemostasis was achieved by immunosuppression with cyclophosphamide only.

Institut f. Exp. Haematologie und Transfusionsmedizin und *Medizinische Klinik der Universität Bonn, Sigmund-Freud-Str. 25, 5300 Bonn 1

MOL33AR ANALYSIS AND IN VITRO EXPRESSION OF A HEREDITARY FACTOR X DEFICIENCY WITH A SEVERE BLEEDING DISORDER. H.N.Wetzke, A.Wallmark, N.Nameuchl, D.W.Stefford and K.A.High.

FX"Santo Domingo" is a hereditary FX deficiency which is clinically characterized by a severe bleeding diathesis. The proposita has a F V activity of <1% and her FX antigen is reduced to 5%. We have determined the molecular base of the defect in the FX"Santo Domingo" gene by amplification of all eight exons with PCR and subsequent sequence analysis. A G to A transition in exon I at codon -20 (numbering the alanine of the start site of the mature protein as +1) results in the substitution of arginine for glycine in the carboxy-terminal part of the signal peptide. The amino acid change occurs near the presumed cleavage site of the signal peptidase. It was therefore speculated that the mutation might prevent cleavage of the signal peptidase which in turn would impair proper secretion of the FX protein. To test this hypothesis we compared the expression of a wild type and the mutant FX cDNA in a human kidney cell line. A normal FX cDNA was cloned into the plasmid pMAC 254. The FX"Santo Domingo" genotype was introduced into the plasmid by site directed mutagenesis using oligonucleotide hybridization and the gapped duplicon method. The mutant and the wild type cDNA were cloned into the expression vector pCMV4 Sequence analysis confirmed the correct orientation of the insert and the presence of the point mutation in the FX"Santo Domingo" construct. The human embryonic kidney cell line 253 was transfected with the wild type and the mutant plasmid in a transient expression system. The cell supernatant and the cell lysate of both transfections were analyzed for the presence of human FX by an ELISA. FX antigen levels in the supernatant of the cells containing the FX"Santo Domingo" construct were <5% of those harboring the wild type construct. No FX antigen was detected in the cell lysate of the SD construct. To insure that the difference in protein levels was not due to a difference in shaping state levels of RNA Northern analysis was performed on the cell lysates of both constructs. A transcript of the same size and in roughly equal amounts was detected in both cases. Thus, the defect in the signal sequence in FX"Santo Domingo" exerts its effect posttranscriptionally, at the level of translation. FX"Santo Domingo" is the first example of a human disease due to a mutation in the signal sequence.

Universitätsklinik für Internen I/Hämatologie, Währinger Gürtel 18 - 20, A-1090 Wien

FACTOR X DEFICIENCY IN AL-AMYLOIDOSIS

J. Lin*, A. Bierhaus, R.P. Linke; W. Ki-siel; R. Waldherr*, H. Böhrrer*, V.M. Zhang*, K. Andrassy*, E. Ritzi*, F.P. Nawroth*.

To study the mechanism of Factor X deficiency in amyloidosis, amyloid derived from the spleen of a patient with A-lamba-amyloidosis, kidney failure and rapidly decreasing PT levels (under 10%) was isolated. The coagulation abnormality was due to a shortened halflife of factor X (6 minutes). Prothrombin was normal, indicating specificity of binding. Splenectomy resulted in an increase in factor X levels, which became evident directly after ligation of the splenic artery. Immunofluorescence could demonstrate colocalisation of factor X with the amyloid deposited in the patients spleen, but not in vessels without severe amyloidosis. The association of factor X with the spleen containing amyloid could also be demonstrated in Western blots. The clonality of the amyloid producing cells was indicated by colonal rearrangement studies. Southern blot analysis of DNA extracted from patient spleen and hybridized to the Ja probe showed additional bands not seen in the DNA from control spleen. The purified amyloid was characterized as A-lamba using specific antibodies. Factor X binding to amyloid was demonstrated by Western Blots. These studies help to further elucidate the mechanism of factor X deficiency in A-lamba amyloidosis.

*Univ. Heidelberg; Univ. of New Mexico; Max Planck Inst. München;

FACTOR XII DEFICIENCY IN A BAVARIAN TRAIT is not caused by a gene deletion, as proved by PCR double-exon-screening.

Stefan Rühl and Bernhard Kempfer
Klinikum Großhadern, Institut für Klinische Chemie (Dr. Prof. F. Seidel); Marchioninistr.15, 8000 München 70

We have examined a southern bavarian family, in which one member has a complete factor XII deficiency. Two generations could be investigated and the typical hemostaseological pattern was observed: one patient with no factor XII activity and no detectable Factor XII antigen, who's sisters and children had about 50% of activity and antigen. In order to see, if there was a complete loss of the gene for this factor we analyzed the DNA of the patient. For each of the 14 exons primers were sythesized for both exon-intron borders. Polymerase-chain-reaction (PCR) was performed for each single exon. In addition we did that for any two exons in a row with the intron inbetween, which we call double-exon-screening. By that timesaving technique we could demonstrate that the homozygous patient had the same gene parts (exons and introns) as a normal person and no mayor deletion or insertion, but rather a frameshift or nonsense mutation in the gene (because of the absence of antigen) is the cause of the disease.
Activated protein C (APC) exerts its physiologic anticoagulant role by proteolytic inactivation of the blood coagulation cofactors, Factors Va and Villa. To identify the regions on the surface that mediate anticoagulant activity, 26 synthetic peptides were prepared representing 90% of the human protein C heavy chain primary structure and tested for their ability to inhibit APC anticoagulant activity. Peptide (390-404) specifically inhibited APC activity in APTT and Xa-1-stage coagulation assays in normal, in protein S-depleted and Factor VIII deficient plasma with 50% inhibition at 5 µM peptide. Polyclonal antibodies raised against this peptide and immunoaffinity-purified on a protein C-Sepharose column inhibited APC anticoagulant activity in APTT and Xa-1-stage assays in normal, protein S-depleted and Factor VIII deficient plasma with half-maximal inhibition at 30 nM anti-(390-404) antibody. Neither the peptide (390-404) nor the anti-(390-404) antibodies inhibited APC amidolytic activity or the reaction of APC with recombinant [Arg8]-antitrypsin. Furthermore in a purified system, peptide (390-404) inhibited APC catalyzed inactivation of Factor Va in the presence as well as in the absence of phospholipids with 50% inhibition at 4 µM peptide. These data suggest that the region containing residues 390-404 in APC is essential for anticoagulant activity and is available to interact with antibodies or with other proteins such as the macromolecular substrates Factors Va or Villa.

The Scripps Research Institute, La Jolla, CA, USA
*R Present address: Medizinische Klinik und Poliklinik der Universität, Abteilung Innere Medizin A, Albert-Schweitzer-Straße 33, D-4400 Münster

Activated protein C (APC) exerts its physiologic anticoagulant role by proteolytic inactivation of the blood coagulation cofactors, Factors Va and Villa. To identify the regions on the surface that mediate anticoagulant activity, 26 synthetic peptides were prepared representing 90% of the human protein C heavy chain primary structure and tested for their ability to inhibit APC anticoagulant activity. Peptide (390-404) specifically inhibited APC activity in APTT and Xa-1-stage coagulation assays in normal, in protein S-depleted and Factor VIII deficient plasma with 50% inhibition at 5 µM peptide. Polyclonal antibodies raised against this peptide and immunoaffinity-purified on a protein C-Sepharose column inhibited APC anticoagulant activity in APTT and Xa-1-stage assays in normal, protein S-depleted and Factor VIII deficient plasma with half-maximal inhibition at 30 nM anti-(390-404) antibody. Neither the peptide (390-404) nor the anti-(390-404) antibodies inhibited APC amidolytic activity or the reaction of APC with recombinant [Arg8]-antitrypsin. Furthermore in a purified system, peptide (390-404) inhibited APC catalyzed inactivation of Factor Va in the presence as well as in the absence of phospholipids with 50% inhibition at 4 µM peptide. These data suggest that the region containing residues 390-404 in APC is essential for anticoagulant activity and is available to interact with antibodies or with other proteins such as the macromolecular substrates Factors Va or Villa.

The Scripps Research Institute, La Jolla, CA, USA
*R Present address: Medizinische Klinik und Poliklinik der Universität, Abteilung Innere Medizin A, Albert-Schweitzer-Straße 33, D-4400 Münster

Pericellular plasminogen activation - function and regulation in tumor cell invasion and metastasis
G. Brunner, A. Meissauer, L. J. Erkell, M. D. Kramer and V. Schirrmacher

Tumor cell metastasis is considered to be a multistep process involving the breakdown of biological matrices followed by the invasion of host tissues by tumor cells. These degradative processes appear to be catalyzed by various proteolytic systems, e.g. plasminogen activators (PAs) produced by the tumor cells activate the inactive proenzyme plasminogen. The end-product plasmin is able to degrade almost all components of extracellular matrix and activate other proteolytic systems. Plasminogen activation appears to be restricted to the pericellular microenvironment and PA-catalyzed generation of cell-associated plasmin is sufficient to mediate tumor cell invasion in vitro. Pericellular plasminogen activation as well as cellular invasion are modulated by the interaction of proteases (PA, plasminogen/plasmin), protease inhibitors (PA inhibitor), and protease-regulating growth factors (basic fibroblast growth factor, transforming growth factor-β) with specific receptors on the cell surface, with components of the extracellular matrix, or with soluble macromolecules.

The Scripps Research Institute, La Jolla, CA, USA
* Present address: Medizinische Klinik und Poliklinik der Universität, Abteilung Innere Medizin A, Albert-Schweitzer-Straße 33, D-4400 Münster

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German Cancer Research Centre, Institute of Immunology and Genetics, Im Neuenheimer Feld 280, D-6900 Heidelberg

The Scripps Research Institute, La Jolla, CA, USA
* Present address: Medizinische Klinik und Poliklinik der Universität, Abteilung Innere Medizin A, Albert-Schweitzer-Straße 33, D-4400 Münster

Identification of a sequence in human activated protein C (residues 311-325) essential for its interaction with Factor Va
R. M. Mesters* and J. H. Griffin

Identification of a sequence in human activated protein C (residues 311-325) essential for its interaction with Factor Va
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Structural and functional properties of the extracellular matrix
K.T. Preissner

Extracellular matrix (ECM) of variable composition and properties at various sites in the organism is crucial for the organization of cells and the integrity of tissues and functions as highly specialized barrier for a number of physiological processes. In general, the structure-giving elements of ECM mainly consist of different types of collagens whose biosynthetic processing may vary according to the adhesive cell phenotype (e.g. during smooth muscle cell proliferation), the collagen network together with other structural proteins such as laminin, fibronectin, thrombospondin and others as well as proteoglycans is produced by most adhesive and polarized cells and constitutes their anchorage support or growth substratum. These ECM proteins are recognized in a diverse but rather cell-specific manner by a number of ubiquitous, hetero-dimeric adhesion receptors (termed integrins) and other cell surface receptors resulting in different types of tight or loose cell-ECM connections. Due to a variety of binding sites in the ECM compartment (e.g. the subendothelial basement membrane), various cell-specific or cell-independent effector molecules (e.g. plasminogen activator inhibitor-1, growth factors) may become deposited also dependent on the cell phenotype and thus determine the functional repertoire of the ECM. In particular, the adhesive, proteolytic - antiproteolytic, mitogenic or chemoattractant potential of a given ECM is relevant for its role to support platelet adhesion, induce or prevent ECM-destruction, release proliferative signals or promote cell migration and invasion. Effective control of these processes that are crucial in haemostasis and inflammation as major defense mechanisms requires the cooperation between other cell surface receptors (e.g. urokinase receptor) or ECM-associated multifunctional proteins such as vitronectin. More work is needed to clarify the role of these molecules in a physiological setting and in pathological situations of thrombosis, bleeding or tumor metastasis where among other events pericellular proteolysis appears to be out of control.

Haemost. Res. Unit, Korkhoff-Klinik, MPG, 6350 Bad Nauheim

Identification of a sequence in human activated protein C (residues 390-404) essential for its anticoagulant activity
R. M. Mesters* and J. H. Griffin

Identification of a sequence in human activated protein C (residues 390-404) essential for its anticoagulant activity
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Structural and functional properties of the extracellular matrix
K.T. Preissner

Extracellular matrix (ECM) of variable composition and properties at various sites in the organism is crucial for the organization of cells and the integrity of tissues and functions as highly specialized barrier for a number of physiological processes. In general, the structure-giving elements of ECM mainly consist of different types of collagens whose biosynthetic processing may vary according to the adhesive cell phenotype (e.g. during smooth muscle cell proliferation), the collagen network together with other structural proteins such as laminin, fibronectin, thrombospondin and others as well as proteoglycans is produced by most adhesive and polarized cells and constitutes their anchorage support or growth substratum. These ECM proteins are recognized in a diverse but rather cell-specific manner by a number of ubiquitous, hetero-dimeric adhesion receptors (termed integrins) and other cell surface receptors resulting in different types of tight or loose cell-ECM connections. Due to a variety of binding sites in the ECM compartment (e.g. the subendothelial basement membrane), various cell-specific or cell-independent effector molecules (e.g. plasminogen activator inhibitor-1, growth factors) may become deposited also dependent on the cell phenotype and thus determine the functional repertoire of the ECM. In particular, the adhesive, proteolytic - antiproteolytic, mitogenic or chemoattractant potential of a given ECM is relevant for its role to support platelet adhesion, induce or prevent ECM-destruction, release proliferative signals or promote cell migration and invasion. Effective control of these processes that are crucial in haemostasis and inflammation as major defense mechanisms requires the cooperation between other cell surface receptors (e.g. urokinase receptor) or ECM-associated multifunctional proteins such as vitronectin. More work is needed to clarify the role of these molecules in a physiological setting and in pathological situations of thrombosis, bleeding or tumor metastasis where among other events pericellular proteolysis appears to be out of control.

Haemost. Res. Unit, Korkhoff-Klinik, MPG, 6350 Bad Nauheim
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BOTH, THE UROKINASE-TYPE PLASMINOGEN ACTIVATOR (uPA) AND ITS INHIBITOR PAI-1 ARE STONG AND INDEPENDENT PREDICTORS OF RELAPSE AND SURVIVAL IN BREAST CANCER.

F Jänkke, M Schmitt, K Uhl*, H Graeff. Frauenklinik und "Institut für Med. Statistik und Epidemiologie der Technischen Universität München, FRG

The capacity of breast cancer cells for tissue invasion and early hematogenous metastasis is closely related to the action of the receptor-bound tumour-associated protease uPA (urokinase-type plasminogen activator). In 1989, we first reported in THE LANCET that uPA antigen content determined in tumor tissue extracts (+1% Tdton X-100) is a strong and independent prognostic factor in breast cancer. However, tumors also exhibit the plasminogen activator inhibitor type 1 (PAI-1) which blocks the enzymatic activity of free and receptor-bound uPA. Thus the presence of both, uPA and PAI-1 will modulate invasive and metastatic phenotype of cancer cells.

To evaluate the relative prognostic impact of uPA and PAI-1 in breast cancer, PAI-1 antigen (in cytosols) was measured in parallel with uPA antigen (+1% Triton X-100) in extracts of primary tumors of breast cancer patients (n=205) and benign lesions (n=41). In addition to elevated values of uPA (median 2.6 ng/ml versus 0.22 ng/ml protein), PAI-1 was found to be fifty times higher (ELISA) in tissue extracts of breast cancer compared to benign breast (median 1.9 ng/ml versus 0.02 ng/ml protein; both p<0.001).

uPA antigen (cut-off value 2.69 ng/ml) was the strongest independent predictor of relapse and also of overall-survival after median observation time of 30 (16-50) months. Moreover, patients with high PAI-1 content in their primary tumors (PAI-1 > 1.3 ng/ml protein) also displayed a significantly higher incidence of relapse and also shorter survival than patients with low PAI-1 content, similar to uPA. Multivariate regression analysis (Cox) revealed that the impact of uPA on prognosis (relapse-free and overall survival) was stronger than that of the classical prognostic factors and also than that of PAI-1, while PAI-1 remained an independent predictor. On the basis of uPA and PAI-1 antigen determination, combination of the two independent variables allows an even more individualized delineation of those patients having a low or high risk of relapse and death, irrespective of the established risk factors. This is of particular interest concerning patients without axillary lymph node involvement with respect to the decision, whether adjuvant chemotherapy should be given or not.

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NEW ASPECTS OF HEMOPHILIA TREATMENT - UPDATE OF CLINICAL TRIALS WITH RECOMBINANT F. VIII

I. SCHRAMM

After the occurrence of numerous problems in the treatment of hemophilia with plasma-derived (pd) clotting factors, the synthesis of recombinant factor VIII (rF VIII) may raise new hope in the life of hemophiliacs. Both F VIII and pd F VIII are quite similar in their structure and function. During the manufacturing process many safety regulations for F VIII producing human cells and many purification procedures are demanded. The resulting F VIII is pure and has a specific activity of 3000 units/mg. F VIII has similar pharmacokinetisk and in vivo recovery properties to those of pd F VIII.

Based on these findings, multinational international clinical studies have been organized by Baxter and by Cutter to test the long-term safety and efficacy over a period of 16 months. As of October 91, a total of 274 patients worldwide have been treated with r F VIII of Baxter and Cutter. Of these 132 patients have received Cutter r F VIII (33 previously treated adults, 2 previously treated children and 67 previously untreated children, RPA’s). 122 patients have enrolled in the Baxter study (38 previously treated, 43 previously untreated patients). Based on these preliminary experiences it may be suggested that r F VIII is comparable with pd F VIII in terms of safety and efficacy in treatment on demand, in prophylaxis, in home treatment and in surgery. Acute adverse reactions occurred only rarely. Inhibitors have been identified in 12 of 66 RPA’s in the Cutter trial. 8 of the 12 inhibitor-negative patients were reported in 27 RPA’s in the Baxter study. 2 of the 3 have been low level. As present the significance of the rather high incidence of mostly low level inhibitors remains unclear.

ZENTRUM DER INNERN MEDIZIN, DER UNIVERSITÄTSKLINIKEN FRANKFURT/MAIN THEODOR-STEIN-KAT 7, 6000 FRANKFURT/MAIN

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THERAPY OF ANTI-FACTOR-VIII INHIBITORS

R. Zimmermann

Most patients with severe haemophilia A have a good response to factor VIII concentrate. But in up to 20% of the patients an anti-factor VIII antibody may develop and makes bleeding harder to stop. As one cause of inhibitor formation the absence of the factor VIII protein was estimated. Treatment of acute bleeding episodes has to be individually considered. Patients with low level inhibitors (<10 BU) often respond to repeated infusions of factor VIII. At anti-factor VIII levels of 10-50 BU prothrombin complex concentrates or porcine factor VIII are of therapeutic benefit while at higher antibody titers activated prothrombin complex concentrates present the most therapeutic value. Another exciting developments are the use of factor VII a and in future possibly recombinant tissue factor. Induction of immune tolerance may be achieved by repeated small doses of factor VIII in patients with low-level inhibitors and by massive doses in high responder patients. Intravenous lgl, cyclophosphamide and factor VIII are only of small benefit given alone but the combined use has proven effective in most of the patients.

Rehabilitationsklinik Heidelberg und Hämophiliezentrum, Stiftung Rehabilitation, Bonhoeffer Straße, D-6900 Heidelberg

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PROCEDURES FOR VIRUS INACTIVATION IN PLASMA AND PLASMA DERIVATIVES: PRESENT STATUS AND FUTURE DEVELOPMENT

L. Dörrler

Inactivation of infectious agents in plasma has to follow the balance of either being potent enough to destroy viral structures and especially their capability of cell attachment and replication and either being mild enough to preserve enzyme activity and protein structure of the specific plasma components. The following procedures have been found to be valid for virus inactivation:

Physical: Heat inactivation at 60°C in the liquid state in 18 months. As of October 91, 91% of 274 patients worldwide have been treated with r F VIII of Baxter and Cutter and many level inhibitors have been low level. 3 inhibitors were reported in 27 RPA’s in the Baxter study. 2 of the 3 have been low level. As present the significance of the rather high incidence of mostly low level inhibitors remains unclear.

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Most patients with severe haemophilia A have a good response to factor VIII concentrate. But in up to 20% of the patients an anti-factor VIII antibody may develop and makes bleeding harder to stop. As one cause of inhibitor formation the absence of the factor VIII protein was estimated. Treatment of acute bleeding episodes has to be individually considered. Patients with low level inhibitors (<10 BU) often respond to repeated infusions of factor VIII. At anti-factor VIII levels of 10-50 BU prothrombin complex concentrates or porcine factor VIII are of therapeutic benefit while at higher antibody titers activated prothrombin complex concentrates present the most therapeutic value. Another exciting developments are the use of factor VII a and in future possibly recombinant tissue factor. Induction of immune tolerance may be achieved by repeated small doses of factor VIII in patients with low-level inhibitors and by massive doses in high responder patients. Intravenous lgl, cyclophosphamide and factor VIII are only of small benefit given alone but the combined use has proven effective in most of the patients.

Rehabilitationsklinik Heidelberg und Hämophiliezentrum, Stiftung Rehabilitation, Bonhoeffer Straße, D-6900 Heidelberg

Future will be the use of recombinant produced clotting factors. While their action has been proved their immunogenicity has to be elucidated in long term studies. Since monoclonal antibodies are used to purify recombinant antigens an inactivation procedure has to be included. From the recent epidemiology of the human immunodeficiency virus (HIV) and the bovine spongiform encephalitis virus (BSE) we have to learn that new viruses can spread at any time.

Max von Pettenkofer Institut, University of München, Pettenkofer Str. 9a, D-8000 München 2
ABNORMALITIES OF MEMBRANE GLYCOPROTEINS IN INHERITED AND ACQUIRED PLATELET DISORDERS
B. Kehrel, J. Kardoeus-Kehrel, and K. J. Clemetson

Recent advances in flow cytometry have made it possible to study rapidly the quantity and function of membrane proteins on intact platelets. The most profound defects in platelet aggregation occur in patients with the autosomal recessive hereditary disorder Glanzmann's thrombasthenia caused by deficiency or abnormality of the membrane glycoprotein IIb/IIIa complex. Flow cytometry using different monoclonal antibodies against GPIb/IIa has been shown to be a successful technique for rapid diagnosis of Glanzmann's thrombasthenia. Moreover it is a useful tool for identifying heterogeneities in the disorder and for carrier detection of type I thrombasthenia. With some monoclonal antibodies it is even possible to distinguish in platelets of some thrombasthenia variants whether the defect is in agonist-mediated fibrinogen receptor activation rather than in fibrinogen binding or events distal to binding. In flow cytometry platelets have been identified and distinguished from other cell types by their light scatter profile. In certain diseases like the Bernard Soulier's syndrome platelets or platelet aggregates may have a light scatter overlap with erythrocytes or even leukocytes. In such cases two-color immunofluorescence staining has to be applied so that platelets can be discriminated from other cells on basis of specific fluorescence. Usually antibodies against GPIb have been used for this purpose except in Bernard Soulier's syndrome which can be diagnosed by flow cytometry very easily. Flow cytometry has also been used to show deficiencies in the GPIa/IIa complex and in GPIIb and is useful to measure the amount of membrane glycoproteins in some disease states like uremia and myeloproliferative disorders.

Medizinische Klinik A der Westfälischen Wilhelms-Univer-
sität Münster, Exp. Hämostaseforschung, Domagkstr. 3,
D-4400 Münster

DETECTION OF ACTIVATED CIRCULATING PLATELETS IN CLINICAL DISORDERS. *A.T. Nurden, P Hourdill6, C. Durrieu, L. Macchi, D. Lacaze, J. Duchary, R. Sanchez, G. Vezon and P. Besse.

We have applied flow cytometry to the detection of activated platelets in patients with severe haemostatic defects. Platelets from freshly drawn blood were washed and incubated with one of the following antibodies: Bx-I (anti-GP Ib), AP-2 (anti-GP IIb-IIIa complex), VH10 (anti-GMP-140, a glycoprotein of the α-granule membrane), SG11 (anti-thrombospondin, TSP; a secreted α-granule protein), or PAC-1 (directed against an activation-dependent determinant on GP IIb-IIIa complexes, from Dr. Shattil). Bound antibody was quantitated by flow cytometry after the addition of FITC-conjugated anti-immunoglobulin.

We first examined patients with severe burns, chosen to represent a condition with widespread damage to the microvasculature. Here, platelet counts fell dramatically in the hours after the accident. Recovery was seen after 2 to 3 days, and it was at this time that the number of circulating activated platelets started to reach a maximum. Situations where 30-40 % of platelets were activated were often observed. Yet, even in these cases, activation was mostly partial. The levels of antigen expressed by individual platelets rarely reached those seen when control platelets were stimulated with thrombin in vitro. Furthermore, when platelet activation was at its maximum, the circulating platelets expressed a storage pool-like functional defect (a situation more related to a haemorrhagic rather than a thrombotic tendency).

Our second model concerns patients with coronary artery disease undergoing transluminal angioplasty. Initially, platelets were examined at different periods after surgery. Levels of activated platelets were variable, remaining in the 2 to 4 % range of control donors for some, but increasing to 10 to 30 % for others (despite the patients having been given heparin and aspirin). Maximum levels were again delayed, being detected at 24 or 48 h. Once more only a partial release reaction had occurred. Results with VH10 and PAC-1 often, but not always, correlated suggesting different pathways of platelet activation. No correlation has so far been established between the level of platelet activation and a tendency for thrombosis or restenosis.

*URA 1464 CNRS, Hôpital Cardiologique, 33604 Pessac, France.

ABNORMALITIES OF MEMBRANE GLYCOPROTEINS IN INHERITED AND ACQUIRED PLATELET DISORDERS
B. Kehrel, J. Kardoeus-Kehrel, and K. J. Clemetson

Recent advances in flow cytometry have made it possible to study rapidly the quantity and function of membrane proteins on intact platelets. The most profound defects in platelet aggregation occur in patients with the autosomal recessive hereditary disorder Glanzmann's thrombasthenia caused by deficiency or abnormality of the membrane glycoprotein IIb/IIIa complex. Flow cytometry using different monoclonal antibodies against GPIb/IIa has been shown to be a successful technique for rapid diagnosis of Glanzmann's thrombasthenia. Moreover it is a useful tool for identifying heterogeneities in the disorder and for carrier detection of type I thrombasthenia. With some monochlonal antibodies it is even possible to distinguish in platelets of some thrombasthenia variants whether the defect is in agonist-mediated fibrinogen receptor activation rather than in fibrinogen binding or events distal to binding. In flow cytometry platelets have been identified and distinguished from other cell types by their light scatter profile. In certain diseases like the Bernard Soulier's syndrome platelets or platelet aggregates may have a light scatter overlap with erythrocytes or even leukocytes. In such cases two-color immunofluorescence staining has to be applied so that platelets can be discriminated from other cells on basis of specific fluorescence. Usually antibodies against GPIb have been used for this purpose except in Bernard Soulier's syndrome which can be diagnosed by flow cytometry very easily. Flow cytometry has also been used to show deficiencies in the GPIa/IIa complex and in GPIIb and is useful to measure the amount of membrane glycoproteins in some disease states like uremia and myeloproliferative disorders.

Medizinische Klinik A der Westfälischen Wilhelms-Univer-
sität Münster, Exp. Hämostaseforschung, Domagkstr. 3,
D-4400 Münster

CHANGES OF MEMBRANE EPITOPES DURING PLATELET STORAGE H.K. Nieuwenhuis.

The progressive decline in platelet function during storage is a major problem. With the availability of monoclonal antibodies (MoAbs) against platelet surface glycoproteins, the investigation of membrane glycoproteins becomes possible. We studied the effect of platelet storage on the expression of several platelet membrane glycoproteins using flowcytometry and a panel of MoAbs. A gradual loss of GPIb binding sites during the storage period was observed, with a decrease of 76 % after 9 days. This may be caused by internalization of the GP Ib/IX complex upon activation. The expression of markers for platelet degranulation the α-granule membrane proteins GMP-33, P-selectin (CD62), and the lysosomal membrane glycoprotein LIMP-CD63 slowly increased from day 0 to 5 with a stronger increase till day 9. There was no change in the expression of GPIIia and the CD31 antigen. These changes were similar to the changes observed after in-vitro stimulation with thrombin. In contrast, UV-irradiation of platelet concentrates did induce the exposure of fibrinogen binding sites on GPIb/IIa (as measured with Moab 2.41 reacting with GPIIb/IIIa after a conformational change in the complex has occurred by the binding of fibrinogen), but not exposure of α and lysosomal granu-

Dep. of Haematology, University Hospital Utrecht, P.O.
Box 85500, 3508 GA Utrecht the Netherlands.
INCREASED GP IIb AND GP IIb/IIIa EXPRESSION ON DIABETIC PLATELETS
- Quantitative evaluation of antibody binding sites using the Duesseldorf protocol for single platelet flow cytometry (SPFC)
D. Tacke

Platelet hyperreactivity is considered to be the key signature of the prothrombotic state in diabetes with patients contributing to the early pathogenesis of microangiopathic organ lesions and to the vascular excess mortality. Conventional techniques were not able to identify the underlying molecular alterations. From the severely reported increased platelet volume in diabetes we postulated a primary platelet abnormality resulting from the release of more potent platelets by an altered megakaryocytic thrombopoiesis. In order to further explore this hypothesis we standardized the method of single platelet flow cytometry (SPFC) for constitutive membrane glycoproteins which represent megakaryocytic lineage markers and are essential for the adhesion and aggregation behaviour of the platelets. This technology allowed to report for the first time an increased expression of GP IIb and GP IIb/IIIa (20-30%) on platelets of IDDM and NIDDM patients as a possible molecular mechanism for the severely reported increased adhesion and aggregation derived from global function tests. Moreover, increased GB III/IIIA expression was directly confirmed on megakaryocytes of insulin treated BB-rats, but did not depend on metabolic parameters. Taken together, these data are confirmative for a primary change in the thrombopoiesis. The megakaryocyte-platelet system is activated in diabetic subjects leading to an increased circulating thrombocytic mass.

The analysis of diabetic platelets for the expression of activation dependent functional markers (CD61, CD63) with the Duesseldorf III protocol showed that the increased thrombotic potential may also result in an increased intracellular activation. The practicability and the value of flow cytometric tests for the clinical handling of patients at a prethrombotic state such as diabetics will be discussed.

"Cellular Haemostasis Group", Diabetes Research Institute at the Heinrich Heine University, Duesseldorf, Aufrm Hennekamp 65, D-4000 Duesseldorf 1.

FLOW CYTOMETRY IN THE EVALUATION OF THROMBOCYTOPENIC DISORDERS
J. Klaes

Fluorescence activated flow cytometry has important applications in the evaluation of thrombocytopenic disorders. Several methods have been devised to measure platelet-associated immunoglobulins and complement in autoimmune and alloimmune thrombocytopenia by flow cytometry with either whole blood or separated platelets. Modifications of these techniques using single donor platelet preparations or frozen-thawed UCLA-typed pooled platelets have proven useful to screen serum samples for the presence of allo- and auto- and allo-antiplaatlet antibodies. The diagnostic utility of flow cytometry also offers new perspectives for cross-matching of donor platelets with sera of recipients who respond poorly to transfusion of random donor platelet preparations.

We have recently described an additional application of flow cytometry to the diagnostic evaluation of thrombocytopenic disorders (J. Klaes and G. Schmitz, Blood 75:116-121,1990). This assay is based on platelet staining with the fluorescent dye thiolane orange (TO), which is characterized by a 3,000-fold fluorescence enhancement upon binding to RNA. TO readily permeates live blood cell membranes. Using standardized staining conditions (50 ng/ml of TO in staining solutions; incubation time 2.5 hours) followed by flow cytometric analysis of the platelet population, the assay allows to identify a subset of platelets with increased residual RNA. In haematologically normal controls, this subset amounts to 4.6 + 2.4 % (n=50) of total platelets. In thrombocytopenic patients whose bone marrow contains normal to increased numbers of megakaryocytes, the percentage of TO-positive platelets is significantly increased to 26.3 + 10.9 % (n=21) (P < 0.0001). In contrast, the proportion of positively stained platelets in patients with thrombocytopenia due to impaired platelet production does not significantly differ from normal controls. Thus the sensitivity and the specificity of this method is in distinguishing between these categories of thrombocytopenia rather high.

Overall, the main advantages of flow cytometric techniques applied to platelet analysis in thrombocytopenic disorders reside in their relative timeliness, the ease of performance and their high sensitivity.

Med. Klinik and Poliklinik der Westf. Wilhelms-Universitat, Albert-Schweit-zer-Str. 33, 4400 Münster

TISSUE MEASUREMENTS OF VITAMIN K IN RELATION TO ITS METABOLISM AND NUTRITION
M. J. Shearer

During the last decade sophisticated methods have evolved for the measurement of the very low endogenous tissue concentrations (down to 1ppb) of phylloquinone (K1) and menaquinones (K2 or MKs). All are based on high performance liquid chromatography (HPLC) using different detection methods. Early UV detection methods have now been superseded by more sensitive and selective methods based on either electrochemical or fluorescence measurements. Despite much progress, attention should be drawn to the present limitations which is reflected, for example, in the lack of agreement in published values for plasma concentrations of K1 and the future need for even greater sensitivities to measure cord plasma K1 or adult plasma levels of MKs.

Measurements of K vitamins are being increasingly used to study the physiology and biochemistry of vitamin K in early and adult life. Some knowledge of tissue levels has already been gained including the importance of K1 as the major circulating form, the prevalence of MKs in adult livers and the reduced reserves of the newborn. Many major questions still surround the transplacental transfer, intestinal absorption, intermediary metabolism, plasma transport, bioavailability, and storage of K vitamins including the relative importance of K1 versus MKs to human nutrition. The continuing development and critical assessment of tissue assays for K vitamins and their metabolites should undoubtedly provide the basis to answer many of the above questions.

Haematology Dept., Guy's Hospital, London SE1.

THE ROLE OF VITAMIN K-DEPENDENT CARBOXYLATION FOR THE BIOLOGICAL FUNCTION OF COAGULATION PROTEINS
K. T. Preissner

Vitamin K-dependent coagulation proteins including prothrombin, factor IX, factor X, factor VII, protein C, and protein S represent structurally related proteins of hepatic origin which fulfill an important biological role in haemostasis. The functional activity relies on undisturbed post-translational modification during endoplasmatic reticulum passage resulting in vitamin K-dependent carboxylation of 10-12 glutamic acid residues in the amino terminal portion of these proteins. The selectivity of carboxylation is believed to depend on the presence of a specific recognition sequence in the pro-peptide portion of the Gla-proteins, which also interacts with a recognition site on the carboxylase. Since the Gla residues may act as chelating groups mainly for calcium ions, the Gla-domain is thereby involved in fixation of these coagulation proteins to lipid membranes. Consequently, localization of Gla-proteins is one crucial step in the assembly of the multicomponent enzyme complexes (e.g. tenase, pro-thrombinase) which express maximal catalytic efficiency in the presence of the respective cofactors (e.g. Villa, Va). In contrast to the activated forms of the other Gla-proteins, thrombin has lost the Gla-domain during activation and may distribute between fluid and cell surface (thrombomodulin) phase. Subsequent activation of protein C by thrombin-thrombomodulin-complex is not dependent on the intact Gla-domain portion of the proenzyme, although effective efficiency of activated protein C depends on the presence of the vitamin K-dependent cofactor protein S in its free form. These diverse interactions are crucial for the physiological role in the haemostasis system. Consequently, oral anticoagulation with vitamin K-antagonists influences the carboxylation of both pro- and anti-coagulant Gla-proteins resulting in a desired suppression of haemostatic activation in the patient.

Haemosostat, Res. Unit, Kerckhoff-Klinik, MPG, 6350 Bad Nauheim,
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INFLUENCE OF ANTIBIOTICS ON VITAMIN K METABOLISM
K. Uchida

Newer β-lactam antibiotics containing an N-methyltetrazolethiol (NMTT), thiadiazolethiol (TDTT), or methylthiadiazolethiol (MTDT), induce hypoprothrombinemia. These antibiotics or side chains do not inhibit either vitamin K reductase or γ-glutamylcarboxylase, but decrease vitamin K epoxide reductase activity and thus increase the plasma vitamin K epoxide level. When vitamin K intake from foods is reduced or menaquinone producing intestinal bacteria are decreased by treatment with antibiotics, reuse of vitamin K through the vitamin K cycle becomes important in the synthesis of blood coagulation factors. Since the side chains, NMTT, TDT and MDT, block the vitamin K cycle at the step of vitamin K epoxide reductase, the antibiotics containing these side chains promote the manifestation of hypoprothrombinemia in vitamin K deficient humans and rats. However, vitamin K administration can quickly normalize the hypoprothrombinemia since neither vitamin K reductase nor γ-glutamylcarboxylase are inhibited by these side chains.

Diagnostic Division, Shionogi & Co., Ltd.
Settsu, Osaka 566, Japan

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VITAMIN K-DEPENDENT PROTEINS NOT RELATED TO BLOOD COAGULATION
C. Vermeer, B.A.M. Soute and K. Hamulyák

The only known function of vitamin K in vertebrates is that it serves as a coenzyme for the microsomal enzyme γ-glutamylcarboxylase. This enzyme is involved in the posttranslational processing of secretory proteins, and belongs to the standard machinery of most types of cells and tissues. The vitamin K-dependent reaction is the conversion of glutamate into γ-carboxyglutamate (GLA) residues. The six GLA-containing proteins (GP’s) involved in blood coagulation are all synthesized in the liver. Examples of extrahepatic GP’s are: osteocalcin and matrix GLA-protein (from bone), and plaque GLA-protein (from atherosclerotic plaques). Almost all mammalian extracellular GP’s known today are associated with calcified tissues, and there is strong evidence that some of them play a role in calcium metabolism. The diet forms the major source of vitamin K, and is generally sufficient to maintain the normal synthesis of blood coagulation factors. Our results demonstrate, however, that the vitamin K requirement of non-hepatic tissues is higher than that of the liver, and that GLA-deficient osteocalcin is common in substantial parts of the population, notably in newborns and in elderly women. The physiological impact of these findings will be discussed. Consensus should be reached about the definition of vitamin K-deficiency and its recommended daily allowance.

Department of Biochemistry and Cardiovascular Research Institute, University of Limburg, PO Box 616, 6200 MD Maastricht, The Netherlands

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EFFECT OF LOW AND NORMALLY DOSED VITAMIN K-ANTAGONISTS ON PLATELET-INDUCED THROMBIN GENERATION (PITT)
H.K. Breddin and M. Basic-Micic

PITT (Platelet-Induced Thrombin Generation Time) is a new sensitive test system, in which the time of aggregation (Ta) and coagulation (Tc) of platelet rich plasma (PRP) are measured. PRP from partially anticoagulated blood samples is rotated in the light beam of a photometer. Low concentrations of hirudin (0.7 µg/ml), Fraxiparin (7.0 µg/ml) or other thrombin inhibitors are used as anticoagulants. Thrombin is generated at the cuvette walls mediated by a large air-plasma interface.

In patients receiving normally dosed phenprocoumon treatment (prothrombin time 15-25 % of normal) the PITT-parameters Ta and Tc are prolonged to 20-30 min and more (normal: Ta 7.2 + 2.5 min, Tc 9.1 + 3.4 min). In patients receiving low dose vitamin K-antagonist treatment with the aim of constant prolongation of Tc. With low dose phenprocoumon treatment there is only a poor correlation between PITT parameters and prothrombin time.

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Phlebography: Method of reference for diagnosis of deep vein thrombosis
W. Hach

There have been various evaluations concerning phlebography and its development using it as a method of reference for diagnosing thrombosis. The first stages of research have been done before the second world war, they were followed by a scientific period in the 6th and 7th decade of this century. There was a lack of practical therapeutic consequences as well as a low level of interest in venous diseases. This situation changed considerably with the introduction of surgical thrombectomy and then, rapidly spread on a wider level, with the use of fibrolysis. New contrast mediums with very little side effects advanced this tendency on a larger scale.

The actual acceptance of phlebography as an invasive technique depends on 3 conditions. The first condition is the minimization of the risk. With the use of modern non-ionic contrast mediums and a well developed, standardized technique the risk of phlebography is as good as non existent. Also the second condition with the demand for a painless examination has been fulfilled. The third aspect is of most importance for practical and scientific interests, whether imaging sonography or phlebography gives more specific information in deep venous thrombosis. Phlebography and sonography most definitely have differences in their diagnostic preference. They harmonize optimally, where a precise diagnosis is demanded. One method can be used without the other. For the question "obtusive thrombosis yes or no" sonography gives information with a high sensibility and specificity. Although this would be sufficient for a basic decision it is however not enough for a differentiated diagnosis.
Studies of the last years have shown that assessment of compressibility of venous lumen is of prominent significance in diagnosis of deep venous thrombosis of the leg by ultrasound (compression-sono-graphy): unlike arteries, open veins collapse under gentle pressure applied by the probe whereas thrombosed veins are not or only par-tial compressible. In the tigh v. femoralis and v. poplitea lie in vicinity of the artery which can be visualized easily in cross section scanning. In the calf normal unobstructed veins can usually not be viewed in the supine patient. In thrombosis however they be-come distended and can be seen as incompressible echopoorse cords in typical place.

Diagnostic accuracy of compression-sonography in acute venous thrombosis of the tigh is well proved in literature with a sens. of about 95 % and spec. near 100 %. In our experience and according to results of other authors diagnosis of acute calf vein thrombosis is possible with a sens. of 90 % (spec. near 100 %). Location and ex-istence of thrombosis can be determined with high accuracy. Typical positive sonographic findings need no confirmation by phlebography. A negative sonographic examination rules out thigh thrombosis with sufficient accuracy. Thrombosis of muscle veins, proximal thrombo-sis of v. saph. magna in danger of growing into v. fem. com. and occlusions of v. fem. prof. can be missed by routine phlebography but are easily visible in ultrasound. Complementary to phlebography soft tissue process mimicking deep vein thrombosis (ruptured Baker cyst, phlegmon) can be distinguished. Compression sonography is a highly accurate method in diagnosis of acute venous thrombosis of the leg. It can be done with every high resolution ultrasound scanner (5 MHz probe). Adequate equipment is available in almost all clinics and many medical practice and wait to be used with appropriate knowledge and skill in diagnosis of venous thrombosis.

Medizinische Universitätsklinik, Josef-Schneider-Str. 2, D-8000
München 2
The present study reports on 176 deep vein occlusions which were treated by streptokinase. The UHSK scheme consisted of one or multiple I.V. infusion courses with an infux rate of 1.5 million units per hour (one course - 9 million I.V. SK over 6 hours). The number of UHSK courses varied one to four, but in most cases a 2-course scheme was administered. Without exception one UHSK course was given on one day, hence UHSK courses were identical with the number of treatment days. Of the 176 deep vein occlusions the most proximal locations were: One calf vein, 109 femoral veins, 45 iliac veins, and two subclavian veins. The therapeutic result was as follows: Total clearance 74/176 = 42.0%, partial clearance 65/176 = 36.3%, and no clearance 38/176 = 21.6%. The occlusion history was an important factor for venous thrombosis removal. Thrombi older than 14 days did seldom respond to lytic treatment; thus length was a factor correlated with lytic success in that the shorter the occlusion was the more frequent their removal rate became. The simplicity of the UHSK technique and the rate of hemorrhagic complications indicate that this new SK regimen is a valuable modality in the treatment of venous occlusions. However, because of several pulmonary embolism accidents originating from iliac thromboses under this therapy we have proposed that iliac thromboses should not be treated by UHSK in the first instance but rather with a less aggressive agent such as urokinase (100 000 IU/h over one week). After removal of the iliac blocking lysis of occlusions further distally located (femoral, calf veins) could be treated with UHSK in the above outlined fashion.

Geriatric Department and Radiology Department
Städtische Kliniken, 4100 Duisburg, W. Germany

Dietrich C. Gulba, Medizinische Hochschule, Konstanty-Gutschow-Str. 8, D-3000 Hannover 61, F.R.G.

Differential Therapeutic Aspects of Pulmonary Embolism.

Inspite of heparin prophylaxis, pulmonary embolism (PE) remains one major hazard to in hospital patients, the long term prognosis being related to the degree of obstruction of the pulmonary arterial tree and the consecutive hemodynamic changes. In case of minor obstruction (10-30%), or submassive (<50% ob), a most conservative therapeutic strategy is indicated. These emboli generally are succumable without permanent damage. Only in cases with repetitive emboli thrombolyis might be expected beneficial.

When massive (>50% ob) or fulminant (>65% ob) PE are causing life threatening hemodynamic deteriorations [mortality >70% and >90%] more aggressive strategies are mandatory. The decision for surgical or thrombolytic interventions must be based on the most rapid availability. Modern surgical techniques have strongly reduced the risk of embolektomy. In our hospital 15/27 patients survived embolektomy. If technical reasons prevent immediate embolektomy, immediate thrombolysis should be the intervention of first choice. Once the decision for thrombolysis has been made, the treatment regimen should be selected. In our hands with 120mg of rt-PA/2h (20 mg as a bolus up front), between 1987 and 1991, 13/27 lives of patients with life threatening PE (mainly post op surgery or under cardio-vascular or pulmonary resuscitation) were saved; 4 additional patients died from repeat PE 4 to 17 days later. If patients start bleeding blood lossses are substituted until the success of therapy is indicated and thrombolysis is stopped with aprotinin and or tranexamic acid thereafter.

Michael Martin and B.J.Othmar Fiebach

LONG-TERM TREATMENT IN PATIENTS WITH THROMBO-PHILIA
I. Pabinger-Fasching

Oral anticoagulants are currently the therapy of choice in patients with deep vein thrombosis for 5-12 weeks after the thrombotic event. Low dose unfractionated heparin or aspirin have not been proven to be effective in long-term prophylaxis. Studies on low molecular weight heparin used as a long-term treatment are lacking.

In patients with recurrent venous thrombosis and/or pulmonary embolism who do not have malignant disease, long-term treatment with oral anticoagulants (INR>2.0) has been proven to be highly effective.

In patients with a known deficiency of a natural anticoagulant (antithrombin III, protein C or protein S) oral anticoagulants are able to prevent thromboembolic events. However, the question of duration of treatment must be leaved unanswered. Retrospective studies have shown, that part of the deficient patients not on oral anticoagulation treatment had no thromboembolic events over long periods of time (up to 60 years), although they had a previous history of venous thrombosis. To clarify the indication of long-term treatment in those patients prospective studies are needed. In those studies not only the risk of thrombosis but also the risk of bleeding of long-term treatment has to be evaluated.

Klinik für Innere Medizin I, Währinger Gürtel 18-20, A - 1090 Vienna

D. Petersen, M. Barthels, G.Schumann, J.Böttner

HPLC METHOD FOR ROUTINE DETERMINATIONS OF PHEN-PROCOUMON CONCENTRATIONS IN SERUM AND SERUM WATER

HPLC Instrument used is an HP 1090 liquid chromatograph equipped with an HP 1046 fluorescence detector set at 320 nm excitation and 390 nm emission wavelength (Hewlett Packard, Bad Homburg, FRG). The analytical column (Hyperl-C18, 5 µm, 200×4.6mm) is purchased from Hewlett Packard. A phosphate buffer (pH 4) is used for isocratic elution. Calibrators for S-PPC are prepared by spiking drug free pool serum with PPC. Aqueous calibrators are used for SW-PPC analyses. A linear relationship was observed between S-PPC concentrations and the corresponding peak areas (r = 0.995, 0.992, 0.995). A linear relationship was observed between SW-PPC concentrations and the corresponding peak areas (r = 0.995). The intraday and interday precision for S-PPC was determined with spiked serum samples. The intraday coefficients of variation (CV) were 5.8, 1.6, and 2.1% for concentrations of 0.1 mg/L, 1.0 mg/L, and 10.0 mg/L, respectively (n=12). The intraday CV was 2.7% at a concentration of 2.5 mg/L (n=7). A linear relationship was observed between SW-PPC concentrations and the corresponding peak heights (r = 0.995). The intraday CV was 3.3% at a concentration of 5.0 mg/L and 3.3% at concentrations of 0.1 mg/L, 1.0 mg/L, and 10.0 mg/L, respectively (n=12). The interday CV was 4.3% and 3.9% for concentrations of 15 µg/L and 60 µg/L, respectively (n=8). No interferences could be detected.

The sensitivity of the HPLC procedure for the monitoring of S-PPC and SW-PPC. Sample preparation for S-PPC determinations consists of a single precipitation step using acetonitrile. For SW-PPC proteins are removed by ultrafiltration. The HPLC Instrument used is a HP 1090 liquid chromatograph equipped with an HP 1046 fluorescence detector set at 320 nm excitation and 390 nm emission wavelength (Hewlett Packard, Bad Homburg, FRG). The analytical column (Hyperl-C18, 5 µm, 200×4.6mm) is purchased from Hewlett Packard. A phosphate buffer (pH 4) is used for isocratic elution. Calibrators for S-PPC are prepared by spiking drug free pool serum with PPC. Aqueous calibrators are used for SW-PPC analyses. A linear relationship was observed between S-PPC concentrations and the corresponding peak areas (r = 0.995, 0.992, 0.995). A linear relationship was observed between SW-PPC concentrations and the corresponding peak areas (r = 0.995). The intraday and interday precision for S-PPC was determined with spiked serum samples. The intraday coefficients of variation (CV) were 5.8, 1.6, and 2.1% for concentrations of 0.1 mg/L, 1.0 mg/L, and 10.0 mg/L, respectively (n=12). The intraday CV was 2.7% at a concentration of 2.5 mg/L (n=7). A linear relationship was observed between SW-PPC concentrations and the corresponding peak heights (r = 0.995). The intraday CV was 3.3% at a concentration of 5.0 mg/L and 3.3% at concentrations of 0.1 mg/L, 1.0 mg/L, and 10.0 mg/L, respectively (n=12). The interday CV was 4.3% and 3.9% for concentrations of 15 µg/L and 60 µg/L, respectively (n=8). No interferences could be detected. In 5 serum samples spiked with PPC (range: 1.0-7.5 mg/L) the fraction of SW-PPC was between 0.57 % and 0.79 %. The described method is easy to perform and permits the specific and sensitive determination of S-PPC and SW-PPC. It is suitable for routine measurements of patient samples.

Institut für Klinische Chemie, Medizinische Hochschule Hannover, Konstanty-Gutschow-Str. 8, D-3000 Hannover, FRG
ANALYSIS OF PHENPROCOUMON AND ITS METABOLITES IN HUMAN PLASMA AND URINE BY SOLID PHASE EXTRACTION AND HPLC
E. Schmitz-Kummer, J.X. de Vries, E. Weber

Instead of conventional liquid-liquid extraction (LLE) used previously for the purification of biological samples prior to the assay of phenprocoumon (PH) and its metabolites by HPLC, a solid phase extraction (SPE) method was developed. Plasma samples were extracted using octadecyl-silane (ODS) modified silica columns; urine samples were hydrolysed and filtered successively through quaternary ammonium and ODS modified silica columns. Reversed phase gradient elution HPLC with UV detection was used. Recovery yields of PH and 4'-OH-, 6-OH-, 7-OH-PH metabolites using SPE were high and reproducible; a good linearity (range 0.2-5.0 μg/ml, r²=0.9899), precision (CV: 2-6.7%) were found; detection limits: 50 ng/ml; other drugs and endogenous substances did not interfere. The advantages of the SPE with respect to the LLE method are: higher recoveries of PH and metabolites; the avoidance of using chlorinated solvents; the assay can be carried out using internal as well as external standards; PH and metabolites can be quantitated in urine, which was not possible previously using LLE, owing to interference. SPE extraction was shown to be a reproducible, accurate and sensitive, as well as a fast, simple, convenient and selective purification method for the quantitation of PH and metabolites in plasma and urine for drug monitoring and kinetic studies.

Med. Universitätsklinik, Abt. f. Klin. Pharmakologie, Bergheimerstr. 58, D-6900 Heidelberg.

INR-PROFICIENCY TESTS BY ÖQUASTA FOR THE PROTHROMBIN TIME: USE OF PLASMA FROM ORAL ANTICOAGULATED DONORS.
H. Lang, B. Moritz, V. Hinger, E. Legenstein*, M. Fischer**, E. Kaiser**

In 1983, the ISI/INR scheme for the standardization of the prothrombin time was established in order to check oral anticoagulant therapy.
Since 1983, ÖQUASTA (Austrian Society of Quality Assurance and Standardization of Diagnostic Medical Investigations) performs prothrombin time proficiency tests using control plasmas from oral anticoagulated donors in order to evaluate the results of the participants.
The data of 13 proficiency tests were presented. Using the laboratory-specific 100% values of prothrombin times and the ISI values indicated by the manufacturers of thromboplastin reagents, the mean INR show a coefficient of variance of about 10% after elimination of outliers. Using control plasmas in the normal range as 100% values, lower coefficients of variance were obtained.
Although there are some statistical differences depending on thromboplastin and endpoint determination, the mean INR after outlier elimination can be used as the assigned value.
Using hypothesized control plasmas, the INR provides a suitable means for assessing the data of the participants in proficiency tests.

IMMUNO AG, Industriest. 67, A-1220 Vienna, Austria; ÖQUASTA*; Central Laboratory, Municipal Hospital Linz**; Department of Medical Chemistry, University***; Vienna, Austria.

PASSAGE OF PHENPROCOUMON (MARCUMAR®) INTO HUMAN MILK
R. von Kries, D. Nöcker, E. Schmitz-Kummer*, J.X. de Vries*

As the passage of Phenprocoumon into human milk has not been studied yet, mothers on oral anticoagulation with Phenprocoumon are advised to stop breastfeeding in order to avoid the potential hazards of vitamin K deficiency haemorrhage in their babies. We analysed the passage of Phenprocoumon into human milk in a breastfeeding mother of a premature baby (gestational age 32 weeks), who required oral anticoagulation on day 19 post partum. The mother was advised to continue collecting her milk with an electric pump, and to resume breastfeeding if a significant passage of the drug was excluded. Milk sampling (fore and hind milk pairs (n=2), formilk (n=4), 24 hours pooled collections) for the Phenprocoumon analyses with an HPLC method was performed on days 27, 28 and 31 when the Quick's Prothrombin time was stable in the therapeutic range (Phenprocoumon plasma concentrations: 1.7 - 2.2 μg/ml). Results:Phenprocoumon was higher in hind than in foremilk. With constant plasma concentrations the variability between different foremilk samples was 28 - 76 ng/ml. The Phenprocoumon concentration in the 24 hours pooled sample was 33 ng/ml. Conclusions: Estimates of the Phenprocoumon secretion into human breast milk should be from pooled milk samples of a 24 hours collection. Phenprocoumon in human milk is only about 1/60 of the corresponding maternal plasma concentrations. The daily Phenprocoumon intake from maternal milk in a baby of 3 Kg bodyweight drinking 500 ml of milk is estimated at 5 - 6 μg/kg. This is much less than the average maintenance requirement for anticoagulation with Phenprocoumon in children (about 60 μg/kg/day). Mothers on oral anticoagulation with Phenprocoumon - like those on Warfarin treatment - may be allowed to breastfeed their babies but monitoring of the clotting times and supplementation with vitamin K1 accordingly is recommended. Unfortunately this mother had weaned off breastfeeding shortly after termination of the milk sampling for the analyses.

Kinderklinik der Heinrich Heine Universität Düsseldorf; * 1 Med. Universitätsklinik Heidelberg, Abt. f. Klinische Pharmakologie
INFLUENCE OF LIPOPROTEINS ON VITAMIN K TRANSPORT IN BLOOD - PRELIMINARY DATA

J. Sauge, M.J. Shearer and M. Kohlimair

There is no consensus whether correction of serum-concentrations of vitamin K (K) by using lipid concentrations give more reliable information about K-availability for target cells. Therefore, we investigated transport of K in blood of haemodialysis patients. In this preliminary report the data from 20 patients are presented. K was significantly correlated with triglycerides (Tg) in serum (r=0.73) and in VLDL (r=0.72), and cholesterol (C) in HDL (r=0.49) and VLDL (r=0.67). Nonsignificant correlations were seen with C in serum (r=0.38) and LDL (r=0.27). Measurements of K in VLDL, LDL, and HDL-tractions showed that more than 75% of K is transported in VLDL whereas the remainder is distributed equally among HDL and LDL. To assess possible genetic differences in the K-transport system we also determined apolipoprotein E phenotypes and calculated correlations of K and lipoprotein concentrations. Means (standard errors in brackets) are given in the table notwithstanding the small number of patients tested. Concentrations of K were inversely dependend on apoE allele designation (E4=E3=E2). The same nonsignificant trend was seen in respect to the in-vitro hydroxylapatite binding capacity (HBG) of osteoclast (OC) which is an index for the grade of K-dependent carboxylations in nonhemat K-dependent proteins.

| apoE-phenotype | E2 | E3 | E4 | H-Test |
|----------------|----|----|----|--------|
| (n)            | (3) | (13) | (4) |        |
| VLDL-Tg/[mg/dl]| 493(219) | 184(61) | 80(26) | 0.012  |
| LDL-C/[mg/dl]  | 85(29)  | 122(12) | 150(22) |        |
| HDL-C/[mg/dl]  | 29(4.23) | 36(4.3) | 45(14)  |        |
| HBC of OC [%]  | 61(4.4) | 60(2.6) | 52(4.4) |        |

ILL. Inn. Abt. KH Moebel, Tumstrasse 21, W-1000 Berlin 21, FRG.

ISCHEMIC CEREBRAL INFARCTION - A NEW TYPE OF COUMARIN NECROSIS IN PROTEIN C-DEFICIENCY ?

B. Enekes-Matthes, K.J. Matthes

Introduction: Patients with protein C deficiency have an elevated risk to suffer from coumarin necrosis during the initial phase of coumarin treatment. Up to now, coumarin necrosis was only observed in the skin region. We describe a protein C-deficient patient suffering from cerebral ischemic infarction after a very high initial dose of 18 mg phenprocoumon. The day after, the patient fell into cerebral coma and bilateral ischemic infarction of Aa. cerebri posteriores as well as a cerebellum ischemic infarction were found. The patient recovered and protein C deficiency type I was diagnosed. Under protection with 500 - 1,000 IU protein C concentrate (IMMUNO) daily, coumarin treatment was initiated again with 2 - 4 mg acenocoumarol daily and no further complications occurred. Conclusion: A very severe cerebral ischemic complication during the initial phase of coumarin treatment was observed - probably a "new" type of coumarin necrosis. Therefore: 1. Coumarin treatment should be initiated carefully in all patients.

HEMISYNTHESIS OF ACYLATED HIGH AND LOW MOLECULAR WEIGHT HEPARIN DERIVATES *

R. Malsch, J. Harenberg and D. L. Heep

New high and low acylated heparin derivates were prepared in order to modify their physicochemical properties and biological activities. Acid anhydrides of ethanoic-, butanoic-, decanonic- and octodecanonic-acid in different concentrations were used. Antifactor Xa (aXa) activity ranged from 20 to 150 U/mg for heparin and from 70 - 240 U/mg for heparin-derivates. Antifactor IIa (aIIa) activity varied from 9 to 31.7 U/mg for heparin and from 109 to 440 U/mg for heparin-derivates. Anti-aPTT was measured from 18 to 80 U/mg for LMW-heparin and from 110 to 560 U/mg for heparin-derivates. aXa, aIIa and anti-aPTT activity was higher for high molecular weight heparin derivates than for low molecular weight heparin derivates. The derivates were soluble in water and were analyzed by high performance gel permeation chromatography (GPC). The time for elution and the kav coefficient increased by the grade of acylation up to 20 per cent. Reversed phase high performance liquid chromatography (RP) showed no difference in retention if the derivates were in aqueous solution but they differed in activity.

The acylated lipophilic heparins have altered anticoagulant activities in vitro and chromatographic parameters.

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Zentrum Innere Medizin, Klinikstr.36, 63 Giessen
ANALYSIS OF OLIGOSACCHARIDES BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY, HIGH PERFORMANCE GEL PERMEATION CHROMATOGRAPHY AND AFFINITY CHROMATOGRAPHY *

R. Malisch and J. Harenberg

Samples of high and low molecular weight heparin, dermatan sulphate and dermatan sulphates were eluted by different chromatographic methods in order to study their differences. High performance gel chromatography (GPC) was used to determine molecular weight distribution (Mn, Mw, Mz) and polydispersity Q obtained by controlled chemical degradation of native glucosaminoglycans. Each compound can be eluted with a concavalin column. Retention times varied from 1.0 to 1.46 min. Spectral maxima ranged from 205 to 220 nm. GPC, RP-HPLC, HPAC are valuable instruments to analyse the physiochemical properties of glucosaminoglycans. Each compound can be eluted at optimized conditions and the spectral data are selective for the identification of the compounds.

* Supported by grants from Deutsche Forschungsgemeinschaft Ha 1164/3-1

I. Medizinische Klinik der Fakultät Klinische Medizin Mannheim der Universität Heidelberg, Theodor-Kutzer-Ufer, D-6800 Mannheim

PHARMACOKINETICS OF LOW MOLECULAR WEIGHT DERMATAN SULPHATE

M. Jeschek, M. Acker, M. Schäfer, J. Harenberg and Dl. Heene

Low molecular weight dermatan sulphate has been obtained by controlled chemical degradation of native dermatan sulphate. LMW-DES acts by inhibiting heparin cofactor II rather than by inhibiting antithrombin III. The present study was designed to investigate the pharmacokinetics of 2 intravenous doses in healthy persons on global coagulation tests and specific anti-factor Xa (aXa), anti-factor Xa-like and heparin cofactor II (HC-II) assays.

100 mg and 200 mg LMW-DES has only minimal effect on activated partial thromboplastin time and on thrombin clotting time. Inhibition of (aXa) chromogenic S 2222 assay increased from 1.3 μg to 17 μg after i.v. administration of 100 mg LMW-DES and normalized within 8 hours. HC-II activity showed the similar pharmacokinetic pattern. In contrast heptest coagulation values increased from 2.25 μg/ml to 28.0 μg/ml and were 3.4 μg/ml after 12 hours. After administration of 200 μg LMW-DES the aXa chromogenic S 2222 method and HC II activity returned to normal within 12 hours. However, using heptest assay 2.8 μg/ml was still detectable at 24 hours after administration.

The data demonstrate that LMW-DES acts by inhibiting both heparin co-factor II and antithrombin III. Heptest coagulation values determine some anticoagulant activities which are not detected with other methods.

I. Medizinische Klinik der Fakultät Klinische Medizin Mannheim der Universität Heidelberg, Theodor-Kutzer-Ufer, D-6800 Mannheim

INTERACTION OF TISSUE-FACTOR PATHWAY INHIBITOR WITH HEPARINS AND PROTAMINE *

J. Harenberg, M. Siegele, M. Schäfer, G. Stehle and D. L. Heene

Recently Extrinsic Pathway inhibitor has been described to inhibit specifically the factor VII-Tissue Factor Pathway Inhibitor (TFPI). The antithrombotic effect of heparins is partially mediated by the release of TFPI after administration into man. The present study was designed to investigate the interaction of protamine and heparins on the release of TFPI in order to get indications whether the antithrombotic effect of heparins are mediated by TFPI when they are antagonized by protamine.

The results demonstrate that after administration of unfractonated and fractionated heparin the release of TFPI is independent of the compound injected. The administration on protamine results in an immediate decrease of TFPI to values before administration of heparin. There were no differences between unfractionated and fractionated heparins.

The data indicate that TFPI activity is not responsible for the anti-factor Xa-activity which remains after administration of protamine and subsequent injection of protamine and 2. that heparins release TFPI from its binding sides and that protamine binds heparins and by that TFPI returns to its receptor sides.

* Supported by grants from Sandoz-Stiftung für therapeutische Forschung

I. Medizinische Klinik der Fakultät Klinische Medizin Mannheim der Universität Heidelberg, Theodor-Kutzer-Ufer, D-6800 Mannheim

NEUTRALIZATION OF HEPARIN-OLIGOSACCHARIDES AND HEPARINOIDS BY POLYBRENE AND PROTAMINE *

S. Alban*, J. Kraus*, J. Harenberg# and G. Franz*

The anti-factor Xa activity of high and low molecular heparin can be neutralized by protamine (PR) and polybrene (PO), respectively. The present study was designed to define the interaction of heparin-oligosaccharides, other glycosaminoglycans (GAG) and glycans with PR and PO. Because of their different chemical structure PO neutralizes independently of the charge density, molecular weight and the affinity of the GAG to antithrombin III (AT III), whereas the required PR excess increases with the affinity to AT III and is more than twofold enhanced compared to PO. In contrast to the specific binding of genuine heparin and the heparin-related oligosaccharides to AT III, only non-specific electrostatic forces are involved in their interaction with PO and PR. The anti-factor Xa activity and anti-factor II a activity of Org 10172, dextran sulfate, dermatan sulfate and the sulphated 8-1,3-glucan-derivatives can be completely neutralized by PO at a weight ratio of 1:1 and 1:2, respectively, independently of the specific activity and degree of sulfation. Using PR larger excess is needed for neutralization. As a consequence, PO can be used for the gravimetric quantification of GAG in plasma samples with the intention to determine the concentrations not by means of measuring the pharmacodynamic effects but quantitatively analyzing its kinetics.

Department of Pharmacy, University of Regensburg, D-8400 Regensburg*, Ist Department of Medicine, Faculty of Clinical Medicine, University of Heidelberg, D-6800 Mannheim#
DETERMINATION OF LOW LEVELS OF HEPARIN IN PATIENTS WITH LOW DOSE PROPHYLAXIS BY THROMBIN TITRATION. J. Keute, R. Seitz, L. Lerch, A. Immel, R. Egbring.

Thrombin-titration to test the thrombin time (TT) for therapeutic administration of heparin had been performed by J. Jürgens in 1950. Lowering the test-concentration from 0.3 to 0.2 U thrombin/0.1 ml solution the test will be sensitive to lower heparin levels as found during "low dose thrombose prophylaxis" (LDThrPr).

Methods: Thrombin-titration was performed with 0.2 U thrombin per instead of 0.3 U (standard concentration). For heparin determination Berichrom-Heparin test kit was performed with Behring-Chromotimer according to the test-instruction. In in vitro experiments Heparin was added in increasing amounts to normal plasma and thrombin time and Anti-Xa activity were determined. Additionally these parameters were measured in several plasma samples of patients under LDThrPr.

Results: The TT increased according to the plasma heparin level from 20 seconds to nearly 40 seconds if the heparin level increases from zero to 0.2 U Heparin/ml plasma. In patients with LDThrPr the TT correlates to heparin level equally to the experiments. Time courses of sc. administrated low dose heparin show that both parameters are significantly altered. But some patients with possible high tolerance against heparin did not demonstrate elevation of both parameters.

Summary: The thrombin-titration from 0.3 to 0.2 U thrombin enables the control of LDThrPr. The Anti-Xa activity was in the range between 0.05-0.2 U heparin/ml plasma to be held sufficient for LDThrPr. Additionally patients with possible high tolerance against heparin could be withdrawn as non-responders requiring higher doses of heparin.

Department of Haematology, University of Marburg, D-3550 Marburg.

STUDIES ON THE POSSIBLE PATHWAY OF VARIOUS HEPARINS IN PLASMA VIA INHIBITION OF ALPHA AND BETA FACTOR XII.

In previous studies we described chromogenic peptide substrate (CS) assays for plasma inhibitors of β-FXIIIa. We also reported that some heparins reduced β-FXIIIa inhibition. This effect was reversed in a dose-dependent manner by aprotinin. We subsequently developed CS assays for determining inhibitors of alpha-FXIIIa in plasma and in the present study compared the effects of heparins with and without aprotinin on the plasma inhibition of alpha- and β-FXIIIa (alpha-FXIIIa and β-FXIIIa). 4 unfractinated and 4 fractionated heparins were studied. These were added to pooled normal plasma in a range of anti FXa activities from 0 to 4 U/ml. The samples were diluted and tested at plasma dilutions equivalent to the 100 % value used for preparing the standard curve and the percentage inhibition calculated from a standard curve prepared from the pooled plasma without heparin. The effect of aprotinin was investigated by adding aprotinin (0 - 400 KIU/ml) to the heparinised plasmas. All of the heparins reduced β-FXIIIa at 1 or 2 U/ml. One fractionated heparin reduced alpha-FXIIIa, another had no effect whilst the other four heparins increased alpha-FXIIIa. In the absence of aprotinin aprotinin had no effect on alpha-FXIIIa or β-FXIIIa. Where inhibition of β-FXIIIa was reduced aprotinin counteracted the heparin effect to some extent but had no effect on the heparin which reduced alpha-FXIIIa. With the heparins which potentiated alpha-FXIIIa aprotinin gave further dose-dependent increases in inhibition. Our results show that at similar anti FXa concentrations various heparins have different effects on the plasma inhibition of alpha- and β-FXIIIa.

Abt. Thorax-, Herz- und Gefäßchir., Univ. Tübingen, Calwerstr.7, D-7400 Tübingen.

EFFECT OF HEPARINS ON PLATELET-INDUCED THROMBIN GENERATION TIME AND PLATELET ADOPTION.

A. Basic-Mitic, M. Basic-Mitic, R. Corin, H. Merkens, D. Deschner, H. Sadeghi and H.K. Breddin.

PITT (Platelet-Induced Thrombin Generation Time) is a new sensitive test system, in which the time of aggregation (Ta) and coagulation (Tc) of platelet rich plasma (PRP) are measured. PRP from partially anticoagulated blood samples is rotated in the light beam of a photometer.

8 volunteers received a single s.c. injection of a low molecular weight heparin (LMNH), Fraxiparin (Fx), Sanofi, Munich (36 mg). Ex vivo effects of Fx on PITT and platelet adhesion.

After an interval of at least 7 days the same volunteers received unfractionated heparin (UFH, Liquemin) in a single s.c. dose of 5000 IU. Fx significantly inhibited PITT and platelet adhesion. Thrombin inhibition occurred 3 hours after the s.c. injection following a slow normalization. 21 hrs after the injection the preinjection values were reached. In contrast the inhibition of platelet adhesion lasted longer with the highest reduction between 8 and 15 hrs after injection. UFH showed similar results.

26 surgical patients without antithrombotic prophylaxis showed a significantly stimulated platelet adhesion and PITT during and 1 day after the operation with normalization 5-6 days postoperatively.

16 patients undergoing similar surgical interventions received Fx (36 mg s.c. in a single daily dose) did not show increased platelet adhesion. From the 3rd to the 7th postoperative day adhesion was significantly reduced. Thus monitoring of antithrombotic treatment may be improved by measuring platelet function parameters as platelet adhesion or a very sensitive clotting assay.

Zentrum der Inneren Medizin, Abt. für Angiologie, J.W. Goethe-Universität, Theodor-Stern-Kai 7, 6000 Frankfurt/ Main 70.
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STUDIES IN HEPARIN-INDUCED THROMBOCYTOPENIA IN CORONARY HEART DISEASE AND PLATELET DAMAGE DURING EXTRACORPOREAL CIRCULATION

W. Heller

Today the side-effects of heparins on the platelets and the lipolytic effect seemed to be sufficiently well-known. For a long time the possibilities for improving the heparin treatment were limited by insufficient knowledge of various aspects such as heparin structure, conditions for structural activity, the exact mechanism of the anticoagulatory effect and the exact pharmacokinetics. On the basis of our model studies with high and low molecular heparins (4 HMW-a,b,c,d and 4 LMW-A,B,C,D-heparins) it seemed reasonable to carry out comparative investigations regarding efficiency, or possible side-effects on cellular components of the blood. The model studies were conducted with fresh blood in a closed-loop heart-lung machine system (ECC model). Blood sampling from the system was carried out according to the following scheme: Patients (HMW-heparin) were studied during ECC, before recirculation and heparinisation, after 1, 5, 10, 20, 30, 60, 90 min. recirculation. Noticeable damage to the platelet membrane can be observed in the recirculation phase through the application of heparin B and C already after 10 min. the values are distinctly higher than in the other investigated heparins. This damage is most marked after 90 min. with heparin C. The other two LMW-heparins behave similarly to the HMW-heparin. If we consider the 1-minute value, i.e. after the addition of heparin to the stored blood, already at this point we can observe the release of PF4. From these results it must be concluded that heparin B and C have a considerable membrane-damaging effect on the platelets in the recirculation phase. This then means that in our study with B a strong heparin-activating process occurs. On account of our findings the heparins can again be divided into two further groups, namely those with high affinity towards AT III and those with low affinity.

Abb. Thorax-, Herz- und Gefäßchir., Univ. Tübingen, Calwerstr. 7, D-7400 Tübingen

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HEPARIN ASSOCIATED THROMBOCYTOPENIA (HAT): CROSSREACTIVITY OF LMW HEPARINS AND PROSPECTIVE SELECTION OF A COMPATIBLE HEPARINOID BY HIPA ASSAY

A. Greinacher, I. Michels and C. Mueller-Eckhardt

Diagnosis of HAT type II and treatment of thromboembolic complications in these patients are difficult. Recently we have developed the heparin induced platelet activation (HIPA) assay which allows a rapid confirmation of the tentative diagnosis of HAT type II.

In vitro studies with sera of 30 patients revealed crossreactivity to the LMW heparin Fragmin, Fraxiparin and Clexane whereas a LMW heparinoid, Org 10172 (Lomparan), did not.

In a prospective study this heparinoid was selected for 10 HAT patients, for whom further parenteral anticoagulation was required. In 7 of these patients who received LMW heparins prior to laboratory investigations low platelet counts persisted under treatment with LMW heparins and 2 patients developed additional thromboembolic complications.

Upon treatment with Org 10172 platelet counts normalized in 8 patients, in 1 patient thrombocytopenia was unrelated to parenteral anticoagulation, in 1 patient platelet count normalized after discontinuation of Org 10172.

We conclude that the HIPA assay allows the laboratory diagnosis of HAT type II and the selection of a compatible heparin or heparinoid for further parenteral anticoagulation.

Institut für Klinische Immunologie und Transfusionsmedizin der Justus-Liebig-Universität, Langhansstraße 7, D-6300 Gießen

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HEPARIN ASSOCIATED THROMBOCYTOPENIA IS NOT CAUSED BY A HEPARIN SPECIFIC ANTIBODY

A. Greinacher, I. Michels and C. Mueller-Eckhardt

Heparin associated thrombocytopenia (HAT) is the most common drug-induced thrombocytopenia caused by an immunologic mechanism. The hypothesis was assessed whether HAT may be caused by an antibody dependant on polysulfated oligosaccharide epitopes, present not only on heparin but also on different polysulfated substances such as dextran sulfate and pentosan polysulfate.

We found that the major factor for eliciting platelet activation with sera of HAT type II patients is neither the structure nor the AT III binding capacity of an oligosaccharide, but rather its grade of sulfation. This was investigated by in vitro cross reactivity studies with sera of HAT type II patients using unfractionated heparins, LMW heparins (Fragmin, Fraxiparin), enoxaparin, LMW heparinoid (Org 10172), de-N-sulfated heparin, dextran sulfate, pentosan polysulfate and dextran. Platelet activation was measured by the heparin induced platelet activation (HIPA) assay and the serotonin release assay (5-HT). The platelet activating factor was isolated with the IgG fraction, but did not bind to heparin and dextran sulfates fixed to a solid phase.

We conclude that heparin-associated thrombocytopenia is not caused by a heparin-specific antibody and that a major factor contributing to the pathomechanism is the high grade of sulfation present in a variety of polysulfated oligosaccharides.

Institut für Klinische Immunologie und Transfusionsmedizin der Justus-Liebig-Universität, Langhansstraße 7, D-6300 Gießen

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RADIOPHARMACOLOGY OF A HOMOGENOUS HEPARIN OLIGOSACCHARIDE IN RATS

G.Stehle*, E.Friedrich*, H.Simm*, H.Schrenk*, J.Harenberg*, D.L.Heene*

The pharmacokinetics of heparin are not yet fully understood. Radiochemical studies with 35-S, 3-H, 125-Iodine suggest for the liver, the endothelium and the kidneys a role in inactivating and degrading heparin. However difficulties in interpretation of the studies arise due to the heterogeneous structure of heparin and to tracer instability. In this study we overcame these problems with newly synthesized compounds. A HPLC purified narrow molecular weight range heparin (3600 kD) was used, and the newly developed "cumulative labelling" technique was applied. This label allows to identify in vivo the degradation sites of heparin. 24 rats received different dosages of the biologically active heparin oligosaccharide tracer (10, 50 and 100 μg). With in 10 minutes about 30 - 40 % of the tracer activity appeared in the liver, however only about 5 to 15 % was found in the urine, and the rest of the tracer was likely to be metabolized by the endothelium of the vessel wall. Comparing conventionally labelled heparin with cumulatively labeled heparin allows to draw the conclusion that 1) considerable amounts of heparin are metabolized in the liver within minutes after application and that 2) conventionally labelled tracers are not reliable due to rapid degradation.

* Universität Heidelberg, Klinikum Mannheim, I. Medizinische Klinik, Theodor Kutzer Ufer, D-6800 Mannheim,
+ Abteilung für Radiochemie und Radiofarmakologie, Deutsches Krebsforschungszentrum, D-6900 Heidelberg
OPTIMIZATION OF A DRUG TREATMENT SCHEDULE (UF-HEPARIN FOLLOWED BY LMW-HEPARIN) FOR POST-PTCA REDUCTION OF RESTENOSIS USING A NON-HUMAN PRIMATE MODEL

P. Bachr, O. Iqbal, D. Hoppensteadt, J. M. Walenga and J. Fareed

Percutaneous transluminal angioplasty (PTCA) approaches are increasingly used to treat chronic and acute coronary artery stenosis. Unfortunately, due to a variety of pathophysiologic processes and restenosis occurs in 30-50% of patients after a successful procedure. Currently, studies are in progress to investigate the influence of low molecular weight heparin (LMWH) prophylaxis on the patency rate after PTCA. Our aim was to determine an optimal schedule to start the LMWH prophylaxis after therapeutic heparinization with UFH. The alterations of the hemostatic parameters during the drug regimens change-over were evaluated. Non-human primates (Macaca mulatta) were divided into 6 treatment groups (n=3/group). Three groups received UFH i.v. at a dose of 15 U/kg to mimic the end phase of treatment in patients after PTCA with a constant therapeutic infusion for 12-24 hrs. Three groups were started with full heparinization (250 U/kg i.v.) to mimic patients without the above interim phase. Following heparinization, LMWH (Sandoparin) (1 mg/kg, s.c.) was started at various intervals. The group initially treated with 15 U/kg UFH exhibited a continued anticoagulant effect when LMWH was started 30 min after the heparin injection. However, when LMWH was started after 2 hours the measurable anticoagulant effect was lost during 1 and 3 hours after the heparin injection. When LMWH was started 2 or 4 hours after the full heparinization, the anti-coagulation response sustained. There was no significant difference between these two regimens. Although our data shows definite differences between the studied drug treatment schedules, further studies are warranted prior to an optimal drug regimen can be suggested for clinical use.

Hemostasis Research Lab., Loyola University Medical Center, 2160 S. First Avenue, Maywood, IL 60153, U.S.A.

THROMBIN-ANTITHROMBIN III COMPLEX FORMATION IN PATIENTS WITH DEEP-VEIN THROMBOSIS RECEIVING EITHER LOW MOLECULAR WEIGHT HEPARIN OR UNFRACTIONATED HEPARIN

W. Polacheck, E. Halasz, K. Gutierrez*, G. Kuner*, J. Sharr* and P. Hoppeort

It is still a topic of discussion whether low molecular weight heparin (LMWH) can be recommended for the treatment of deep-vein thrombosis. In this connection, we studied thrombin formation as reflected by the measurement of thrombin-antithrombin III complexes (TAT) in patients with phlebographically recorded acute venous thrombosis, who were treated either with LMWH (group I, n = 6, age: 61.7 ± 14.6 years) or with unfractionated heparin (UFH) (group II, n = 6, age: 62.7 ± 5.2 years).

Dosage: Patients in group I were given LMWH subcutaneously (Fragmin, Kabi Vitrum, Sweden) (0.6-100 IU/kg b.w. x 10 000 IU/d, if >80 kg b.w. 2 x 12.000 IU/d); the plasma level was kept between 0.5-4.5 mu/ml (bottom value) and 1.0 X aA/ml (peak value). Patients in group II received UFH (Heparin Immuno, Immuno, Austria) intravenously (initially 100 IU/kg b.w. followed by an infusion of 1.000 IU/h). TAT were measured by immunoassay (Enzymostat TAT, Behring, Germany).

Results: During the initial phase of heparin therapy, subcutaneous high-dose LMWH is less effective in inhibiting thrombin formation than an intravenous infusion of UFH.

Central Laboratory and 2nd Med. Dept. (*), KA Rudolfstiftung, 1030 Vienna.
NEW DEVELOPMENTS IN THE MECHANISM OF GLYCOSAMINOGLYCANES AND LOW MOLECULAR WEIGHT HEPARINS

W. Heller, H.P. Wendel, R. Klaffenholz, H-E. Hoffmeister

Recently lactic acid (LBA) was introduced as an anticoagulant and we compared its pathway with some LMW- and HMW-heparins in an recirculation model (CPB) under ex vivo conditions.

We first studied a range of LBA concentrations in the model (10, 25, 50, 75 µg/ml) monitoring fibrinopeptide A (FPA) and thrombin-antithrombin complexes (TAT). At 10 µg/ml LBA clot formed in the CPB machine whilst at 25 µg/ml significantly elevated levels of FPA and TAT were seen after 30 minutes recirculation. The results with 50 and 75 µg/ml showed equally good anticoagulation. We then compared 50 µg/ml LBA with heparin (3 U/ml) in the model (10 runs with each anticoagulant).

Blood samples were taken before CPB and at various times during recirculation and plasma samples used for determining various components of the plasma calilric system together with antithrombin III (AT III) and platelet factor 4. The activation of the KK system as evidenced by raised kallikrein-like activities and reduced prekallikrein and B-FXIIa inhibition was markedly lower in the LBA group. Also platelet factor 4 levels were significantly lower in this group.

These results indicate that 50 µg/ml LBA is a suitable concentration for anticoagulation in a CPB model and that this anticoagulant reduces the contact activation and platelet damage seen with heparin.

On the basis of our investigations we were able to show that through measuring the activity of FXII, FXII inhibition as well as alpha-FXIIa and B-FXIIa inhibition, taking into account the FXa and FXIIa activity it is possible to demonstrate the pathway of these anticoagulants in detail. Thus it can be shown that LBA acts mainly via the fibrinolytic pathway.

Abt. Thorax-, Herz- und Gefälschir., Univ. Tübingen, Calwerstr.7, D-7400 Tübingen

SUCCESSFULL OUTCOME OF LUPUS PREGNANCY - A CASE REPORT

V. Hach-Wunderle and I.Scharrer

Several therapy regimens have been recommended to improve the rate of live births in women with lupus anticoagulants. Recently ROSOVE et al. (1990) reported a success rate of 93% in 14 women, treated with full-dose heparin and a daily dose of 50mg acetylsalicylic acid. At 28 weeks' gestation she was found in the coagulation and fibrinolytic system.

Being pregnant again, she was immediately treated with unfractionated heparin subcutaneously (3x 10000 U or 2x 12500 U/day). APTT was prolonged twice as long as normal, directly before the next heparin injection. At 25 weeks' gestation the patient was treated with heparin and a daily dose of 50µg acetylsalicylic acid. At 28 weeks' gestation she gave birth to a boy (weight 700g) by caesarian section. The 10 month-old child is now in a good physical condition.

William Harvey-Klinik, D-6350 Bad Nauheim; #Universitätsklinik, Abt. Angiologie, D-6000 Frankfurt/Main

IN VITRO METHODS FOR MONITORING RECOMBINANT HIRUDIN (rH)

J. Hägel, I. Stefanidis, N. Maurin

Monitoring by means of a simple coagulation method is necessary to control administration of rH for anticoagulation in extracorporeal circulation, the aim being to achieve rH concentrations of 3.5 to 9 µg/ml. We investigated the suitability of a number of tests which can be carried out routinely.

Blood from 20 healthy subjects was used. Citrated platelet-poor plasma was mixed with varying rH concentrations (Knoll AG, 15000 ATU/ml) for prothrombin time (Thrombin's value), activated partial thromboplastin time (aPTT), thrombin time (TT) and Heptest®. For activated clotting time (ACT, ACTester®) blood was extracted in syringes with differing hirudin concentrations.

At low hirudin doses (1-2 µg/ml), there was an exponential rise in TT. With Heptest® an exponential rise was also observed in a range between 5 and 10 µg/ml. With the Quick's value, a concentration-dependent fall was observed in the 0-50 µg/ml range. For aPTT, there was a virtually linear rise between 0 and 20 µg/ml, which then flattened off. ACT showed a clear linear rise between 0 and 10 µg/ml.

Taking the intended therapeutic dose range into account, ACT and aPTT appear suitable for hirudin therapy monitoring, by virtue of their linear correlation, whereas the Heptest® and TT are rather unsuitable, owing to the exponential curve.

Medical Clinic II, University Hospital Aachen, Pauwelsstr. 30, D - 5100 Aachen

HIRUDIN CONSUMPTION RESULTING FROM SNAKE VENOM-INDUCED ACTIVATION OF PROTHROMBIN IN RATS

E. Bucha and G. Nowak

Bleeding after overdosage of hirudin or its impaired renal elimination in case of nephropathies represents the most undesirable side effect of this tight-binding selective thrombin inhibitor from medicinal leeches or its recombinant variants. So far, no efficient hirudin antidote has been found. In this context, meltothrombin, an stable intermediate product of the Echis carinatus-induced prothrombin-thrombin conversion was studied for its suitability as hirudin antidote.

The meltothrombin formation was recorded in highly diluted human plasma by a chromogenic substrate method. Hirudin is able to completely inhibit the formation of meltothrombin in a dose-dependent manner.

In vivo, bilateral nephrectomy in Wistar rats of either sex was followed by administration of hirudin (1 mg/kg). One hour later, the hirudin blood level remained constant at 3.5 - 4.2 µg/ml. An E. carinatus venom dose of 50 µg/kg x h was infused 180 min after hirudin administration. After starting the infusion the hirudin blood level dropped sharply reaching significantly reduced values of 2.2 µg/ml. Platelet count and fibrinogen level in plasma remained unchanged in all experiments.

Hence follows that ecarin, the purified protease from E. carinatus represents a remarkably efficient hirudin antidote.

Institute of Pharmacology and Toxicology, Medical Academy Erfurt, FRG.
PLATELET MEDIATED VASOCONSTRICTION IS NOT SOLELY DEPENDENT UPON THROMBIN. AN EXPERIMENTAL STUDY WITH R-HIRUDIN AND A POLYDESOXYRIBONUCLEOTIDE DERIVED DRUG (DEFIBRODITE).

G. Kindel, F. Bacher, O. Eqbal, B. Lojewski, J.M. Walenga and J. Fareed

The hypothesis that thrombin plays the key role in the activation of platelets and its related vasoconstriction was investigated with platelet aggregation studies and a coupled platelet activation/rabbit aortic strip technique. Recombinant hirudin (r-H) (1-5 mg/kg) and defibrotect D (D) (1-10 mg/kg) were injected i.v. at various doses to New Zealand White rabbits. D was also administered p.o. at 10 mg/kg in one group. Blood was drawn at baseline, 15, 30, and 60 minutes followed by the dissection of the thoracic aorta. Citrated platelet rich plasma (PRP) from the same animals was also prepared. PRP from r-H treated animals exhibited similar response to saline treated animals in the aggregation assays. r-H treated PRP did not inhibit AA induced vasoconstriction of the aorta. In contrast, D treated PRP exhibited a significant inhibitory effect on AA induced vasoconstriction. All dosages of D (i.v. or p.o.) produced the same degree of inhibition. Collagen activated PRP obtained from either r-H or D treated animals did not reveal any significant difference from PRP obtained from saline treated animals. Thus, the agonist mediated platelet aggregation is modified by D and vasculoactive mediators associated with platelets upon activation are also markedly inhibited by this agent. For the specific inhibitor of thrombin, r-H, this was not found at the dosages studied. This study suggests that thrombin is not the sole mediator of platelet mediated vascular pathology.

Hemostasis Research Lab., Loyola University Medical Center, 2160 S. First Avenue, Maywood, IL 60153, U.S.A.

RECOMBINANT HIRUDIN AS A HEPARIN SUBSTITUTE IN A CARDIOPULMONARY BYPASS MODEL

J.M. Walenga, M. Koza, M. Wallock, D. Poppensteadt, N. King, M. Bahkos, J. Fareed and R. Pifarre

We have studied the anticoagulant efficacy of recombinant (r) hirudin, a thrombin inhibitor, in cardiopulmonary bypass (CPB) in a dose–finding study (n=10/group). An intracardiac (i.c.) bolus of 1-2 mg/kg r-hirudin was administered followed immediately by an intravenous infusion of 1-2 mg/kg/hr (control, 1.66 mg/kg heparin i.c.). Male mongrel dogs were placed on bypass for 60 min. No antagonist was used to reverse the effect of r-hirudin. The dogs remained anesthetized for 180 min post-CPB. For all groups coagulation parameters were markedly elevated during CPB but returned to normal within 30 min after CPB. Bleeding times were 7-8 min during CPB (>15 min, heparin group). Hematocrit, platelet count and fibrinogen showed an initial decrease due to hemodilution but no further change during CPB. No difference in blood loss was observed between the various r-hirudin and heparin groups. Pump-line filters contained significantly higher amounts of fibrin deposits for the 1-1.5 mg/kg/hr r-hirudin dosages (p<0.02) but equivalent deposits as the heparin group for 1.5-2.0 mg/kg/hr dosages. The bolus plus infusion was also more effective than bolus alone based on amount of filter deposits. No microthrombi were observed in vital organs in all groups. These data suggest that r-hirudin may be as safe and effective an anticoagulant as heparin for use in CPB and that further studies are warranted.

Hemostasis Research Lab., Loyola University Medical Center, 2160 S. First Avenue, Maywood, IL 60153, U.S.A.

THE THROMBIN INHIBITOR HIRUDIN IS SIGNIFICANTLY MORE EFFECTIVE IN PREVENTING THROMBUS FORMATION OR CORONARY STENTS THAN HEPARIN.

C. Unterberg, A. B. Buchwald, D. Sandrock, R. NebenGall, D. L. Munz, J. Schrader, V. Wiegand.

Deposition of 111-Indium labeled platelets (PLT) and 125-Iodine labeled fibrinogen were measured 12 hours after coronary stent implantation in minipigs. Prior to stenting, animals in group A (13 stents) received a bolus of 1 mg/kg Hirudin (HIR) and 250 mg acetylsalicylic acid (ASA), followed by an i.v. infusion of 1 mg/kg HIR per hour. In group 2 (11 stents) heparin (HEF, 100 U/kg, ASA (250 mg) and dextran (500 ml) were given i.v. before stenting followed by 50 U/kg HEF per hour i.v. FTT was prolonged to above 2x control values in both groups. After 12 hours, hearts were excised and stented coronary segments were counted for 111-Indium and 125-Iodine activity. Histologic examination (5 slices/stent) showed presence of medial tear in 6 stents in group 1 and in 6 stents in group 2. In group 1, 19.7 ± 2.4 x 106 PLT/stent were present, as compared to 64.3 ± 16.7 x 106 PLT in group 2. (p<0.05) In stents with intimal tear, PLT deposition amounted to 24.4 ± 4.1 x 106/stent in group 1 (n=6), and to 103.2 ± 18.9 x 106/stent in group 2 (p<0.01). Likewise, fibrin deposition in group 1 was significantly lower compared with group 2 (138.4 ± 34.8 vs 315.4 ± 119.6, p<0.05). It is concluded that HIR significantly reduces thrombus formation on coronary stents compared to heparin, particularly so in the presence of medial dissection.

Abteilung Kardiologie, Universit~t Göttingen, Robert-Koch-Str. 40, 3400 Göttingen
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RECOMBINANT DESULFATO HIRUDIN, A SPECIFIC ANTIMITHROMBIN, IN PATIENTS WITH CHRONIC, STABLE CORONARY DISEASE: EFFECT ON HEMOSTATIC PARAMETERS P. Zoldhelyi, J.H. Whe;s, M.W., Web.ster, D.J. Drill, G. G, D. J. Edwards, C.P. Broat, V. Fust.r

In animal studies, recombinant desulfato hirudin (GFP53953 'hirudin') prevents fibrin and platelet-rich arterial thrombosis at activated partial thromboplastin times (aPTT) of 2-3x baseline. We evaluated the hemostatic parameters in 39 patients with chronic, stable coronary artery disease in a single-blind, ascending dose, placebo-controlled study. Thrombin time (TT), aPTT, prothrombin time (PT), and bleeding time (BT) were measured in sec before, during and for 18 hours after a 6 hour infusion of hirudin or placebo. Antiplatelet drugs were stopped 7 days prior to the study. Partial data (mean±SD) are shown below:

| Dose (mg/kg/h) | TT (sec) | aPTT (sec) | BT (sec) |
|---------------|---------|-----------|--------|
| Placebo       | 30      | 50±3      | 18±2   |
| 0.05          | 40±5    | 60±5      | 20±3   |
| 0.10          | 50±5    | 70±5      | 22±3   |
| 0.20          | 60±5    | 80±5      | 24±3   |

The only side effect was mild bruising at a venipuncture site in one patient receiving 0.1mg/kg/h. No antibodies to hirudin were detected 2 weeks after the infusion. The anticoagulant effect of hirudin was best reflected in the prolongation of the aPTT with a near linear dose-response at the dosages tested.

A short-term infusion of hirudin is safe and well-tolerated at dosages of up to 0.3mg/kg/h which prolonged the aPTT to 3.0 x baseline (or 2.2 x the upper normal value). Hirudin offers a potentially high benefit to risk ratio in the treatment of arterial thrombosis.

Mayo Clinic, 200 S.W. 1st Street, Rochester, MN 55905 USA

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ANTITHROMBOTIC AND HEMORRHAGIC PROPERTIES OF A SYNTHETIC TRIPPEPTIDE (DUP 714) IN COMPARISON TO RECOMBINANT-HIRUDIN

D. Celuss, P. Bacher, O. Iqbal, D. Hoppensteadt, J.M. Walenga and J. Farbrook

The pharmacologic activities of a newly developed synthetic tripeptide (DUP 714) for protease inhibition was investigated in ex vivo animal models. The antithrombotic effect was studied in vivo in rabbits using a modified Wessler stasis thrombosis model (STM). The hemorrhagic effect was tested in a rabbit ear blood loss model. During these experiments blood was drawn for ex vivo testing to determine the coagulation profile (PT, APTT, 10 U TT, 10 U Ca++TT, anti-IIa, anti-Xa) and to calculate the plasma concentrations using postconstrued calibration curves. A clear dose-dependent antithrombotic effect was observed in the STM from 6.3 to 50 mg/kg for DUP 714 (n=3/group). On an equimolar dose basis DUP 714 exhibited a 2-fold stronger antithrombotic effect than recombinant-hirudin.

A significant bleeding effect in the rabbit ear bleeding model was observed but only at doses of 125 mg/kg and above (n=3/group). In contrast, APTT was not potent anticoagulant which acts mainly by inhibiting thrombin but also possesses other antiprotease actions. It exhibits a strong antithrombotic effect with low bleeding properties in ex vivo animal models which could indicate a broad therapeutic range. However, further studies need to be performed to validate these conclusions.

Hemostasis Research Lab., Loyola University Medical Center, 2160 S. First Avenue, Mayowd, IL 60153, U.S.A.

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ANTICOAGULANT AND ANTIARTHROMBIC ACTIVITIES OF APROSULATE SODIUM (LW 10082), UNFRACTIONATED AND LOW MOLECULAR WEIGHT Heparins.

Fumitoshi, A., Sugidachi, A. and Kojike H.

Aprosulate sodium (LW 10082, developed by Luitpold-Werk, Munich) is a novel synthetic anticoagulant agent which is currently under clinical evaluation. The anticoagulant and antithrombotic effects of aprosulate sodium (AS), unfractionated heparin (UH) and low molecular weight heparin (LH) were evaluated on a weight basis and were related to their effects on hemostasis. All of three agents exhibited concentration-dependent anticoagulant activities in aPTT assay in vitro. AS, but not UH and LH, showed a species specificity with a greater potency in rat and human plasma. I.v. bolus injection of these agents in rats produced an immediate prolongation of the aPTT, but the effects of AS (0.9 mg/kg) were more long-lasting than those of UH (0.2 mg/kg) and LH (0.5 mg/kg) at equipotent doses indicated in the parentheses. The dose-response relationship of anticoagulant effect was less pronounced for AS than UH and LH. UH and LH exhibited a dose-related anti-Xa activity, whereas AS did not show anti-Xa activity even at the highest dose used (80 mg/kg). The antithrombotic effects were assessed using a rat A-V shunt model, where all of these agents inhibited thrombus formation in a dose-dependent manner. At the antithrombotic dose, UH prolonged the bleeding time measured by the rat tail transection method, but AS and LH did not. The bleeding time on different animal models (Wessler-test, Harhauer-model, laser induced coagulation, and epinephrine) on platelet aggregation was observed. In different animal models (Wessler-test, Harhauer-model, laser induced coagulation, and rat jugular vein clamping model) in rabbits and rats, the compound demonstrated interesting antithrombotic activities. In contrast to its anticoagulant activity, the antithrombotic activity of aprosulate sodium was neutralized by protamine. In exaggravometric doses protamine neutralized 70 % of the Heptest clotting time and the thrombin time. When the protamine dose was increased to 2.0 times the dosage of aprosulate, a complete antagonism to the anticoagulant and antithrombotic effects took place. The mode of action of aprosulate is not fully enlightened, but by preliminary results it is supposed that it acts on the intrinsic pathway.

Department of Pharmacology and Chemistry, Luitpold-Werk, Chemisch-pharmazeutische Fabrik, Zielstellstrasse 9, W-8000 Mlnchen 70, FRG
PHARMACOLOGICAL STUDIES ON A SYNTHETIC THROMBIN INHIBITOR OF THE TRIPEPTIDE TYPE
B. Kaiser, J. Hauptmann, M. Richter and E. Glusa

The synthetic thrombin inhibitor D-phenylalanyl-L-prolyl-L-arginine nitrile (PPACN) was studied with respect to its toxicity, pharmacokinetics, pharmacodynamics as well as anticoagulant and antithrombotic actions. In mice the LD₅₀ after i.v. injection was 30-40 mg/kg. After i.v. administration into rats PPACN reduced the arterial blood pressure up to 70-80% of initial values. The bleeding time after standardized incision of the rat tail was not significantly prolonged and at tolerated doses. The pharmacokinetic behaviour of PPACN was characterized by a biologic half-life of 12 min after i.v. injection, no measurable plasma levels after p.o. or i.d. administration of high doses, prolonged time course of plasma concentrations after s.c. injection and pronounced binary excretion of the active compound. PPACN was anticoagulantly effective in common test assays such as TT, APTT, PT and thrombelastography; it prolonged clotting times in a concentration-dependent manner and inhibited thrombin-induced aggregation of human blood platelets. The antithrombotic potency of PPACN could be demonstrated in rats using models of stasis-induced venous thrombosis, arterial thrombosis after electrically induced damage of the vessel wall, extracorporeal a-v shunt thrombosis as well as thrombin-induced microthrombosis. Maximum antithrombotic effects were seen at doses ranging from 0.3 to 1 mg/kg/min.

Institute of Pharmacology and Toxicology, Medical Academy, Nordhäuser Str. 74, D-5010 Erfurt, FRG

DESIGNING OF NOVEL THROMBIN INHIBITORS, J. Stürzebecher, H. Vieweg and P. Wikström

Thrombin is the key enzyme in coagulation and its inhibitors are of therapeutic interest since they are potential anticoagulants. Thrombin cleaves the C-terminal bonds of the basic amino acids Arg and Lys. Inhibitors of this enzyme have been found not only among Arg and Lys derivatives but also with structurally related benzanilides. The most potent inhibitor of benzamidine type is Na-(2-naphthylsulfonyl)-4-amidinophenylalanine piperidide (α-NAPAP). However, α-NAPAP and other substances designed so far do not fulfill all pharmacological and toxicological requirements. Obviously, the undesired effects of benzamidines are due to the basicity of the amidino moiety. This prompted us to synthesize novel isosteric derivatives with other basic groups containing phenylalanine as key building block.

Some of the newly synthesized compounds possess a high affinity for thrombin and exert Kᵢ-values near nanomolar range. They are selective inhibitors of thrombin. First studies indicate improved pharmacokinetic properties. Thus, significant blood levels were observed after s.c. and p.o. administration. Enteral absorption was never observed with thrombin inhibitors derived from benzamidine thus far.

*Institut für Pharmakologie & Toxikologie, Medizinische Akademie Erfurt, D(O)-5010 Erfurt Pentapharm Ltd., CH-4002 Basel, Switzerland;

1Luitpold-Werk, Chemische-pharmazeutische Fabrik, Zeltstatatstr. 5, 8000 München 70; 2Inveresk Clinical Research, Edinburgh; 3Loyola University, Chicago; 4Technische Universität, München
**ACTIVATED PROTEIN C**

H.J. Ehrlich1,2 and N.U. Bang2

Human protein C is the zymogen of a vitamin K-dependent plasma serine protease with potent anticoagulant properties. Activation of the zymogen is accomplished through proteolytic removal of a 12 amino acid activation peptide from the NH2-terminus of the heavy (COOH-terminal) chain, a reaction physiologically catalyzed by the thrombin-thrombomodulin complex. Activated protein C (APC) in turn proteolytically degrades coagulation cofactors Va and VIIIa, thereby playing a critical role in the regulation of thrombin generation. A potential therapeutic utility of APC in the prevention and/or treatment of micro- and macrovascular thrombosis is suggested by the following observations: the i.v. administration of APC a) prevented the extension of venous thrombi in dogs and rhesus monkeys; b) prevented platelet-dependent thrombus formation under arterial flow conditions in baboons; and c) protected the animals from an otherwise lethal E.coli infusion in a baboon model of septic shock. Presently, both plasma-derived as well as recombinant APC are rapidly approaching clinical trials. Basic research already focusses on second generation molecules, e.g. on mutants of human protein C that do not need the cofactor activity of thrombomodulin for efficient activation by thrombin.

1Kerckhoff-Klinik, Dept. of Hemostasis and Blood Transfusion, Sprudelhof 11, 6350 Bad Nauheim, FRG; 2Lilly Laboratories for Clinical Research, Eli Lilly and Company, and Indiana University School of Medicine, Dept. of Medicine, 1001 W. 10th Street, Indianapolis, Indiana, 46202, USA; 3present address: Sandoz AG, Dept. of Clinical Research, Deutschherrenstr. 15, 8500 Nürnberg, FRG

**BLOCKADE OF PLATELET AGONIST RECEPTORS**

H. Patscheke

Platelet agonists like thromboxane A2 (TXA2), platelet-activating factor (PAF), ADP and serotonin are ligands of specific surface receptors that are activated by an occupation mechanism. In contrast, thrombin cleaves its receptor by a proteolytic attack that unmasks a tethered ligand within the thrombin receptor, thereby effecting receptor activation. Adhesion to collagen fibers comprises a multisite binding which greatly differs as a consequence of the different agonistic efficacy of the corresponding stimuli. Since thrombin is the most potent platelet agonist, inhibitors of the thrombin-platelet interaction have the highest potential for a platelet inhibition. TXA2 receptor antagonists strongly prolong the bleeding time but spontaneous bleeding has not been reported. Since PAF, ADP and serotonin are still less effective, their antagonists have weak effects on processes which involve also more potent agonists. Mediators like TXA2 or PAF may reach high local concentrations, e.g. in a septic lung. Hence, they can only be overcome by even higher concentrations of a competitive antagonist. Therefore, efforts to develop receptor antagonists with a non-competitive effect are justified in order to increase their potency and duration of effect. An attractive approach to antagonists with a non-competitive inhibitory effect is provided by competitive binding antagonists with a low dissociation rate. This can be demonstrated with some highly potent TXA2 receptor antagonists which TXA2 cannot displace rapidly enough to elicit receptor activation.

Institute of Medical Diagnostics, Klinikum Karlsruhe, 7500 Karlsruhe 1

**SELECTIVE INHIBITION OF PLATELET-VESSEL WALL INTERACTION: GLYCOPROTEIN (GP) RECEPTORS, MONOCLONAL ANTIBODIES, AND SYNTHETIC PEPTIDES**

R.E. Scharf1 and Z.M. Ruggeri2

Platelet adhesion to vessel walls and subsequent aggregation require binding of adhesive proteins (e.g. von Willebrand factor, fibrinogen) to GP receptors on the platelet plasma membrane. Consequently, inhibition of binding to these GPs by monoclonal antibodies or synthetic peptides could provide a useful antithrombotic strategy. Because of the key role of the GP IIb-IIIa complex in platelet aggregation, most of the studies that have used this approach have focussed on the interruption of binding of adhesive molecules to this receptor. Injections of monoclonal antibodies raised against GP IIb-IIIa cause an increase in bleeding time and a decrease in ADP-induced platelet aggregation, both indicative of impaired platelet function. Bleeding and thrombocytopenia are undesired side effects of this therapy. There is some evidence that anti-GP IIb-IIIa antibodies are more effective at reducing platelet adhesion than anticoagulants or thromboxane synthetase inhibitors. Furthermore, as GP IIb-IIIa binds several adhesive proteins, many of which bear the amino acid sequence Arg-Gly-Asp, it may be possible to prevent arterial thrombus formation by the use of synthetic peptides containing this sequence. However, the low affinity of most of the compounds studied so far represents a severe limitation to their potential pharmacological use. The substitution of residues on the amino terminal side of the Arg-Gly-Asp recognition sequence with basic residues has been shown to result in greater affinity of the peptide molecules. Thus, it is possible to tailor molecules with characteristics of specificity and affinity necessary for a pharmacological inhibition of the receptor function of GP IIb-IIIa. Recent experimental and clinical data will be discussed.

1Institut für Exp. Hämatologie und Transfusionsmedizin, Univ. Bonn, Sigmund-Freud-Str. 25, D-5300 Bonn 1
2The Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA

**NEW DEVELOPMENTS IN GLYCOSAMINOGLYCAN AND RELATED AGENTS.**

J. Fareed

The fundamental knowledge on heparin's polyelectrolyte nature, recognition sequence for humoral and cellular sites, biologic amplification role and signal transduction mediation have led to the appreciation of the biologic roles of non-heparin glycosaminoglycans (GAGs). Currently, several natural, semisynthetic and synthetic homologues of glycosaminoglycans are clinically developed. These include dermatans, heparans, chondroitin sulfates, native and depolymerized mixtures of GAGs, sulfated dextran, pentasulfonated polysulfate and marine polysaccharides. Knowledge of the structure activity relationships of these agents has led to the development of synthetic analogues of biologically active GAGs such as the chemically synthesized pentasaccharide, lactobionic acid amides and sulfated compounds with defined structural domains. Interestingly, these non-heparin GAGs exert profound biologic actions independent of antithrombin III and heparin cofactor II. Endogenous modulation of cellular functions, promotion of fibrinolysis, modulation of eicosanoids and cytokine function are some of the proposed mechanisms of these actions. Thus, beside the application in the prophylaxis and treatment of deep venous thrombosis, these agents have found their way in the prophylaxis and treatment of arterial thrombosis, cardiovascular disorders, adjunct usage for thrombolysis and prevention of post-coronary intervention restenosis. Major developments in identifying the endogenous role of non-heparin GAGs are expected to occur during the next few years. Several new drugs derived from non-heparin GAGs will become available for the prophylaxis and treatment of thrombotic disorders.

Hemostasis Research Lab., Loyola University Medical Center, 2160 S. First Ave., Maywood, Illinois USA
Clinical investigations with low molecular weight heparinoid: efficacy and safety.

J.W. ten Cate, Michael T. Nurmohamed, Harry R. Büllier.

Low molecular weight heparinoid (molecular weight range: 1000-10,000 d) has introduced into clinical research since 1982. A total of six randomized controlled clinical investigations have been enrolled since then in patients at high risk for venous thromboembolism, i.e. in patients with stroke and limb paralysis, in patients undergoing elective total hip replacement surgery or undergoing orthopedic surgery for fractured hip. A single study was undertaken in patients with malignancy undergoing other abdominal or thoracic surgery. The efficacy of heparinoid was assessed by objective tests for the diagnosis of deep vein thrombosis and pulmonary embolism.

Risk reductions (RR) in placebo controlled studies ranged between 73-86 percent, and in comparison with other accepted modes of anticoagulant prophylaxis the RR ranged between 35-62 percent. Hence, a satisfactory further reduction of the postoperative risk of venous thromboembolism in these patients at high risk. This RR was not associated with bleeding enhancement.

Of interest is the apparent safety of heparinoid in patients with heparin induced thrombocytopenia, which now emerges as a particular indication for the heparinoid.

Dir. Hemostasis, Thrombosis, Atherosclerosis and Inflammation Research, Academic Medical Center, Amsterdam.

Haemostaseologic and haemorheologic effects of garlic and fish oil

H. Kiesewetter, F. Jung, E.M. Jung, E. Wenzel

University of the Saarland, Dept. of Clinical Hemostasiology and Transfusion Medicine, 6650 Homburg-Saar

Mortensen (1983), Sanders (1983) and Green (1985) found a significantly increased bleeding time 2 to 3 weeks after the intake of 1.8 to 4 g/day eicosapentaenoic acid (in the form of fish oil capsules given over 2 to 6 weeks). After 6 weeks, however, the bleeding time did not differ from the placebo group. The compliance concerning the intake of 6-10 capsules/day is generally unsatisfactory. This could explain the decrease in bleeding time after 6 weeks of therapy. Rylance (1986) observed an increased coagulation time. The induced platelet aggregation was not influenced in almost all studies although Li & Steiner found an increased and Sanders and Rylands a decreased platelet aggregation. Hay shows a decrease in factor VIII antigen, other authors a reduced plasma viscosity and erythrocyte rigidity. In all, the haemostaseologic effects of fish oil capsules are not clear, but a slight improvement of blood fluidity seems to be assured.

Almost all investigators found reductions of all induced platelet aggregations and plasma viscosity after the intake of 600 mg powdered garlic/day or higher dosages of fresh garlic, oils, oil maceration products or alcoholic or aqueous tinctures. Increased blood coagulation times were found by Arora and Bordia. A fibrinolytic activity was found by almost all investigators (shortened euglobulin lysis time, increased IPA-activity). The plasmatic coagulation was not influenced.

INVESTIGATIONS ON THE COAGULANT PROFILE OF DERIVATIZED HEPARINS

A. Stemberger, P. Bader, S. Haas, J. Walenga, G. Bülilmen

Blood compatible biomaterials can be prepared by heparin fixation onto suitable biomaterials. Various procedures are described in the literature. Attempts were performed to fix heparin onto collagen using glutaric- and adipic dichloride. Following these procedures, collagen induced aggregation of thromocytes disappeared. However, little attention has been paid to the action of these chemical procedures on the pharmacological profile of heparin. Chemical modifications were performed with the divalent halides and acetylchloride. The available data demonstrate a loss of the anticoagulant properties. Alterations of the anticoagulant action of heparin can be performed with minute amounts of di- and monovalent reagents. The coagulation profiles were investigated with various clotting test such as: aPTT, Héparin, anti Xa- and anti IIa chromogenic substrate tests. The antithrombin III dependent effects were studied in a plasma system by the addition of specific antibodies directed to this inhibitor. As a result of these investigations, chemically modified heparins are devoid in enhancing the antithrombin III related inactivation of thrombin and factor Xa. The activity via heparin Cofactor II is not altered. Unfractionated and low heparins demonstrate the same reaction pattern, independent of the used chemical procedures. In the coagulation tests modified heparins and the heparinoid SP 54 behave in a similar way. As demonstrated by aPTT tests in plasma systems following addition of antibodies directed against antithrombin III, the heparin induced anticoagulant activity, is greatly diminished. However, a relevant activity is still detectable. When the modified heparins were tested without the addition of the anti-antithrombin III antibody, similar anticoagulant activities could be demonstrated. Evaluations on the chemical nature of the modifications are in progress. Crosslinking of heparin by the divalent halides is unlikely, HPLC analysis failed to demonstrate the nature of the modifications are in progress. Crosslinking of heparin by the divalent halides is unlikely, HPLC analysis failed to demonstrate.
THE LONGETEST ANTICOAGULATION WITH A COUMARIN DERIVATION LASTING FOR MORE THAN 10 YEARS IN PATIENTS HAVING SURVIVED A MYOCARDIAL INFARCTION

Christel Meinicke

The prevention of relapses following a myocardial infarction presents a still unsatisfactorily solved problem. As atherogenicity and thrombogenicity mutually induce each other and both additionally are strong causes, mediators an interruption of these deleterious functional circles and an efficiency in treatment become attainable only by control but not without considering computable reduction of the thrombogenic potential. On account of this we have - in spite of a wanted large acceptance - some years continued very successfully the follow-up treatment of myocardial infarction by the oral anticoagulant FaliThrom (TM), a coumarin derivation. We analysed exclusively patients who had survived a myocardial infarction and undergone this therapy for 10 and more years. Altogether 2 669 years of treatment respectively 15.3 (±/- 3.6) years per patient were analysed. The reliability of this treatment strategy is to be obtained only by a complex control of therapy taking into consideration secondary- and tertiary-illnesses as well as medicamentos interactions, and simultaneously taking for granted a good patient-compliance as well as a stable and an exact laboratory control. Under these conditions an effective anticoagulation will be possible to be practised up to an advanced age. The duration of this therapy of 60-70 years old patients suffering from a myocardial infarction amounts to 1 011 therapeutical years, that one of 70-80 years old comes to 818 years and that one of patients older than 80 years to 239 years. The duration of therapy of patients suffering from infarction older than 80 years on an average amounts to 15.9 (±/-2) years. Quick's values >30-35% are to be stated but seldom. In case suchlike will be found out, the patient will get about 30 minutes after blood-taking a subcutaneous injection of heparin.

Krankenhaus Friedrichshain, Abt. Hämostaseologie-Angiologie, Leninallee 49, D-1017 Berlin

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INHERITED SEVERE PROTEIN S DEFICIENCY IN A YOUNG BOY WITH CONSUMPTIVE COAGULOPATHY

F. Bergmann, P.F. Hoyer, C. Osterreich*, M. Barthels*

A previously healthy eight year old boy with a short history of sore throat, cervical lymph node enlargement and fever, treated with cephalicin was hospitalized. During the course the patient developed purple discoloration and painful induration on the lower legs. Laboratory data showed signs of infection (CRP 150 mg/l, platelets 33 000-10 000, elevated antistreptolysin titer). Coagulation parameters suggested a DIC. The normal antithrombin III of 117%, fibrinogen 0.5 g/l, platelets 33 000/l, fibrin degradation products 1 462 µg/l, thrombin-antithrombin III complex >70 µg/l. A mild toxic shock syndrom was suspected and treatment was started with antibiotic, heparin and FFP. He improved shortly but consequently developed hemorrhagic blisters in his face and on the lower limbs and fibrinogen became undetectable despite aprotinin infusions to prevent hyperfibrinolysis. He developed massive thrombosis of superficial veins and despite heparinization and replacement therapy with FFP pulmonary emboli were suspected. Further investigation revealed a severe deficiency of total Protein S (5%). Despite continuous replacement therapy with FFP an increase of free Protein S was not detected even when measured 15 min post infusion. During heparin therapy at 40-50% replacement therapy with FFP was continued for about 4 weeks. During this time the boy recovered. The total level of total Protein S was about 35% and free Protein S about 6%. Phenprocoumon therapy was started and tolerated without complications.

Kinderklinik, Division of Haematology, MMH Konstanty-Gutschow-Str. 8, 3000 Hannover 61

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MOLECULAR GENETIC ANALYSIS TECHNIQUES: THEIR APPLICATION FOR THE DIAGNOSIS OF COAGULATION DEFECTS

C. Mannhalter, G. Miitterbauer

During the last few years molecular genetic analysis techniques became increasingly important for the diagnosis of monogenic disorders. For coagulation defects, a much more accurate diagnosis of carriers of the disorder can be obtained, and prenatal diagnosis can be carried out at an early stage of pregnancy. Different technical approaches are currently available. The goal is, of course, the direct identification of the genetic defect (deletion, point mutation). However, some diseases, e.g. hemophilia A are caused by a large number of different mutations. Thus, direct identification of the mutation is laborious and impractical for diagnostic laboratories. An alternative approach, gene tracking, by which a defective allele can be followed within a given family, has proven very useful. Southern blot techniques and amplification of polymorphic regions by the polymerase chain reaction (restriction fragment length polymorphisms and other length polymorphisms, e.g. variable number tandem repeats) can be applied. Very recently, the use of single strand conformation polymorphism analysis was established for the localization of point mutations in larger groups of patients. Once the area carrying the mutation is localized, it can be sequenced and the mutation can be identified. Examples for each method will be presented.

Klinisches Institut für medizinische und chemische Labordiagnostik, Universität Wien, Währinger Gürtel 18-20, A-1090 Wien.
GENE DIAGNOSIS AND GENE THERAPY IN HEMOPHILIA
H. H. Weitzke

Molecular biology and gene technology are currently applied to many fields in medicine. Pharmaceuticals engineered by means of gene technology are routinely used by almost every physician. Gene technology is also used for the diagnosis of genetic disorders and represents a major breakthrough in genetic counseling. Gene therapy, in contrast, is currently not applicable on a routine basis but is gaining more and more attention as the first clinical trials on gene therapy in humans are underway. Molecular biology has a tremendous impact in blood coagulation. It enhances our knowledge on structure-function relationship of the proteins involved in hemostasis. The analysis of the genetic basis of hereditary coagulation defects allows the identification of the defect on the amino acid level. The correlation of the molecular defect with the functional defect elucidates the function of the normal protein. Functionally altered molecules can be generated by site-directed mutagenesis and in vitro expression. This results in specifically altered proteins with distinct functional characteristics which again enhances our knowledge on the interaction of the coagulation factors. For the diagnosis of hereditary defects in blood coagulation gene analysis is performed routinely. Some defects (Hemophilia B) are diagnosed by sequence analysis of the respective gene. In others (Hemophilia A), the diagnosis is still dependent on linkage analysis of affected genes (restriction fragment length polymorphism). Hemophilia is an ideal model for gene therapy for many reasons: coagulation factors are constitutively secreted into the plasma not requiring specific regulation processes; elevation of as little as 10% of circulating F VIII or F IX would tremendously improve the clinical outcome. Finally, there is an animal model which perfectly suits the needs of gene therapy. Clinical application of gene therapy in hemophilia is however limited by the fact that the currently used replacement of coagulation factors is an effective established and clinically useful therapy. Thus, gene therapy is clinically only used in genetic disorders without established treatment (ADA deficiency).

Universitätsklinik für Innerne Medizin, Klinik für Hämatologie, Währinger Gürtel 18-20, A-1090 Wien

 IMMUNE THROMBOCYTOPENIA: DIAGNOSTIC STRATEGIES

V. Kiefel, S. Santosa, and C. Mueller-Eckhardt

Platelet specific antibodies bring about thrombocytopenia through accelerated platelet destruction in different conditions. Autoimmune thrombocytopenia (AITP), caused by platelet specific autoantibodies (aab) occurs as "idiopathic" AITP or "secondary" AITP, accompanying diseases as SLE, malignant solid tumors, CLL, lymphomas, or HIV infection. Neonatal alloimmune thrombocytopenia (NAIT) is equivalent to hemolytic disease of the newborn. In NAITP, thrombocytopenia of the fetus and the neonate is the consequence of maternal immunization against incompatible platelet alloantigens. Another condition always associated with platelet alloantibodies is post transfusion purpura (PTP), a rare transfusion reaction characterized by severe immune-mediated thrombocytopenia.

Discrimination between platelet specific auto- and alloantibodies and immunoglobulin reacting with HLA class II antigens, Fc receptors or Ig trapped in platelet alpha granules is possible with glycoprotein-specific immunoassays using monoclonal antibodies for antigen isolation (MAIPA), immunoblot or radioimmunoprecipitation.

Most platelet specific antibodies characterized so far react with monomorphic (aab) or polymorphic determinants (alloantibodies) on glycoproteins IIb/IIIa, Ib/IX, Ia/IIa or IV of the platelet membrane.

Institut für Klinische Immunologie und Transfusionsmedizin der Justus-Liebig-Universität, Langenhensalstr. 7, D-6330 Gießen

Chronic idiopathic thrombocytopenic purpura (ITP) - treatment results

M. Winkelmann, P. Leifeld, W. Schneider

We are reporting the treatment results of 161 patients suffering from ITP (115 female, 46 male; ration 2.5:1, age between 17 and 93 years). Complete remission was defined as at least 6 months of platelet counts over 150,000/ul without any therapeutic intervention. Results: only 2 patients died initially of cerebral bleeding, 10 patients (6.2%) achieved complete remission without therapy. 24 patients (14.9%) had platelet counts over 30,000/ul without therapy. 120 (74.6%) patients were primarily treated with glucocorticoids (in fact prednisolone). 29 of them reached complete remission after one therapy cycle, 9 patients had to be treated with glucocorticoids a second time and then reached complete remission (total remission rate 31.7%). An outstanding result of this regimen is the significant correlation between the initially applied glucocorticoid dose and the initial increase of platelet count (p < 0.02) and the rate of complete remissions (p < 0.001). 34 patients (21%) underwent splenectomy. 22 of them reached complete and 4 partial remission (total success rate 76.5%), only 8 needed further therapy. However, 19 (glucocorticoid treatment failures) out of 31 non-splenectomised patients have to receive permanent treatment. Our study reveals the following aspects about therapy and the cause of ITP:

1. In individual cases, the risk involved in splenectomy should be weighed against the risk of a second cycle of glucocorticoid treatment.
2. The initial glucocorticoid dose correlates significantly with the rate of complete remission.
3. The number of patients having to undergo treatment after glucocorticoid and splenectomy regimen is clearly below the 10% mark.

Department of Hematology, Oncology and Clinical Immunology, Heinrich-Heine-University, Medical Center, Moorenstr. 5, 4000 Düsseldorf 1.