6th Congress of the GTH
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ACTIN DETERMINATION IN PLATELETS ADHERED TO PLASTIC AND IN CULTURED ENDOTHELIAL CELLS
P. Spangenberg, A. V. Mavros, M. C. Lukashev, U. Till

Actin determination in cells requires lysis of cells which is carried out more easily in cell suspensions compared to lysis of cells attached to artificial surfaces. Here we report our data of actin analysis in platelets adhered to plastic and of cultured endothelial cells (EC). Analysis of the actin tails in platelets and EC was performed using the ONase I inhibition assay.

During adhesion of platelets to plastic walls (Nunc™, Denmark) a shift in the G- to F-actin equilibrium occurred. An increase of 22.0 ± 6.7% (n = 5) F-actin was found compared to control washed platelets. This increase during adhesion is moderate in comparison to that found during thrombin-induced platelet aggregation.

To find out if human umbilical veins were cultured on gelatin-coated plastic dishes. They contain 12.9 ± 2.3 μg actin/10^6 EC (n = 10), 57.4 ± 9.1% F-actin for one single centrifugation. The F-actin level nearly returned to values found in unstimulated platelets.

A 231B7 or lomocin also induce actin polymerization, but the degree is smaller (12-25% more cellular F-actin compared to controls). The increase in cytosolic Ca²⁺ induced by the ionophore precedes the actin polymerization. EDTA inhibits partially ADP- or collagen-induced actin polymerization. From both results and the finding that procol ester (0.8 μM PMA) leads to an increase of about 45% F-actin in platelets we conclude tentatively that Ca²⁺ and protein kinase C seem to be involved at least in part into the initial actin assembly process in platelets.

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ACQUIRED THROMBOPATHY ASSOCIATED WITH AUTOANTIBODIES AGAINST MEMBRANE GLYCOPROTEIN IIb-IIIa COMPLEX. 2. AGGREGATION AND ALTERATIONS IN THE MICROFLAMENAL SYSTEM
P. Spangenberg, C. M. Kirchmaier, A. Schirmer, M. Meyer, K. Breddin

Platelet functions in a 63 years old woman with autoantibodies against the GP IIb-IIIa complex (A. Schirmer et al., part 1) were severely impaired. Aggregation could be induced only after depolymerization of the filaments on the surface, indicating that filaments stick tightly to the plastic.

Patient's platelets contain only 473 μg actin/10^6 platelets and an extremely low F-actin value (3% of total platelet actin). Stimulation of these platelets with 0.1 U/ml thrombin for 3 min and measurement of the G-/F-actin equilibrium resulted in an increase of only 5% F-actin, whereas ADP and collagen did not induce any actin polymerization. Addition of patient's serum to normal platelets leads to an inhibition of thrombin-induced actin polymerization.

Both effects of patient's serum on normal platelets (inhibition of aggregation and actin polymerization) points to receptor blockade by platelet autoantibodies found in the patient's serum.

Ca²⁺ movement in the patient's platelets is severely impaired after ADP or collagen stimulation, whereas a normal Ca²⁺ movement was induced by 0.1 U/ml thrombin. However, normal Ca²⁺ movement by thrombin but inhibited actin polymerization by this agonist does not necessarily point to an independence of actin polymerization from Ca²⁺ ions, but emphasizes the receptor-mediation for actin polymerization.

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ACTIN POLYMERIZATION DURING AGONIST-INDUCED ACTIVATION OF HUMAN BLOOD PLATELETS
P. Spangenberg, U. Till, S. Heptinstall, C. M. Kirchmaier, A. Schirmer, M. Meyer, K. Breddin

Actin exists in platelets in two main forms, the monomer G-actin and the filamentous polymeric form, F-actin. The equilibrium of G- to F-actin in resting platelets is maintained at a constant ratio, but upon stimulation F-actin can readily polymerize to form filaments.

After irreversible aggregation induced by ADP, collagen, adrenaline, arachidonic acid, PAF or thrombin, a significant increase in F-actin was found (30-50% more cellular F-actin compared to controls). Actin polymerization is a very fast process reaching a significant level already with shape change and well before aggregation commenced or release of serotonin was significant.

During reversible aggregation the degree of actin polymerization is smaller and at the end of deaggregation the F-actin level nearly returned to values found in unstimulated platelets.

In conclusion, hereditary thrombopathies with similar clinical and laboratory abnormalities of platelet GPs. He reports on patients from three families with normoblastic anemia, Bernard-Soulier syndrome, and severe bleeding. In the third family, the patient's platelets contain a molecular abnormality of the GP IIb-IIIa complex, which is suggested by the finding that platelets with this abnormality show a complete lack of aggregation. The family's patients' platelets show decreased aggregability in response to ADP and contain only 25-30% of GP IIb-IIIa complex. In addition, the complex may have a different mobility in crossed immunoelectrophoresis.

In a second family a structural abnormality of the GP IIb-IIIa complex is suggested by the finding that platelets with this abnormality show a complete lack of aggregation. In the third family the patient's platelets contain a molecular abnormality of the GP IIb-IIIa complex, which is suggested by the finding that platelets with this abnormality show a complete lack of aggregation.

In conclusion, hereditary thrombopathies with similar functional defects may be caused by a variety of structural abnormalities of platelet GPs.
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PLATELET DISORDERS

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FLOW CYTOMETRIC ANALYSIS OF PLATELETS FLUORESCENTLY LA-

BELED WITH THEAZOLE ORANGE IN PATIENTS WITH QUANTITATIVE

PLATELET DISORDERS

J. Kienast* and G. Schmitz§

The fluorescent dye thiazole orange (TO) is characte-

rized by a large fluorescence enhancement and high quan-

tum yield upon binding to nucleic acids, particularly 

DNA. In addition, the dye readily permeates live cell

membranes. Whole blood samples from hematologically nor-

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A NEW VARIANT OF HEREDITARY THROMBOPENOCYTOSIS - THE SEBASTIAN-PLATELET SYNDROME

A. Greinacher(1), H.K. Niewehohu(2), J.G. White(3)

A new variant of hereditary thrombocytopenia combined with giant platelets and inclusion bodies in the polymorphonuclear neutrophils (PMN) is presented.

In a 26 year old woman thrombocytopenia was diagnosed by chance. Family investigations revealed a bleeding history in 10 of 28 family members. 1 out of them died of bleeding complications (intestinal, cerebral). 7 had macrothrombocytopenia and inclusion bodies in the PMN, a mild bleeding tendency (easy bruising, ecchymosis, menorrhagia), no muscle or articular bleeding, no hearing problems or kidney disease. Bleeding time was slightly prolonged. Rumpf-Leede test (negative). Platelet counts varied from 20 x 10^9/l to 120 x 10^9/l, by phase contrast microscopy. The cell counter does underestimate the actual platelet count by 40-60%, mean platelet volume was 16.9 fl. Platelet function test, clotting tests, von Willebrand factor, ATP/ADP ratio and platelet serotonin are normal.

Bone marrow examinations revealed normal or slightly increased megakaryo
topoiesis. Giant platelets were seen in peripheral blood smears. Except for the large size and the relative spongial shape ultrastructural appearance of the platelets was not different from that of normal controls. If blood smears were stained within 4 hours after venipuncture, small inclusion bodies could be detected in PMN. By electron microscopy they were differentiated from those found in the May-Heiglin anomaly and from Dohele bodies. Serostics and i.v. IgG do not elevate the platelet count in this syndrome. The Sebastian-Platelet-Syndrome represents an unique syndrome with giant platelets and neutrophil inclusions in the absence of other congenital anomalies.

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A DOMINANT INHERITED GIANT PLATELET SYNDROME IN PREGNANCY - THE SEBASTIAN-PLATELET SYNDROME (SPS). PREGNATAL DIAGNOSIS AND PERINATAL MANAGEMENT

A. Greinacher, V. Jovanovic, V. Kiefl and C. Mueller-Eckhardt

The first case of a prenatal diagnosis in pregnancy, complicated by a dominant inherited giant platelet syndrome combined with inclusion bodies in the leukocytes, but normal platelet function, is presented. In the patient the diagnosis of a SPS was made before pregnancy. Except for the low platelet count (plt) count of 26 x 10^9/l the pregnancy was uncomplicated. At week 28 of pregnancy a screening for platelet antibodies was positive in the PAIF. MAIPA assay showed positive reactions with GP Ib/IIa and Ib/IX. EDT revealed additional LA antibodies. To avoid any additional risk for the fetus by the maternal antibody, i.v. IgG treatment (1 g/kg/week) was started at week 30 of pregnancy. At week 38 the patient had a sudden onset of painless vaginal bleeding which stopped spontaneously.

After preparing a platelet concentrate from a single crossmatch negative donor, an uncomplicated umbilical cord sampling was performed at the end of the week 39. Fetal blood was confirmed by a HbF stain. plt count in the fetus was 26 x 10^9/l (phase contrast microscopy).

Hb 13.8 g/l, blood smears revealed giant platelets and inclusion bodies in the neutrophils characteristic for the SPS. By unconnected in the selection section were normal healthy female infant was delivered the same day.

Postpartal blood count: plt 80 x 10^9/l, Hb 19.8 g/l. We conclude that the pregnancy in hereditary thrombocytopenias might be complicated by additional platelet reactive antibodies. At the end of pregnancy umbilical cord blood sampling may help to choose the appropriate way of delivery, as it allows to diagnose the giant platelet syndrome in the fetus. A platelet concentrate from a compatible donor should be prepared before to treat possible bleeding complications in the mother.

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EXPERIMENTALLY INDUCED THROMBOSIS AT ARTERIOULAR BRANCHING.
D. Seiffge; E. Kremer; V. Laux; P. Reifert

Blood flow at vessel segments with complicated geometry are the objects of fluid-dynamically oriented investigations concerning atherogenesis and thrombosis. The development of atherosclerosis in large arteries is determined by two pathogenetic factors: the arterial wall and the contents of the vessel. Both factors should be considered as pathogenetic equals. The areas of predilection for atherosclerosis and thrombosis in the vascular system are characterized by curvature, branching, bifurcation or embarking. We have investigated induced thrombus formation at arteriolar bifurcations with migrating stagnation points and local vortex flow. Thrombogenic endometrial lesions were produced by argon laser injury or by photochemical reactions (using FITC-dextran 70). The following items have been evaluated:
1. local red blood cell velocity
2. geometry of the vessel
3. localisation of the first thrombus growth
4. estimation of the extent of thrombogenic stimulus in relation to the extent of thrombus area or volume (video densitometry).

The obtained results of increased thrombus formation at arteriolar vessel segments with complicated geometry are in accordance with findings in artificial tubes or human arteries. We could demonstrate that the onset of thrombus formation will not appear at stagnation points, but at areas of complicated flow within the branching vessel segments (80 to 90%). Whether those areas are reattachment points or areas of recirculation and local vortex flow remains to be open. Specific three dimensional flow dynamic calculations to determine those points were just carried out.

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PREVENTION OF DEEP VENOUS THROMBOSIS AFTER MAJOR GYNECOLOGIC OPERATIONS
L. Heilmann

The efficacy and safety of Low Molecular Weight Heparin (LMWH, Sandoz, FRG, -1000 aPTT units once daily = 2 placebo injections daily) and a conventional Unfractionated Heparin (UFH 3 x 500 IU daily) were compared. In a prospective double blind investigation of the prophylaxis of deep vein thrombosis undergoing major gynecologic surgery, 300 patients were randomized into two groups. Treatment was initiated 2h preoperatively in both groups and continued for 7 days. Screening for the Deep Vein Thrombosis (DVT) was performed by impedance plethysmography. The following parameters were investigated: Anti Xa-activity, aPTT, fibrinogen, D-Dimer, F VIII, AT III, Protein C, Erythrocyte aggregation and plasma viscosity.

Results: Two patients (1.2%) treated with LMWH and 6 patients (4%) receiving UFH developed a DVT. Haemorrhagic complications occurred more often in the UFH-group Intraoperative high blood loss 8% vs. 7.3%, blood transfusion 24.1% vs. 21.3%, drainage bleeding 70.5% vs. 77.4% and wound haematoma 18% vs. 12.6%. The acceptance of the LMWH-regimen was decisively better. Concerning the results of the laboratory tests, the most striking effect of the LMWH was a significantly 2-3 times higher Anti Xa-activity in the postoperative period in comparison to the UFH. The aPTT was only minimally affected by both groups. There results show that once daily prophylaxis is equally as effective and as safe as the three daily regimen using UFH in patients undergoing elective, major gynecologic surgery.

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Changes of Hemostatic Potential in Comparison of 4 Methods for Plasmapheresis

H. Neuremyer, B. Schulte, S. Quentin and J.U. Wiedling

In the course of this study, separation of plasma using the systems 'Plasmapur Monitor' (Fa. Organon), 'Autopheresis-C' (Fa. Baxters) and 'PCS' (Fa. Haemonetics) was compared with the conventional blood bag centrifugation. In 18 apheresis per method, parameters mainly reflecting the blood coagulation were examined in addition to other criteria. Blood/plasma samples were drawn from the donor before, during and after the separation run as well as from the used device during the separation and from the collected plasma. Thrombin-antithrombin-complexes and the fibrinogen-derivatives, soluble fibrin and fibrinopeptid A, proved to be sensitive indicators of a slight activation of the coagulation system. This activation was less pronounced by separation through centrifugation than through filtration; however, even the filtration devices activated the coagulation system less than common bag centrifugation.

Furthermore, a decrease in blood coagulation factors and inhibitors were most pronounced using bag centrifugation. The number of cells remaining in the collected plasma was highest after separation by centrifugation alone, whereas less than 1/10 as many were seen using filtration methods.

Considering the fact that presently most of the daily transfused fresh frozen plasma is collected by centrifugation of blood bags, the comparatively slight activation of blood coagulation by plasma separation machines seems to be negligible. However, the number of remaining cells in the collected plasma may be of greater interest: a total elimination of cells from transfused plasma should be the goal to avoid activation of blood coagulation in recipients.

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Urokinase-Type Plasminogen Activator Gene: Transcriptional Control of Plasminogen Activator Synthesis and Regulation of Expressed Activity

D. von der Ahé, A. Müllermeine and G. Müllermeine

The genes coding for the two plasminogen activators urokinase-type (uPA) and tissue-type (tPA) have been identified. They are located on different chromosomes. Both genes are organized with separate exons. The homology between the two PAs at the amino acid level is only 40%, but the enzymes are highly similar in their basic structures, which demonstrates a close evolutionary relationship. uPA is implicated in various aspects of cellular functions such as fibrinolysis, cell migration, tissue reorganization during morphogenesis and invasive growth in both normal and pathological conditions linked to increased plasmin activity. uPA synthesis is modulated by a variety of factor molecules such as phorbol esters, growth factors, peptide and steroid hormones, retinoids and others. The regulation of uPA synthesis occurs mainly at the level of gene transcription. To elucidate the mechanisms of regulation, we studied the human and pig uPA genes including their putative regulatory 5'-flanking regions. For this purpose, we constructed a series of hybrid genes with uPA-5'-flanking dation mutants and the E. coli chloramphenicol acetyltransferase as a reporter gene. Using gene transfer techniques, this hybrid gene was stably integrated in the host cell genome and the activity of the reporter gene measured. This analysis revealed that sequences proximal to the transcription start site as well as sequences far upstream play an important role in hormonal regulation through the protein kinase A pathway and by phorbol esters using the protein kinase C pathway. Furthermore, we identified the protein-binding sequences within the regulatory regions by DNase I footprint and gel band shift analysis. A comparison of regulatory sequences of human and pig uPA genes will be discussed.

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Comparative Studies on the Anticoagulant and Antithrombotic Effects of Heparin, LMWH, Synthetic Heparin-Pentasacharide and Dermatan Sulfate

K. Krupinski, H.K. Breddin, J.M. Walenga, J. Fareed, B. Ca-su and J. Choay

Seven glycosaminoglycans (GAGs) were studied for their antiocoagulant effects in vitro, platelet adhesion to bovine extracellular matrix (ECM) and antithrombotic effects in a rat thrombosis model. Unfractionated heparin (UFH), low molecular weight heparin (CF 216), a synthetic heparin-pentasacharide (PS) and dermatan sulfate (DS) were obtained from Institute Choay, Paris, France and a sulfated low molecular weight heparin (SAG 669) and heparin in which the binding site for antithrombin III had been inactivated (SAG 262) were obtained from Prof. Casu (lst. Stefisico di Chimica "G. Ronzon", Milano, Italy). A series of in vitro assays including aPTT, TT, heptest, anti Xa and anti IIa assays using chromatographic substrates were performed in both human and rat platelet poor plasma. All agents inhibited thrombus formation in vessels at minimal effective doses from 0.01 to 5 mg/kg 30 min after a single i.v. injection. The dose-dependent antithrombotic effect was not positively correlated with the inhibition of F. Xa or F. IIa in human or rat plasma. These data suggest that inhibition of thrombosis at the endothelial surface does not directly correlate with current ex vivo coagulation assays. Higher doses were required to observe antithrombotic effects after subcutaneous injections and their duration lasted far longer after s.c. administration than after i.v. injection. The antithrombotic effects persisted longer than detectable ex vivo anticoagulant activities. The low molecular weight heparins, pentasacharide and dermatan sulfate showed in vitro inhibitory effects on platelet adhesion to bovine ECM. This inhibitory effect was not observed with two unfractionated heparins (SAG 262 and Liquemin). Further studies are needed to clarify a possible correlation between inhibition of platelet adhesion and antithrombotic effects of GAGs.

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Effect of VWF Content of the Culture Medium on the VWF Production of Cultured Endotelial Cells

I. Altornsay, C.M. Kirchner, Zs. Vigh, K. Krupinski, I. Scharrer, H.K. Breddin

The exact regulation of synthesis of von Willebrand factor by endothelial cells is still unclear. The effect of the VWF content of the culture medium on the production of VWF by cultured human ECs was studied.

The amount of VWF produced and released by ECs in the presence of ECM. The latter correlated well with the amount of VWF produced in the presence of a serum-free culture medium. The multimeric structure of VWF released by ECs was the same in all cases.

If human ECs were cultivated in a medium containing normal serum the adhesion of platelets to the matrix produced by these cells was higher than the adhesion to matrix produced by ECs which had been cultivated in a medium containing serum from a patient with severe VWS.

This finding may be correlated with the higher VWF content of ECM.

A stimulating effect of von Willebrand factor contained in the culture medium on the VWF synthesis of cultured ECs is supposed.

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IN VITRO EFFECTS OF UK IN PLASMA AND THEIR PREVENTION BY DIFFERENT INHIBITORS.

M.D. Oethinger and E. Seifried

We investigated dose-, time- and temperature-dependent effects of urokinase (UK) on normal citrated plasma in vitro. When 5 µg/ml UK were added to pooled normal plasma and incubated for 30 minutes at ambient temperature (20° C), a-antiplasmin decreased to 8% of the control value. Incubation on ice yielded a decrease to 45% of control, whereas a-antiplasmin was fully consumed at 37° C. Fibrinogen and plasminogen fell to 46% and 39%, respectively, after a 30 minutes incubation at 25° C. Thrombin time prolonged to 182% of control.

Various inhibitors were studied with respect to their suitability and efficacy to prevent these in vitro effects. Aprotinin exhibited a good protective effect on fibrinogen at concentrations exceeding 500 KIU/ml plasma. Its use however was limited due to interferences with some haemostatic assays. We could demonstrate that L-6-nitro-1-L-glycyl-L-arginyl-2-naphthylmethylketone (GGACK) and a specific polyclonal anti-UK-antibody effectively inhibited urokinase-induced plasmin generation without interfering with haemostatic assays. The anti-UK-antibody afforded full protection of a-antiplasmin at therapeutic levels of UK.

It is concluded that UK in plasma samples from patients during thrombolytic therapy may induce in vitro effects which should be prevented by the use of a suitable inhibitor such as GGACK or specific anti-UK-antibody.

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ALTERATIONS OF COAGULATION PARAMETERS IN THE EARLY PHASE OF POLYTRAUMA: A PROSPECTIVE STUDY.

E. Seifried, L. Lampl, D. Ellbrück, M. Tisch, K.H. Bock

Changes of the blood coagulation system were investigated in twenty adult polytrauma patients with a mean injury severity score (ISS) of 37 (20 - 50) and a NACA-score between IV and VI. Citrated blood samples were taken without delay at the site of the accident with a median time interval between trauma and sampling of 18 min (range: 10 - 29 min). No patient had received more than 500 ml of cristalloid or colloidal fluid before sampling. Blood samples were centrifuged and snap frozen immediately using a portable centrifuge and cool aggregate. A second blood sample was taken in the emergency room at hospital.

The following parameters were determined in both blood samples (mean results are given, sample taken in hospital in brackets): Plasminogen 93% (72); Antiplasmin 68% (48); t-PA-antigen 14.5 mg/ml (3.2); t-PA-activity 21.6 mg/ml (4.7); PAI 8.0 IU/ml (19.1); D-dimer 13.7 µg/l (15.4); FgDP 8.9 µg/ml (6.7); TDP 13.4 µg/ml (19.2); Thrombin 27.4 µg/ml (12.5)

The meaning of these changes of the fibrinolytic system are unclear. Longitudinal studies of the fibrinolytic system after polytrauma have to be performed to answer the question whether therapeutic use of inhibitors of fibrinolysis i.e. aprotinin have a positive effect on the outcome of these patients, especially with regard to the frequency of acute respiratory distress syndrome (ARDS).

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THE CLINICAL COURSE OF 3 PATIENTS SUFFERING FROM COUMARIN NECROSIS.

D. Ellbrück, H. Wankmüller, K.D. Oethinger, A. Gabelmann, E. Seifried

coumarin necrosis is a rare but clinical important complication of treatment with oral anticoagulants. We report three cases which we observed over the last years.

First case: A 75 year old woman was admitted with a deep vein thrombosis (DVT) of the femoral and iliac veins. After heparin therapy oral anticoagulation with phenprocoumon was initiated. The patient developed a coumarin necrosis at the right breast. Second case: A 21 year old obese woman (weight: 100 Kg, height: 160 cm) presented with an acute DVT which was complicated by a subsequent pulmonary embolism of grade III. The patient had a longstanding history of severe allergic bronchial asthma. She was a heavy smoker and took the o.c. pill. I.v. heparin was started, followed by Marcumar®. After a few days, the patient developed a large necrosis at the anterior abdominal wall which finally had to be treated with skin grafting. Third case: A 25 year old obese patient was admitted with PE. He had a 10 years history of hay fever and allergic bronchial asthma and smoked 40-60 cigarettes/day. The patient received heparin followed by Marcumar®. On the 5th day a large coumarin necrosis developed on the right hip with involvement of the muscles; the patient had to undergo five operations. No abnormalities in the haemostatic system were detected in any of the patients, in particular no protein C-deficiency. It is concluded that an allergic diathesis may play an important role in the pathogenesis of coumarin necrosis.

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THROMBOPROPHYLAXIS IN GENERAL SURGERY WITH A LOW MOLECULAR WEIGHT HEPARIN (LMW 21-23).
A PROSPECTIVELY RANDOMIZED CLINICAL STUDY
E. Seifried, D. Ellbrück, J. Limmer, A. Schwarz, H. Müller, E. Eisele, H.G. Beger

Study design: A prospective randomized clinical study was performed to compare the efficacy of low molecular weight heparin (LMW 21-23), bivalirudin and unfractionated heparin (UFH, Heparin Braun) in 203 patients undergoing general surgery. 100 patients received a single daily injection of 2500 anti-factor Xa units of LMW 21-23 and 100 patients received 3 x 5000 IU UFH/day. A radio-active fibrinogen uptake test was used for screening of deep vein thrombosis (DVT). If DVT was suspected either clinically or by leg scanning the diagnosis was verified by phlebography.

Results: No patients of either group presented clinical signs of DVT. In the LMWH group we observed a tendency to less hemorrhagic complications (n.s.). There was a statistically significant difference between anti-Xa levels in both groups (median results of SEM are given, values of the UFH group in brackets; samples were taken on the 3rd day post-op): 0.10 ± 0.02 anti-Xa U/ml (0.02 ± 0.008). Values for PT, FTT, TT, fibrinogen, AT III, protein C, plasminogen, antitrypsin and t-PA antigen did not differ significantly. The results indicate that a single injection of 2500 anti-factor Xa units of LMW 21-23 is as effective and at least as safe as 5000 IU of UFH given daily.

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LEVELS OF FIBRIN(ogen) DEGRADATION PRODUCTS AND OF INTACT FIBRINOGEN IN PATIENTS RECEIVING THROMBOLYTIC THERAPY WITH RT-PA
E. Seifried, E. Hoegee-de Nobel, D. Ellbrück, W. Haerer, P. Tanswell, W. Nieuwenhuizen

In 12 MI-patients treated with 100 mg rt-PA/3 hours in 18 healthy subjects (0.25 mg/kg, 0.50 mg/kg rt-PA and placebo over 30 min) fibrinogen, antiplatelet and t-PA inhibitory activity and its derivatives were measured in serial plasma samples containing citrate and the t-PA inhibitor PPACK. Fbg was measured with clotting assays and a new ELISA for intact fibrinogen (Thromb Haemost 60, 415, 1988). D-dimer, degradation products of Fbg (FgDP), fibrin D-dimer (FDP) and total DP (TDP) in plasma were assessed by specific ELISA. FDP in serum were also measured.

In MI-patients D-dimer, FgDP, FDP, TDP and FDP in serum increased from 0.1, 0.4, 0.4, 0.8 and 6.0 to 2.3, 20, 8, 30 and 126 mg/ml at the end of the infusion, respectively. In normal subjects FDP and D-dimer increased from 0.5 to 0.9 and from 0.1 to 0.5 mg/ml. Mean Fbg levels were 51%, 61% (Clauss and photometric assay) and 81% (ELISA) of the preinfusion values. After 3 days all fibrinogen (ogen) degradation product values were normal whereas Fbg levels were 120 - 140% (Clauss, photometric) and 105% (ELISA). The discrepancies between the functional and ELISA levels of Fbg suggest that about 25% of Fbg is converted to (slowly clotting, i.e. less functional) LMW-Fbg and that rt-PA induces less Fbg degradation than expected from functional assays. The levels of FDP and D-dimer are too high to be derived exclusively from a coronary thrombus and probably originate from another fibrinolytic system. Medizinische Klinik und Poliklinik, University of Ulm, Robert-Koch-Str. 8, D - 7900 Ulm, F.R.G.

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SYSTEMIC EFFECTS OF INTRAVENOUS RT-PA AND UROKINASE IN ACUTE MYOCARDIAL INFARCTION
E. Seifried, E. Limmer, A. Schwarz, H. Müller, E. Eisele, H.G. Beger

Recombinant tissue-type plasminogen activator (rt-PA) was described as affecting a more specific thrombolysis avoiding a systemic fibrinogenolysis as well as serious bleeding complications. The systemic activation of the fibrinolytic system by rt-PA and urokinase was studied in 69 patients presenting with symptoms of acute myocaridal infarction of less than 6 h duration. 34 patients randomized to the rt-PA group received a bolus injection of 10 mg rt-PA followed by 60 mg rt-PA over 90 min. 35 patients randomized to the urokinase group received a bolus injection of 1.5x10^6 IU urokinase and 1.5x10^6 IU urokinase over 90 min. Blood samples were obtained before therapy, after 90 min, 4, 12 and 24 h. Both groups showed a significant systemic reaction after 90 min fibrinogen (Clauss), plasminogen and antithrombin decreased considerably, while fibrin(ogen) degradation products, reptilase time and plasmin increased accordingly. Even though the average systemic effects of rt-PA were lower than those of urokinase, in later blood samples a significantly more rapid return to normal coagulation status was observed in the rt-PA group. However, dispersion of parameters in the rt-PA group were quite remarkable: There were considerable fibrinogenolysis in the rt-PA group which may lead to serious hemorrhagic complications. No serious individual reactions have not been sufficiently explained. Therefore, as in any other thrombolytic therapy, careful monitoring of patients treated with rt-PA is strongly advisable.

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ROLE OF FIBRINOGEN-MEDIATED CONTACTS FOR THE ACTIVATION OF POLYMORPHONUCLEAR LEUKOCYTES (PMNLs) BY STIMULATED PLATELETS. A. Ruf, R. Schlenk, A. Maras, E. Mengenstern* and H. Patscheke.

Cell suspensions containing human washed platelets (2 x 10^9/ml) and human PMNLs (2 x 10^6/ml) were stimulated with the platelet agonists thrombin (0.06 IU/ml, U 66191), ADP 10 μM, or ADP 10 μM. Platelet aggregation and the luminol-enhanced chemiluminescence (CL) of the PMNLs were simultaneously monitored in a multichannel luminometer. In contrast, ristocetin and U 66191 were able to trigger platelet aggregation and the CL in the absence of exogenous fibrinogen, whereas ADP required the presence of added fibrinogen to aggregate aspirin-treated platelets and to induce the CL. EDTA and no stirring suppressed the platelet aggregation and the CL in the mixed suspensions. Interference and electron microscopy revealed mixed platelet-PMNL aggregates. Cell-bound fibrinogen was localized by immunofluorescence and immunoelectron microscopy. It was found in the contacts between platelets, between platelets and PMNLs, at the free surfaces of both cell species and in the platelet α-granules. Neither thrombin, U 66191 and ADP nor the supernatant or a filtrate of thrombin-stimulated platelets induced any CL in pure PMNL suspensions. In aspirin-treated whole blood, U 66191 and ADP did not stimulate the CL, but a filtrate of thrombin-stimulated platelets induced any CL in pure PMNL suspensions. In aspirin-treated whole blood, U 66191 and ADP did not stimulate the CL, but a filtrate of thrombin-stimulated platelets induced any CL in pure PMNL suspensions.
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BINDING AND INTERNALIZATION OF SOLUBLE FIBRIN BY THROMBOCYTES

H. Würmann, V. Jelinić and H. Richter

Gelfiltered human thrombocytes fail to bind soluble 125-I-fibrin unless they had been supplemented with activated plasma transaminase (coagulation factor XIIIa). In addition, a free 30 kDa-domain from the N-terminus of the fibronectin subunit chains is required as a cofactor. Fibrin binding is inhibited by the transaminase competitor putrescine and histamine. There is evidence that the free fibronectin domain is circulating in plasma, although its quantitative determination meets some difficulties.

Centrifuged platelets still containing their halo of adsorbed plasma proteins bind 125-I-fibrin to a variable extent. Binding is improved by the 30 kDa-domain at lower concentrations than required for fibrin binding to gel-filtered platelets suggesting that some free fibronectin domain is among the adsorbed proteins. On the other hand, binding is incompletely inhibited by putrescine indicating that still a second pathway for fibrin binding is possible.

Platelets in a thrombocyte concentrate even internalize 125-I-fibrin within few hours. The trapped activity is only detectable for about 6 hours indicating intracellular degradation and release of the fragments. Internalization is promoted by the free fibronectin domain and is improved by factor Xlla. However, other unknown plasma compounds are still essential.

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IS THROMBOLYSIS IN VIVO MORE EFFECTIVE WHEN rtPA IS GIVEN BY INFUSION THAN AS A BOLUS?

K.S. Herrmann, H. Kreutzer and U. Tobbe

Acute myocardial infarction is treated by parenteral application of thrombolytic compounds. Different drugs are available, mode of application and dosages are still under discussion. Clinical studies to standardize therapy are time consuming and expensive; animal experiments can shorten the way to find a therapeutical standard.

We developed a method to induce local endothelial trauma in vivo by employing a contactless, photochemical process. Thus, a reproducible damage can be initiated and stable arterial thrombi are produced in a highly reproducible way. As thrombogenesis and thrombolysis take place under direct visual control, direct information about blood flow can be obtained and quantified.

After occlusive thrombi were formed, thrombolysis was induced by (A) bolus (B) or infusion of rtPA at different dosages or (C) solvent alone. Patencies were compared consecutively and for dosages of 0.5 and 1.4 mg/kg rtPA.

2.4 mg/kg rtPA induced reperfusion to 80% (A) or 60% (B) (SEM = 16 or 14%, n.s.). 0.5 mg/kg rtPA was more effective when given as an infusion than when applied as a bolus (49 or 19%, SEM = 12 or 3%; p < 0.01). (C) was without efficacy.

We conclude that similar dosages of rtPA obtain better patencies when the drug is applied as infusion rather than as a bolus.

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MONITORING OF INTRAVENOUS HEPARIN THERAPY BY A MODIFIED CHROMOGENIC ANTI-XA-ASSAY

A commercial chromogenic heparin assay (Coatest heparin; Boehringer Mannheim, FRG) was modified for the measurement of anti-Xa-activities from patients treated with different heparin regimens. The most important modification was the addition of the chromogenic substrate S-2222, which was modified by adding tris(hydroxymethyl)aminomethane (Tris) buffer to the reaction mixture. This modification resulted in a more sensitive assay that could detect lower levels of anti-Xa-activity than the original assay.

Anticoagulant activities were measured in 50 patients undergoing coronary-artery bypass surgery. The modified assay was compared with the Coatest heparin assay and the activated partial thromboplastin time (APTT). The results showed that the modified assay had a higher sensitivity for detecting anticoagulant activity and was more accurate in determining the appropriate dose of heparin.

The modified assay was also compared with the anti-IIa assay, Coatest heparin, and Coatest anti-IIa. The correlation between the two assays was weak or nonexistent, indicating that the modified assay provides additional information not covered by the traditional methods.

In conclusion, the modified chromogenic anti-Xa assay is a valuable tool for monitoring heparin therapy, especially in patients undergoing coronary-artery bypass surgery. It provides a more sensitive and accurate measurement of anticoagulant activity, which helps in maintaining the therapeutic range of heparin doses without over- or underdosing.

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PERFORMANCE OF A CHROMOGENIC AT III TEST ON A HITACHI 717 ANALYZER

We have applied the manual AT III reagent (Boehringer Mannheim, Cat.No. 739376) using Tos-Gly-Pro-Aro-Arg-PNA (Chromosoy® TH) as substrate on a Hitachi 717 analyzer. At 25 and 30°C reaction temperature 10µl sample (diluted 1:50) and 250µl of a buffered (Tris/HCl,100 mmol/l, pH 8.1) thrombin solution (0.024 U/l) are incubated for 5 minutes. Then the chromogenic substrate solution is added (50µl) and the increase of absorbance is measured kinetically during a time interval of 2 minutes after another minute. At 37°C reaction temperature half of the sample volume and half of the thrombin concentration are used.

Results (25°C): Within run CVs (n=20) ranged from 1% to 3.3% between 14 and 5.6 IU/ml (112-45% AT III), day to day CVs (n=10) from 1.2% to 3.7% using the same specimens (control plasma and frozen human plasma). No deviation from linearity was observed between 3 and 25 IU/ml (25-200% AT III) using +/- 5% as acceptance criteria. The recovery of assigned values in two controls (PreciChrom® I and II) was 98% and 101%.

Method comparison in 50 deep frozen plasma specimens with AT III activities from 2 to 25 IU/ml showed an excellent agreement with the manual method: y = 0.2 + 1.00 x. The median AT III activity in fresh frozen plasma samples from healthy volunteers was 12.8 IU/ml (102%). Using the same reagent in the analyzer the calibration factor was found to be acceptable for at least 3 weeks. Results of the same quality were obtained at 30 and 37°C.

We conclude, that the described procedure is well suited for routine analysis.

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PHARMACOLOGICAL AND PHYSIOLOGICAL PROPERTIES OF RECOMBINANT TISSUE-TYPE PLASMINOGEN ACTIVATOR PRODUCED IN ESCHERICHIA COLI (BM 06.021)

The aim of our studies was to investigate the pharmacokinetic and pharmacodynamic properties of a recombinant tissue-type plasminogen activator (t-PA) produced in E. coli. The aim was to determine if there were differences between recombinant t-PA produced in E. coli and a thrombolytic agent that was well known as a thrombolytic agent.

Rabbits were infused with 200 000 IU/kg b.w. of BM 06.021 or Alteplase for 30 minutes. Plasma samples were assayed for t-PA activity by a spectrophotometric method with Chromozym® PL. In comparison with Alteplase (n=5), BM 06.021 showed about a three-fold longer initial half-life (2.1±0.6 vs. 0.5±0.2 min, n=5), a three-fold slower plasma clearance (22±7.6 vs. 7.5±1.7 ml/min, n=5), and a three-fold increased area under the curve (133±44 vs. 452±105 IU · ml-1 · min). In a rabbit model of jugular vein thrombosis BM 06.021 was infused for 4 hours at at least 3 dose levels. The specific activity of BM 06.021 was comparable with that of Alteplase. The analysis of the dose-response curves showed that BM 06.021 has a two-fold increase in thromboembolic potency (90.25 mg/kg b.w., vs. 0.55 mg/kg b.w.). At equieffective doses there were no statistically significant differences either in the plasma levels of t-PA-activity, fibrinogen, plasminogen or alpha2-antiplasmin between BM 06.021 and Alteplase at the end of the experiments or in the hemodynamics during the infusion of the two agents.

It is concluded that glycosylated t-PA produced in E. coli is biologically active. Furthermore, BM 06.021 shows improved pharmacokinetic properties and an increase in thromboembolic potency. At equieffective thrombolytic doses BM 06.021 is comparable with Alteplase in its effect on the hemostatic and hemodynamic systems.

Therefore, we anticipate that non-glycosylated recombinant t-PA from E. coli exhibits superior pharmacological properties.

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CHANGES IN PLATELET PARAMETERS AFTER CORONARY-ARTERY BYPASS SURGERY MAY HELP DETECT THROMBOTIC AND SEPTIC COMPLICATIONS

A.A. von Ruecker, R. Dickerhoff, P. Hufnagel, D. Murday, and F. Bidlingmaier

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A.A. von Ruecker, R. Dickerhoff, P. Hufnagel, D. Murday, and F. Bidlingmaier

Patients with coronary heart disease undergoing coronary-artery bypass surgery represent a well-studied group in which changes of a number of hematological, mostly platelet parameters have been described. New electronic cell counting and sizing techniques in hematology enable us to analyze most of these partially uncommon parameters such as mean platelet volume (MPV), platelet volume distribution width (PDW), and PBC-distribution width (RDW) on a fast scale. It is important to be aware of the extent of normally occurring changes so that misinterpretation of findings can be avoided.

In this study, our first aim was to generate diagrams showing the average response (and 99% confidence intervals) of those hematological parameters that showed characteristic postoperative changes in patients undergoing coronary-artery bypass grafting without complications (n=50). Secondly, we examined if by means of hematological parameters postoperative disease states such as thrombosis, infection, or reinfarction could be differentiated from changes that normally occur during the postoperative period.

Our investigation shows that the monitoring of platelet count and MPV during the postoperative phase with the aid of the mentioned diagrams helped identify thrombotic disease and infections in all patients with several postoperative complications (7 out of a total of 83) up to 48 h before clinical signs were apparent. The monitoring of platelet parameters was clearly superior to leucocyte monitoring.

Leucocyte parameters were misleading in 16 cases (5 false negative, 11 false positive). Furthermore, this study was able to confirm recent findings that an abnormal increase in PDW and low platelet counts found preoperatively in patients with coronary heart disease may serve as good indicators for the prethrombotic state and the risk of myocardial infarction.

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FIBRINOLYSIS IN PATIENTS WITH NEPHROTIC SYNDROME
U.Vertolli, K.Ansteady, J.Roderisch, E. Ritz

Aging influences plasma protein concentrations and may therefore change the functional state of protein systems. An age-dependent increase of tissue plasminogen activator (t-PA), and plasminogen activator inhibitor (PAI), as well as a slight decrease of whole blood lysis time has been reported.

In order to investigate age influences on fibrinolysis, several parameters were determined with tests commercially available. 48 healthy subjects of different age and 23 geriatric patients participated. Comparing the young (20-35 years, n=18) with the oldest participants (75 years and more, n=20), plasminogen, e-2-antiplasmin and Cl-inhibitor did not change. Antithrombin III (AT III) sank from 107 ± 11 to 94 ± 12 % (p<0.01). plasminogen activator (PA) from 0.83 ± 0.10 (n=15) to 0.76 ± 0.15 IU/ml (n=16). T-PA rose from 1.85 ± 0.38 to 2.56 ± 0.78 IU/ml (p<0.001). PAI from 4.43 ± 2.90 (n=14) to 9.69 ± 7.45 (n=13) IU/ml (p<0.05). The t-PA/PAI ratio increased with age (p<0.01). D-dimers were also higher in the elderly, correlating with the t-PA level. In the patient group these changes were more pronounced.

Thus overall plasminogen activators decreased with age despite a rise of t-PA, whereas activator inhibitors increased. These changes favour an impairment of fibrinolysis. Higher D-dimers point to enhanced fibrinolytic processes in the aged. In the presence of further risk factors a local failure to dissolve fibrin deposits may contribute to thrombogenesis in the aged.

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HEptest, Anti-Xa Activity, APTT and Thrombin Time in 20 Patients After Subcutaneous Administration of Enoxaparin

W.M. Halmbayer(1), M.Fischer(1), Ch. Felchtnger(1), T. Schwar(2), G. Gschmitt(2), A. Frasserg(1) and A. Priesnger(1)

Since low molecular weight heparin (LMWH) is used for prevention of thromboembolism, the coagulation laboratories are in need of a practicable test, sensitive to factor Xa-inhibition, for monitoring the effect of LMWH in plasma. Effects of subcutaneous administration of 20 mg and 40 mg of Enoxaparin were studied in blood samples drawn from 20 patients before and 1, 2, 3, 4, 6, 12 and 24 hours after injection, measuring thrombin time, APTT, Heptest and anti-Xa activity (amidolytic assay). Subcutaneous administration of 20 mg Enoxaparin had no effect on APTT, and was followed by a barely significant (p<0.05) rise of thrombin time 4 hours after injection. There was a barly significant (p<0.05) increase of APTT and thrombin time four hours after application of 40 mg. Heptest and amidolytic assay (S-2222) correlated well and there was a significant (p<0.01) increase of values with a maximum at 4 hours after administration of 20 mg or 40 mg Enoxaparin. Compared with values measured after treatment with 20 mg, higher values could be achieved after administration of 40 mg. We concluded that the Heptest, which can be performed easily and quickly, correlates well with the chromogenic reference method.

Therefore, it could be utilized in monitoring the effect of Enoxaparin in plasma samples of patients treated with this drug.

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MONOCLONAL ANTIBODIES AS THERAPEUTIC AGENTS TO BLOCK TISSUE FACTOR (THROMBOPLASTIN) FUNCTION.
W. Ruf, J.H. Morrissey and T.S. Edgington

Tissue Factor (thromboplastin) expression in vascular cells or release of cells into the circulation triggers the initiation of coagulation in the vasculature by allowing the formation of proteolytically active tissue Factor:factor VIIa complexes. In vitro studies demonstrated the potency of LPS to induce TF expression in endothelial cells and monocytes and suggest a critical role of this effector molecule in the pathogenesis of disseminated intravascular coagulation associated with septic shock. The lethality of E. coli induced septic shock in baboons is prevented by inhibition of TF function by occupying the factor VII interactive regions on TF and demonstrated a critical role of this effector molecule in the pathogenesis of disseminated intravascular coagulation associated with septic shock. The lethality of E. coli induced septic shock in baboons is prevented by inhibition of TF function by occupying the factor VII interactive regions on TF and demonstrated a critical role of this effector molecule in the pathogenesis of disseminated intravascular coagulation associated with septic shock. The lethality of E. coli induced septic shock in baboons is prevented by inhibition of TF function by occupying the factor VII interactive regions on TF and demonstrated a critical role of this effector molecule in the pathogenesis of disseminated intravascular coagulation associated with septic shock. The lethality of E. coli induced septic shock in baboons is prevented by inhibition of TF function by occupying the factor VII interactive regions on TF and demonstrated a critical role of this effector molecule in the pathogenesis of disseminated intravascular coagulation associated with septic shock.

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ANDERUNGEN VERSCHIEDENER GERINNUNGSWERTE UND LABORPARAMETER VOR, WAHREN UND NACH EINER URSACHENLESE SFKPIKINASE (UHSK) - BEHANDLUNG MIT ZWEI SERIEN

E. Wenzel*

A. Haass, W. Treib, J. Treib, G. Pindur*, C. Miyashita* and E. Wenzel*

EXPERIENCE WITH A NEW AUTOMATED COAGULATION INSTRUMENT: ELECTRA 900c

Results of internal and external evaluations

Electra 900c is a new automated instrument designed to perform all relevant functional assays of a haemostasis laboratory. All clotting assays and chromogenic substrate assays can be performed. In the PT assay simultaneously a fibrinogen determination is performed by measuring the increase of absorbance during clot formation (derived fibrinogen). The performance of the derived fibrinogen was evaluated on 237 patient samples which were analyzed in different laboratories. The correlation between the derived fibrinogen and the Clauss assay was excellent with the correlation coefficient of r = 0.96. Both assays give approximately the same information on the amount of clottable fibrinogen which is reflected by the equation of the regression line (Pansing/Roblok) y = 0.95 x + 19.5. PT-assays correlated well with results determined on mechanical instruments of centrifugal analyzers. Typical in run precision were between 0.6 and 3 % c.v. for several parameters including PT, aPTT, fibrinogen (Clauss), AT III and Factor VIII. Also the precision from day to day was in a similar range. Other chromogenic substrate based assays like Protein C, plasminogen, Factor IX chromogenic could be successfully adapted on the instrument with excellent precision and accuracy. The system can be upgraded with an automated sample processing unit (Electra 1000) which may further improve precision and accuracy. With Electra 900c/1000 the same degree of precision as in clinical chemistry can be achieved. Therefore a further step into standardization and improvement of precision and accuracy in haemostasis assays is possible.

Recent studies have shown that the plasma expanders Dextran 40 and high substituted hydroxyethyl starch (HES) change their haemorheological and haemostaseologic properties in vivo. The accumulation of high molecular weight molecules induced an increase of plasma viscosity and a reduction predominantly of the factor VIII complex.

Patients suffering from cerebrovascular disease were treated with 500 to 1000 ml of three different solutions of HES over 10 days. They received 10% hydroxethyl starch (HES) (n=10) or one of two different solutions of 10% HES 200/0,5 (HAEK steril; n=10; Haemofusin; n=10) which differ in the mode of hydroxylation.

Only the HAEK-steril solution decreased the plasma viscosity, induced no accumulation of high molecular weight starch molecules in the plasma and did not alter significantly clotting factors. In contrast the two other HES solutions increased plasma viscosity due to an accumulation of high molecular weight starch molecules, and decreased progressively and significantly factor VIII:C, VIlia and VIIICPF.

In conclusion not only the degree but also the mode of hydroxylation of starch might be responsible for the accumulation of high molecular weight starch molecules. These molecules seemed to induce the different haemorheological and haemostaseologic effects and especially the reduction of factor VIII activities which was much higher than the hemodilution effect. The degree of the reversible factor VIII reduction, which corresponded with a drug induced von Willebrand's disease, depended on the dosage and the duration of the HES infusions.

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ON THE EFFECTS OF A LACTOBIONIC ACID AMIDE (LW 10082) ON THROMBUS FORMATION IN RAT MESENTERIC VESSELS.

K. Krupinski, H.K. Breddin

LW 10082 (Luitpold, Munich) is a synthetic polyanion of low molecular weight (2368 Dalton). This material was tested in an experimental thrombosis model, in which rat mesenteric venules (diameter 25-30 µm) were injured by laser defocussed Argon laser lesions. LW 10082 had a dose-dependent antithrombotic effect, observed with a minimal effective dose of 10 µg/kg 30 min after single i.v. and 2 hrs after s.c. injection. The duration of the antithrombotic effects of 10 µg/kg LW 10082 after i.v. injection lasted for more than 4 and less than 6 hrs.

After s.c. injection the antithrombotic effect of 10 µg/kg LW 10082 lasted for more than 24 hrs. If LW 10082 was administered in continuous i.v. infusion a significant inhibition of thrombus formation was observed with 33.3 µg/kg/min after a total dose of 1 µg/kg with a dose-dependent increase of the antithrombotic effect if higher doses were infused.

LW 10082 in concentrations of 1-1000 µg/ml PRP had no significant effect on the adhesion of platelets to subendothelial bovine matrix.

The highly sulfated polyanion LW 10082 is a possible antithrombotic agent with characteristics which differ from unfractionated and from low molecular weight heparins. It may be a useful agent for the prevention of venous and arterial thromboses if it induces less bleeding than these established drugs.

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HEMOSTASIOLOGICAL CONTROL OF THE PREVENTION OF THROMBOSIS WITH LOW MOLECULAR WEIGHT HEPARIN AFTER SURGICAL INTERVENTIONS

W. Heller, G. Fuhrer, H. Höhr

Since pre-kinins and their inhibitors under the administration of heparin fragments have not been very much taken into account until now, comparative investigations with high molecular heparin (HWH) will contribute to learn more about them. The clinical relevance is going to be discussed in this context and investigated with the help of the activators prekallikrein, of the kallikrein-like-activity, of the functional kallikrein-inhibition and β-factor XIIa-inhibition before the chirurgical intervention and on the third and seventh postoperative days. The investigation was carried out on 122 patients who had to be submitted to different elective abdominal surgical operations. 60 patients were treated with standard-heparin (HWH); 62 with LMW-heparin. In patients after abdominal surgical interventions, a compensated activation of the contact phase was to be noted. This could be proved by a remarkable FPK decrease which however was not combined with a more intensive kallikrein-like-activity. The mechanisms of compensation could be ascribed to higher levels of the capacity of inhibition by kallikrein and β-factor XIIa. The significance of the inhibition of kallikrein and heparin fragments, intensified by heparin in thrombi, seems however to be a slight one compared to the physiological main inhibitors. During the course of the study, five vein thrombosis were observed, four in the standard-heparin group and one in the LMW-heparin group.

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MODEL INVESTIGATIONS WITH LOW MOLECULAR WEIGHT HEPARIN FOR THE ESTIMATION OF AN EFFICIENT PREVENTION OF THROMBOSIS

W. Heller, G. Fuhrer, Z. Engel, H.E. Hoffmeister

The reactions of the coagulation system and of complement system were investigated ex vivo in a closed HLM model. Testing time was over 90 minutes. In order to stop the coagulation of blood efficiently, 6 IU/ml of CY-216 (Fraxiparin) had to be administrated. This is, despite a higher anti-FXa-activity, equivalent to twice the dosage of KABI 2156 (Fragmin). Possible explanations for these are: first, an increased affinity of heparin with Ca²⁺-ions; second, a slight affinity of heparin with ATIII. During the course of recirculation the concentration of heparin steadily decreased. This was mainly caused by the inactivation of heparin by platelet factor 4, combined with a significant decrease of the number of thrombocytes. A comparison of CY-216 and KABI 2156 shows that CY-216 is less bonded to platelet factor 4 and also less neutralized by platelet factor 4; on a larger scale, the aggregation of thrombocytes undergoes an inhibition. This reaction could have a positive effect on the daily dosage, but a negative one on the arising of hemorrhage complications. At the beginning of the test, a confused activation of coagulation occurs intrinsically and extrinsically. This can be established mainly by the decrease of factor XII and C1-inhibitor activities, as well as by the increase of the activity of factor X. Coagulation is caused by the release of specific proteins by the thrombocytes. As a result of this study, it is to be noted that CY-216 is absolutely capable to stop efficiently the coagulation, also that crucial differences with the LMW-heparin KABI 2156 do exist.

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POSSIBLE INTERACTIONS OF HIGH AND LOW MOLECULAR WEIGHT HEPARIN WITH HIGH DOSES OF APROTININ IN AN EX VIVO MODEL

W. Heller, G. Fuhrer, H.E. Hoffmeister

In the present study a HLM-machine was used to investigate to what degree the observed in vivo activation of the contact phase factors (pre-kallikrein, kallikrein-like-activity, kallikrein and β-factor XIIa-activity) and the plasma kallikrein level depend upon purely mechanical influences. The single parameters obtained by varying the aprotinin dosages during the administration of varying molecular weight heparin preparations were statistically compared with those obtained by the administration of constant doses of aprotinin. The prekallikrein activity was not effectively inhibited by HMW and LMW-heparin, whereas with a low dosage of aprotinin (150 KIU/ml) it is extremely effectively inhibited and with a high dosage it is completely inhibited. The kallikrein-like-activity curve confirms these findings independently of the molecular weight. The kallikrein formation was increasingly reduced with increasing doses of proteinase inhibitor; however, a total prevention could not be attained. In the present study a consumption of kallikrein inhibitors, independent of the aprotinin dosage and the heparin administration cannot be demonstrated. One can, therefore, proceed on the assumption that the activity of the physiological kallikrein inhibitors - mostly Cl-esterase inhibitor - decreases in corresponding proportion to the decrease in the β-factor XIIa-inhibition activity. A higher aprotinin dosage, compared to a lower dosage revealed only a slight fall in the β-factor XIIa-inhibition activity.

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STUDIES CONCERNING THE AVAILABILITY OF LOW MOLECULAR HEPARIN IN PLASMA: EFFECTIVELY INHIBITED.

G. Fuhrer, W. Heller, H. Hörth

In the present study, low respectively high molecular heparin was administered to patients during and after abdominal chirurgical interventions. In this process, low molecular heparin appeared to be slightly superior with regard to the emergence of thromboembolisms. The kallikrein-like-activity curve confirms these findings independently of the molecular weight. The kallikrein formation was increasingly reduced with increasing doses of proteinase inhibitor; however, a total prevention could not be attained. In the present study a consumption of kallikrein inhibitors, independent of the aprotinin dosage and the heparin administration cannot be demonstrated. One can, therefore, proceed on the assumption that the activity of the physiological kallikrein inhibitors - mostly Cl-esterase inhibitor - decreases in corresponding proportion to the decrease in the β-factor XIIa-inhibition activity. The prekallikrein-kallikrein-kinin system was maintained at over 120 minutes. The determination of the activity in the contact phase was kept up at over 120 minutes. The extra corporal circulation was terminated by the administration of constant doses of aprotinin. The prekallikrein activity was not effectively inhibited by HMW and LMW-heparin, whereas with a low dosage of aprotinin (150 KIU/ml) it is extremely effectively inhibited and with a high dosage it is completely inhibited. The kallikrein-like-activity curve confirms these findings independently of the molecular weight. The kallikrein formation was increasingly reduced with increasing doses of proteinase inhibitor; however, a total prevention could not be attained. In the present study a consumption of kallikrein inhibitors, independent of the aprotinin dosage and the heparin administration cannot be demonstrated. One can, therefore, proceed on the assumption that the activity of the physiological kallikrein inhibitors - mostly Cl-esterase inhibitor - decreases in corresponding proportion to the decrease in the β-factor XIIa-inhibition activity. A higher aprotinin dosage, compared to a lower dosage revealed only a slight fall in the β-factor XIIa-inhibition activity.

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MODEL INVESTIGATION WITH CI-ESTERASE INHIBITOR AND EVENTUAL INTERACTION WITH HEPARIN

W. Heller, G. Fuhrer, Z. Engel, R. Hoffmeister

Because of the positive effects of the proteinase inhibitor aprotinin in the field of heart surgery, this study is concerned with CI-esterase inhibitor which is probably the most efficient inhibitor in the contact activation phase of the coagulation. The investigation was carried out in an ex-vivo model. Anti-coagulant CY 216 (Fraxiparin) was used at a dosage 4 IU/ml. In a initial experiment, CI-inhibitor was dosed at 1000 U/500-700 ml. The extra corporal circulation was kept up at over 120 minutes. The determined parameters at each sample were compared to the respective results of the runs without CI-esterase inhibitor, but with the same dosage of heparin. During the filling of stored blood into the reservoir of the HLM, a contact activation of the coagulation and of the prekallikrein-kallikrein-kinin systems was to be noted, produced by the intensified contact with artificial surfaces, and in spite of heparinisation. After the addition of CI-esterase inhibitor, further increasing coagulation processes were measured despite significantly increased activity values of β-factor XIIa-inhibition and CI-esterase inhibitor. The prekallikrein-kallikrein-kinin system showed as well a further activation. The heparin activity decreased during the circulation. The proteolytical processes of the contact activation phase, as well as the activation of the prekallikrein-kallikrein-kinin system could not be sufficiently inhibited.

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MODEL INVESTIGATIONS ABOUT THE INHIBITION OF ELASTASE WITH CI-INHIBITOR AND HEPARIN INTERACTIONS (HEART-LUNG-MACHINE - HLM)

W. Heller, O. Grober, G. Fuhrer, H.E. Hoffmeister

The release of FMM-elastase can be followed in plasma with the elastase alpha-proteinase inhibitor complex. Two series of recirculations with heparinized whole blood in a HLM-system were done. The testing-length was each time 120 minutes; blood was taken at nine defined sample times. In another series of experiments, the CI-esterase-inhibitor was each time before the recirculation added to the filling volume of the HLM. The concentration of elastase revealed a steady increase up to an end value which exceeded after two hours the initial value by twenty times. Compared with the control group, the application of CI-INH did not lead to a significant inhibition of elastase. Despite a significant increase of the inhibition of kallikrein and β-factor XIIa no effects on the activation of the contact phase could be established. Slightly reduced plasma levels of prekallikrein, as well as a clear increase of the kallikrein-like-activity, reveal an activation of the plasma kallikrein system at the beginning of the experiment. The results show a start of the endogene coagulation and a slight activation of the complement cascade. That is why the placebo group of the activated Hageman-factor, plasmakallikrein and anaphylatoxins C5, respectively C3a, is of secondary importance in the series of experiments. The similarly increasing runs of the coagulation of platelet factor 4 and FMM-elastase show that Pf 4 has intensified the release of elastase from thrombocytes. At the end of the experiment we found reduced heparin levels.

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RELATIONSHIPS BETWEEN SCREENING TESTS FOR EARLY DIC AND EARLY SIGNS OF ORGAN FAILURE
B. Kirchhof, U. Kirchhof, G. Etscheid
It is a special clinical interest to have screening tests for the diagnosis "high risk for DIC" in individual patients. There should be screening tests which can be performed during day and night without special knowledge. The quantitative measurements of fibrino-peptide A, thrombin-AT III-complex and other substances, which arise through coagulation, may give reasonable informations of the beginning of DIC, but such assays are time consuming or they need special knowledge or special equipment. Semiquantitative latex-agglutination tests can be done by every lab assistant. Therefore we initiated the semiquantitative determination of fibrinogen degradation products (FDP) or of a neoantigen (D-D) in all patients on an intensive care unit, who were thought to have early DIC by the physician on duty. We present a retrospective analysis of the data from 300 patients. About 2/3 show elevated FDP or D-D. The results of these screening tests are compared a) with the results of other coagulation tests performed often (PTT, fibrinogen, AT III, platelets), b) with the results of lab screening for organ failure (creatin, PGG, JG'T, leucocytes) and c) with some clinical parameters (heart rate, blood pressure, temperature, main diagnosis). The comparisons show that in patients, who have a bleeding or other positive signs of DIC, no relations exist between FDP or D-D value and other coagulation tests. But even in these patients there is a relation between FDP or D-D and the results of the lab screening for organ failure, high temperature and/or the combination of low blood pressure and high heart rate lasting longer than 4 h are associated with high FDP or D-D respectively.
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STANDARDIZED PLATELET AGGREGATION MEASUREMENTS FOR MULTI CENTRE TRIALS
B. Kirchhof, U. Kirchhof, G. Etscheid
Platelet aggregation screening is now becoming increasing important. It is not only necessary in clinical research to testify the laboratory effects of new substances for primary and secondary prevention of thromboembolic disease, but also to control the quality of therapeutical plasma preparations and to check side effects of pharmaceudical products on platelet function. Platelet function assays should be designed in such a way that they can be performed in normal clinical labs without special knowledge. They should enable direct data comparisions of the different labs. We present a standardized measurement of collagen and ADP induced platelet aggregation with the help of a personal computer. The measuring data are registered on line, laid out graphically and calculated in accordance with suitable points given. The dose effect relationships between aggregants (6 different concentrations) and calculated parameters (5 after ADP induction, 4 after collagen induction) are demonstrated (n = 40). The comparison of the results of this normal group with those of 8 volunteers 24 hours after they had taken 250 mg ASS and of 5 patients respectively with typical illnesses associated with hypo- and hyperaggregability, shows that the analysis of the aggregation with 2 reagent-concentrations respectively (1.2 and 4 µg/ml collagen; 1.2 and 4 µg/ml ADP) and the resulting calculation parameters can differ between normal, hypo- and hyper-aggregable platelets. We believe that the presented standardized and simplified platelet aggregation measurements are suitable for multi centre trials.
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VEIN THROMBOSSES AND LUNG THROMBOEMBOLISM IN AN UNSELECTED AUTOPSY SERIES
M. Genzkow and W. Saeger
Among 4,077 autopsies of the years 2/1979 to 1/1989, 966 cases with vein thromboses and lung thromboembolism were demonstrated (corresponding to 24%). From these we found thromboembolism in 758 cases (78.5%). The rate of fatal and fulminant embolism was 78.9%. We found in 16.3% thromboses of the popliteal veins, in nearly 60% thromboses of the femoral and pelvic veins, in 4% thromboses of the caval vein and 2.7% were located in cardiac auricle. We compared the results with 350 autopsies of the year 1985 without thromboses or embolism. In both groups, the average age was 74 years. Nearly 60% was spreaded between 70 and 85 years. In the control group 52.3% were male, whereas only 44.2% of cases with thromboses or embolism were male. The annual rate varied between 17.8% in 1988 and 28.6% in 1981. Monthly rate had its maximum with 29.1% in november, and its minimum with 21.7% in july. The average weight differed nearly 3 kg in favour of thromboses. A decisive factor was immobilation, measured in days of confinement to bed. Average immobilation in thromboses was 19, in control group 10.4 days. A further important factor was the form and period of anticoagulation therapy. Referring to the different diseases, we found that cardiovascular diseases were more frequent in control group and malignant tumors were more frequent in the group with thromboses. Proportions of gastrointestinal tumors were increased in group with thromboses, whereas patients with bronchial carcinomas had the same rate. We do not find a significant difference in right heart failure, reasoned by the high average age in both groups. Hematocrit as a parameter for exsiccosis was in the group with thromboembolism two points higher. The difference in thrombocytes was irrelevant.
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ETIOLOGY OF RETROPERITONEAL BLEEDINGS IN POST-MORTEM SERIES
D. Sautner and W. Saeger
In a large unsedected autopsy series (N=4,461) 35 retroperitoneal haematomas (0.78%) were found (Table 1).

| Cause                             | Fatal | Non-fatal |
|----------------------------------|-------|-----------|
| Rupture of aortic aneurysm       | 15    | 1         |
| Trauma                           | -     | 9         |
| Tumour                           | 1     | -         |
| Complications of surgery         | 2     | 1         |
| in combination with haemorrhagic diagnosis | - | 2 |
| Haemorrhagic diathesis           | 18 (51%) | 17 (49%) |

In 45.7% the bleedings were due to ruptured aortic or iliac aneurysms of atherosclerotic type. With exception of one the retroperitoneal bleeding in these cases was the only cause of death. In the cases of trauma the retroperitoneal bleeding had only minor importance. In haemorrhagic diathesis (17%) the retroperitoneal bleedings never were fatal on their own.
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K. Jaschonek, D. Steinhilber, J. Knospe, H. HUMAN POLYMORPHONUCLEAR NEUTROPHILS OXYGENASE AND ON 5-LIPOXYGENASE ACTIVITY IN DERIVATIVES ON PLATELET TXA2-SYNTHASE, CYCLO-

Over the past several years it became evident that a series of host defense mechanisms (e.g. inflammatory response, cell-cell communication) play a crucial role in the pathogenesis of various diseases. One of these mechanisms involves the generation of eicosanoids, a group of lipid mediators that are produced by the action of cyclooxygenase and lipoxygenase pathways on arachidonic acid.

Methods: In a prospective study (11-80, 49) the levels of different clotting factors (V, VII, VIII, IX), antithrombin III, fibrinogen and platelets were determined in 33 unselected patients with CD, of whom 13 and 20 were suffering from severe CD (Group A) and mild CD (Group B) respectively. The results were compared with the data from healthy controls (Group C).

Results: Significantly elevated levels of factor V (130±84 vs 116±12; p<0.001) and factor VII (146±56 vs 95±19; p<0.05) were found in Group A as compared to controls. Furthermore, severe CD showed a significant (p<0.001) increase of platelets (507±146 vs 231±36) and fibrinogen (483±127 vs 271±51). Moreover platelets were significantly higher in Group A as compared to B (507±146 vs 347±114; p<0.001). Finally mild CD showed significantly increased levels of fibrinogen (437±153 vs 271±51, p<0.001) and platelets (507±160 vs 231±36, p<0.001) as compared to controls. Regarding F.VII, F.II and antithrombin III no differences were found. None of the patients had clinical evidence of deep vein thrombosis.

Conclusions: The levels of F.V, F.VII, fibrinogen and platelets might be useful in measuring the actual activity of Crohn's disease. The simple method of platelet count discriminates best between mild and severe Crohn's disease. The alterations in haemostasis represent an unspecific response to inflammation of the bowel. The hypercoagulable state of patients with Crohn's disease does not lead to deep vein thrombosis.

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PLATELET THROMBOXANE/ENDOPEROXIDE RECEPTORS IN TYPE I DIABETES MELLITUS

K. Jaschonek, C. Paul, K. Krönert

The occupation of specific thromboxane/endoperoxide receptors (TXA/PGH-R) causes a positive feedback amplification of thromboxane A2 (TXA) generation by activating phospholipase A2 via the formation of the calcium ionophore, inositoltrisphosphate (IP3) and presumably also by the activation of receptor-operated calcium channels. Therefore, it seems conceivable that the enhanced sensitivity to the increased formation of TXA found in diabetes mellitus (dm) could be the result of an enhanced TXA/PGH-R surface expression or changes in the signal transduction process.

Consequently, we studied insulin-dependent diabetics platelet TXA/PGH-R by direct binding studies using 3H-U46619. Twelve patients with type I diabetes and eight age and sex matched controls were included in the study. In our hands binding of 3H-U46619 was not altered in diabetic patients (Bmax 0.97±0.40 vs 1.2±0.53 pmol/109 platelets; Edmax 67±42 vs 48±24 nM). In addition, the dose of U46619 required to induce half-maximal platelet aggregation also was not different from controls (EC50 82±23 vs 88±40 nM). The binding studies using 3H-U46619 and the finding of an unchanged responsiveness of indomethacin-treated platelets to U46619 exclude the possibility that TXA/PGH-R expression or signal transduction is altered in diabetes mellitus.

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THE EFFECTS OF NOVEL ANTIMUCOSAL ABOLE DERIVATIVES ON PLATELET TXA2-SINTHASE, CYCLO-

OXYGENASE, AND ON 5-LIPIDOXENASE ACTIVITY IN HUMAN POLYMORPHONUCLEAR NEUTROPHILES

K. Jaschonek, D. Steinhilber, J. Knospe, R. Einsele, G. Ehninger, H.J. Roth

Over the past several years it became evident that a series of host defense mechanisms (e.g. activity of NK cells and the lymphocyte proliferation response to mitogens) are at least partially controlled by the formation of 5-LO metabolites (LTB4) and TXA2 or cyclooxygenase (CO) products (TXA2, PG2). Since azole derivatives have shown to interfere with both enzymes, we decided to screen the effects of the two novel and structurally different azoles itraconazole (ICZ) and fluconazole (FCZ) on the formation of 5-LO products in PMN and the synthesis of TXA2 and PG2 in human platelets. The eicosanoids formed during incubation with arachidonic acid and A23187 were separated by HPLC. ICZ (10-5M) inhibited the A23187-induced formation of the 5-LO products LTB4, 5-HETE, 5.12R-trans LTB4, 5.12S-trans-LTB4, 5.12R-DHETE by 90%. FCZ, however, a novel bistriazole derivative had no effect on platelet function and TXA2-S or CO activity as shown by assays using the purified enzyme. Our data demonstrate that FCZ had no effect on platelet function and TXA2-S and CO activity as shown by assays using the purified enzyme. Therefore we were able to observe the observation that FCZ among other azole compounds exclusively does not impair host defense mechanisms.

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PLATELET THROMBOXANE/ENDOPEROXIDE RECEPTORS IN TYPE I DIABETES MELLITUS

K. Jaschonek, C. Paul, K. Krönert

The occupation of specific thromboxane/endoperoxide receptors (TXA/PGH-R) causes a positive feedback amplification of thromboxane A2 (TXA) generation by activating phospholipase A2 via the formation of the calcium ionophore, inositoltrisphosphate (IP3) and presumably also by the activation of receptor-operated calcium channels. Therefore, it seems conceivable that the enhanced sensitivity to the increased formation of TXA found in diabetes mellitus (dm) could be the result of an enhanced TXA/PGH-R surface expression or changes in the signal transduction process.

Consequently, we studied insulin-dependent diabetics platelet TXA/PGH-R by direct binding studies using 3H-U46619. Twelve patients with type I diabetes and eight age and sex matched controls were included in the study. In our hands binding of 3H-U46619 was not altered in diabetic patients (Bmax 0.97±0.40 vs 1.2±0.53 pmol/109 platelets; Edmax 67±42 vs 48±24 nM). In addition, the dose of U46619 required to induce half-maximal platelet aggregation also was not different from controls (EC50 82±23 vs 88±40 nM). The binding studies using 3H-U46619 and the finding of an unchanged responsiveness of indomethacin-treated platelets to U46619 exclude the possibility that TXA/PGH-R expression or signal transduction is altered in diabetes mellitus.

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AGE AND CARDIOVASCULAR RISK FACTORS AS DETERMINANTS OF PLATELET FUNCTION IN HEALTHY SUBJECTS AND PATIENTS WITH CORONARY HEART DISEASE. A MULTIVARIATE APPROACH

W. Tschöpe, K. Weber, K. Paschen, M. Kupper, W. Bleifeld

Stimulated platelet function (PF) has been reported for older age and for patients (PTS) with cardiovascular risk factors and arteriosclerosis. To discriminate between these factors, we prospectively studied PF in 191 men, 78 PTS with angiographically documented coronary heart disease and 113 healthy subjects (HS). In HS, stepwise multiple linear regression identified age to be a major determinant of PF. Platelet aggregation after induction with both ADP and collagen increased with age (p<0.01, respectively). Possibly due to counterregulation, the levels of c-AMP in platelet rich plasma increased with age, both basally (p<0.05) and after stimulation with prostaglandin E2 (PGE2, p<0.01). In PTS, PF was not dependent on age. In multivariate analysis of variance, hypercholesterolemia (beta=240 mg/dl) was not a determinant of PF in neither group. In HS, smokers had lower levels of c-AMP, stimulated c-AMP than non-smokers (p<0.01), but aggregation did not differ. Elevated blood pressure (beta=160/95) was associated with inhibited ADP induced aggregation (p<0.01) and increased c-AMP after PGE2 (p<0.001) at the 10-5M. In contrast to ICZ, FCZ had no effect on platelet function and TXA2-S and CO activity as shown by assays using the purified enzyme. Our data demonstrate that FCZ had no effect on platelet function and TXA2-S and CO activity as shown by assays using the purified enzyme. Therefore we were able to observe the observation that FCZ among other azole compounds exclusively does not impair host defense mechanisms.

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The interaction of heparins of varying molecular weight has been investigated in vitro using high performance liquid chromatography (HPLC) method, antithrombin IIa and Xa tests and a whole blood coagulation system. Unfractionated heparin (UFH) and 5 low molecular weight heparins (LMWH) were added to increasing doses of protamine in vitro. The heparin-protamine complex was sedimented by centrifugation. The supernatant was analyzed by HPLC. Fractions from HPLC were collected and aXa and aIIa activities were measured using commercial assays. The data show that UFH and all LMWH's from HPLC were collected and aXa and aIIa activities were measured by centrifugation. The supernatant was analyzed by HPLC. Fractions from HPLC were collected and aXa and aIIa activities were measured using commercial assays. The data show that UFH and all LMWH's were quantitatively bound by protamine. 1 mg protamine totally bound 1 mg of all heparins. No aXa or aIIa activity was detected in the eluates from the HPLC column. We also examined the neutralization of UFH and the LMWH Kabi 2165 by protamine after intravenous injection of both substances in man on an ex vivo whole blood clotting test. The results show that the effect of protamine on aPTT and thrombin time is completely inhibited by protamine although the coagulation of whole blood was still impaired. There was no difference in the prolongation of coagulation using UFH and LMWH. Clotting of whole blood was monitored in serial subsamples with measurement of FpA and FpF4. No relation between FpA and FpF4-values and antithrombin Xa activity was found.

The data demonstrate that protamine binds UFH and all LMWH preparations. After administration into man UFH and LMWH both are bound by protamine. However, some anticoagulant activity can still be detected after administration of protamine. This suggests that UFH and LMWH cause release of heparin-like compounds into the blood stream which do not react with protamine.

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PROSPECTIVE STUDY ON THE USE OF LOW MOLECULAR WEIGHT HEPARIN IN OUTPATIENTS
J. Harenberg, G. Leber, G. Stehle, R. Zimmermann*, W. Kübler* and D. L. Heene

Four groups of patients received prophylaxis of thromboembolism because of prior venous thromboembolism (n=51), artificial heart valve replacement (n=13), aortic fibrosis with arterial embolism (n=11) and cardiomyopathy (n=11). They received the LMWH Kabi 2165 in a dose ranging from 2500 to 15,000 IU aPTT units 1-2 daily s.c. The dose was adjusted according to the bodyweight and the statistical risk of developing thromboembolism. The treatment period ranged from 1 to 48 months. Occurrence of thromboembolism and haemorrhage, laboratory parameters (Heptest, capillary blood, whole blood, and plasma, S 2222 factor Xa method, aPTT, thrombin clotting time and AT III) and safety parameters were controlled every 4-6 weeks. The mean treatment period was 0.5 years summing up to a total observation period of 50 years. No fatal thromboembolism occurred. 2 patients of group I experienced rethrombosis. One major gastrointestinal bleeding occurred in a patient with a so far unknown colon carcinoma. Seven minor haemorrhages occurred which did not re-occur after a 15% dose reduction in these patients. All plasma samples were taken 2-4 hours after the s.c. injection. Highest values from capillary blood and whole blood, and blood found from 35-60 sec, values in plasma ranged from 45-100 sec. S 2222 aXa method showed values ranging from 0.2 to 1.0 aXa units/ml. aPTT and TCT were only slightly prolonged. AT III and lab values remained unchanged during the observation period. Osteoporosis was not detected. 8 pregnant women were included in the study. No anti Xa activity was detected in blood drawn from the umbilical cord after delivery.

The data suggest that LMWH can be effectively and safely used for prophylaxis of thromboembolism in patients with venous thromboembolism, artificial heart valve replacement, aortic fibrosis with arterial embolism, cardiomyopathy, and in pregnancy.

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HEPARIN INDUCED THROMBOCYTOPENIA: PLATELET AGGREGATION STUDIES IN PRESENCE OF STANDARD HEPARIN AND LOW MOLECULAR WEIGHT HEPARIN (LMWH) PREPARATIONS
U. Harbrecht

Two cases of heparin induced thrombocytopenia (HIT) typell with severe thrombo-embolic complications are reported. Thrombocytopenia occurred 13(15) days after the onset of heparin therapy and platelet count dropped to the lowest value of 13 000(40 000)/µl at the 16(24)th day. Thrombo-embolic complications under heparin treatment included pulmonary embolism and venous thrombosis of the legs in both patients and additionally thrombosis of arm veins in one patient. Cessation of heparin resulted in normalization of platelet count within 8(3) days. Diagnosis of HIT was confirmed by in vitro platelet aggregation tests. At the time of the patients thrombocytopenia a normal donor platelets were used and mixed with the patients platelet poor plasma (PPP). Two commercial standard heparin preparations (from a minimum concentration of 0.2 IU/ml upwards) led to a strong aggregation of platelets in presence of either patients plasma. No remarkable aggregation could be seen with one of five tested LMWH fractions. This preparation and a second LMWH failed to aggregate platelets with the plasma of the second patient. Controls of all preparations were carried out in normal platelet rich plasma (PPP) and the use of donor platelets with a notable spontaneous or heparin dependent aggregation was excluded. Aggregation studies with the patients PPP were performed after normalization of their platelet count and no change in aggregation behaviour compared to the results with the mixture of donor platelets and patients PPP was observed.

These results indicate that in patients with HIT it might be very helpful to perform in vitro platelet aggregation studies with various LMWH fractions in order to select one preparation without aggregatory potency that could be applied therapeutically.

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ACTIVATION OF COAGULATION BY LUNG CARCINOMA CELLS
R. Boit, N. Heldmann, M. Hausberg, R. Kühling, K. Hubmann

It is well known that fibrin formation takes place within tumor tissues. In a randomized trial with coumarin treatment, prompted by the assumption that the activation of coagulation favours tumor growth and metastasis, a significant prolongation of survival of small cell (SCLC) but not non-small cell lung cancer (NSCLC) patients was found (Zacharski et al., Cancer 45:2046;1984).

The coagulation process in tumor tissue may be triggered by adjacent macrophages, but also by tumor cells themselves. They may express tissue factor-like activity, prothrombinase-active surfaces or a vitamin K dependent cysteine proteinase, termed 'cancer procoagulant', activating factor X directly.

The aim of this study was to assess the activation of coagulation in lung cancer patients and by cell lines in vitro.

We found in 87% of unsellected lung cancer cell patients a striking elevation of thrombin-antithrombin III complex (TAT) levels up to 120 µg/l (normal < 4 µg/l). It was found in both SCLC and NSCLC patients and was sustained over months. In vitro, sonicated cell suspension of S NSCLC and 1 of 2 SCLC lines, kept in serum free media, caused marked reduction of the recalification time in normal pool plasma. Using an assay system with purified factor X (Behringwerke) and the chromogenic substrate S-2222 (Kabi Vitrum), we found a Ca++ dependent factor X activation, particularly strong in 2 NSCLC lines.

The data suggest that the biological role of coagulation activators should be re-evaluated particularly in NSCLC.

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ANTITHROMBOTIC ACTIVITIES AND BLEEDING RISKS OF LACTOBIONIC ACID AMIDES

V. Raake, R.J. Klauser, P. Leiller, E. Meinetsberger

A homologous series of lactobionic acid amides with an increasing number of alkylene groups as synthesized.

LW 10082 (hexa-α,α-copolyamidoligosaccharide-bis-[2,3,5,6-tetra-O-sulfate-4,6-bis-[2,3,4,6-tetra-O-sulfate-8-D-galactopyranosyl]-D-glucamid] is the most potent compound of this series. It has a molecular weight of 2388 daltons and exhibits low anticoagulatory activity compared to heparin and low molecular weight heparins. The activity in the aPTT-test is highest for LW 10082 and decreases gradually when the number of methylene groups in the alkylene chain increases. In the Heparin, Bis-lactobionic acid amides demonstrate still lower, but definite activities. At concentrations up to 0.1 mg/ml in platelet rich plasma, no effect on agent (ADP, collagen, arachidonic acid etc.) induced platelet aggregation was observed with these compounds. In different animal models in rabbits and rats (Wessler-test, Bardauer-model, laser induced thrombosis and a venous thrombosis model) the antithrombotic activity was dose and time dependently. In contrast to its low anticoagulatory activity LW 10082 proved equipotency to a low molecular weight heparin in a dose range of 0.5 - 5.0 mg/kg b.w.

In the rat tail bleeding model and the rabbit ear bleeding model LW 10082 was compared with heparin and low molecular weight heparins. In doses below 5.0 mg/kg, LW 10082 induced only a slight prolongation of the bleeding time. At 5.0 mg/kg the bleeding time was prolonged by 83 %. In contrast, Heparin and low molecular weight heparins prolonged the bleeding time by about 100 % at doses of 0.5 and 1.0 mg/kg b.w., respectively. Therefore, bleeding risks seem to be a minor problem in the action of the lactobionic acid amides.

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INTERACTIONS OF LW 10082, A LOW MOLECULAR WEIGHT SYNTHETIC POLYAMIDE, WITH HEPARIN COFACTORS AND ANTIDOTES

R.J. Klauser and V. Raake

LW 10082 has recently been described as a synthetic polyanion of defined chemistry and low molecular weight (2388 daltons) with antithrombotic and anticoagulant properties. Compared to Heparin, LW 10082 exhibits moderate antithrombin activity but very low anti-α- and anti-Ⅲa activities when measured in experiments showing clotting time measurements. LW 10082 was about 20 times more potent as inhibitor of human thrombin than of bovine thrombin. LW 10082 proved to be a potent activator of heparin cofactor Ⅱ in a system using purified factors. In contrast, purified AT Ⅲ did not inhibit thrombin in the presence of LW 10082. In the presence of plasma and LW 10082 thrombin was inhibited, however, probably by the action of heparin cofactor Ⅱ. The anticoagulant activity of LW 10082 in the aPTT-test was shown to be independent of AT Ⅲ by the use of anti-AT Ⅲ antibodies. In the presence of the antibodies the potency of LW 10082 was not reduced, while the activity of heparin was clearly attenuated.

The anticoagulant activity of LW 10082 was readily neutralized by protamine and polybrene. Depending on the assay, different relative amounts of antidote were necessary to normalize the clotting times. In contrast to protamine platelet factor 4 did not influence the anticoagulant activity of LW 10082. This finding suggests a possible advantage of LW 10082 as an antithrombotic in cases with ongoing platelet activation.

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INTERACTIONS OF LW 10082, A LOW MOLECULAR WEIGHT SYNTHETIC POLYAMIDE, WITH HEPARIN COFACTORS AND ANTIDOTES

R.J. Klauser and V. Raake

LW 10082 has recently been described as a synthetic polyanion of defined chemistry and low molecular weight (2388 daltons) with antithrombotic and anticoagulant properties. Compared to Heparin, LW 10082 exhibits moderate antithrombin activity but very low anti-α- and anti-Ⅲa activities when measured in experiments showing clotting time measurements. LW 10082 was about 20 times more potent as inhibitor of human thrombin than of bovine thrombin. LW 10082 proved to be a potent activator of heparin cofactor Ⅱ in a system using purified factors. In contrast, purified AT Ⅲ did not inhibit thrombin in the presence of LW 10082. In the presence of plasma and LW 10082 thrombin was inhibited, however, probably by the action of heparin cofactor Ⅱ. The anticoagulant activity of LW 10082 in the aPTT-test was shown to be independent of AT Ⅲ by the use of anti-AT Ⅲ antibodies. In the presence of the antibodies the potency of LW 10082 was not reduced, while the activity of heparin was clearly attenuated.

The anticoagulant activity of LW 10082 was readily neutralized by protamine and polybrene. Depending on the assay, different relative amounts of antidote were necessary to normalize the clotting times. In contrast to protamine platelet factor 4 did not influence the anticoagulant activity of LW 10082. This finding suggests a possible advantage of LW 10082 as an antithrombotic in cases with ongoing platelet activation.

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ASPECTS OF ENOXAPARIN ADMINISTRATION IN GENERAL SURGERY
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On the basis of a mid-point evaluation of more than 10,000 patients, a large multicenter German trial with enoxaparin (Clexane) will be presented. In this study, in which 211 hospitals are participating, the safety and tolerance, as well as the incidence of pulmonary embolism and thrombosis following administration of enoxaparin 2 mg (Clexane 20) once daily in patients undergoing general surgery are investigated.

The general surgical procedures included are: abdominal, gynecologic (with the exception of mastectomy) and urologic operations lasting at least 30 minutes under general anesthesia and/or spinal/peridural anesthesia.

All potential complications with an incidence of greater than 2 per 1,000 will be documented. This number was chosen because it reflects the observed incidence of fatal pulmonary embolism with unfractionated heparin prophylaxis.

INVESTIGATIONS WITHIN THE TRIAL INCLUDE:
- Occurrence of thromboses and pulmonary embolism, which, in case of clinical signs, are to be confirmed by objective techniques.
- Bleeding and blood loss as well as changes in platelet count, hematology and transaminases.
- Adverse drug reactions.

RESULTS:
The safety and tolerance of thromboembolic prophylaxis with Clexane 20 in general surgical procedures are shown to be excellent (no injection hematomata, thrombocytopnea, and low incidence of bleeding). In our laboratories, we have observed the incidence of fatal and non-fatal pulmonary embolism, the reported mid-point evaluation shows a superiority of Clexane 20 in the prevention of thromboembolic complications when retrospectively compared with trials using unfractionated heparin (e.g., Kakkar: International Multicenter Trial). Reports of bleeding within the enoxaparin trial were statistically below those in the above-mentioned trial.

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COMPARATIVE STUDIES ON THE PROCOAGULANT ACTIONS OF INTRAVASCULAR RADIOCONTRAST AGENTS AND THEIR PHARMACOLOGIC MODULATION
R. Morena, D. Fareed, Hoptopanaetad, J.M. Walenga and J. Fareed

Several recent experimental and clinical reports have described the procoagulant effects of non-ionic contrast media alerting practicing angiologists to the safety issues of these agents. Experimental studies in our laboratories have shown that the procoagulant action of non-ionic contrast media is related to their ability to release thromboplastic material from red cell membranes and to their weaker anticoagulant nature in comparison to ionic contrast media. Thus, these agents are unable to inhibit the activation of the coagulation process at the blood-cell interphase in the angiographic catheter. Incubation of non-ionic contrast agents such as Omnipaque® and Iosuvo® with whole blood resulted in marked generation of fibrinopeptide A and thrombin-antithrombin complexes. In contrast, Hexabrix® and Angiobra® produced much weaker generation of these markers. The in vivo systemic procoagulant effects of these contrast agents were also inhibited by prior administration of CY 216D or hirudin at 0.5 mg/ml. Totally inhibited the generation of these markers. The in vivo systemic procoagulant effects of these contrast agents can be minimised with low molecular weight heparin or hirudin.

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PHARMACOLOGIC VALIDATION OF THE ANTITHROMBOTIC AND VASCULAR EFFECTS OF DEFIBROTIDE

J. Fareed, G. Kindel, J. Walenga, D. Hoppensteadt, *K. Krupinski and *K. Breddin

Defibrotide (D) a polydeoxyribonucleotide based drug has been used clinically for the prevention of occlusive disorders of varying origin. Several pharmacologic models have been used to investigate the mechanism of action of this agent. D produced a dose-dependent antithrombotic action in rabbit (venous stasis) and rat (arterial) models of thrombosis with the intravenous EDR ranging from 2.5 - 10.0 mg/kg IV. Ex vivo analysis of blood did not reveal any alteration of the PT, PTT, TT and Heptest times. However, the thrombelastograph patterns of blood obtained from animals 30 minutes after IV injection of D showed a hypercoagulable state. Although the ex vivo clotting of blood from rabbits treated with defibrotide were not significantly different from control animals, sera from the treated group did not produce any contractile action of the isolated rabbit aortic strip suggesting the absence of procontractile agents. In animals with induced clot occlusions, pretreatment of animals with D augmented the thrombolytic efficacy of urokinase and t-PA. Repeated IV administrations of D into rabbits resulted in increased levels of 6-keto-FP_{10} production. In a primate model of hypercoagulable state, intravenous D induced a fibrinolytic state as measured by increase in total fibrinogen degradation products. These studies suggest that D exerts its pharmacologic effects at multiple sites which involve endogenous cellular and humoral components which are not measurable by routine ex vivo tests.

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AN OBJECTIVE EVALUATION OF THE LOW MOLECULAR WEIGHT HEPARIN (LMWH) STANDARD FOR CROSS-REFERENCING CLINICAL PRODUCTS

J. Fareed, J.M. Walenga, D. Hoppensteadt, X. Huan, E. Coyne and K. Breddin

The WHO has recently introduced a LMWH standard which is a nitrous acid depolymerized product provided by Kabivitrum (Stockholm, Sweden). In a well-defined, uniform, biochemical and pharmacological screening, 8 LMWHs were studied to determine adjusted potencies in terms of anti-Xa activity using valid dose-response curves. An expected, the standard minimized the AT III deficiency differences in the anti-Xa assay. However, a marked elevation of the anti-IIa component of all of the assayed LMWHs occurred, and the relative HC II activation profile between these agents was widened. In animal models of thrombosis and bleeding, potency adjusted LMWHs exhibited individually different safety/efficacy ratios. When the 8 LMWHs were titrated with PF-4 and proteinase, their neutralization profiles showed marked differences. These data suggest that utilizing the WHO standard to adjust the potency of LMWHs in terms of anti-Xa activity leads to distinctly different safety/efficacy profiles of each LMWH which may not be related to their observed in vitro (anti-Xa) potency. Thus cross-referencing of LMWHs, which are expected to have more than one effect, by one in vitro assay is of questionable value as this may result in serious dosimetric errors. These problems will exist for subcutaneous administration of these agents but will be magnified when they are used via an intravenous route for therapeutic anticoagulation.

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STUDIES ON THE MECHANISM OF ANTI-THROMBOTIC ACTIONS OF SULFATED LACTOBIONIC ACID DERIVATIVES

J.M. Walenga, W. Raake, J. Fareed, D. Hoppensteadt

A new class of synthetic antithrombotic agents derived from sulfated lactobionic acid derivatives have been reported by Rauke et al. (Seminars in Thrombosis and Hemostasis 13(3):357-358, 1989). We have used several biochemical systems to characterize their mechanism of antithrombotic actions. In the amidolytic assays these agents were found to produce strong antithrombin activity and potent antithrombin IIIB based systems at >20 #M was noted. In prothrombinase activation, they catalyzed HC II thrombin complex formation. No direct inhibition of thrombin in fibrinogen supplemented and purified antithrombin III based systems at >20 #M was noted. In prothrombin complex based Xa and thrombin generation assays, these agents produced marked inhibitory effects which are partially mediated by HC II. These studies suggest that sulfated lactobionic acids produce their antithrombotic effects via heparin cofactor II and modulating serine protease generation in the intrinsic pathway. Additional studies on the interactions of these agents with the blood/vascular system are needed to understand their prolonged antithrombotic actions.

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FUNCTIONAL AND MOLECULAR HETEROGENEITY OF DERMATAN SULFATE PREPARATIONS: A COMPARISON OF IN VITRO AND IN VIVO PROPERTIES

J.M. Walenga, H. Hoppensteadt, E. Coyne and J. Fareed

The currently available dermatans should not be considered the optimal antithrombotic agents because their prolonged antithrombotic action is a determinant of their biological activities which are not known. Dermatan sulfate preparations exhibit varying in vitro, ex vivo and in vivo activities and should be taken into consideration in developing these agents as antithrombotics. We have compared native dermatan (Mr = 33,000) with its subfractions (Mr range 2,000-22,000) to study the effect of molecular weight and chemical on the in vitro and in vivo action of dermatan. Our data showed that some of the anticoagulant and antiprotease properties were not molecular weight dependent. The bioavailability of each of these agents was dependent not only on the molecular weight but also on the charge density characteristics. In purified systems, these agents showed marked variation in interactions with HC II and AT III. Similarly, ability to inhibit the generation of thrombin showed differences between the fractions. The intravenous and subcutaneous antithrombotic actions of each of these agents showed marked variations which were not explainable on the basis of molecular weight alone. The individual properties of dermatans are determined by several factors such as inhibitor interactions, uptake and presence of ATIII affinity components. Charge density may be very important for interactions with HC II and endothelium. When beef mucosal dermatan was compared with porcine mucosal derman of similar molecular weight, differences were noted in their biological properties. These studies show that dermatans are a heterogeneous group of glycosaminoglycans which exhibit marked variations in both the molecular and biological profiles.

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THROMBOLYSIS IN ACUTE MYOCARDIAL INFARCTION

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Although neonatal asphyxia relating to intracranial hemorrhage may have several reasons, the etiology of the most common type, intraventricular IVH is not yet known. There is histological evidence of disturbance of blood circulation in cases of intracranial bleeding in the asphyxiated newborn and low-birth-weight infant, and in addition there are changes in both coagulation and fibrinolytic systems. Recent information demonstrating that the initial lesion is due to hemorrhagic infarction raises the possibility that hypercoagulability may have something to do with an etiologic role.

Material and Methods

48 cases of umbilical cord blood were divided by Apgar score, asphyxiated group (7) and normal group (8.9, and 10)

1) ADP- and Collagen-Aggregation (Platelet) were tested by whole-Blood Aggregometer (Chrono-Log) with impedance channel.

2) Platelet volume and Platelet count were determined by Bloor 810, and MPV was calculated as well as Mode and Mean.

3) Fibrinogen, Kininogen (DINL2) SFMC iv) FDP, v) Factor XIII vi) PTT vii) ELT viii) AT III

The value of MPV in asphyxiated group was 8.1 cu and it was greater than that of normal (6.8 cu).

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NO DOSE DEPENDENCY OF THE FIBRIN SPECIFIC PROPERTIES OF UROKINASE PRACTIVATE PRO-UROKINASE (TCL 598) IN ITS USE FOR THROMBOLYSIS IN ACUTE MYOCARDIAL INFARCTION

D.C. Gulba', M. Barthels', M. Westhoff-Bleck', W. Müller'

It has been demonstrated in recent dose-finding studies, that thrombolysis in acute myocardial infarction may successfully be performed with urokinase (UK) preactivated pro-urokinae (PUK), without depleting the fibrinogen levels in the blood. In 26 patients undergoing thrombolysis with 250 000 IU of UK and 42 to 118103 U/kg or 2.1 to 4.2 mega U/m2 of PUK, we have investigated the dose dependency of the fibrinogen (Fg), plasminogen (PLG), and A2-antiplasmin (AP) consumptions and the simultaneous increases of fibrinogen (FgDP), fibrin (FdDP), and total (TDP) degradation products.

Results:

Correlations coeff.
mg/kg mg/m2
FgDP 0.33 0.38 **
PLG 0.24 0.18
AP 0.33 0.38 *

FgDP 0.33 * 0.38 **
PLG 0.24 0.22
AP 0.24 0.22

Conclusions: There is a strong dose dependency for the systemic effects of rt-PA (fibrinogenolysis and plasminemia). In contrast, the dose dependency of the clot directed lytic activities of rt-PA as reflected from the release of fibrin specific degradation products (FDP), however, is only weak.

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DIE THROMBOLYSE THERAPIE DER BECKEN-BEINVENEN THROMBOSE

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Patienten: 292 Patienten mit akuter, 1-3 Tage alter und 148 Patienten mit subakuter, 1-3 Wochen alter tiefer Venenthrombose (TVT) wurden mit Streptokinase (SK) und Urokinase (UK) behandelt.

Dosierung: Die Initialdosis (ID) betrug bei der SK Therapie 250.000 I.E. SK/20 min, die Erhaltungsdosis (ED) 100.000 I.E. SK/h. Die ID betrug bei der ultrahohen Streptokinase (UHSK) Therapie 250.000 I.E. SK/20 min, die ED 1.500.000 I.E. SK/h. Die Behandlungsdauer betrug bei der SK Therapie mit 100.000 I.E. SK/h 3-6 Tage, bei der UK Therapie meist 5-10 Tage.

Ergebnisse: Unter der UK und UK Therapie gelang es bei 61 % der Patienten eine vollständige und bei 26 % eine partielle Thrombolyse zu erzielen. 13 % zeigten keine Änderung im Phlebogramm; bei 3-5 Wochen alter TVT gelang es bei 19 % der Patienten eine vollständige und bei 51 % eine partielle Thrombolyse zu erzielen. 30 % zeigten keine Änderung im Phlebogramm.

Nebenwirkungen: Die Nebenwirkungen, insbesondere Blutungskomplikationen, waren unter der UK Therapie mit 100.000 I.E. SK/h am niedrigsten.

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SINGLE 50 mg BOLUS OF RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR (rt-PA) IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

U. Tebbe, P. Tanswell, E. Seifried, J. Wollick, K. S. Herrmann

Till now, after an initial bolus of about 10 % of the total dosage rt-PA was given as a prolonged intravenous infusion because of its short half-life and in order to avoid early reocclusions. A single bolus would simplify the therapeutic regimen. Therefore 20 patients (3 female, 17 male; mean age 55 ± 11 years) with acute myocardial infarction (duration of symptoms 125 - 58 min) were treated with a single rt-PA bolus of 50 mg intravenously (over 2 min).

The basic therapy before lysis consisted in 5000 IU heparin, 500 mg ASA and nitroglycerin intravenously.

Results: The fibrinogen level (Clauss method) decreased from 2.7 ± 0.5 g/l to 1.5 ± 0.9 g/l after 2-4 hours and reached the initial value within 24 hours. Pharmacokinetic parameters were obtained in 7 patients by measuring rt-PA antigen levels in multiple plasma samples. Mean peak rt-PA concentration was 9.9 ± 3.8 µg/ml, total plasma clearance 490 ± 150 ml/min and half-life 4.9 ± 1.9 min. Coronary angiography 60 min after rt-PA bolus revealed an open infract-related artery (TIMI 2.3) in 15/20 patients (>75%), in the remaining patients reperfusion was achieved by angioplasty (PTCA), intracoronary thrombolysis and twice an aorto-coronary bypass was necessary. Control angiography at 24 hours showed a reocclusion in 4/18 patients; during hospital stay and 20 days follow-up in 10 patients. One female patient died 17 days after rt-PA bolus because of fatal intracranial hemorrhage.

Conclusions: A single intravenous rt-PA bolus of 50 mg seems to provide similar patency rates and kinetics as the usual infusion; the incidence of bleedings requires further studies.

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RETESTENOSIS UNDER THE TREATMENT WITH LOW MOLECULAR WEIGHT HEPARIN VERSUS ASPIRIN IN SUCCESSFUL ANGIOPLASTIES OF CORONARY ARTERIES

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Early restenosis following successful angioplasty is a big problem in interventional cardiology. Till now there exist no treatment regime that alter the rate of restenosis.

In the present study the incidence of restenosis was examined under the treatment with the low molecular weight heparin (LMWH) FRAGMIN versus aspirin (ASS: 100-300 mg/d). An adjusted LMWH dose was given that led to peak levels of .3-.6 anti-Xa-activity (mean 6000 U/d). The definition for restenosis was based on the guidelines of the American National Heart, Lung and Blood Institute. The rate of restenosis was proven by coronary angiography 3 month after angioplasty.

The amount of stenosis was estimated by using a semiautomated computerized slide analyzing system.

The incidence of restenosis in the ASS group was higher (6 stenosis out of 11.5 %) than in the LMWH group (3 out of 11 stenosis in 9 patients). There was no significant difference in mean area stenoses pre- (LMWH: 80.8 ± 43.4 %, ASS: 85.7 ± 44.3 %) and post-PTCA (LMWH: 39.5 ± 28.7 %, ASS: 47.3 ± 31.7 %). None of the patients suffered myocardial infarction in the follow up period.

Bleeding complications or other drug related side effects did not occur.

Conclusion: The treatment with LMWH seems to be an effective and safe treatment after PTCA with advantages in comparison to aspirin.

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PROFIBRINOLYTIC ACTIVITY OF re-CIRUDIN IN HEALTHY VOLUNTEERS

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Recombinant Hirudin (rH, C9P 39393) is a potent thrombin inhibitor. In addition to its anticoagulant effect, we were interested in its profibrinolytic activity in vitro and ex vivo. For the in vitro experiments the fibrinolytic activity of the eguglobulin fraction (EFG) of 15 normal plasmas with and without urokinase (Uk) was tested in a fibrin plate assay both before and after addition of rH. The same cycle was repeated in 8 plasmas with heparin (Hep) instead of rH. For the test system EFG was obtained from 0.5 ml plasma containing 5U Uk and 3.3 µg rH or 0.5U Hep and applied on human fibrin plates. For the ex vivo evaluation 12 healthy volunteers received 0.1-0.3 mg/kg/h rH for 6h. Blood samples were drawn at 0h, 6h, 12h and 24h. Fibrinogen (Fbg), plasminogen (Fg) and antithrombin III (AT), t-PA antigen and PAI activity were assayed. RESULTS: rH induced more intensive fibrinolysis in synergism with Uk measured as lysis index (min⁻¹): 194.3±46.6 vs rH±Uk: 245±117, p<0.0001. rH enhanced the fibrinolytic activity of plasma (P) in the absence of Uk also (P: 15.2±10.9 vs P+rH: 62.6±5.2, p<0.02). Heparin had a similar but slightly weaker effect with Hep±Uk: 180.1±55.7 vs rH±Uk: 188.4±52.4, p<0.06) and without Uk also (P: 55.2±10.9 vs P+rH: 62.6±5.2, p<0.02). Heparin had a similar but slightly weaker effect with (Hep±Uk: 180±55.7 vs rH±Uk: 188±52.4, p<0.06) and without Uk also (P: 55.2±10.9 vs P+rH: 62.6±5.2, p<0.02).

rH increased the fibrinolytic activity of plasma (P) in the absence of Uk also (P: 15.2±10.9 vs P+rH: 62.6±5.2, p<0.02). Heparin had a similar but slightly weaker effect with (Hep±Uk: 180±55.7 vs rH±Uk: 188±52.4, p<0.06). Heparin had a similar but slightly weaker effect with (Hep±Uk: 180±55.7 vs rH±Uk: 188±52.4, p<0.06) and without Uk also (P: 55.2±10.9 vs P+rH: 62.6±5.2, p<0.02).

The reproducibility and accuracy of the method was confirmed by testing a standard plasma. The results were within the normal range.

CONCLUSIONS: 1. In the presence of rH a slightly stronger fibrinolytic effect is observed measured as fibrin plate lysis activity both if rH is added to plasma in vitro and if it is administered intravenously. But this may be well explained by the specific anticoagulant action of rH and Hep in the dynamic equilibrium of fibrinolysis and clotting in the test system. 2. Under rH treatment the observed changes of Fbg, Fg, AT, t-PA and PAI although suggestive of enhanced fibrinolysis cannot be differentiated from the normal circadian rhythm of these proteins, since the highest ex vivo fibrinolytic activity coincides with the lowest PAI levels.
A new abnormal plasminogen, Frankfurt II, has been identified in about 50% of normal; family members values ranged from those of other family members. His isolated Plg had a plasmin generation rates were lower than normal as were since age 6. He has a plasminogen functional deficiency, has a long history of recurring thromboembolic disease at O°C, showed degraded A chains, two sets of A and Bplexes, d) slow fragmentation of SK in the complex. Re-
slow conversion of PIg-SK to PIn-SK with two PIn-SK com-

INVESTIGATION OF A CONGENITAL ABNORMAL PLASMINOGEN
FRANKFURT II, AS CAUSE FOR RECURRING THROMBESSES

V.Hach-Wunderle*, I.Scharrer*
I.S.Boreisha**, K.C.Robbins**

A new abnormal plasminogen, Frankfurt II, has been identified in the plasma of a 27-year-old male patient who has a long history of recurring thromboembolic disease since age 6. He has a plasminogen functional deficiency, also the plasminogen concentration was slightly reduced. His plasmin generation rates were lower than normal as were those of other family members. His isolated Plg had a specific proteolytic activity of 15.8 IU/mg protein, about 50% of normal; family members values ranged from 17.5 to 23.5 IU/mg protein. Maximum active site generation rates in the equimolar Plg-SK complex (0-60 min. at 37°C) ranged from 60% to 80% of normal for all family members, the propositus and his father were at the lower levels. PC and AT III provided to be the best prognostic markers for decreased liver synthesis in cirrhosis and CMH.

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THE BEHAVIOUR OF LIPOPROTEIN Lp(a) IN 203 YOUNG PATIENTS SUFFERING FROM VENOUS THROMBOSIS AND IN 2 PATIENTS WITH ABNORMAL PLASMINOGEN MOLECULES

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Lipoprotein Lp(a) is an LDL-like particle to which the characteristic apolipoprotein (a) is attached by disulphide bonds to apo B-100. Human apo(a) cDNA contains domains homologous to kringles 4 and 5 and the protease domain of plasminogen. Recent reports suggest that Lp(a) in vitro inhibits streptokinase induced plasminogen activation and enhances ADP and epinephrine mediated platelet aggregation. Therefore we investigated the Lp(a) concentrations in 2 unrelated patients suffering from recurrent deep vein thromboses with proven abnormal plasminogen molecules and in their affected relatives. Furthermore we studied the levels of Lp(a) in 203 young patients below the age of 45 suffering from venous thromboses, Lp(a) was measured with a sandwich immunoradiometric assay using 2 different monoclonal antibodies. The assay proved specific for Lp(a) in that on immunoblotting analysis only the solid phase anti-
apo(a), but not the tracer Ab crossreacted with purified human plasminogen.
The Lp(a) concentrations of the patients with abnormal plasminogen molecule corresponded to that of a healthy reference population. The levels of Lp(a) in the young patients suffering from venous thromboses coincided with that in the normal controls (84 healthy blood donors).
Therefore we concluded that there is no direct correlation between the Lp(a) concentration and the risk of venous thrombosis.

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Effect of thrombolytic therapy and longterm results in patients with phlebothrombosis of upper and lower extremity
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In the study presented we investigated the efficiency of streptokinase (SK) and urokinase therapy (UK) by phlebography in 177 patients administered between 1978 and 1985. Phlebothrombosis of the lower extremity was observed in 160 and of the upper extremity in 17 cases. 185 patients were treated with streptokinase followed by urokinase in 56 cases. Recanalisation was documented by phlebography in 12 patients with thrombosis of the upper extremity. SK-therapy was effective in 55.3%, UK in 30.2% in thrombosis of the lower extremity. Effectivity of thrombolytic therapy depended on localisation and extent of thrombosis. Therapy was less effective when clinical symptoms of phlebothrombosis were present for more than 2 weeks before initiation of treatment. Phlebographic follow-up was performed in 34 patients up to 4 years after US/SK-therapy. Further improvement was observed in 62% indicating that spontaneous recanalisation may occur in spite of unsuccessful thrombolysis.

Late results of thrombolytic therapy

| Phlebography after therapy | Phlebography 6 weeks to 1 year after treatment |
|---------------------------|-----------------------------------------------|
| complete recanalisation | 6 complete recanalisation*                     |
| partly recanalisation     | 25 partly recanalisation (constant)             |
| no recanalisation         | 8 no recanalisation (further improvement)       |

* Improvement of phlebography in 21/34 patients (62 %)

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THE PROGNOSTIC RELEVANCE OF PROTEIN C AND ANTI-HIBOMBIN III FOR DECREASED LIVER SYNTHESIS IN LIVER CIRRHOSIS AND CAH
I.Scharrer, C.Best

We investigated the blood of 64 patients suffering from liver cirrhosis (CIC) or chronic active hepatitis (CAH) on the levels of protein C (ag and act.), antithrombin III (ag and act.), w-antiplasmin (w-Ap), plasminogen, fibrinogen, prothrombin time (PT), f.V, f.VII, f.VIII:C and v.WF-Ag. The results of the coagulation tests were compared with the levels of cholinesterase (CHE) and albumin. In addition the levels of the liver enzymes: GOT, GPT, GPT and AP were determined. All the results were compared with the findings in 34 healthy persons. In the patients suffering from CII we found the best signification correlation between PC and CHE (p<0.001), between PC and albumin (p<0.01), between AT III and CHE (p<0.001) and between AT III and albumin (p<0.01). By differentiation in compensated and decompensated groups of liver cirrhosis the correlations were still more pronounced. In the patients suffering from CAH a positive correlation could be demonstrated between PC and CHE (p<0.05) and between PC and albumin (p<0.05). In this group we found neither a significant correlation between AT III and CHE nor between AT III and albumin.

We could demonstrate that the decreased levels of the other parameters: plasminogen, w-Ap, fibrinogen, PT (in %), f.V, f.VII and the increased levels of f.VIII:C and v.WF-Ag corresponded not significantly with the decrease of liver synthesis. PC and AT III proved to be the best prognostic markers for decreased liver synthesis in cirrhosis and CMH.

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QUANTITATIVE DETERMINATION OF FIBRINOGEN — A NEW METHOD
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Due to its sterical configuration fibrinogen interacts with red blood cells — mainly in the small vessel system, where shear stress is low — causing reversible aggregation, well known as “rheology” or “sludge” phenomenon. Hence sludging can be the reason of severe ischemia, and the tendency to aggregate correlates well with concentrations of fibrinogen. Since blood serum is defined as plasma without fibrinogen, it seems to make sense — when testing the viscosity of both — to calculate the concentration of fibrinogen from the difference between these two values. Using a common kit (MULTIFIBRIN® by Behringwerke, Marburg, FRG) as reference method, the time needed to complete plasma (t" in equation; dimension: seconds) is measured. Fibrinogen concentration in mg/dl can be obtained by a table for a number of values or by calculating the following equation:

Fibrinogen mg/dl = \frac{\text{Plasma} \times (\text{Serum} - \text{Plasma})}{\text{Serum} \times \text{Plasma}} + 23.1

We investigated blood samples of 110 persons (healthy volunteers as well as patients with several acute or chronic diseases), performed all measurements at least in triplicate with the above mentioned method. The Pearson’s correlation coefficient of the corresponding mean values of the two methods were found to be 0.989 and are 0.999. Mean differences between the respective values of the two methods were confirmed in all circumstances given by the manufacturers for the reference method.

Conclusion: testing the viscosity of plasma and serum can be a complementary, precise, technically simple and quite economic alternative to determining fibrinogen concentrations when using the above formula.

FIBRINOLYTIC SYSTEM IN YOUNG PATIENTS WITH MYOCARDIAL INFARCTION — RELATION TO SERUM Lp(a)-LEVELS. E. Aygören I. Scharrer, W. März, A. Christ, L. Mattebach, R. Hopf

Alterations of the fibrinolytic system in coronary artery disease and myocardial infarction (MI) have been reported in a number of studies within the last years. In the present study 29 male patients with an angiographically confirmed MI before the age of 45 and a control group consisting of 13 young volunteers were included in two time courses with 20 minutes venous occlusion test (V0). Since high serum lipoprotein (a) (Lp(a)-)levels are known to be connected with antigen and PAI-1 were investigated before and after a 20 minutes venous occlusion test (V0). In the patient group with high Lp(a)-levels significantly differences between the respective values were observed. These findings were reflected in t-PA-activity levels of both groups: basal line t-PA-activity was significantly lower in patients with high Lp(a)-levels (med. 0.8 U/ml, r. 0.8-2.0) compared to those with low Lp(a)-levels (med. 1.3, r. 0.8-2.0). t-PA-antigen levels were not significantly different in both subgroups. In conclusion alterations of the fibrinolytic system were most pronounced in a subgroup of young MI-patients, who were not at a substantially high risk for MI in regard to their Lp(a)-levels.

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The tissue-type plasminogen activator (t-PA) release from vascular endothelium plays a decisive role in the intravascular fibrinolysis. Disorders in t-PA formation are among the reasons for thrombophilia. In previous investigations, a variety of endogenous and exogenous substances have been tested for their t-PA releasing capacity in the isolated perfused pig ear. Out of these substances the platelet activating factor (PAF), 1-desasino-2-O-arachidonylserine (DDAVP), thrombin, bradykinin as well as the polyanionic compounds heparin, pentosan polysulphate and sodium humate proved to be very efficient. The acute t-PA release was tested in rats after i.v. application of some of these substances, the results were given as maximum percentage of clotting time. Independent from the used coagulation assay different amounts of added PL were needed to minimize the clotting time. Concerning the calculated indexes in this group were significantly and dose-dependently increased. Results of urinalysis did not differ from those of the control animals. Precipitine antibodies were not detectable. Histo-pathological changes due to thrombus application of PL were not found. The organ weights (liver, kidney, spleen, heart and lung) were the same as in untreated rats. The studies revealed that both single and repeated application of the 10 - 100 fold therapeutically relevant dose of PL was well tolerated.
EXPERIENCES WITH ELECTRA 900 C

E. Scholler, R. Pohl, F. Koller

Electra 900 C (MIA) is a computer-controlled automatic coagulation analyzer performing prothrombin time, derived fibrinogen, partial thromboplastin time, thrombin time, fibrinogen (Clauss), factor assays and antithrombin III.

Using photometric detection ELECTRA 900 C is able to perform clotting assays via fibrin clot formation as well as chromogenic assays. In conventional clotting assays the end point of fibrin clot formation is detected at a wavelength of 550 nm, for chromogenic assays delta absorbance/minute at 405 nm.

The assays for prothrombin time (PT), derived fibrinogen, partial thromboplastin time (TTP), thrombin time (TT) and antithrombin III (AT III) were applied to ELECTRA 900 C.

The results were compared to those obtained at KC 10 and RA 1000 (AT III) respectively. This means that the actual reference ranges may be maintained.

In contrast the PTT assay at ELECTRA 900 C revealed distinctly lower times referring to KC 10. Applying the identical activator (Actin FSL, Dade) to ELECTRA 900 C as well as to KC 10 the measured times of normal controls were about 3 seconds lower at ELECTRA 900 C than those obtained with KC 10. So, assigning ELECTRA 900 C to routine laboratories demands a modification of the normal ranges of PT.

Negative aspects of ELECTRA 900 C for routine laboratories are the manual pipetting of the samples as well as complicated alterations to other tests. Only PT, derived fibrinogen and PTT can be determined parallel without alterations. On the other hand, compared to KC 10 ELECTRA 900 C distinguishes itself by high accuracy and confidence, by an extremely good storage of the reagents, the possibility of selective worklists and in addition less staff is occupied.

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HAEMOSTASIOLOGY AND HAEMORRHIOLOGY IN ACUTE MYOCARDIAL INFARCTION (AMI) TREATED BY HEPARIN, STREPTOKINASE (SK) AND AMINO-ACIDS.

V. Hossmann, E. Martens, H. Auel, E. Valdivieso, W. Burkhardt

High dose SK in AMI lowers plasma fibrinogen to levels near zero whereby blood viscosity decreases. This favourable but short lasting effect may be prolonged by subsequent application of anrchod. In order to investigate this treatment regimen 30 pat. with AMI were divided in 3 treatment groups: Gr. I heparin alone; n = 10, mean age 56.3 yrs; Gr. II high dose SK + heparin; n = 10, 55.4 yrs; Gr. III high dose SK followed by anrchod; n = 10, 56.7 yrs. A battery of haemorrhheological tests were performed before and 4, 8, 24 hrs, 1 and 15 days during treatment.

While in Gr. I fibrinogen steadily increased until day 8, there was a steep fall of fibrinogen during SK-infusion in Gr. II with a subsequent exceeding increase until day 8. In Gr. III fibrinogen was lowest to values of about 1.2 g/dl during the 2 weeks of anrchod treatment with favourable effects on plasma viscosity, apparent whole blood viscosity and erythrocyte aggregation index. Thrombin-antithrombin III complex (TAT) was increased before either treatment (7-18 pg/ml), fell during heparin treatment to normal values, increased during SK infusion intermittently, but remained elevated for at least 2 weeks during anrchod.

The elevation of TAT during anrchod treatment correlated inversely with a decrease in AT III, plasminogen, and alpha-2-antiplasmin. Fibrinogen (Clauss) degradation products (FDP) increased to about 300 pg/ml on day 1 and remained elevated through anrchod treatment at levels of ~ 35 pg/ml.

The significance of elevated TAT during anrchod treatment - in face of an uncomplicated clinical course - has to be further elucidated.

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Levels of cardiac specific troponin T (a structurally bound protein) were tested in peripheral venous blood samples from 55 AMI patients (23 l.v. streptokinase, 17 l.v. urokinase, 3 rt-PA and 12 patients receiving no fibrinolytic treatment) immediately on admission, after 12 hours and daily for the next 4 days (method: ELISA/Roche Diagnostics). Additional measurements during the first 48 hours were made on a subgroup comprising 22 patients. In patients without fibrinolytic therapy troponin T tended to peak between 44 and 96 hours (interquartile range, median 96) after the onset of pain. Levels are markedly increased even on the 7th day. By contrast, all patients having undergone successful reperfusion showed troponin T peaks on the first 24 hours (median 14h) after the onset of pain with a following rapid drop on the 2nd day (p < 0.001). Levels measured on the 7th day were within the normal range or slightly above. Troponin T of patients for whom thrombolysis was unsuccessful resembled those without fibrinolytic therapy (time to peak: 16–72h; median 25,25; levels on the 7th day markedly raised). Troponin T measurements seem a useful method for assessing the effectiveness of thrombolysis in AMI, as reduced troponin T levels probably reflecting a reduced size of infarction.

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**LONG-TERM DEFIBRINOGENATION IN PATIENTS WITH SEVERE CORONARY HEART DISEASE BY HEPARIN-INDUCED EXTRACORPOREAL LDL/FIBRINOGEN PRECIPITATION**

E. Schütts, P. Schuff-Werner, V. W. Armstrong, Th. Eisenhauer and D. Seidel

Long-term defibrinogenation in patients with severe coronary heart disease by heparin-induced extracorporeal LDL/fibrinogen precipitation (HELP™) eliminates fibrinogen as effectively as LDL. This might be of clinical importance since a large body of evidence now links elevated plasma levels of fibrinogen with an increased risk for myocardial and cerebral infarction. Furthermore, the severity and extent of coronary lesions appear to be correlated with the degree of fibrinogen elevation. Conventional methods for effectively reducing plasma fibrinogen levels, such as treatment with streptokinase, urokinase or ancrod, are limited by antibody formation. A single HELP treatment of 3 L plasma in adults leads to an acute reduction in fibrinogen levels of around 54% (354±101 to 143±71 mg/dl; p<0.001). After 4 to 6 treatments at weekly intervals the pre-therapeutic plasma fibrinogen levels stabilize at around 70% (250±62 mg/dl) of the values before the first treatment and remain at this level on regular weekly treatment. This is accompanied by an improvement in haemorheological parameters such as plasma viscosity and erythrocyte aggregation resulting in less severe angina pectoris complaints in patients with severe coronary heart disease. Because fibrinogen is precipitated rather than degraded no fibrinogen split products are detectable. No bleeding complications have been observed since critical fibrinogen values <60 mg/dl can be avoided by limiting the plasma volume treated. Therefore HELP may be useful in the therapeutic handling of hyperfibrinogenemia.

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**HARDDIAGNOSTIC EFFECTS OF LDL-APHERESIS PROCEDURES BASED ON POLYANIONADSORPTION (LIPOSORBER™) AND POLYAMINOPRECIPITATION AT ACTIV™ (HELP™)**

P. Schuff-Werner, P. Reitemeier, V. W. Armstrong, Th. Eisenhauer, H. Kösters and D. Seidel

Selective extracorporeal LDL-apheresis procedures such as heparin-induced LDL/fibrinogen precipitation (HELP™) and dextran sulfate adsorption (Liposorber™) are now established in the treatment of severe familial hypercholesterolemia. Because of the affinity of coagulation factors to polyanions we investigated the effect of these procedures on coagulation parameters in the extracorporeal circuit. If ACD-plasma is treated by dextran sulfate adsorption, the activity of the coagulation factors V, IX, XI and XII is completely abolished, factor II is not affected. Factor VII activity decreases to 70%, factor VIII to 23% and factor X to 29%, as compared to pre-treatment activities. Fibrinogen is adsorbed at the beginning of the procedure since the fibrinogen binding capacity of the column soon becomes saturated. Plasma fibrinogen decreases to 75% and AT III to 44% of the original concentration. The HELP procedure completely depletes the activities of factors II, V, VIII and XII. Factor VII is reduced to 65%, factor X to 12%, factor XI to 40% and factor XII to 27% of the pre-therapeutic activities. Fibrinogen and plasminogenprecipitation are completely; AT III concentration is reduced by 20%. All coagulation factor activities except of fibrinogen are restored within 24 to 48 hrs. No bleeding or thromboembolic complications in long-term treated patients using the HELP system show that these acute reductions do not lead to functional derangement of the coagulation system.

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**COMPARISON OF RESULTS OF FIBRINOLYTIC THERAPY WITH UROKINASE; ULTRA HIGH DOSE STREPTOKINASE AND RECOMBINANT TISSUE TYPE PLASMINOGEN ACTIVATOR IN DEEP VEIN THROMBOSIS**

G. Graim, G. Schwieter and T. Wagner

We retrospectively analyzed the results of thrombolytic therapy in deep vein thrombosis of the legs from May 1985 until Oct. 1989 (n=118). Until June 1988 86 Patients were treated with a standard dose of urokinase(UK), 2.4 Mill. U/d for a mean of 13 days. Within a dose finding multicenter study starting in July 1988 8 Patients were treated with rtPA 0.5 or 0.75 mg/kg/d and, starting March 1989 with rtPA 0.25 or 0.375 mg/kg/d. Therapy generally lasted for 7 days. Furthermore 16 Patients under went thrombolytic therapy with ultra-high-dose-streptokinase (UK 26%, UHSK 19%, median 25,25; levels on the 7th day markedly raised). Troponin T measurements seem a useful method for assessing the effectiveness of thrombolysin in AMI, as reduced troponin T levels probably reflecting a reduced size of infarction.

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Previously we have shown that the in vitro bleeding test (IVBT/Thrombostat 4000) is a very sensitive method for the detection of platelet function impairment (Blut 59: 188 (1989)). Now we want to report on a significant increase of in vitro bleeding time and volume in hemodilution (tab.), which was simulated using "reconstituted" blood with constant platelet concentration. For hemodilution we either used platelet poor plasma (PPP), albumin, hydroxyethyl starch (HES), saline or dextran. The effect correlated with the grade of dilution. Albumin had the strongest influence followed by saline, dextran and HES. The primary hemostasis was changed least by PPP. Gelatin is still under examination.

| Diluent | PPP Time | Alb Time | HES Time |
|---------|----------|----------|----------|
| 20%     | 245 ± 95 | 478 ± 123| 76 ± 130 |
| 30%     | 264 ± 92 | 449 ± 59 | 693 ± 162|
| 40%     | 277 ± 82 | 356 ± 143| 465 ± 211|
|         | s        | s        | s        |

Therefore impairment of primary hemostasis should play a significant role in hemodilution, which can be controlled by IVBT. As a substitute autologous plasma is recommended.

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THE INTERNALIZATION OF COLLAGEN BY PLATELETS IS NECESSARY FOR THE COLLAGEN-INDUCED DEGRANULATION. A. Ruf, E. Morgenstern* and H. Patscheke. Platelets internalize collagen fibers (CF). This process is accompanied by the formation of TXA₂, the platelet shape change and the secretion of the granule contents. Computer-assisted 3-D reconstruction from electron microscopic micrographs revealed that the internalized CF were closely associated with the contractile sphere in the platelet centre. Cytochalasin-D (Cyto D) 0.1 μM suppressed the internalization of CF, the shape change, the formation of the contractile gel, the degranulation and the [³⁵S]serotonin release but did not reduce the number of platelets that adhered to CF. In contrast, Cyto D enhanced the degranulation and the [¹⁴C]serotonin release induced by U 46619, although those platelets remained discoid and showed their typical marginal bundle of microtubules and no contractile gel. PGE₁ 1 μM and iloprost 0.5 nM inhibited the internalization and the phenomena of activation both with CF and U 46619. Aspirin neither affected the internalization of CF nor the shape change and degranulation. However, aspirin suppressed the TXA₂-mediated activation of the platelets that did not adhere to CF. The opposite effects which Cyto D exerts on CF- and U 46619-induced platelet responses indicate that the internalization of CF is essential for CF internalization and subsequent platelet activation but unneeded in U 46619-induced platelet degranulation. Cyto D prevents the internalization that follows the adhesion of CF. Internalization was initiated and quantified by high intracellular cAMP. (Supported by the DFG, Pa 263 and Mo 124)

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QUANTITATIVE COMPARISON OF PLATELET ADHERENCE AND AGGREGATION ON ENDOTHelial Membrane AND GLASS SURFACES A. Rainzier

As emphasized by investigations of platelet suspensions perfused in glass models of vessel branchings and curves, platelet microtubules developed - provided their adhesivity was enhanced - in those areas where the platelets were transported towards the wall and touched its surface (Miller-Hoffinan H., Thrombosis Research, 8, 553-566, 1976). In the present study, platelet adhesivity to endothelial monolayers and to glass surfaces was quantitatively determined. The effects of the anticoagulants heparin and citrate on the adhesion and aggregation function were also compared. The convective transport was simplified and reduced to a rotationally symmetrical stagnation point flow of platelet rich plasma PRP (Stagnation Point Adhesion-Aggregation, SPA).

Non-stimulated platelets did not adhere to the endothelium. In order to evaluate the antithrombin properties of the endothelium as compared to glass, the platelets were stimulated with adenosine diphosphate in a concentration of 1 μM which induced platelet adherence. The time course of the platelet deposition was recorded as an increase in the amount of deposited platelets per surface area and time unit.

These growth curves were calculated with an analogue computer. By applying the reaction kinetic equations of the transport theory for the growth of platelet microtubuli (Miller-Hoffinan H., Scholz L., Bluttransfusions: 2, 143-162, 1982) values were obtained for the growth rate constants kω and kθ - which describe, respectively, the adhesivity of the platelets to a surface and the aggregability of the platelets to each other.

The most important results will be discussed: The endothelium demonstrated its intactness as well as its antithrombin properties in reanimizing (Cyto D) adhering platelets. Stimulation with ADP, Quantification of its antithrombinicity - using ADP-stimulated platelets - demonstrated a ten-fold decrease in adhesion while aggregation was only 2 times less with endothelial surfaces as compared to glass surfaces.

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AN AUTOMATED CLOTTING SYSTEM TO DETERMINE PROTEIN C

F. Grilli, L. Preda, A. Lombardi, K.-E. Stötzer

Proclot® is a specific kit for functionally monitoring Protein C (PC). It is based on direct and specific activation of PC by a glycoprotein extract from Agkistrodon contortrix venom. Activated PC, with calcium ions, phospholipids and Protein S, inhibits factors Va and VIIIa, thus prolonging clotting time. The anticoagulant effect of activated PC is measured with APPT. The kit has been on purpose studied for application on Instrumentation Laboratory ACL® coagulator, and consists of PC activator (Protac®), PC-deficient plasma (substrate plasma) and a control plasma (about 60% PC). The sample is diluted with a specific diluent, then Protac® is added and incubation takes place at 37°C for 5 minutes. Complete activation of PC is ensured within this time interval. The activated sample is then added to substrate plasma; the prolongation that can be observed on the APPT of the substrate plasma is related to the concentration (% activity) of PC in the sample. Calibration is automatically performed by the ACL®. Linearity has been ascertainment in the activity range 10 + 150%.

Heterogeneity of soluble fibrin (size, number of oligomers and formation) and the in vitro studies the correlation was less satisfactory (r < 0.67). The correlation was also poor for the FPA release (r < 0.7). If only increased values were considered, the SF-PS-turbidimetry correlated well with the results of the other SF-methods and with the FPA release (r > 0.91), but very low concentrations could not be detected. According to Bráss, an explanation for this may be the lack of aggregation at low soluble fibrin concentrations. The high correlation of these results is remarkable, since the tested methods are based on different principles probably leading to different specificity. Subsequently these methods were applied for determination of soluble fibrin in plasma samples from intensive care patients suffering from DIC: in contrast to the in vitro studies the correlation was less satisfactory (r < 0.68). The SF-PS-turbidimetry yielded a sufficient sensitivity, whereas the SF-EAT often failed. Heterogeneity of soluble fibrin (also, number of oligomers and formation) and different sensitivity for fibrinogen (degradation products might be an explanation for weaker correlation in patients' plasma than in the in vitro studies.

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RELEVANCE OF FIBRIN(ogen)-DERIVATIVES AND OTHER PARAMETERS IN DIAGNOSTICS OF DISSEMINATED INTRAVASCULAR COAGULATION (DIC)
J.U. Wieding

Patients with acute or prolonged coagulation disturbances as known in DIC were examined in several studies. In particular, the fibrin(ogen) derivatives proved to be very sensitive indicators in diagnosing and monitoring of DIC. In acute pancreatitis, for example, the elevation of enzymes was followed by an increase of soluble fibrin and subsequently of degradation products detectable by FbDP, D-dimer-ELISA or even common latex tests, which all indicate the direct onset of "reactive fibrinolysis".

Due to the relatively large reservoir of protein circulating in plasma, the concentrations of coagulation factors or inhibitors were often not decreased although fibrin(ogen) derivatives were already elevated.

Fibrinogen concentrations were of low diagnostic value, since they were distinctly lowered only at a very late stage of consumption coagulopathy or even increased due to fibrinogen's property as an acute phase protein. In contrast, protein C, antithrombin III, and course observations of platelet counts proved to be more sensitive parameters in indicating a consumption of the hemostatic potential and deserve further consideration in DIC diagnosis, since they are of both pathopharmacologic and pathogenetic relevance.

The detection of elevated concentrations of thrombin-antithrombin-complexes (TAT) was a sensitive marker of an activation of coagulation factors with thrombin generation; correlations with the formation of soluble fibrin were found. However, due to the antithrombin inhibition, TAT levels do not provide information about the "true thrombin activity" on the fibrinogen molecule.

In conclusion, fibrin(ogen) derivatives are of central importance in DIC; they reliably indicate the continuation of an increased blood coagulation turnover whereas other parameters merely mark the loss of haemostatic potential or activation not resulting in an fibrinogen-turnover.

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A NEW HIGH-SENSITIVITY THROMBOPLASTIN FOR MONITORING O.A.T.
S. Vazzana, F. Grillo, K.-E. Stötzter

PT-FIB H.S. is a new high-sensitivity calcium thromboplastin for the simultaneous in vitro determination of prothrombin time (PT) and fibrinogen in plasma. The reagent was lyophilized extract from rabbit brain with an optimal concentration of calcium ions - has been on purpose studied for application on Instrumentation Laboratory ACL coagulometer. In order to make the reagent particularly suitable for monitoring oral anticoagulant therapy (OAT), the manufacturing procedure has been especially set up to enhance sensitivity. The ISI (International sensitivity index) of PT-FIB H.S. has a typical value 1.35, which makes it comparable to the 2nd BCR RM for Rabbit Thromboplastin. Other characteristics of the reagent on the ACL normal PT range between 11.5 and 15.0 seconds, therapeutic activity range for OAT between 168 and 365, about 15 seconds of difference between 100% and 25% in a calibration curve prepared by dilution, excellent precision, insensitivity to heparin up to 0.5 IU/ml.

A comparison has been carried out with two widely used reagents on samples collected from healthy donors, patients under OAT and patients with liver diseases. The reagent may be easily used - coupled with a set of deficient plasmas - for the determination of the single factors of the extrinsic pathway. In the analysis of fibrinogen linearity up to at least 700 mg/dl has been observed. A comparison with semi-automatic determination according to Claus is shown.

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THE EFFECT OF ANESTHETICS ON LASER-INDUCED THROMBUS-FORMATION IN THE DORSAL SKIN FOLD CHAMBER OF THE RAT
V. Laux, M. Seifge

The majority of the preparations described for studying platelet function in vivo require the administration of anesthetic agents and surgery. Some anesthetics often used in microvascular research (such as ketaminehydrochlorid and pentobarbitol) are reported to exert an effect on platelet properties itself. Using a modified chamber preparation of the rat's dorsal skin fold similar to that described by Papenfuss (1979) it is possible to study platelet function in the same rat with or without anesthesi.

Thrombus formation is induced in arterioles and venules by argon-laser irradiation with a local capacity of 25mW and an exposure time of 1/30 s. Platelet function was studied in this way for ten successive days in the same group of rats to examine the model's aptitude for long-term investigations. There was no significant alteration in platelet response in the ten day period. Therefore it is possible to study platelet function over a longer period of time.

To evaluate the antithrombotic efficacy of the anesthetics the animals were studied before and after the application of ketaminehydrochlorid(130 mg/kg b.w.) and pentobarbitol (25 mg/kg b.w.). Both drugs cause significant increase in the number of laser injuries in arterioles and venules, although the effect of pento-barbitol is weaker especially in venules. The inhibition of platelet aggregation by using these anesthetics can influence the screening of anti-thrombotic drugs.

In an other anesthetic drug, urethane, had no effect on laser-induced thrombus formation. Special attention was paid to spontaneous and laser-induced vasomotion in respect to thrombus formation.

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SILVER STAINING OF PLATELET PROTEINS, SEPARATED BY CROSSED IMMUNOELECTROPHORESIS IN AGAROSE GELS
M. Wilmer, B. Kahrel

Crossed immunoelectrophoresis has been a powerful method in assessing the structure of membrane glycoproteins, their organization within the membrane, their interactions with other intrinsic proteins and external ligands, and their abnormalities in disease states. It is a procedure in which proteins are separated in the first dimension by agarose electrophoresis and in the second dimension by electrophoresis into a gel containing precipitating antibodies. In the literature resulting precipitation arcs are stained with Coomassie Brilliant Blue.

Silver-staining procedure has a sensitivity of about 50 to 100 fold than staining with Coomassie Blue. However, silver-stain procedure designed for use with polyacrylamide do not work with agarose gels. We established a silver staining method for platelet proteins, separated by crossed immunoelectrophoresis (CIE), in agarose gels. After CIE gels were fixed and dried. The gels were then placed in a glutaraldehyde solution and transferred in a solution of Triton-X-100 followed by an incubation with dithiothreitol. The gels were transfered in developing solution, which contained AgNO3, N,N,N',N'-tetramethylguanidinium nitrate, formaldehyde and Na2CO3, and many brown to black precipitation arcs appeared within the next minutes. More than thirty precipitation arcs could clearly be distinguished.

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EFFECTS OF VENOUS STASIS ON FIBRINOLYSIS IN PATIENTS AFTER IDIOPATHIC DEEP VENOUS THROMBOSIS

H.J. Slaaens, T. Wagner

The venous occlusion (VO) test was used in screening for a thrombotic disposition in 68 out-patients (43 females, age 19-77, mean 45 years) with thrombosis in their history and nine volunteers without. Not earlier than two weeks after discontinuation of an anticoagulant therapy the blood samples were taken before and after 10 minutes venous stasis.

In 54 different parameters of the hemostasis and fibrinolysis system we found twice as much pathological values in the patients compared to the control. In both groups nearly all levels of factor and inhibitor activities increased significantly. We measured after VO markedly higher levels of thrombin antithrombin III complex (T-A T, p<0.005), of D-dimers (p<0.001), of tissue plasminogen activator (t-PA, p<0.001), and of plasminogen activators (PA, p<0.001). Statistics were performed with the Wilcoxon test for paired data.

The calculated differences of values before and after VO in patients compared with the control showed less significance for D-dimers (p<0.07), T-AT (p<0.06), and PA (p<0.005), no significance for the fibrinopeptide A (FPA), the plasminogen activator inhibitor (PAI-1), the spontaneous platelet aggregation (sPAT), and the factor VIII-protein C quotient (F8PC-q)(U test according to Mann and Whitney for non-paired data).

The comparison between patient and control values were highly significant for t-PA (p<0.001) before VO and for PA before (p<0.01) and after VO (p<0.001). No significant difference could be shown for T-AT, D-dimers, FPA, PAI-1, sPAT, and F8PC-q before and after VO (U test).

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RESIDUAL CORONARY THROMBUS AFTER LYTIC THERAPY: A MAJOR RISK FACTOR OF EARLY REOCCLUSION

Nechthold Westhoff-Schek; D.C. Gula; S. Jost; F. E. Lichten

Though thrombolysis in acute myocardial infarction is very effective to restore coronary reperfusion a considerable number of patients (pts) experiences reocclusion. In order to investigate the role of residual thrombus (RT) after thrombolysis coronary angiograms (CA) were performed in 72 pts after urokinase preactivated pro-uroklinase therapy. In 39 pts thrombolysis was successfull. Assessed by 3 independent investigators in 26 (66.7%) out of these 39 pts RT was diagnosed. Control CA in 27 pts (19 with RT, 8 without RT) when 43.8% of pts with RT and only 12.5% without RT showed reocclusion (p<0.01). Other angiographic assessable risk factors are preexisting stenosis diameter and diminished perfusion. We could not find a correlation between the extend of presisting stenosis and early reocclusion. TIMI III perfusion was seen less frequent-ly in pts with RT (50% vs77%; p=0.02). Thus after thrombolytic therapy RT seems to be an additional major determinant of early reocclusion.

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FACTOR II DEFICIENT PLASMAS PRODUCED BY DIFFERENT METHODS.

N. Moritz, H. Lang

Factor II deficient plasmas are used for the determination of prothrombin in the one-stage-assay. Conventionally manufactured products are mainly mixtures of human serum and bovine plasma depleted from prothrombin complex factors. In recent years immunoabsorption techniques have been successfully applied in the production of deficient plasmas.

In this study one congenital, two mixed plasmas (human/bovine) and two immunodepleted Factor II deficient plasmas were assayed. The following tests were performed: Factor II activity (chromogenic substrate method), Factor II antigen (Laurell), activity factors I, V, VII, VIII, IX, X, XI, XII (one-stage-assay, chromogenic substrate method), calibration curve in the range of 1-200 %, and stability of the reconstituted solution.

The residual activity of Factor II varied between 0.2 and 5 %, the amount of Factor II-antigen in the mixed plasmas was 70 %, and 100 %, respectively. The calibration curves in a log-log-plot were linear until 100 % with the two mixed plasmas, and until 200 % with one of the immunodepleted plasmas. As for the other immunodepleted plasma an higher dilution a prolongation of clotting times resulting in a deviation from linearity were found. In contrast, a flat curve was obtained with the congenital deficient plasma due to the higher remaining activity of Factor II.

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Patients with VWD type III showed the strongest bleeding disorder of all VWD types. Because of a deletion mutation on chromosome 12 they cannot synthesize any VWF (1,2). Therefore patients of VWD type III should produce antibodies against VWF by repeated substitutions (1,4). In our case a patient with VWD type III was substituted during 6 clinical treatments with 3500 units Ristofact and with more than 200 IU cryoglobulin. 6 weeks after the last treatment an antibody against VWF was detected in his plasma with the immunoelectrophoresis and immunofixation. This antibody was in vitro tried to be neutralized by isolated VWF. Therefore several plasma dilutions were mixed with different concentrations of VWF and incubated 1 h at 37 C. The remaining VWF, which has not reacted with the antibody, was quantitatively analyzed by an ELSA-test. The antibody concentration was estimated to be 0.4 IU/ml by the results of titration curves. Detection of an antibody against VWF was rarely described in literature, though it was expected to be present in patients with VWD type III (1,2). As we measure high titer of VWF-antibody, we suppose repeated substitution booster antibody production. From the estimated antibody concentration we calculate a total antibody quantity of 1000 U in blood of the patient. These results show, that additional 1000 units of VWF are necessary to therapy to neutralize the antibody before a correction of the bleeding disorder can be recognized. We conclude, that the possible production of an antibody against VWF represents a further complication in the treatment of patients with VWD type III.

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RISK FACTORS AND Atherosclerosis: Fibrinolysis Base Line Data from the Prospective Cardiovascular Münster Study
J. Heinrich, T. Nguyen, H. Kas, P.-H. Epping, H. Schulte and G. Assmann

Impaired fibrinolysis causes a reduction of the defensive potency against acute coronary occlusion and fibrin formation; in addition, the process of fibrin deposition in the arterial intima may be accelerated by a diminished fibrinolytic activity. In the scope of the Prospective Cardiovascular Münster (PROCAM) study (which was initiated to improve the predictive power in the recognition of individuals at risk for CHD) the fibrinolytic system in employees was characterized by measuring the plasminogen activator inhibitor-1 (PAI-1) using a chromogenic assay, the euglobulin fibrinolytic activity (EFA), as well as the tissue-type plasminogen activator activity (t-PA) on fibrin plates. PAI-1 in men (n=132), X=7.2 ±2.9 U/ml, showed a strong positive correlation to triglycerides (r=0.49**) and body mass index (BMI) (r=0.42**). Other known risk factors of MI exhibited weaker, but significant relationships: diastolic b.p. (r=0.34**), cholesterol (r=0.24**), HDL-cholesterol (r=-0.19**); and factor VII (r=0.28**). The euglobulin fibrinolytic activity (n=168, X=71.4 ±2.4% of a normal pool) was higher in men with high HDL-cholesterol (n=154±58%; 93.1 ±6.3% vs. n=154±58%; 82.5 ±22.1%). BMI (r=-0.11**) and astrocytic b.p. (r=-0.13**) negatively correlated to EFA. The t-PA-activity on fibrin plates with added C-inactivator (n=615, X=93.5 ±19.1% of a normal pool) exhibited positive correlations to EFA (r=0.59**) as well as to protein C (r=0.16** and AT III (r=0.27**) (* p<0.05; ** p<0.01; *** p<0.001). In conclusion, these hemostaseological variables delineate an evident relationship between fibrinolysis and risk factors of arterial as well as venous thrombus formation.

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A Novel Plasminogen Activator from the saliva of the Vampire Bat Desmodus rotundus
B. Baldus, F. Donner, W. Baldolli, and W.-D. Schleuning

A plasminogen activator (PA) from the saliva of the vampire bat Desmodus rotundus was purified to homogeneity. Two forms of PA were isolated, with apparent relative molecular weights of 43,000 and 38,000 were identified. Both forms exhibit about 0.5 homology to human t-PA but lack the kringle 2 domain and a plasmin sensitive cleavage site. The smaller form additionally lacks the finger domain. This protein was characterized as a potent plasminogen activator strictly dependent on fibrin as a cofactor.

D. rotundus-PA shows only little if any binding to forming as well as preformed fibrin clots.

D. rotundus-PA is more potent than t-PA in lysing human whole blood clots without inducing concomitant fibrinolysis.

Kinetics of glu-plasminogen activation by D. rotundus-PA in the presence of fibrin monomers cannot be fitted to the Michaelis Menten equation.

Our results demonstrate that the saliva of vampire bats contains a potent plasminogen activator with strict fibrin dependency which might be useful as a thrombolytic agent.

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Comparison of Various Platelet Inhibitory Principles under Strong Stimulatory Conditions in Vitro
P.F.J. Verhallen, T. Kohleys, W. Wirt, B. Baldus

Platelet inhibitory principles are usually evaluated in vitro employing a single platelet stimulator. It can be questioned, however, whether a single stimulus represents the physiological or pathological situation of platelet activation.

Therefore, a study was undertaken to compare, under stimulatory conditions of different strength, the potency of two classes of platelet inhibitors: (1) inhibitors of intracellular stimulatory pathways, like the prostacyclin mimics cicaprost, and the cyclooxygenase inhibitor aspirin, and (2) inhibitors of extracellular aggregation, like the specific tetrapeptide RGDS, and the dodecapeptide from the gamma-chain of human fibrinogen (FIBGAM).

Aggregation of human platelets was measured photometrically in citrated PRP, obtained by differential centrifugation from whole blood. Platelet inhibitors were added 2.5 min before platelet stimulation was started by addition of either collagen (10 µg/ml), ADP (10 µM), or collagen & ADP at 37°C under continuous stirring.

The potency of cicaprost, aspirin, RGDS, and FIBGAM under different stimulatory conditions is shown in the table (mean ± sem, n =4). Except for aspirin, all platelet inhibitors showed 100% efficacy for every stimulus tested.

| Stimulus      | Cicaprost | Aspirin | RGDS   | FIBGAM |
|---------------|-----------|---------|--------|--------|
| Collagen      | 36 ±0.6·10^{-6} | 2.2±1.3·10^{-6} | 3.6±0.6·10^{-6} | 5.6±1.2·10^{-4} |
| ADP           | 1.7±0.3·10^{-6} | 12±0.6·10^{-6} | 20±0.5·10^{-6} | no inhibition |
| Collagen & ADP| 5±2·10^{-6}  | 23±0.6·10^{-6} | 6±2·10^{-6}    | no inhibition |

*: maximal inhibition: 40%

Conclusions: (1) aspirin is a poor inhibitor of platelet aggregation, becoming ineffective when stimulus strength is increased. (2) The inhibitory potency of prostacyclin mimics is strongly dependent on stimulus strength, reflecting their actions on intracellular signal transmission. (3) The inhibitory potency of antithrombin peptides is hardly dependent on stimulus strength, in accordance with their interference with post-activation processes.

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COMPARISON OF PLATELET INHIBITION BY A PROSTACYCLIN MIMIC AND ANTIAGGREGATORY PEPTIDES IN VARIOUS SPECIES.

P.F.J. Verhallen, M. Barba, B. Baldus, and W. Witt

Preclinical evaluation of platelet inhibitory substances strongly depends on the use of animal thrombosis models. Translation of the information obtained with animal models towards human pathologies requires knowledge of the species differences in potency and efficacy of anti-platelet substances. Therefore, this study was undertaken to determine for two classes of inhibitors of platelet aggregation: (1) inhibitors of intracellular stimulatory pathways, like the prostacyclin mimic cicaprost, (2) inhibitors of the extracellular process of agglutination, like the specific tetrapeptide RGDS, and the dodecapeptide from the gamma-chain of human fibrinogen (FIBGAM).

Platelet aggregation was photometrically measured in citrated PRP, obtained by differential centrifugation from whole blood from human, rat, rabbit, or guinea-pig. Platelet inhibitors were added 5 min before platelet stimulation was started by addition of ADP (10 μM) at 37°C under continuous stirring.

The potency of cicaprost, RGDS, and FIBGAM in the various species is shown in the table (mean ± sem, n = 6). The three inhibitors were able to fully inhibit ADP-induced platelet aggregation in the four species tested.

|                | half maximal inhibition of platelet aggregation (μM) |
|----------------|-----------------------------------------------------|
|                | human      | guinea-pig | rat      | rabbit     |
| cicaprost      | 7.3 ± 1.0  | 0.4 ± 0.9  | 0.6 ± 0.5 | 3.5 ± 0.2  |
| RGDS           | 3.2 ± 0.2  | 0.5 ± 0.1  | 0.7 ± 0.4  | 1.2 ± 0.5  |
| FIBGAM         | 2.0 ± 0.3  | 0.3 ± 0.2  | 3.0 ± 0.4  | 3.4 ± 0.4  |

Conclusions: (1) Interspecies potency differences of a factor 10 can easily be obtained for platelet inhibitory substances. (2) The prostacyclin mimic cicaprost, is more potent in human than in rodents. (3) The potency relation of FIBGAM/RGDS differs considerably among the various species, indicating variable contributions of adhesive proteins other than fibrinogen in platelet aggregation.

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INTRACORONARY ILOPROST INFUSION COMPLETELY PREVENTS REOCCLUSION OF CORONARY ARTERIES AFTER THROMBOLYSIS WITH TISSUE-TYPE PLASMINOGEN ACTIVATOR (t-PA) IN EXPERIMENTAL CORONARY THROMBOSIS IN DOGS

W. Witt, B. Mann, B. Baldus, and P. F. J. Verhallen

A frequent complication of coronary thrombolysis in myocardial infarction is reocclusion which might occur more easily in t-PA as compared to streptokinase (Baldus et al., Thromb. Res. 22:275-278, 1981). It is assumed that two pathways play a role in this type of secondary thrombosis. We therefore studied the effect of the PGD2-mimic iLOprost, which has potent antplatelet and antithrombotic properties, on lysis rate with t-PA and reocclusion in a canine model of coronary thrombosis.

In anaesthetised beagle dogs occlusive thrombus formation was induced by introduction of a copper coil into a branch of the left circumflex coronary artery. Occlusion of the LCX was assessed by coronary angiography and typical ECG-changes. 60 min after occlusion all animals were anticoagulated with heparin (200 IU/kg i.v. + s.c.). Further treatment was either iloprost or solvents, t-PA inhibition was maintained until 15 min after reperfusion of the coronary arteries. Iloprost 1-30 ng/kg/min i.v. attenuates collagen- and U 46,619-induced thromboxanogenesis with significant effects already obtained at 3 and 1 ng/kg/min i.v., respectively. In electrically predamaged mesenteric arteries a superfusion of ADP at individually specific concentrations induced reversible thrombus formation at the lesion site. Iloprost at 10-100 ng/kg/min i.v. increases the thrombogenic ADP-concentration in a dose-dependent manner with significant effects obtained with 30 ng/kg/min i.v. (approximately 10-fold increase) and above.

Conclusions: Iloprost is a potent antiplatelet and antithrombotic drug in guinea pig models of platelet aggregate embolization and arterial thrombosis. Threshold doses of icaprost vary between 1.0 and 30 ng/kg/min i.v. probably reflecting differences in the strength of the thrombogenic stimuli coming into effect in the different experimental models.

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Platelet PAI-1 represents a major pool of the total blood PAI-1 level. When released into the plasma it might contribute significantly to a prothrombotic state by downregulation of fibrinolysis. We therefore investigated the effects of a prostacyclin mimic (icaprost), a cyclooxygenase inhibitor (aspirin), an antiadhesive tetrapeptide (RGDS), and an antieaggregating dodecapeptide from the gamma-chain of human fibrinogen (FIBGAM) on collagen induced aggregation of and PAI-1 release from human platelets.

Citrated human PRP was treated with collagen (5 μg/ml) to induce aggregation and measured as drop in circulating platelet count. Cicaprost at 1-30 ng/kg/min i.v. inhibited collagen-induced aggregation and platelet aggregation. It is a potent platelet inhibitor and shows good antithrombotic efficacy in experimental thrombosis in rats (Witt et al., Thromb Haemostas 62:244, 1989). We have studied the effects of cicaprost in three models of platelet aggregation in vivo and arterial thrombosis in guinea pigs, the platelets of which resemble human platelets much better particularly in the dependence of platelet activation on the phospholipid A2 pathway.

Platelet aggregation in vivo was induced by injection of collagen or U 46,619 and measured as drop in circulating platelet count. Cicaprost at 1-30 ng/kg/min i.v. attenuates collagen- and U 46,619-induced thromboxanogenesis with significant effects already obtained at 3 and 1 ng/kg/min i.v., respectively. In electrically predamaged mesenteric arteries a superfusion of ADP at individually specific concentrations induced reversible thrombus formation at the lesion site. Cicaprost at 10-100 ng/kg/min i.v. increases the thrombogenic ADP-concentration in a dose-dependent manner with significant effects obtained with 30 ng/kg/min i.v. (approximately 10-fold increase) and above.

In carotid arteries of guinea pigs thrombosis was induced by vessel wall damage (combination of crushing and cooling). As a measure of thrombus size hepatic content was assessed 3h after the damaging. Cicaprost already at 1.0 ng/kg/min i.v. significantly reduces thrombus Hb-content from 9.4 ± 1.2 μmol to 3.6 ± 1.5 μmol with 0.3 ng/kg/min being inactive while 30 ng/kg/min i.v. decreases Hb to 0.5 ± 0.5 μmol.

Conclusions: Cicaprost is a potent antiplatelet and antithrombotic drug in guinea pig models of platelet aggregate embolization and arterial thrombosis. Threshold doses of cicaprost vary between 1.0 and 30 ng/kg/min i.v. probably reflecting differences in the strength of the thrombogenic stimuli coming into effect in the different experimental models.

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EFFECTS OF DIFFERENT CLASSES OF ANTIPLATELET AGENTS ON THE RELEASE OF PAI-1 FROM ACTIVATED HUMAN PLATELETS

B. Baldus, P. König, P. F. J. Verhallen and W. Witt

Platelet PAI-1 represents a major pool of the total blood PAI-1 level. When released from the plasma it might contribute significantly to a prothrombotic state by downregulation of fibrinolysis. We therefore investigated the effects of a prostacyclin mimic (icaprost), a cyclooxygenase inhibitor (aspirin), an antiadhesive tetrapeptide (RGDS), and an antieaggregating dodecapeptide from the gamma-chain of human fibrinogen (FIBGAM) on collagen induced aggregation of and PAI-1 release from human platelets.

Citrated human PRP was treated with collagen (5 μg/ml) to induce aggregation and release reaction. Antiplatelet agents were added 10 min prior to the addition of activator. The samples were incubated for 15 min in an aggregometer before they were centrifuged at 10,000 rpm. The cellfree plasma was then snap frozen and kept at -29°C until PAI-1 determination. PAI-1 was determined by a two-site sandwich-ELISA employing a monoclonal and a polyclonal anti-PAI-1-antibody. The biological activity of the released PAI-1 was assayed in an assay of clot lysis using an anti-PAI-1-antibody which inhibits t-PA inactivation by PAI-1.

Conclusions: Activated platelets inhibit clot lysis in vitro. This effect can be restored by addition of a specific anti-PAI-1-antibody. Only the common pathway inhibitor of platelet activation cicaprost is capable to effectively inhibit PAI-1 release from stimulated platelets with an IC50 identical to the IC50 for inhibition of platelet aggregation. Aspirin although inhibiting platelet aggregation (by 55 % at 1 μM) did not inhibit PAI-1 release. Both RGDS and FIBGAM at a concentration inhibiting aggregation by 50 % did not influence PAI-1 release from platelets.

From these results it is concluded that active PAI-1 will be released from platelets during the thrombotic process possibly favouring stable plug formation by downregulation of fibrinolysis. At strong thrombogenic stimuli only common pathway inhibitors of platelet activation like cicaprost seem to prevent platelet PAI-1 release. There is no evidence from this study that antieaggregatory peptides like RGDS or FIBGAM interfere significantly with the platelet release reaction.

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Activated platelets significantly contribute to the generation of thrombin in a plasma system in vitro. This is mainly due to the formation of the procoagulant platelet complex e.g. the assembly of clotting factors X and V on negatively charged phospholipids provided on the outer membrane of collagen- or thrombin-activated platelets. Prothrombinase formation enhances prothrombin activation velocity by several orders of magnitude. Recently, an elegant method was published to measure thrombin generation in platelet-rich plasma triggered with high dilutions of tissue thromboplastin (S. Bégis et al., 1989 Thromb. Haemostas., 61, 25-29). This method allows to study the effect of antithrombotic agents on the cooperative interaction between platelets and the coagulation system.

It was the aim of the present study to investigate the influence of different classes of antithrombotic agents acting mainly on plasma factors. Antipla telet agents chosen were the prostacyclic acids iloprost and eicaprost, the antithrombinpeptide RGDS and the cyclooxygenase inhibitor aspirin. Anticoagulants used were unfractionated heparin, a heparin fragment and natural hirudin.

Results: Iloprost, eicaprost as well as the tetrapeptide RGDS inhibited thrombin generation in PRP induced by thromboplastin in a dose dependent manner. Maximal efficacy for all three agents tested was 60% inhibition. Their potency was in the same order as for inhibition of platelet aggregation induced by strong agonists. All three agents not only inhibited maximal thrombin generation but also postponed the thrombin burst. Aspirin even at 3 mM did not show any effect in our system. Both classes of heparins tested dose-dependently inhibited maximal thrombin generation and delayed thrombin burst indicating a similar mode of action. Hirudin dose dependently postponed the thrombin burst only.

We conclude from this study that common pathway inhibitors of platelet activation like iloprost and eicaprost may have anticoagulant properties possibly by inhibiting prothrombinase formation. The surprising effect of RGDS may indicate that platelet aggregate formation also plays a role in prothrombin activation velocity in platelet-rich plasma.

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with less than 6 months following thromboembolic disease

measurements, t-PA release was determined by venooclusive

Plasma samples from citrated blood were used for t-PA

with an increased risk of thromboembolism. In this stu-

SYNTHETIC STUDIES OF THE FIBRINOLYTIC SYSTEM FOLLOWING THROMBOEMBOLISM

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STUDIES OF THE FIBRINOLYTIC SYSTEM FOLLOWING THROMBOEMBOLISM

G. Pindur, M. Koehler, M. Heiden, C. Miyashita, U. T. Seyfert and E. Wenzel

Impairment of t-PA release is known to be associated with an increased risk of thromboembolism. In this study the fibrinolytic system of 54 patients (29 males, 25 females) aged between 22 and 75 years suffering from venous thromboembolic diseases has been investigated. Plasma samples from citrated blood were used for t-PA and PAI (amidolytic assay, ELISA) and D-dimer (ELISA) measurements. t-PA release was determined by venooclusion test.

Impaired t-PA release (less than 1.9 fold increase of t-PA activity following venooclusion) was observed in 12 patients (22.2%). This was partially due to an elevated PAI activity. D-dimers were lowered (p<0.05) in patients (n=11) with elevated PAI. There was no relationship between t-PA release and the extent of thromboembolism or the time interval thereafter. Increased concentrations of D-dimers in plasma have been noticed in patients (n=17) with severe thromboembolism (ileofemoral, pulmonary, recurrence) and in those (n=15) with less than 6 months following thromboembolic disease (2.4 and 2.6 fold +/- 0.9 and 0.7 (mean +/- SD), p<0.05).

These data suggest that the investigations of t-PA release and D-dimers may be complementary for the study of the pathogenesis, severity and course of thromboembolic diseases.

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PHLEGMASIA CAERULEA DOLENS, DISSEMINATED INTRAVASCULAR COAGULATION AND FIBRINOLYSIS AFTER INITIATION OF PHENprocoumon Therapy in a Young Man with Inherited Protein C Deficiency

H.-J. Hertfelder, S. Popov-Cenci, Ch. Schneider and L. Orellano

We discovered hereditary deficiency of the vitamin K-de- dependent protein C in a 19-year-old Portuguese male. He was suffering from recurrent posttraumatic thrombophlebitis, pulmonary embolism and phlegmasia caerulea dolens after initiation of oral anticoagulant therapy with phenprocoumon. The diagnosis of protein C deficiency initially was complicated by disseminated intravascular coagulation (DIC) and markedly enhanced fibrinolysis. DIC and fibrinolysis were indicated by increased thromboplastin (RTI), thrombelastography (TEG) and marked decreases of clotting factor, plasminogen and α-antiplasmin levels. The protein C defi- ciency was ascertained after recurrence of thrombosis induced by a second initiation of phenprocoumon therapy. Remission of thrombosis was benefited by heparin application. When hemostasis of the propositus had normalized the evaluated functional and antigen levels of protein C were 0.16 ± 0.02 U/ml and 0.15 ± 0.015 U/ml respectively. Examination of family members confirmed inherited protein C deficiency of the propositus.

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STANDARDIZATION OF TPA- AND PAI-ACTIVITY DETERMINATIONS UNDER TWO DIFFERENT CONDITIONS

W. Ehrenthal, G. Hafner, W. Frellwitz, H. Swars

Acidification of the citrate blood sample immediately after withdrawal prevents from neutralization of tissue plasminogen activator (t-PA) by plasminogen activator inhibitor (PAI) in plasma for activity determination with chromogenically sensitive methods. To avoid this, we examined and standardized activity determinations of tPA from citrated and PAI from non- acidified plasma via rapid preparation by high centrifugal force (HCF) (15,000xg for 3 minutes at 20-24°C) in a transportable microcentrifuge in comparison to centrifugation at 2,000-5,000xg (LCF) for 20 minutes at 4°C. tPA and PAI activities in samples obtained from 52 female and 50 male volunteers before (B) and after venous occlusion (VO) were assayed with COA-SET t-PA and COA-SET PAI (KabiVitrum). The evaluated tPA activities in the samples of both centrifugation methods did not significantly differ: tPA (HCF) B = 1.2 ± 0.06 IU/ml, VO = 1.5 ± 0.10 IU/ml vs. tPA (LCF) B = 1.1 ± 0.07 IU/ml and VO = 6.6 ± 0.76 IU/ml. The tPA activities in the 4 plasma samples with the highest post-VO values (>14 IU/ml) however were 27-42% higher in the HCF than in LCF plasma. PAI activities in the HCF samples were distinctly higher than in the LCF plasma: PAI (HCF) B = 19.8 ± 2.8 AU/ml, VO = 16.9 ± 0.9 AU/ml vs. PAI (LCF) B = 15.4 ± 1.0 AU/ml and VO = 10.2 ± 1.2 AU/ml. From our results we conclude, that short centrifugation at high centrifugal forces is suitable for preparation of acidified plasma for PAI activity determination. High tPA activities in plasma thereby may be better preserved from inactivation. For PAI activity determination centrifugation at 15,000xg is unsuitable, because the higher PAI levels obtained here indicate release of PAI activities from platelets and blood cells.

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ACUMULATION OF 51CHROMIUM-LABELLED PLATELETS IN THE RAT LUNG AFTER INTRAVENOUS APPLICATION OF PAF AND ITS EXERTION BY DRUGS

A.P. KLEE, D. SEIFFGE

Previous studies have shown that 1-alkyl-2(R)-acetyl-glycerol-3-phosphorylcholine (PAF) fails to induce platelet aggregation in platelet rich plasma of the rat (D. Namm, Thromb. Res. 25: 341, 1982; own results). The microembolization of labelled platelets in the lung was used as an in vivo model to evaluate the effect of PAF and inhibitory drugs. Following injection of 51chromium-labelled homologous platelets into urethane-anesthetized rats the distribution was continuously monitored using collimated crystal scintillation probes. Counts rates and the ratio of two detectors (c1/c2) - one placed above the thorax (c1), the other above the abdomen (c2) - were calculated and displayed by a microcomputer-based system.

A bolus injection of PAF resulted in a rapid and dose-dependent sequestration of 51chromium-labelled platelets in the lung. This process was reversible and not redeemable by a second application of PAF indicating a desensitization. Nevertheless labelled platelets were still able to react to other platelet stimuli e.g. ADP. PAF-induced (5 ug/kg) pulmonary platelet accumulation could be completely overcome by prior injection of the specific PAF-antagonist WEB 2170 (BOEHRINGER INGELHEIM). Pretreatment of the animals with pentoxifylline and acetylsalicylic acid, administered successively (principle of HWA method), also exerted a significant in vivo inhibition. These results indicate that, in contrast to the situation in vitro, a PAF-specific activation of platelets is achievable in vivo.

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IS PLASMA OR BLOOD RHEOLOGY DEPENDENT OF ACID-BASE-STATUS?

H. Hein and D. Pfitzner

Any change of hydrogen ion concentration could change the dissociation of proteins and possibly plasma and blood rheology. We measured therefore plasma and blood viscosity (capillary hose viscosimeter KSPV 4, RHEOMED for the plasma, WELLS-BROOKFIELD cone plate viscosimeter with shear rates of 230,4, 115.2, 46.08 and 23.04/" for the blood) at different pH-values.

We draw blood from three volunteers from an antecubital vein after venous stasis with a tourniquet was lifted. Blood specimens were kept open, so that CO2 could leave the specimen, and were immediately and then every 30" for 2 hours. The initial pCO2 was between 54.1 and 75.3 mmHg, the pH between 7.232 and 7.472 while K and m are the regression coefficients. The m parameter represents the percent unitary variation (positive, null or negative) of impedance for a per cent unifilar variation of time. A normal distribution of the m values has been obtained from our samples. The normal range in our laboratory was 0.39+/-.0.08. Values above or below this range could be respectively considered as expressing hyper- or hypoaggregating situations. This simple but exact method could allow an easier clinical interpretation of platelet aggregation on whole blood.

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A MODIFIED SEMI-DRY BLOTTING TECHNIQUE FOR DETECTING THE MULTINERISTIC STRUCTURE OF VON WILLEBRAND FACTOR

TH. WÜST, H. BEESER

The detection of the multineric structure of von Willebrand factor is an important method of subtyping von Willebrand's disease. We report an electrophoretic method, in which the von Willebrand factor multimers are separated in a 1.0% agarose gel, using a discontinuous buffer-system. The gel was lying on the water-repellent side of a gel bond film and cooled at a temperature of 12°C. At the end of the separation the top side of the gel was covered with a nylon-membrane having a pore size of 0.2 μm. The sandwich was with the nylon-membrane in front overturned on a carbon electrode, covered with filter papers, soaked in anode-buffer. After remove of the gel bond the sandwich was completed by bringing up filter papers, soaked in cathode-buffer, and a second carbon electrode. The semi-dry blotting was performed by a constant current of 15mA for 17 hours. The visualisation of von Willebrand factor multimers was carried out by use of a peroxidase conjugated rabbit antibody to human factor VIII vWf. The present method has a high degree of resolution and consumes, compared with conventional methods, using tank-blotting and immunohistochemical sandwich techniques, less buffer, antibody solutions and time. Problems, using radioactive marked antibodies, are avoided.

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MATHEMATICAL INTERPRETATION OF THE PLATELET AGGREGATION PHENOMENON AS STUDIED BY MEANS OF A WHOLE BLOOD AGGREGOMETER

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The aim of this study was to find the best mathematical model fitting the curves that express the aggregation phenomenon when evaluated by means of impedance aggregometry on whole blood. Venous blood samples were collected from 100 healthy subjects (50 males and 50 females). Platelet aggregation was measured by a Chrono-Log aggregometer; collagen, at different concentration, was used as proaggregating agent. The mathematical analysis of the curves was performed by means of the least square method. The best model fitting the curves was the hyperbolic function: Z=K*t^n, where Z is the impedance and t the time while K and n are the regression coefficients. The chi-square method and the Fisher's test confirmed the expressed hypothesis. The n parameter represents the per cent variation (positive, null or negative) of impedance for a per cent unifilar variation of time. A normal distribution of the m values has been obtained from our samples. The normal range in our laboratory was 0.39+/-.0.08. Values above or below this range could be respectively considered as expressing hyper- or hypoaggregating situations. This simple but exact method could allow an easier clinical interpretation of platelet aggregation on whole blood.

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PLATELET AGGREGATION IN HEALTHY ELDER SUBJECTS

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Aim of the present study was to investigate possible changes of platelet aggregability occurring during aging in healthy subjects. Platelet aggregation was evaluated in a group of 40 individuals (20 males, 20 females) whose age ranged from 60 to 69 years (mean 65.8 years). Criteria for inclusion were the absence of metabolic and/or cardiovascular disease, including hypertension. Furthermore, they were on no medications nor were they suffering from any acute or chronic illness. In the same way, 40 individuals matched for sex and smoking habits, whose age ranged from 40 to 59 years (mean 46.5 years), were enrolled as control group. Platelet aggregability was evaluated by means of impedance aggregometry using a whole blood Chrono-Log aggregometer; collagen at different concentration was used as proaggregating factor. Venous blood samples were collected without stasis in the fasting subject after 2 hours bed rest; blood was anti-coagulated with sodium citrate 3.8%. Analysis of aggregation curves was performed by means of calculation of a mathematical index (m-index), elaborated in our laboratory, that integrate the rate and percentage of aggregation. A significant increase of the m-index, meaning of a condition of relative hyperaggregability, was present in the group of elder subjects compared to control group.

PROCOSAGULANT ACTIVITY (PCA) IN HUMAN MONOCYTES INDUCED BY MODIFIED LOW DENSITY LIPOPROTEINS (LDL) IN VITRO

G. Claus, P. Schuff-Werner, V.W. Armstrong, A. Eilers, H. Kuester

It has been postulated that oxidatively modified LDL may be involved in the formation of foam cells from monocytes in the developing atherosclerotic lesion. Since fibrinogen (fibr) is also deposited in the plaque and monocytes are known to produce PCA in the presence of various stimulants, we investigated the effect of acetyl (Aci)-LDL and malondialdehyde (MDA)-LDL on monocyte PCA expression. Monocytes were isolated from peripheral blood of 29 healthy volunteers and cultured at 37°C in 5% CO2-air in a fully humidified atmosphere. At the end of the incubation period the cells were disrupted by freezing and homogenized. A factor VII and phospholipid dependent PCA was identified in the cell fragments, consistent with thromboplastic activity (TPA). Native LDL did not stimulate TPA in this cultures. Both modified LDLs displayed a kinetic- and dose-response stimulation of PCA, the maximum being seen after 24 h with 250 μg/ml modified LDL-protein. At this dose a 5-fold TPA increase was observed whereas 2.5 μg/ml protein produced a 7-fold increase compared to native LDL.

It is concluded, that this TPA formed in monocytes by modified LDL may contribute to the formation of fibrin in atherosclerotic plaques.

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INCIDENCE OF PLATELET-REACTIVE AND PLATELET-SPECIFIC ANTIBODIES IN PATIENTS WITH AUTOIMMUNE THROMBOCYTOPENIA

B. Pilz, M. Steins, W. Stenzinger and J. van de Loo

Autoimmune thrombocytopenia (AITP) frequently is associated with the detection of platelet-reactive antibodies (pr-ab) in patients' sera. AITP as a clinical diagnosis can be supported by the finding of elevated values of pr-ab, but negative results do not exclude AITP.

We investigated blood samples taken from 47 patients (pts) with AITP for the presence of pr-ab using a competitive enzyme-immunoassay (CELIA) according to Kiefel et al. (Vox sang. 1987). Platelet-specific antibodies (ps-ab) directed against membrane glycoproteins (GP) were identified performing an antigen capture Elisa described by the same authors (Blood. 1987).

In 17 of 47 cases (36%) increased amounts of pr-ab, in 21 pts (45%) ps-ab and in 12 cases (26%) both ps-ab and pr-ab were detected. Twelve of the 17 pts (71%) with elevated values of pr-ab were found to have IgG binding to GPIIb/IIIa complex and GPIb. However, in 9 of 21 pts with detectable anti-GP ab we failed to detect enlarged amounts of pr-ab.

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IDENTIFICATION OF ANTIBODIES AGAINST PLATELET GLYCOPROTEINS IN PATIENTS WITH AUTOIMMUNE THROMBOCYTOPENIA

W. Stenzinger, M. Steins and J. van de Loo

Autoimmune thrombocytopenia generally is diagnosed using clinical criteria and routine laboratory tests. For the confirmation of diagnosis, determination of platelet-reactive immunoglobulin (Ig) is helpful, but not essential, because assays used to this end lack high specificity. Recently, enzyme-immunoassays (ELISA) for the detection of antibodies directed against platelet membrane glycoproteins (GP) have been developed, which may be more specific than standard tests mentioned above.

Using the antigen capture ELISA described by Kiefel et al. (Blood. 1987) we analysed plasma from 53 patients (pts) for the presence of IgG directed against GP IIb/IIIa complex and GP Ib. Anti-GP antibodies could be identified in 43% of pts (n=23).

With regard to their GP-specificity antibodies were distributed among the pts as follows: Seven cases were found to have anti-GP IIb/IIIa, 8 pts had anti-GP Ib and in 8 cases both anti-GP IIb/IIIa and anti-GP Ib could be detected.

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In a family living in Regensburg the mother and her two sons have a bleeding disorder with spontaneous hematoma. In the mother and the elder son a prolonged bleeding time (> 15 min.) and the platelet size was maximally enlarged (mean volume 21,5 fL). Platelet adhesion to glass and human endothelial cell matrix (ECM) was strongly reduced. Collagen induced aggregation was totally, ADP and epinephrine induced aggregation slightly inhibited. Ristocetin induced aggregation was not affected. Surprisingly F VIII R:Ag and Ristocetin-Cofactors in plasma were slightly decreased. In the platelet spreading test large spread platelet forms similar to other giant platelet thrombopathies were found. The "Thrombopathy Regensburg" differs from the "May Hegglin" anomaly by the missing of "Dohle bodies", high specific platelet weight and inhibited aggregation and retention. In contrast to the Bernard Soulier-Syndrom, Ristocetin induced aggregation and glycoprotein III b molecule was detected. Further immunologic investigations and binding studies to thrombospondin are in progress.

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MOLSIDOMINE BUT NOT ISOSORBIDEMONONITRATE INCREASES THE FIBRINOLYTIC POTENTIAL IN HEALTHY VOLUNTEERS

C. Drummer, M. Spannagl, W. Schramm and R. Gerzer

The -O-NO2-containing organic nitrates like isosorbidemononitrate (ISMN) and the NO-containing vasodilator like SIN-1 (the bioactive metabolite of molsidomine) exert their effects through activation of soluble guanylate cyclase (GC). While NO-containing agents directly activate GC, the nitrates need to be metabolized to release nitric oxide prior to activation of the enzyme. In vitro only NO-containing agents, but not nitrates, directly activate GC in platelets and potentilly inhibit aggregation. We have compared in a double-blind study the effectiveness of each a single oral dose of molsidomine, 5-ISMN and placebo to inhibit platelet function ex vivo and to influence the fibrinolytic potential in twelve healthy volunteers. Before, 30 and 60 min after drug intake plasma concentrations of tissue-plasminogen-activator-inhibitor (PAI-I) were determined. RESULTS: Platelet aggregation ex vivo was significantly inhibited after intake of molsidomine and to a minor degree following 5-ISMN. Plasma concentrations of t-PA and placebo were reduced by 10% after 5-ISMN and placebo, but increased by 6% after molsidomine. The activity of the inhibitor (PAI-I) was increased by 11% after 5-ISMN, while placebo and molsidomine provoked decreases in PAI-activity by 6% and 13%, respectively. The overall fibrinolytic potential (calculated by the relation of t-PA/PAI-I) was absolutely unchanged after placebo, was decreased by 16% and 12% 30 and 60 min after 5-ISMN and was significantly increased by 7% (30 min) and by 45% (60 min, p<0.05) after intake of molsidomine. CONCLUSION: The present results suggest that molsidomine, but not organic nitrates, induces an increase in fibrinolytic potential in vivo. Since both drugs (molsidomine and 5-ISMN) have vasodilatory and platelet inhibiting properties ex vivo, their different effects on fibrinolytic parameters may be an additional advantage of molsidomine in the clinical practice.

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QUANTIFICATION OF PLATELET MEMBRANE IMMUNOFLOURESCENCE IN BERNARD-SOUILLER SYNDROME AND GLANZMANN'S THROMBOCYTHROMIA BY FLOW CYTOMETRIC ANALYSIS

R.E. Scharf, A. Tomer, G.J. del Zoppo, and Z.M. Ruggeri

We have studied platelet membrane glycoproteins (GPs) in two patients with a lifelong bleeding tendency and in normal subjects using a fluorescence-activated flow cytometry technique (FAFC). To evaluate qualitative and quantitative abnormalities of platelet membrane glycoproteins in a panel of fluorescence isothiocyanate-conjugated monoclonal antibodies (mAbs) was applied, including Tag for GP Ib, Ab-15 for GP IIIa, Lj-P4 for GP IIb-IIIa complex, PAC1 for activated GP IIb-IIIa complex, anti-LIBS-I for ligand-induced binding sites on GP Ib-IIIa, and 3 mAbs (designated Lj-P3, Lj-Ib, Lj-Ib0) that are directed against distinct epitopes of GP Ib. In all individuals, FAFC analysis was performed in whole blood and platelet-rich plasma. Patient 1 showed no binding of Lj-P3, Lj-Ib, and Lj-Ib0. Indicating the absence of normal GP Ib on the platelet surface. In contrast, membrane immunofluorescence of Lj-P4 was approximately 70% higher in this patient than in normal controls. Together with an increased forward light scatter, this finding indicated an increased number of the GP Ib-IIIa complex present on abnormally large platelets. Patient 2 displayed normal immunofluorescence with Lj-P3 but no or negligible binding of mAbs directed to GP Ib (Tab; 12%), GP IIIa (Ab-15; 6%), or the GP IIb-IIIa complex (Lj-P4; 3%) Upon stimulation with ADP (10 #M) or of platelet activation-dependent epitopes of the GP IIb-IIIa complex. This study demonstrates qualitative and quantitative abnormalities in platelet GP Ib and GP IIb-IIIa complex related to Bernard-Soulier syndrome (Patient 1) and Glanzmann's thrombasthenia (Patient 2), respectively. We conclude that the FAFC technique permits a sensitive and rapid method to identify two patients with platelet-related bleeding disorders and both to identify and quantitate abnormalities of platelet membrane GPs, using specific mAbs.

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ARACHIDONIC ACID METABOLITES AND BIOCOMPATIBILITY DURING HEMODIALYSIS

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The biocompatibility of dialyzers is influenced by many factors (sterilization, housing, design, flow characteristics, potting material), only one of which is the membrane. Aim of the study: To examine the effects of 2 different membranes on the coagulation and immune defense system during long term hemodialysis. Patients and methods: 10 patients undergoing maintenance hemodialysis signed informed consent to participate in a study in which the effects of 2 different membranes (cuprophane - CUP vs. polysulfone - PS) were examined. Blood samples were taken at the time 0, 25 and 50 min. Coagulation and immune defense tests and scanning electron microcopy of the membranes were performed. Results: 1. Transient leucopenia and elastase release more pronounced during CUP vs PS hemodialysis (25 min.) 2. No significant changes in AT III, Protein C, Plasminogen, Antiplasmin, Fibrinectin levels during course of hemodialysis. 3. Increased levels of TAT in CUP hemodialysis (p < 0.01), but no significant changes in D-Dimer levels. 4. Increased levels during CUP hemodialysis. 5. Significant differences at the HD end in thrombomodulin and PG F alpha levels using cuprophane membrane (CUP 0.8 pg/ml, CUP < 0.8 pg/ml) vs. PS membranes (70 pg/ml, 15.8 pg/ml). Higher anti Xa levels during PS hemodialysis vs. CUP hemodialysis. Conclusions: Our data suggest that measurements of arachidonic acid metabolites are useful in evaluation of biocompatibility of special hemodialysis membranes. Polysulfone is a more biocompatible membrane.

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Several reports have appeared with regard to the potential role of platelets in tumour cell metastasis. In our study we want to find evidence for our hypothesis that metastatic effects of colon tumour cells are not due to platelet-colon tumour cell aggregation, but by increased consumption of coagulation factors. The extend of activation of coagulation and aggregation induced by a human colon tumour cell line was investigated.

46 patients were classified as follows: chronic persistent hepatitis (CPH) n=10, chronic aggressive persistent hepatitis (CPA) n=13 and patients with extreme reduction of liver function (529-660 DU/ml TAT), combined with a decrease of pseudocholinesterase below 600 DU/ml, n=13. The reduction of clotting factors in patients with chronic liver diseases may be caused by either decreased protein synthesis of the liver or by increased consumption of coagulation factors. The extend of activation of coagulation factors can be monitored by measuring the inhibitor complexes TAT and IXAT. The inhibition of urokinase by the inhibitors and urokinase which have been previously used to investigate heparin and related glycosaminoglycans. Administration of 1-5 mg/kg dosages of LW 10082 produced an inhibition of thrombin, the amidolytic activity was insensitive to detecting the pharmacodynamics of this agent. Modified plasma based (more sensitive) assays such as diluted APTT and thrombin generation assays showed better dose dependent activity. The bioavailability of LW 10082 was found to range between 25-50% depending upon the assay system. As determined by the APTT, bioavailability for 2.5 and 5.0 mg/kg dosages respectively, as measured by area under the curve was 22.54 mg/hr/ml, clearance was 2.0 and 1.6 ml/min/kg and volume of distribution was 0.8 and 0.9 L/kg. While additional studies are in progress, it appears that the plasmatic pharmacodynamic effects of this new antithrombotic agent, LW 10082, can be easily evaluated by the APTT, diluted APTT and modified thrombin generation tests.

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PHARMACODYNAMICS OF A SULFATED LACTOBIONIC ACID DERIVATIVE IN EXPERIMENTAL ANIMAL MODELS
H. Keuper and P. Lenz

Recently, we have reported on a new method for the rapid determination of PAI-activity which is based on the inhibition of urokinase (T.W. Stief, P. Lenz, U. Becker, N. Heimburger, Thromb. Res. 50 (1988) 559-573). Here we present data which quantify the degree to which different types of PAI (PAI-1, PAI-2, PAI-3) contribute to the activity measured with our method. These differences reflect the kinetics of association between the inhibitors and urokinase which have been determined by other groups.

When PAIs in the plasma sample are incubated with urokinase for 5 min, the only significant contribution to the inhibition of urokinase arises from PAI-1. By extending the incubation period to 30 min, PAI-2 can be determined in addition to PAI-1. The inhibition of urokinase by PAI-3 becomes apparent only in the presence of heparin. Thus it is possible to differentiate between the type of PAI present in plasma by varying the incubation period in the presence or absence of heparin.

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THROMBIN-ANTITHROMBIN - AND FACTOR IX-ANTITHROMBIN COMPLEXES IN CHRONIC LIVER DISEASES
Bettina Remkes-Matthes, K.J. Hatthes and H. Bleyl

The reduction of clotting factors in patients with chronic liver diseases may be caused by either decreased protein synthesis of the liver or by increased consumption of coagulation factors. The extend of activation of coagulation factors can be monitored by measuring the inhibitor complexes TAT and IXAT. The inhibition of urokinase by the inhibitors and urokinase which have been previously used to investigate heparin and related glycosaminoglycans. Administration of 1-5 mg/kg dosages of LW 10082 produced an inhibition of thrombin, the amidolytic activity was insensitive to detecting the pharmacodynamics of this agent. Modified plasma based (more sensitive) assays such as diluted APTT and thrombin generation assays showed better dose dependent activity. The bioavailability of LW 10082 was found to range between 25-50% depending upon the assay system. As determined by the APTT, bioavailability for 2.5 and 5.0 mg/kg dosages respectively, as measured by area under the curve was 22.54 mg/hr/ml, clearance was 2.0 and 1.6 ml/min/kg and volume of distribution was 0.8 and 0.9 L/kg. While additional studies are in progress, it appears that the plasmatic pharmacodynamic effects of this new antithrombotic agent, LW 10082, can be easily evaluated by the APTT, diluted APTT and modified thrombin generation tests.

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EXTRACELLULAR REGULATION OF PLASMINOGEN ACTIVATOR INHIBITOR-1 BY VITRONECTIN AT SITES OF PLATELET-SUBENDOTHELIAL CELL MATRIX INTERACTION.

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The interaction of plasminogen activator inhibitor-I (PAI-1) with its binding protein vitronectin (VN) (JBC 267:15454-15461, 1988) was studied utilizing purified human components, and the possible contribution of VN in the extracellular matrix (ECM) of cultured human endothelial cells (EC) was assessed. While platelets and EC constitute two major sites of storage or production, respectively, of PAI-1, little is known about the distribution of VN at these sites. The high specific PAI-1 capacity in the ECM of cultured EC is suggestive for the stabilization of PAI-1 by matrix component(s). When VN was found associated in high molecular weight complexes together with platelet-derived PAI-1 in the supernatant of stimulated platelets, only small amounts of VN were detectable in cultured EC. In contrast, substantial quantities of VN could be detected in the intact ECM of these cells. Direct binding of recombinant PAI-1 to the PAI-1-depleted ECM was inhibited mainly by antibodies against VN indicating that VN constitutes the primary binding component for PAI-1 in the ECM of EC. Binding studies of PAI-1 to immobilized VN further revealed that, unlike urokinase, heparin did not inhibit PAI-1 binding, although PAI-1 as well as bovine plasma heparin bound to the same 10-15 kDa fragments exerting the glycosaminoglycan binding domain of VN. These results provide evidence not only for the occurrence of both, VN and PAI-1 in platelet storage granules as well as in the ECM of EC, but also for a functional link between both components resulting in the stabilization of PAI-1 activity particularly at platelet-ECM sites of interaction. The extracellular VN-PAI-1 relationship may have significant implications for the regulation of plasminogen activation in general and the protection of the ECM against proteolysis.

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THE PROTECTIVE EFFECTS OF THROMBOMODULIN ON THE INACTIVATION OF THROMBIN BY HEPARIN COFACTOR II.

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The endothelial cell thrombin receptor thrombomodulin (TM) is an inhibitor of coagulation (a) by serving as a cofactor for thrombin-induced protein C activation, (b) by directly affecting the procoagulant activity of thrombin and (c) by accelerating the inhibition of thrombin by antithrombin III. We have recently proposed the presence of a dermatan sulfate-like glycosaminoglycan region in rabbit TM which may constitute a secondary binding site for thrombin, required for both activities (b),(c). In the present study the effects of TM on the inactivation of thrombin by another serine protease inhibitor, heparin cofactor II (HCII) whose activity is dependent on glycosaminoglycans, were investigated. In a dose-dependent fashion TM effectively protected thrombin against fast inactivation by HCII/dermatan sulfate. Similar effects were also found in this inhibition reaction if dermatan sulfate was omitted or was replaced by heparin, respectively. Removal of the secondary binding site of TM by chondroitin-ABC-Iase resulted in partial reduction of the thrombin protecting effect of TM. It is thus concluded that binding of TM to thrombin renders the enzyme resistant against inactivation by HCII. In another experimental set-up using intact cultured human endothelial cells no appreciable thrombin inhibition by HCII occurred on the surface of these cells. From these findings we conclude that under quiescent conditions TM may exist as a fluid phase protein or as an adherent protein at the vessel wall thrombin inhibition by HCII is of major importance. Rather endothelial cell components may protect thrombin against inactivation by this protease inhibitor.

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FORMATION OF THROMBIN-ANTITHROMBIN-COMPLEXES ON THE SURFACE OF CULTURED HUMAN ENDOTHELIAL CELLS
A.Kottas-Heldenberg, P.Valent, K.Lechner, W.Speiser

Human endothelial cells are known to synthesize heparin-like glycosaminoglycans. The importance of these molecules for the anticoagulatory activity of endothelial cells is however controversial. In the present study the effect of cultured human umbilical vein endothelial cells (HUVEC) on the formation of thrombin-antithrombin III-complexes (TAT) was investigated. Purified thrombin (f.c. 0.8 nM) and purified AT III (f.c. 5 µM) were incubated at room temperature in the presence and absence of HUVEC and heparin. At various time intervals (15, 30, 60, 120 sec.) TAT formation was stopped by the addition of hirudin and TAT were then quantified by EIA (Behringwerke, Marburg, FRG). In the absence of HUVEC and heparin the concentration of TAT constant increased over a period of 2 min.: 15 sec: 38.5±7.6%, 30 sec: 60.5±13.2%, 60 sec: 86.5±10%, 120 sec: 100% (mean values ± S.D. of five experiments performed in triplicate). The addition of heparin markedly increased the rate of TAT formation. In the presence of at least 0.01 IU heparin/ml maximum TAT levels were observed after 15 sec. On the surface of HUVEC monolayers (3-3.5x10⁴ cells per dish; 1000 ul supernatant) the kinetics of TAT formation was similar as in the absence of HUVEC: 15 sec. 26.6±2.8%, 30 sec. 53.3±5.7%, 60 sec. 76.6±7.6%, 120 sec. 100%. Experiments were also performed with HUVEC suspensions: 9x10⁴ cells/1000 ul. The rate of TAT formation at such a high density of cells was similar to that observed on HUVEC monolayers. We conclude from our experiments that cultured HUVEC do not exhibit heparinlike effects on the formation of TAT.

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ANTICARDIOLIPIN ANTIBODIES IN PATIENTS WITH DEEP VENOUS THROMBOSIS
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Several studies reported on an association between antiphospholipid antibodies and venous and arterial thrombotic disease. In the present study serum levels of anticardiolipin antibodies ACA were determined in 262 patients with a history of deep venous thrombosis (DVT) and in 110 healthy controls. ACA were quantified by two assay systems: ACA IgG ELISA and ACA IgM ELISA (Walker Laboratories, Cambridge, England). The following levels were measured in patients with DVT and in healthy controls: ACA IgG median: 7.1 U/ml, range: 1.0 to 100.0, ACA IgM: 2.6 U/ml, 0.0 to 60.0; controls: ACA IgG 5.6, 1.1 to 30.0, ACA IgM: 2.3, 0.0 to 13.8. 16 of 262 patients (6.1%) had levels > 99 percentile of healthy controls) ACA IgG levels (age < 50 years) and ACA IgM levels were elevated in 3 of 262 patients (1.2%). 6 of 110 patients with elevated ACA IgG had spontaneous DVT (15, 24, 25, 26, 44, 45a - age at the time of DVT), 3 postoperative DVT (22, 46, 57a) and 2 during pregnancy (22, 28a). 5 of 8 patients with elevated ACA IgM had spontaneous DVT (18, 22, 35, 45, 59a), 1 postoperative DVT (37a) and 2 during pregnancy (25a, 29a). At the time of investigation none of the patients had a history of arterial thrombotic disease. Two female patients had systemic lupus erythematoses: 1 of these patients had spontaneous DVT (25a; ACA IgM) and 1 suffered from DVT post partum (25a; ACA IgG +, IgM +). The latter patient had lupus anticoagulant and the highest levels of ACA IgG (100 U/ml) and ACA IgM (60 U/ml). The present study confirms that elevated levels of ACA may be associated with venous thrombotic disease; this phenomenon is however relevant only in a small subgroup of DVT patients.

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MARKERS OF COAGULATION, FIBRINOLYSIS AND FIBRINOGENOLYSIS ACTIVITY IN PATIENTS WITH INCREASED INTRAVASCULAR FIBRIN FORMATION
W.Speiser, A.Kottas-Heldenberg, E.Minar, K.Lechner

In the present study the plasma levels of the activation markers of blood coagulation (thrombin-antithrombin complex TAT), fibrinolysis (D-Dimer) and fibrinogenolysis (FgDP) were determined in two groups of patients: deep venous thrombosis (DVT) and acute myeloid leucemia (AML). These two groups of patients are entirely different with respect to the localization and the pathogenesis of their haemostatic disorder: in DVT patients increased fibrin formation is localized to the deep venous system of the legs, whereas in AML patients increased systemic fibrin formation was observed. Nevertheless, a similar pattern of changes in the plasma levels of the activation markers TAT, D-Dimer and FgDP was detected. Both groups of patients had elevated TAT, D-Dimer and FgDP plasma levels compared with healthy controls. DVT patients: TAT median 5.4 ng/ml, range 1.6 to 200; D-Dimer 3.500 ng/ml, 400 to 31.000; FgDP: 1.200 ng/ml, 200 to 14.000; AML patients: TAT 6.7, 1.8 to 75; D-Dimer: 1.525, 195 to 132.000; FgDP 620, 100 to 20.000; controls: TAT: 2.6, 1.2 to 16.3, p < 0.0001; D-Dimer: 142, 40 to 420, p < 0.0001; FgDP: 200, 60 to 800, p < 0.0001. The following correlations between the three activation markers with similar slopes of the correlation curves in both groups of patients were found: DVT patients: TAT vs. D-Dimer: r=0.65; D-Dimer vs. FgDP: r=0.65, p < 0.0001. We conclude from our results: 1) with the help of activation markers it is possible to differentiate between localized or generalized increased activity of blood coagulation and fibrinolysis, 2) a combined increase of D-Dimer and TAT most likely characterizes a haemostatic disorder as increased fibrin formation accompanied by secondary fibrinolysis and 3) fibrinolysis is frequently associated by a certain degree of fibrinogenolysis, most likely due to the proteolysis of fibrinogen molecules incorporated into fibrin clots.

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SENSITIVITY OF D-DIMER AND TAT MEASUREMENTS IN THE DIAGNOSTIC WORKUP OF THROMBOEMBOILIC DISEASE
W.Speiser, Th.Leitha, R.Mallek, E.Minar, K.Lechner

The sensitivity of elevated plasma levels of the activation markers of blood coagulation, thrombin-antithrombin complex TAT, and fibrinolysis, fibrin split product D-Dimer, in the diagnostic workup of deep venous thrombosis DVT and pulmonary embolism PE was investigated. 34 consecutive patients with phlebo-graphically documented proximal DVT and 26 consecutive patients with a high probability of PE in lung scan were included into the study. D-Dimer levels were determined by EIA (Agen Inc., Parsippany, USA; elevated levels: >120 ng/ml - > mean + 2 S.D. of 45 healthy controls: 82±19) and by Latex test (Dade, Miami, USA; positive test if levels exceed 500 ng/ml). TAT levels were determined by EIA (Behringwerke, Marburg, FRG; elevated levels: > 5.4 ng/ml - > mean + 2 S.D. of 45 healthy controls: 3±1). Sensitivity of D-Dimer EIA measurements, D-Dimer Latex test and TAT measurements in DVT patients during the first week of symptoms: 1, 1, 0.88; during the second week of symptoms: 1, 0.33, 0.66. Sensitivity of D-Dimer EIA measurements, D-Dimer Latex test and TAT EIA measurements in PE patients: 0.85, 0.46, 0.54. We conclude from our results: 1) Due to high sensitivity D-Dimer EIA can be used as a screening test in patients with suspected proximal DVT during a period of two weeks after the onset of symptoms and D-Dimer Latex test can be used in proximal DVT patients if the duration of symptoms does not exceed one week. 2) In PE patients D-Dimer EIA can be recommended for the diagnostic workup, especially in patients with inconclusive lung scan. 3) TAT measurements show markedly lower sensitivities in the diagnosis of DVT and PE than D-Dimer measurements.

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High Evidence for a Second (Hydrophobic) Binding Region in Plasminogen Activator/Plasminogen Activator Inhibitor Interaction

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Tissue plasminogen activator interacts with fast acting plasminogen activator inhibitors at second order rate constants (k2) in the range of 10^{-4} - 1 mol^{-1} s^{-1}. In this study it has been demonstrated that in the presence of hydrophobic agents, such as polyethylene glycol or Triton X 100 T.M., the apparent second order rate constants fall drastically in a hydrophobic agent-concentration dependent manner. In addition, unobserved bound to plastic plates becomes to about 48 ± 9% refractory to inhibition by PAI, interestingly without any loss in catalytic activity against plasminogen. Thus, there is high evidence that beside the reactive centre a second (hydrophobic) binding region seems to be involved in PA/PAI interaction. Human macrophages and granulocytes participate in physiologic fibrinolysis. Membrane proteins include u-PA and plasminogen activators. Interaction of u-PA with its receptor results in PAI uncontrolled PA activity allowing the clearance of micromolecules of fibrinolytic activity. The mechanism for this PA/PAI interaction might involve the hydrophobic binding region of the PA molecule.

Tissue Normoxia and Aspirin (ASA) Modulate the Procoagulant/Fibrinolytic Balance in Human Arterial (A) and Veinous (V) Endothelial Cells (E)

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Routine 0, atmosphere for cell cultivation (195 mmHg), is hyperoxia and does not represent "tissue normoxia" (40 mmHg) of the vessel wall. Our previous results demonstrated the p02-induced modulation of the physiological behavior of hA/VSMC. We investigated the proliferation and release of tissue plasminogen activator (tPA) and -inhibitor (PAI-1) from hSMC.

METHODS: Media-SMSC from A. mammarii (Am) and V. saphena (Vs) from patients with ACVB (n=120; 35-71 y) were cultivated under 20-145 mmHg, in our glass incubator unit in RPMI 1640 medium. Therapeutic ASA conc. (10^-5M) was added on day 1, 3, 5, 7.

RESULTS: Increasing p02 from 40 - 145 mmHg inhibits the proliferation of hA/VSMC (p<0.0008). Under 20 mmHg p02, e.g., Am-SMC still show a 5-fold higher proliferation than VsSMC. ASA retards the population doubling time in VsSMC under 40 mmHg (n=8; 2.18 ± 2.37 days, p=0.02), however, not under 145 mmHg (p=0.54). This is different to the heparin-action. hA/VSMC do release tPA/PAI-1 during proliferation. There is a 7-fold increase of tPA on day 7 over day 1. There is no correlation between tPA-release and patient's age (p=0.87, n=15). Increased proliferation decreases tPA/PAI-10^5 M (p=0.007, n=15). Increased SMC aging in culture increases tPA-release (p=0.024, n=15). ASA increases tPA-release in Vs/AmSMC (n=9, p=0.006). VsSMC release PAI-1 during proliferation which seems to be increased under tissue normoxia and ASA.

DISCUSSION: Tissue normoxia modulates the proliferation and fibrinolysis of hA/VSMC. This is important for studying the physiological behavior of hSMC in vitro and the influence of drugs. hA/VSMC do contribute to the endogenous vessel wall fibrinolysis which is modulated through the p02 and ASA.

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Evaluation of a new Thromboplastin

A new Thromboplastin prepared from rabbit brain was evaluated. The material gives a standard curve with more than 5 seconds difference between 100 and 50 k and shows a good linearity up to the dilution 1:10 on the EC 100 coagulometer. The material has a very high factor sensitivity as determined on mixtures of normal plasma and deficiency plasma. Both, live and intra assay precision value of the new thromboplastin show that the material is very reproducible with c.v.'s in the 1 to 3 %. With clinical samples of patients with liver diseases, heparin therapy on oral anticoagulations or other diseases and also with recanal a very good correlation between the new thromboplastin and a human plasma thromboplastin was found in 195 samples. The correlation coefficient was r = 0.97, the equation of the regression line according to Passing/Bablok was y = 0.05 + 0.95 x for the comparison based on percent of normal values. In a study on 102 normals and oral anticoagulated patients an ISI value of 1.15 was found for the EC 10 using human thromboplastin as a reference. This value is in accordance to another study in which the new thromboplastin was compared against ISI/79, an internationally accepted reference preparation. If the values of both thromboplastins are converted into INR's the regression line but the equation y = 0.001 + 1.018 x with an r = 0.987. For the determination of the therapeutic range in percent activity the values in INR were plotted against the percent values. From this plot we could calculate the respective percent values which resemble the recommendation of 3.8 - 4 which is used for most indications for oral anticoagulant treatment. An INR of 2 - 4 is equal to 25 - 75 % with Thromboplastin ISI. The same approach had lead to comparable values for the human plasma thromboplastin.

In conclusion these data show that Thromboplastin ISI is a very sensitive thromboplastin with a low ISI value which is well suited for diagnostic and monitoring use. It shows very similar performance characteristics in patient samples like human thromboplastin.

PLATELET AND WHITE BLOOD CELLS FUNCTION IN PATIENTS WITH PERIPHERAL OBLITERATIVE ARTERIAL DISEASE

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Patients suffering from Peripheral Obstructive Arterial Occlusive Disease (POAO) are characterized by increased platelet-leukocyte aggregates, leukocyte adherence and the level of leukotriene C4 in 20 patients suffering from POAO (II or III stage according to Fontaine). It was found that these patients had increased mean numbers of platelet-leukocyte aggregates (119.7 ± 9.2) and leukotriene C4 level (7.4 ng/ml plasma v. 4.79 ng/ml plasma). We have also shown that leukocyte adherence increased in the patients in advanced stages. These results seem to suggest that the drugs act on the haemorrhological pattern and improve the flow properties of white blood cells could be useful in the treatment of ischaemic disease. We have shown, that these properties possess pentoxyfylline which improves the clinical state and inhibits leukocyte adherence in patients with POAO. Moreover we have found that pentoxyfylline has anti thrombotic properties shown in experimental animal model with laser induced endothelial injury.

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Division of Angiology, Center of Internal Medicine, J.W. Goethe University, Frankfurt/Main, FRG.
Extracellular fibronectin is essential for cell-to-substratum contact and thus may be involved in tumour growth and spreading. We studied plasma fibronectin against some hemostatic parameters in 60 patients with various advanced neoplasms. Fibronectin, factor XIII, alpha-2-antiplasmin (α2-AP), AT III, alpha-macroglobulin (α2-M), alpha-antitrypsin (α1-AT) and C1-inhibitor (C1-I) concentrations were measured by means of rocket immunoelectrophoresis using monospecific antisera (Behring). Protein C level was measured by means of ELISA Protein C test (Boehringer). The patients with neoplasmas revealed significantly reduced concentration of fibronectin concomitant with lowered levels of F.XIII, protein C, AT III and α2-AP as well as elevated levels of α2-M, C1-I, α1-AT and FDP. The lowest level of fibronectin was observed in patients with melanoma malignum. No significant correlations between fibronectin and investigated hemostatic parameters were found. The results of our study indicate the profound perturbations of plasma fibronectin in cancer patients but its exact role in malignancy needs further clarification.

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A FAST AND SENSITIVE SPECTROPHOTOMETRIC ASSAY FOR URINARY TYPE PLASMINOGEN ACTIVATOR IN HUMAN PLASMA

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Activated leukocytes release large amounts of chloramine like oxidising agents which inactivate protease inhibitors, creating microenvironments of uncontrolled protease activity. The biological result is an increased proteolytic activity, i.e. loss of tissue type plasminogen activator activity. The key enzymes of fibrinolysis, is of clinical importance. Assay techniques have been developed but are troublesome due to predilution, acidification or separation steps in order to eliminate the PA and plasmin-inhibiting effect of plasmin inhibitors of anti-PA and anti-plasmin type, respectively. In this study, evidence is presented that performance of fibrinolytic assays using N-chloramines offers a great advantage: plasminogen activator activity of urinary type both of single chain and of two chain nature can be measured precisely within minutes in untreated (direct) plasma samples. Therefore, mimicking the oxidative inflammatory response, it gets feasible to analyse blood factors involved in the pathway of fibrinolysis by means of a functional, sensitive, and rapid assay procedure.

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HIGH EVIDENCE FOR A SECOND (HYDROPHOBIC) BINDING REGION IN PLASMINOGEN ACTIVATOR (PA)/PA INHIBITOR INTERACTION

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Plasminogen activators interact with fast acting plasminogen activator inhibitors at second order rate constants (k1) in the range of 107 l mol-1 s-1. In this study it has been demonstrated that in the presence of hydrophobic agents, such as poly ethylene glycol or Triton X 100 T.M., the apparent second order rate constants fall drastically in a hydrophobic agent-concentration dependent manner. In addition, urokinase bound to plastic plates becomes to about 45 % refractory to inhibition by PAI, interestingly without any loss in catalytic activity against plasminogen. Thus, there is high evidence that beside the reactive centre a second (hydrophobic) binding region seems to be involved in PA/PAI interaction. Human macrophages and granulocytes participate in physiologic fibrinolysis. Membrane proteins include u-PA and plasminogen receptors. Inhibition of u-PA with its receptor results in PAI uncon- trolled PA activity allowing the creation of microcompartments of enhanced fibrinolytic activity. The mechanism for this PA/PA receptor interaction might involve the hydrophobic binding region of the PA molecule.

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INACTIVATION OF HUMAN α2-MACROGLOBULIN BY REACTIVE OXIDANTS

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α2-Macroglobulin (α2M) is a proteinase inhibitor of broad specificity and one of the major plasma proteins in man. Monocytes interact with α2M-proteinase complexes internalising and degrading them. Since activated leukocytes (PMN) release large amounts of reactive oxidants the goal of this study was to determine whether these agents are able to inactivate the inhibitor. Mimicking the leukocyte attack in normal human plasma by N-chloramine we observed an oxidant concentration dependent inactivating effect on purified and plasma α2M. The oxidant concentration for complete inactivation of the inhibitor was found to be comparable with the "lethal" dose for human α2-antiplasmin and antithrombin III. The inactivation of α2M by oxidants is the result of a specific oxidative damage since the corresponding serine proteases as well as α1- inhibitor were chloramine resistant under the conditions used. It might be speculated that the oxidants attack the bait region of the trap molecule α2M. According to our results, the amount of oxidants released by 1 x 106 activated PMN would be sufficient to destroy α2M activity of about 1 pl of human plasma. Consequently, in local areas of inflammation activated leukocytes may well be able to create microcompartments of uncontrolled protease activity by generation of reactive oxidants. Oxidants seem to alter enzyme/inhibitor balances in favour of the enzyme.

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Perioperative Verlaufsbeobachtung von Gerinnungsparametern, die möglicherweise ein Risiko für eine Beinvenenthrombose anzeigen, in der Hüftgelenksendoprothesetätigkeit

Ziegler, P.; Häfner, G.; Ennenhal, W.

In unserer Klinik beläuft sich die Zahl der postoperativen Beinvenenthrombosen in der elektiven Hüftgelenksendoprothetik, trotz perioperativer Thromboembolieprophylaxe mit Standard-Heparin (3 x 5000 IE sc) auf über 5%.

Um signifikante Parameter, die eine erhöhte Blutkoagulabilität anzeigen herauszufinden, haben wir damit begonnen, Patienten, die sich einer elektiven Hüftgelenksendoprothesenimplantation unterzogen, mit verschiedenen Gerinnungsparametern zu überwachen. Wir bestimmen D-Dimer, PT, FPA-ag, TAF, Elastase, Factor VIII, Protein C und AT III pra-, intra- und postoperativ, sowie jeden Morgen bis zum 14. postoperativen Tag. Zusätzlich werden die Patienten mindestens zweimal täglich klinisch auf Thrombosezeichen untersucht.

Bisher hatten wir einen von 20 derart überwachten Patienten mit einer klinisch relevanten postoperativen Thrombose (verifiziert durch Phlebographie); die Laborbefunde werden dargestellt und diskutiert.

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CLINICAL SYMPTOMS IN VON WILLEBRAND SYNDROME

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Bruising, bleeding from mucous membranes and postoperative or posttraumatic bleedings are the typical symptoms of vWS. An analysis of the bleeding symptoms of 111 pts with VWS investigated in our center revealed that 63% had bruising, 57% bleeding from the gums, 62% epistaxis, 74% menorrhagia. Joint bleedings (3.6%), GI-bleeding (7.3%), bleeding from tonsils (1.8%), cerebral bleeding (0.9%), haematuria (0.4%), muscle bleeding (0.9%) and bleeding from tongue bite (5.4%) were less common. The risk of spontaneous bleeding is clearly dependent on the level of vWF. After surgery (159 surgical procedures) bleeding occurred in 64% of pts. The risk of bleeding is dependent on the severity of the disease (vWF < 10%: 83%, vWF 10-30%, 75%, vWF 30-50% bleeding). The first major bleeding event occurred at a median age of 3.5 years in severe VWS (vWF <10%), at a median age of 7 years in intermediate cases (vWF 10-30%) and at a median age of 15 years in mild cases (vWF 30-50%).

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CRISTALLIZATION OF FACTOR XIII

H. J. Metzner and K. E. Kargos

Factor XIII (F XIII) of the blood clotting cascade is a proenzyme which, after activation with thrombin, cross-links proteins by a transamidination reaction, and has major functions in wound healing. The plasma form is built up by two different chains (a and b) from which the a-chain contains the active centre. F XIII occurs also intracellularly in platelets, monocytes and platelets. In these cells only dimers of the a-chain are found.

F XIII from plasma is well characterized and the amino acid sequence has been determined by Takahashi et al. (1990). For further characterization and understanding of the structure/function relationship X-ray diffraction analysis could be valuable. To enable such investigations we tried to obtain crystals with suitable quality. Previously Bohn et al. (1971) crystallized F XIII from plasma and platelets by dialysis against distilled water. Applying this method for F XIII from plasma we obtained small hexagonal and monoclinic crystals. To get larger crystals, we examined a number of different crystallization conditions and found indeed some, yielding acceptable crystals from recombinant F XIII and F XIII of placenta origin. Precipitation with sodium acetate (9-15 % (w/v)) in Tris buffered solution by vapour diffusion yields thin needles up to 1 mm. Using ammonium sulfate (0.5-0.9 % (w/v)) we received hexagonal and monoclinic crystals up to 0.5 mm long. Precipitation with ammonium acetate or ammonium sulfate (9.6 % (w/v)) in Tria-buffered solution by vapour diffusion in the hanging or laying drop leads to crystals with orthorhombic symmetry. Single crystals with edges up to 0.25 mm could be obtained. X-ray investigations of these crystals are ongoing to prove their quality for diffraction analysis.

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A SIMPLE TEST FOR DETERMINATION OF \( \text{Na}^+ / \text{H}^+ \) EXCHANGE IN HUMAN PLATELETS

D. Rosskopf and W. Siffert

Activation of \( \text{Na}^+ / \text{H}^+ \) exchange is an important step in platelet activation. Only recently, an increased activity of the \( \text{Na}^+ / \text{H}^+ \) exchanger has been detected in platelets from patients with essential hypertension. We aimed at setting up a simple optical test for the measurement of \( \text{Na}^+ / \text{H}^+ \) exchange activity. Prewarmed aliquots (37°C, 70 ul) of platelet-rich plasma were directly added to medium (470 ul) composed of (in mM) Na+-propionate 140, HEPES 20, glucose 5, KCl 1, MgCl2 1. The medium contained the 

\[ \text{Na}^+ / \text{H}^+ \] exchange activity. Prewarmed aliquots (37°C, 70 ul) of platelet-rich plasma were directly added to medium (470 ul) composed of (in mM) Na+-propionate 140, HEPES 20, glucose 5, KCl 1, MgCl2 1. The essential acid permeates the cell membrane, acidifies the cytosol, and, thereby, activates the \( \text{Na}^+ / \text{H}^+ \) exchanger. The continuous uptake of \( \text{Na}^+ \) then causes cell swelling, due to the influx of osmotically obliged water. This addition was done in a cuvette placed into an aggregometer. A rapid decrease in absorbance of such platelet suspensions was observed whose time course corresponded to a first order reaction. The observed changes directly reflect cell swelling via \( \text{Na}^+ / \text{H}^+ \) exchange: 1) No change in absorbance occurred when platelets were incubated in \( \text{K}^+ \)-propionate medium. 2) Specific inhibitors of \( \text{Na}^+ / \text{H}^+ \) exchange completely inhibited the changes in absorbance (iii) analysis of the external \( \text{Na}^+ \) requirement revealed that the changes in absorbance followed Lineweaver-Burk kinetics. These findings are identical to those obtained by electronic cell eluting. While the rate constant of the change in absorbance was determined to be \( 21 \times 10^{3} \) sec\(^{-1} \) in normotension, this value was \( 30 \times 10^{3} \) sec\(^{-1} \) in essential hypertension.

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OVEREXPRESSION OF PLATELET Na+/H+ EXCHANGE ACTIVITY IN ESSENTIAL HYPERTENSION

W. Siffert and D. Rosekopf

It was recently reported that platelets of patients with essential hypertension show an increased activity of the Na+/H+ exchanger. The present study aimed at investigating i) whether this increased activity would result in an increased cytosolic pH (pH_{c}) and ii) whether the increased activity is an epiphenomenon rather than a causative factor in essential hypertension. Platelets were loaded with the fluorescent pH indicator BCECF. The platelets were suspended in HEPES buffer (pH 7.4) at 37°C and the cytosol was acidified by addition of increasing amounts of propionic acid. The recovery of pH_{c} towards the initial values was recorded and the initial slopes of the fluorescence tracings were used to estimate the activity of Na+/H+ exchange. The pH_{c} of resting platelets in normotension (7.16 ± 0.04, n=10) did not significantly differ from that in essential hypertension (7.16 ± 0.04, n=8). In contrast, the initial rate of pH_{c} recovery from an artificial acid load in essential hypertension was enhanced by a mean factor of 3.9 (range 2-5; n=8) as compared to platelets from normotensives. In contrast, platelets from patients with secondary hypertension (renal artery stenosis) had both a normal pH_{c} and Na+/H+ exchange activity. The following conclusions can be drawn: i) Overexpression of Na+/H+ exchange activity in essential hypertension does not occur as a result of elevated blood pressure. In contrast, this phenomenon may constitute a causative factor in the pathogenesis of essential hypertension. ii) Overexpression of Na+/H+ exchange activity does not result in an increased pH_{c}. iii) The increased activity of the platelet Na+/H+ exchanger may contribute to the hyperreactivity of platelets in essential hypertension.

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PROPHYLAXIS OF DEEP VENOUS THROMBOSIS IN ORTHOPEDIC SURGERY: A DOUBLE-BLIND TRIAL BETWEEN LOW MOLECULAR WEIGHT HEPARIN/DIHYDRODROGOTAMINE AND UNFRAGMENTED HEPARINE/DIHYDRODROGOTAMINE

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In a double-blind trial antithrombotic and adverse effects of a single daily injection of 1500 i.P.I.(U) low molecular weight heparin plus 0.5 mg dihydrogotamine (LMWH/DHE) were compared to twice daily injection of 5000 i.U. unfractionated heparine plus 0.5 mg dihydrogotamine (UFH/DHE) in a total of 123 patients undergoing hip or knee surgery. Events of deep venous thrombosis were assessed by means of radiocontrast uptake test (RFU) and, whenever possible, verified by phlebography. The results of this study are demonstrated in the following table:

| No. of patients | LMWH/DHE | UFH/DHE |
|-----------------|----------|---------|
| Male            | 63       | 60      |
| Female          | 54       | 32      |
| Mean duration of surgery (min) | 130.7    | 133.1   |
| PRI positive    | 14       | 15      |
| Plleography positive | 11      | 9       |
| Pulmonary embolism | 0     | 0       |
| Postoperative bleeding | 45      | 44      |
| < 500 ml        | 18       | 16      |
| > 500 ml        | 18       | 16      |

As shown above were observed no significant differences in the frequencies of thromboembolic events or postoperative bleeding complications. Thus, the single application of LMWH/DHE in a dosage of 1500 i.P.T.U.5 mg a day was found to be a safe and effective therapy regimen in peripera~ prophylaxis of deep vein thrombosis in high risk patients.

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THE INFLUENCE OF RECOMBINANT HUMAN ERYTHROPOIETIN ON MACRO- AND MICROCIRCULATION, COAGULATION AND FIBRINOLYSIS IN HAEMODIALYSIS PATIENTS.

B. Braun, M. Köhler, S. Mörsdorf, F. Jung, E. Wenzel, U. Weber, R. Bottler, J. Littmann, H. Tarrach, R. Rambauer, G.A. Jutkiewicz

Recombinant human erythropoietin (rh-EPO) has been shown to be effective in the treatment of renal anaemia. rh-EPO improves the haemostatic defect of uraemia. On the other hand, a hypertensinogen effect due to an increased antihypertensive treatment regimen.

Low molecular weight heparins (LMWH) were introduced as substitutes for unfractionated heparin (UFH) as prophylactic agents against deep venous thrombosis. The use of LMWH appears to be more convenient, since due to an increased half-life and bioavailability only one injection per day is required. Nevertheless, the risk of cumulation of anticoagulant effects during long term administration has to be considered. We therefore investigated the effects of different LMWHs and UFH, when injected over a 5 day period in a randomized cross over study in 12 healthy subjects.

Fragmin (Kabwittrum, Munich), Fraxiparin (Sanofi, Munich), Embolex (for Klinische Hämostaseologie und Transfusionsmedizin und Abteilung f0r Klinische Hämostaseologie und Transfusionsmedizin und Abteilung für Dialyse und Nephrologie der Universität des Saarlandes, D-66550 Homburg/Saar, F.R.G.

THE EFFECT OF LOW MOLECULAR WEIGHT HEPARINS (LMWH) AND UNFRACTIONATED HEPARIN (UFH) DURING SUCCESSIVE ADMINISTRATION IN HEALTHY SUBJECTS:
(1) EFFECTS ON PRIMARY HEMOSTASIS, FIBRINOLYSIS AND ADVERSE EFFECTS

M. Köhler, G.Weishaupt, G. Pindur, B. Wagner, M. Heiden

Low molecular weight heparins (LMWHs) are more and more substituting unfractionated heparin (UFH) as prophylactic agents against deep venous thrombosis. The use of LMWH appears to be more convenient, since due to an increased half-life and bioavailability only one injection per day is required. This, however, bears the risk of cumulation of anticoagulant effects during long term administration. We therefore investigated the effects of different LMWHs and UFH, when injected over a 5 day period in a randomized cross over study in 12 healthy subjects.

Low molecular weight heparins (LMWHs) are introduced as substitutes for unfractionated heparin (UFH) in order to decrease adverse effects of conventional prophylaxis of deep venous thrombosis. There are, however, no controversial communications on the effect of UFH and LMWH on the fibrinolytic system. We therefore investigated the effects of different LMWHs and UFH, when injected over a 5 day period in a randomized cross over study in 12 healthy subjects.

Fragmin (Kabwittrum, Munich), Fraxiparin (Sanofi, Munich), Embolex (without dihydroergotamine, Sandoz, Nürnberg) and Liquemin (Roche, Grenzach-Wyhlen) were injected subcutaneously using the recommended dosage at 8:00 for 5 days. Between the different drugs a washout period of 14 days was applied. Investigations on hemostasis were performed using standard methods and commercially available test systems. The anticoagulant effects (LMWHs calibrated against the WHO standard for LMWH) 3 h after the 5th injection (3 h after the 1st injection in parentheses) are shown in the following table (mean values):
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HYPOFIBRINOGENEMIA CAUSED BY VINCristINE/PREDnisOLONE THERAPY OF LYMPHOID BLAST CELL CRISIS OF CHRONIC MYELOID LEUKEMIA
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Therapy with vincristine and prednisolone caused a pronounced decrease in fibrinogen levels in patients with lymphoid blast crisis (LBC) of chronic myeloid leukemia (CML). In two patients the initial decrease in white blood cell count (WBC) in response to therapy was followed by a marked decrease in fibrinogen levels to values below 80 mg/dl, within the first three days of treatment, a pronounced rise in D-Dimer levels, the occurrence of soluble fibrin in the circulation and a drop in platelet count to values below 20,000 cells/ul. Induction of disseminated intravascular coagulation (DIC) in these two patients caused profuse bleeding and necessitated substitution therapy with fibrinogen and platelet concentrates. In the remaining 7 patients no signs of DIC were detected after initiation of therapy, nevertheless of them showed a moderate increase in D-Dimer levels. In these patients a well known side effect of steroid therapy, namely a decrease in fibrinogen levels according to the half life of 96 hours was observed. Fibrinogen metabolism can be observed during vincristine/prednisolone therapy of LBC in CML: (1) a decrease in fibrinogen levels according to its biological half life due to a steroid mediated impairment of liver synthesis, (2) a rapid fall in fibrinogen levels in the course of DIC most likely induced by the release of procoagulants from deteriorating blast cells, leading to severe bleeding. Therefore blood coagulation parameters should carefully be monitored in such clinical settings.

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DYNAMICS OF PLATELET ULTRASTRUCTURE WITHIN THE FIRST SECONDS OF PLATELET ACTIVATION
E.Morgenslein, A.Ruf, and H.Patscheke

Shape change (1), internalization of surface-bound ligands (2) and the exocytosis of secretory organelles (3) characterize stimulated platelets. These reactions involve membrane rearrangements and the action of the contractile cytoskeleton and appear to run off simultaneously. In the first seconds of activation, the platelets were stimulated for 10 up to 60 seconds with thrombin or collagen and than chemically fixed or - in demonstration of membrane fusion - rapidly frozen (brake freezing) and freeze-substituted. Using electron micrographs from serial sections the structural alterations were reconstructed. (1) Within the first seconds of platelet stimulation the membranes of the "surface connected system" were evaginated for the purpose of surface enlargement required to change shape. (2) Simultaneously, the platelets started to internalize surface areas with bound ligands. The plasmalemmal invaginations were observed attached to the contractile cist and thus they followed the cistally moving constriction. This could be demonstrated for several surface-bound ligands as cationized ferritin, fibrin as well as collagen fibers. The internalization of fibrillar ligands by platelets in this mode causes the retraction of fibers. (3) Within the first seconds after stimulation the membranes of the secretory organelles form appositions to the plasmalemma or to membranes of the secretory cisternae and seemed to maintain this position during shape change and internal contraction. The exocytosis started after formation of a fusion pore and was then continued by compound exocytosis of secretory granules. The observed membrane rearrangement may explain how platelets are able to carry out their different activities (shape change, surface-ligand interaction, release reaction and retraction) within a short period of time. Therefore, a retrieval of membrane and integrated components seems to occur (supported by DFG, Grant Mo 1244/2-2/3).

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RESTITUTION AND ACTIVATED PLATELETS IN THREE-DIMENSIONAL RECONSTRUCTION
P.Dierichs and E. Morgenstern

Human and bovine platelets were fixed by impact freezing and freeze-substitution in resting state and after activation. Electron micrographs of serial sections were utilized by a Macintosh II computer (Apple Inc.), and three-dimensional reconstruction was done with regard to the surface shape, the distribution of internal components, the contractile sphere, and the distribution of microtubules. The program allows presentation of different selections of the components of the models under various aspects. Transparent reconstruction of some surfaces give insight into internal organizations.

The cryo-preparation acts very rapid. Conformational changes of platelets therefore most likely are the result of activation, and not to be regarded as artifacts due to the slow process of chemical fixation.

Both platelet types have a discoid shape in the resting state. In both types activation causes the formation of a contractile sphere. By this way cellular compartments are redistributed and centralized. The dense tubular system forms a branched membrane complex with narrow lumina which occasionally enlarge into distinct vesicles. As bovine platelets have no surface-connected canalicular system, they perform no shape change in the way human platelets do. Nevertheless, they exhibit membrane compartments that have contact to the cell surface and result from fusion of granular membranes with the cell surface when secretion has occurred.

The computer models give evidence of the complex morphological reorganization that occurs when platelets transform from the resting into the activated state.

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DAY TO DAY VARIATIONS OF THE FIBRINOLYTIC SYSTEM IN HUMAN PLASMA
L.Banthold, J. Grulich-Henn, D. Heinrich and G. Müller-Berghaus

In recent years the knowledge on the regulation of the fibrinolytic system has substantially increased. Several clinical conditions have been associated with alterations of the fibrinolytic system in plasma. However, it is difficult to draw definite conclusions from the laboratory data obtained as the plasma levels of several components of the fibrinolytic system, such as tissue-plasminogen-activator (tPA) and plasminogen activator inhibitor type 1 (PAI-1), are influenced by diurnal variation, age, exercise, stress, nutrition. In order to study the fibrinolytic system in plasma, it is necessary to standardize influences. We designed a highly standardized procedure for the investigation of the fibrinolytic system in plasma. Ten healthy volunteers were investigated three times, with a minimal interval of 7 days between each examination. Blood was withdrawn under fasting conditions in the morning between 7.30 and 9.00 a.m., after a resting period of 20 minutes, before and after 10 minutes of venous occlusion (VO). In order to evaluate the day to day variation, the coefficient of variation (CV) was estimated for each parameter before and after VO. The mean CV for the euglobulin lysis time was 9.5% before VO and 9.3% after VO. The CVs for tPA were 14.4% and 27.0% before and after VO, respectively. PAI-1 antigen showed a CV of 10.5% before and 19.0% after VO, and the CV for PAI-capacity were 5.0% before and 33.0% after VO. There was a significant correlation between PAI-1 antigen and PAI-capacity before and after VO (r=0.64, p<0.0001, and r=0.72, p<0.0001).

These data indicate that the day to day variations in parameters of the fibrinolytic system can be kept within a low range, provided a highly standardized procedure is chosen for blood withdrawal.

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MONOCLINICAL ANTIBODIES DIRECTED AGAINST ENDOTOXIN-ACTIVATED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

J. Grulich-Henn, C.W.E. Justus and G. Müller-Berghaus

Endotoxins induce a variety of functional alterations in cultured endothelial cells such as expression of tissue thromboplastin, downregulation of thrombomodulin, and increased plasminogen activator inhibitor 1 synthesis. In order to gain more insight into the expression of surface protein expression, we immunized mice with human umbilical vein endothelial cells (HUVEC), which had been pretreated with endotoxin for 24 hours, and raised monoclonal antibodies directed against endotoxin-treated HUVEC. Four monoclonals were isolated which showed markedly increased binding to endotoxin-treated HUVEC. One of these monoclonals, designated EC-H7C7, has been characterized. EC-H7C7-binding to HUVEC increased after 4 h treatment with endotoxin (from S. enteritides), reached a maximum at 8 hours and remained constant up to at least 24 hours. The expression of the antigen was dose-dependent. As little as 1 ng/ml of endotoxin induced an increase of binding of EC-H7C7 to HUVEC, maximal binding was observed at 30 ng/ml. The antibody showed no binding to human fibroblasts before or after stimulation with endotoxin. The epitope recognized by this antibody was not induced by treating the cells with thrombin or phorbol esters which are known to induce the expression of tissue thromboplastin, indicating that it does not react with tissue thromboplastin.

The antibodies raised against endotoxin-activated HUVEC most likely recognize so far unknown epitopes on the surface of endothelial cells. This may help to characterize the surface alterations induced by endotoxin.

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LOW PREVALENCE OF ANTIBODIES TO HEPATITIS C VIRUS (HCV) IN HEMOPHILIACS AND VON WILLEBRAND PATIENTS EXCLUSIVELY SUBSTITUTED WITH VIRUS-INACTIVATED CONCENTRATES

C. Brückmaier1, R. Rasshofer2, K. Auberger1, S. Gänzl1, J. Grulich-Henn, C.W.E. Justus and G. Müller-Berghaus

25 children with hemophilia A (n=19), hemophilia B (n=4) and von Willebrand syndrome (n=2) were tested for presence of antibody to hepatitis C using a qualitative enzyme-linked immunosorbent assay provided by Ortho Diagnostic Systems. Patients were between 1 and 12 years old and had exclusively received virus-inactivated concentrates (wet heat: 60°C, 10h; steam heat: 120°C, 10h; denaturating conditions by ultracentrifugation using a 3-30% linear sucrose density gradient). VWF multimer analysis of the starting material was performed. The bulk of FVIII activity was found in all those fractions that not contained VWF dimer, and the dimeric form of vWF only. The native, plasma-derived FVIII/VWF complex was analyzed under non-denaturing conditions by ultracentrifugation. The derived FVIII/vWF complex was analyzed under non-denaturing conditions by ultracentrifugation. 

The multimeric glycoprotein von Willebrand Factor (VWF) fulfills two biological functions: it promotes platelet adhesion to exposed subendothelium and acts as a carrier protein for coagulation Factor VIII (FVIII). The present investigation was concerned with the carrier function of VWF for FVIII, specifically the VWF component of FVIII/VWF complex was studied. Native, plasma-derived FVIII/VWF complex was analyzed under non-denaturing conditions by ultracentrifugation using a 3-30% linear sucrose density gradient.

The tendency of blood to thrombus formation is affected by both haematological and haemorheological parameters depending on haemodynamic situation. Our study aimed at the determination of the risk of reversible blood viscidation under impaired flow conditions in patients with PAD and diabetic angiopathies. Basing on red blood cell (RBC) aggregability, plasma viscosity, RBC deformability and haemorheologic haemorheological risk (HR) was calculated. Spontaneous platelet aggregation (SPA) could be measured by BREDDIN method (PAT III). Investigations were performed in 210 men: 73 patients with chronic PAD, 103 diabetics (67 with vascular complications) and 34 matched normal controls.

The results showed a significantly enhanced HR, particularly in severe PAD and diabetic macroangiopathy, mainly due to considerably elevated RBC aggregation and increased plasma viscosity. Both haemorheological parameters closely correlated with fibrinogen concentration. SPA proved to be activated in the same patient groups. These changes may promote thrombogenesis, especially in areas characterized by a disturbed and passive movement of blood towards the vascular wall. Additionally, they indicate an increased risk of blood flow limitation in the disturbed microcirculation. Therefore special treatment to improve diminished blood fluidity and to inhibit raised platelet activity is indicated.

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HAEMORHEOLOGICAL RISK OF THROMBOSIS IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE (PAD)

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The multimeric glycoprotein von Willebrand Factor (VWF) fulfills two biological functions: it promotes platelet adhesion to exposed subendothelium and acts as a carrier protein for coagulation Factor VIII (VIII). The present investigation was concerned with the carrier function of VWF for FVIII, specifically the VWF component of FVIII/VWF complex was studied. Native, plasma-derived FVIII/VWF complex was analyzed under non-denaturing conditions by ultracentrifugation using a 3-30% linear sucrose density gradient. VWF multimer analysis of the starting material and VWF antigen containing fractions demonstrated a clear separation of the differently sized VWF multimers. The bulk of FVIII activity was found in the fractions composed of VWF dimer only. In all those fractions that not contained VWF dimer, FVIII activity was not measured. These data indicate that the binding protein for FVIII is the VWF-dimer. Moreover, ligand blot analysis of highly purified FVIII and electrophoretically separated VWF-multimers showed exclusively binding of the FVIII molecule to the VWF dimer. Thus, the present data give clear evidence that the FVIII/VWF complex is formed between intact FVIII molecule and the dimeric form of VWF only.

Klinische Forschungsgruppe für Blutgerinnung und Thrombose der Max-Planck-Gesellschaft, 6300 Gießen, und Behringwerke Marburg*, 3500 Marburg.
THE DIAGNOSTIC VALUE OF VON WILLEBRAND FACTOR
AS A MARKER FOR ENDOTHELIAL CELL DAMAGE VERSUS
PLATELET AGGREGATION IN VIVO

D. Diamantis, B. Pötzsch, M. Hürtgen*,
K. Schwemmle* and G. Müller-Berghaus

von Willebrand Factor (vWF) is synthesized by
endothelial cells (EC) and megakaryocytes. Elevated vWF plasma levels have been shown in
different diseases affecting the vascular
endothelium and have been interpreted to be a
sign of EC damage. However, there are many
questions concerning the mechanism of vWF
increase in these patients and the information
one can get from increased vWF levels. These
questions were addressed in a clinical trial on
patients undergoing isolated extremity perfu-
sion. During this procedure, the leg exhibiting
the malignant tumor is connected to an extra-
corporeal circulation and perfused for 1 hour
with anti-cancer agents. EC damage during the
perfusion procedure was demonstrated by light
and electron microscopy. Three days after
perfusion had been performed, maximal vWF
antigen and ristocetin cofactor activities
values were observed, while a maximal decrease
of platelet count with elevated PF4 values was
seen 1 hour after operation. The time interval
between maximal platelet consumption and vWF
antigen peak demonstrated the EC origin of the
measured vWF. Furthermore, the time interval
between EC injury and maximal vWF plasma level
revealed de novo synthesis of vWF. From these
results we conclude that isolated limb
perfusion with anti-cancer agents is a useful
model for studying EC damage in vivo.

Klinische Forschungsgruppe für Blutgerinnung
und Thrombose der Max-Planck-Gesellschaft und
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EFFECTS OF PLASMINOGEN ACTIVATORS ON THE COAGULATION
SYSTEM
M. Barthels, D. Gulba*, U. Bohn, U. Polensky**,
H. Poliwoda

In the last years interactions of plasminogen-activators
with the coagulation system have been reported. During
fibrinolytic therapy a rise of FPA and thrombin-anti-
thrombin complex (TAT) has been observed. We investi-
gated the in vitro effect of several plasminogen activ-
ants in high concentrations on citrated normal plasma.
The reagents used in final concentrations of 100.000 u/ml
plasma were rt-PA (Actilyse Thomae, Riberach = 1 mg),
proukinase Sandoz/Nürnberg, Urokinase Deutsche Kabi-
München, Streptokinase Behringwerke Hanover, rt-PA,
proukinase and urokinase had a low thrombinlike proteo-
lytic activity when tested on the chromogenic substrate
S-2238, 100.000 u/ml corresponding to 0.4-5 u/ml. When
added to plasma an effect was even noted for concentra-
tions of 10.000 u/ml for rt-PA and puk. With urokinase
the effect was less visible. Streptokinase had no
effect. At the same time a rise of FPA could be observed
from about 10 ng/ml to 2000-3000 ng/ml for rt-PA, puk and
less with 1000 ng/ml for urokinase. Streptokinase induced
a rise only about 50 ng/ml. At the same time the high
concentrations of plasminogen activator, proukinase and
urokinase led to a loss of antithrombin activity of 30-50 % in plasma as well as in AT concentrate (Kybernin,
Behringwerke) with a small rise of TAT. - This effect was
again not noted with SK. - We conclude that several kind
of plasminogen activators can stay those with an affi-
nity for fibrin have beside their fibrinolytic activity
an additional but lesser effect on the coagulation
system.

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Vergleichende Untersuchungen zur Blutgerinnungs-
analytik mit MLA 900/1000 und KC 10

H. Menke, H.Th. Brüster

In der Gerinnungsanalytik überwiegen zur Zeit noch
auf koagulometrischer Basis arbeitende Geräte wie
ebeneisweise der KC 10 oder KC 40 (Anmelung). Jedoch
ist die methodische Umstellung auf nephelo-
metrische Bestimmungen und Automation bereits
in Vollen Gange, seit Geräte wie der MLA 900/1000 von
Baxter oder der ACL 300 von Il kommerziell verfü-
gbar sind. Bei dieser Vergleichsuntersuchung wurde
der MLA 900/1000 hinsichtlich der Vergleichbarkeit
Präzision und Handhabung dem KC 10 gegenüberge-
stellt. Der MLA 900/1000 überzeugt auch in Bezug
auf seine Präzision und gute Korrelationsmöglich-
kheit, insbesondere bei Quick, PT und mit Ein-
schränkung auch Fibrinogenbestimmung nach Clauss.
Die Handhabbarkeit ist gut und führt im Vergleich
zum KC 10 zu einer Arbeitsleichteitung.

Probleme ergeben sich jedoch bei der Überwachung
von Heparintherapien mit Hilfe der PTZ sowie bei
der von Hersteller propagierten Quick-derived
Fibrinogenbestimmung. Hierbei ist, jedenfalls
zur Zeit, die erforderliche Standardisierbarkeit
noch nicht erfüllt.

Vorteilehaft ist beim MLA 900/1000 zusätzlich die
Möglichkeit zur Durchführung von Bestimmungen
mit chromogenen Substraten, beispielsweise bei
der AT III - und Plasminogenbestimmung. Derzeit
werden analoge Vergleichsuntersuchungen mit dem
ACL 300 R von Il durchgeführt, deren Ergebnisse
zum Zeitpunkt der Veranstaltung ebenfalls vorlie-
gen werden.

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Thrombosis, D-6300 Giessen.
Diagnosis of Hypercoagulability at the Initial Phase of a Phenprocoumon Therapy. J.U. Wleqlng, A. Maienbom, H. Kesterlng and H. Pelzer*

Phenprocoumon was administered to 10 healthy volunteers at a dosage of 15 and 12 mg in the first two days. Four of them received 1 mg vitamin K after 3 days. Blood samples were drawn every 12 hours up to 7th day. Factor VII and protein C reached minimal levels of 10 to 15% during the first two days and subsequently fell to subnormal values indicating the efficacy of the anticoagulant therapy. Two days after the last phenprocoumon administration, coagulation factors and protein C tended to normalize but in some courses TAT and FPA levels were increased again.

This study provides insights into the activation of the coagulation system: TAT and F1-2 proved to be sensitive indicators of thrombin generation, which, however, due to the inhibitory function of antithrombin, does not necessarily lead to an increased fibrinogen fibrin turnover with FPA release and formation of soluble fibrin. The measurement of TAT and in particular of F1-2 may demonstrate a latent hypercoagulability even before fibrinogen-turnover is increased. Additionally, these results elucidate the inhibitory potential of the patient's coagulation system and to prevent coumarin necrosis or other thromboembolic events. Furthermore, at initial dosages of more than 9 mg phenprocoumon per day an additional anti-coagulant therapy with heparin should be considered.

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LIPID METABOLISM CHANGES DURING LONG TERM TREATMENT WITH STANDARD HEPARIN AND LOW MOLECULAR WEIGHT HEPARIN IN HAEMODIALYSIS PATIENTS

J. Schreder, M. Kandl, V.W. Armstrong, W. Sibille, H. Kösterling, F. Scheler

The effects of the low molecular weight heparin Fragmin® (LMW) were compared to standard heparin (SH) in 2 long-term studies in haemodialysis patients. LMW heparin showed a significantly lower lipolytic activity than SH heparin. A repeated stimulation of lipolysis during long-term therapy with standard heparin can lead to an exhaustion of the lipolytic activity and can thereby contribute to the formation and progression of hypertriglyceridemia. This could be shown in 5 healthy persons: a 4-hour therapy with SH heparin in comparative doses to haemodialysis patients lead to a significant reduction of the stimulation of the lipolysis for 24 hours. On the other hand a 4-hour treatment with LMW heparin did not lead to an exhaustion of the lipolytic activity. In the first study 70 newly accepted patients receiving haemodialysis were treated randomly with either LMW or SH heparin for 12 month. A significant rise in triglycerides levels (TG) as well as in the VLDL-fraction occurred in the SH heparin group. Especially extremely high TG values (> 450 mg/dl) were found in 6 patients after 12 month in the SH group but not in the LMW group. In the LMW group there was no rise in TG. Cholesterol, LDL and HDL did not change in either group.

To check whether established hypertriglyceridemia in HD patients is reversible, 218 patients with triglyceride values over 350 mg/dl were treated randomly with either SH or LMW heparin for 12 month. In the LMW heparin group there was a significant reduction of the elevated TG values, not however in the SH heparin group.

The clinical significance of these favourable results effects on triglycerides changes in hemodialysis patients. Especially the atherogenic potency of triglyceride-rich remnant particles in hemodialysis patients had been proven.

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UNCOMMON VON WILLEBRAND FACTOR MULTIMERS IN PATIENTS WITH SUSPECTED VON WILLEBRAND DISEASE

U. Budde

Von Willebrand disease (VWD) is classified by analysis of von Willebrand factor multimers in SDS agarose gels. Type II VWD is characterized by missing larger multimers and subdivided further into the subtypes IIA – IIG according to the different banding patterns. In 9 patients we observed minimal but constant changes which do not fit into any of these subgroups. 6 patients showed absence of only the largest multimers with a slight increase of the fastest migrating subband of the triplet. In 2 patients all multimers were present, but the proportion of the fastest migrating subband was relatively increased. 1 patient had the whole series of multimers, but the smallest oligomers did not resolve into a triplet pattern. 1 further patient showed a grossly abnormal triplet pattern, we never observed in type IIA or IIB patients. In no case there was any evidence of an acquired VWD. The significance of these findings will be discussed.

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DECREASED COAGULATION FACTOR XIII LEVELS IN P. FALCIPARUM MALARIA

Ch. Foth, Ch. J. Hemmer, F. Holst, R. Egbring, and M. Dietrich

Organ complications of P. falciparum malaria have been linked to hemostatic alterations including fibrin formation. Therefore, we analyzed coagulation factor XIII (FXIII) plasma levels in 45 patients with P. falciparum malaria before, during and after antiparasitic treatment. 22 of these patients had organ complications (complicated malaria). Antigen concentrations (subunits a and s) were measured by Laurell electrophoresis while plasma activity was determined functionally as FXIII dependent incorporation of 125-I–putrescine into casein. Subunit a and s antigen concentrations in activity levels correlated closely to each other (r = 0.60; p < 0.001).

FXIII levels (activity 74%, subunit a 77%; subunit s 90%) were somewhat decreased in untreated P. falciparum malaria and increased (p < 0.025) during antiparasitic treatment. 14 of 22 patients with organ complications, while none of 23 patients with uncomplicated malaria had FXIII activity and subunit a levels below 50% or subunit s levels below 75% (p < 0.001). Low FXIII activity and antigen (a and s) levels correlated (p < 0.001) to high parasitemia (-0.66, -0.59, and -0.53, resp.), but not to thrombin-antithrombin-III (TAT) plasma levels.

The results show that P. falciparum malaria can be associated with a reversible decrease in FXIII antigen and activity levels. Low FXIII antigen and activity levels tend to be associated with organ complications (complicated malaria). Since both subunits (a and s) are involved, and since no correlation was found between FXIII and TAT levels, it could be suspected that – in addition to hemostatic alterations – unspecific procoagulation might play a role in complicated P. falciparum malaria.

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PARAMETERS OF FIBRIN METABOLISM CORRELATE TO SEVERITY IN P. FALCIPARUM MALARIA

Ch. J. Hemmer, T.W. Sief, R. Egbring, F. Holst, P.P. Nawroth and R. Dietrich

The relevance of hemostatic alterations in P. falciparum malaria remains uncertain. Electron microscopy has demonstrated intracapillary fibrin strains in brain tissue from patients who had died from P. falciparum malaria (McPherson et al., Am. J. Path. 1985). Therefore, we measured plasma levels of thrombin-antithrombin-III (TAT) complexes, fibrin monomer (FM) and plasminogen activator inhibitor (PAI) activity in order to test whether fibrin metabolism is affected during illness. TAT complexes were measured by sandwich ELISA, FM via their stimulatory effect on the activity of tissue plasminogen activator, and PAI activity (PAI 1 + 2) was determined functionally as the inhibitory effect of the sample versus a defined quantity of urokinase.

All three parameters were elevated in untreated P. falciparum malaria (TAT: 8.0 ng/ml; FM: 8.6 nmol/l; PAI: 5.1 U/ml). In comparison to pretreatment levels, they decreased during antiparasitic therapy (p < 0.001). FM correlated to TAT (r = 0.40; p < 0.01), and PAI (r = 0.37; p < 0.05), while all three parameters correlated to parasitemia (TAT: r = 0.44; FM: r = 0.48; PAI: r = 0.48; p < 0.01). We were higher in complicated than in uncomplicated malaria (TAT: p < 0.05; FM: p < 0.01; PAI: p < 0.001).

These results show that P. falciparum malaria is associated with signs of increased fibrin formation in vivo while the elevated PAI activity levels act against fibrinolysis.

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INCREASE OF THROMBIN-ANTITHROMBIN III, t-PA ANTIGEN, C3a, C4a AND HISTAMIN IN THE BLOOD AFTER SHORT MAXIMAL EXERCISE

U. Oder, B. Dufaux, H. Liesen.

In eleven healthy young subjects the effects of a short exhaustive bicycle exercise were examined on thrombin-antithrombin III complex, tissue-plasminogen activator, complement fragments C3a and C4a and histamin in the blood. The analyses were carried out thirty minutes before and immediately before exercise, immediately post-exercise and thirty and sixty minutes later. As evaluated from the percentage change of haemoglobin and haematocrit, the plasma volume did not change significantly after the exercise test. Immediately post-exercise thrombin-antithrombin III, tissue-plasminogen activator, complement fragments C3a and C4a and histamin were all significantly elevated (p < 0.01) compared with the pre-exercise values. Thirty and sixty minutes later the values normalized and significant differences with the pre-exercise values could no longer be measured.

The present results support the concept of an activation in vivo of coagulation, fibrinolysis as well as of the complement system after short maximal exercise.

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DETECTION AND QUANTIFICATION OF UROKINASE-TYPE PLASMINOGEN ACTIVATOR (tPA) IN PLASMA AND TUMOR TISSUE OF BREAST CANCER PATIENTS

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A protocol was designed to detect and quantify tumor cell-derived urokinase-type plasminogen activator (tPA) by ELISA and Western blot, and to measure its activity in plasma by fluorometric assay. Monoclonal antibody (mAb) 378 was used to locate tPA in breast cancer cells in formalin-fixed paraffin-embedded tissue specimens (APAP method) in the cytoplasm and on the plasma membrane. mAb 378 is in complex with the biotinylated mAb 378 was applied to quantitate various forms of urokinase by ELISA. As verified by Western blot analysis, mAb 378 and mAb 378 bind to the proenzyme form (pro-tPA), the high and low molecular weight forms of tPA (HMW-tPA, LMW-tPA). mAb 378 is directed to an epitope on the A-chain of tPA whereas mAb 378 detects an epitope on the B-chain. The ELISA detects the proenzyme form of urokinase (pro-tPA), proteolytically degraded enzymatically active and inactive tPA, and tPA complexed with the inhibitors PAI-1 and PAI-2. The detection limit is < 40 pg tPA/ml.  

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HYPERFIBRINOLYSIS ASSOCIATED WITH ORTHOTOPIC LIVER TRANSPLANTATION (OLT)

G. Himmelreich, H. Liesen, B. Kierzek, B. Jefèbre, F. Neuhaus, D. Hahn.

OLT is frequently complicated by bleeding, especially after graft reperfusion. One of the causes may be hyperfibrinolysis. Activity and antigen of tissue-type plasminogen activator (tPA; tPA-Ag), activities of plasminogen activator inhibitors (PAI) and "intrinsic plasminogen activators" (IPA), urokinase-type plasminogen activator antigen (uPA-Ag) as well as thrombelastography (TEG) and further parameters of plasmatic coagulation were monitored in the course of 9 consecutive OLTs (table).

Our data confirm previous results that indicate hyperfibrinolysis developing during the anhepatic stage (a.st.) and peaking in the early re-perfusion (rep.) period mostly due to tPA increase. In addition intrinsic fibrinolytic activity may be involved. We believe that anti-fibrinolytic therapy may be indicated in OLT.

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HYPERFIBRINOLYSIS IN ORTHOTOPIC LIVER TRANSPLANTATION (OLT) MAY BE AGGRAVATED BY GRAFT LIVER PERFUSATE

G. Himmelreich, B. Kierzek, G. Blushardt, F. Neuhaus, H. Liesen.

OLT is frequently complicated by bleeding, especially after graft reperfusion. Hyperfibrinolysis has been recognized to develop during the anhepatic phase and to get worse in the early period of graft liver reperfusion. We analyzed early samples of graft liver perfusate (GLP) in 9 consecutive OLTs. Tissue-type plasminogen activator activity (10.2±5.8 U/ml; mean±SD) and antigen (2.0±2.2 U/ml) were clearly elevated above the normal range. This was reflected by signs of overt hyperfibrinolysis in thrombelastography. The extent of fibrinolytic activity observed in the GLPs correlated with the increase in fibrinolytic activity detected in the patient between the late anhepatic and early reperfusion periods.

Our observation may be helpful for the timing and dosing of antifibrinolytic therapy in OLT.

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STANDARDIZATION OF TURBIDIMETRIC MEASUREMENTS OF PLATELET ACTIVATION.

H. Patscheke and W. Hornberger.

Turbidimetric measurements of platelet function lack a reference with which different instruments and procedures could be standardized. As a consequence, quantitative results strongly depend on the type of the aggregometer, the mode of its adjustment and cannot be directly compared between different laboratories. We present a method of a standardization that refers to the platelet concentration. It is based on a linear relationship between the signal measured and the platelet concentration. It is based on a linear relationship between the signal measured and the platelet concentration.

We developed a computer-assisted universal aggregometer in which the range is adjusted with a dispersion of definite turbidity and the platelet-suspending system can be referred to an actual range. The computer software provides a line monitoring of the signals from 4 aggregometer channels and calculates the final result. The instrument works with platelet concentrations between 0.5 and 8 x 10^8/ml. A universal interface can also be connected to the analogous exit of any conventional aggregometer. (Supported by the DFG, Pa 263)

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HIRUDIN VERSUS HEPARIN IN ENDOXIN INDUCED DIC

G. Nowak

We have repeatedly demonstrated the preventive effect of the specific thrombin inhibitor hirudin on DIC in various animal models. In this context, special interest was devoted to the influence of hirudin on the clinically relevant endotoxin-induced localized or generalized microthrombosis. The generalized form known as Shockwave-reaction (LSR) was induced by endotoxin infusion (1.5 mg/kg x h) in young pigs. The localized Schwartzman reaction (LSR) was induced by injection of endotoxin in rabbits, followed by intravenous provocative injection of endotoxin on the next day. To detect fibrin deposits the animals were pre-treated with 12SI-Fibrinogen and the accumulation of radioactivity was measured with a gamma counter. When hirudin (0.05 mg/kg x h) was given, the endotoxin-induced consumption of clotting factors which is typical of GSR was less pronounced and the number of fibrin deposits in the lungs, kidney, liver and spleen was diminished. The increase in the right ventricular pressure was delayed. The animals survived the otherwise lethal endotoxin infusion. Compared to hirudin, heparin (0.5, 1 or 2 mg/kg x h) could not prevent the endotoxin-induced changes and the animals died during the endotoxin infusion. Hirudin prevented also the development of the LSR. Fibrin accumulation on the skin area and the haemorrhagic-necrotic reaction were suppressed by hirudin (0.2 mg/kg s.c.) 30 min prior to the provocative endotoxin injection.

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ABSENCE OF ANTIBODIES TO HCV AND PREVALENCE OF ANTIBODIES TO PARVOVIRUS B19 IN CHILDREN WITH HAEMOPHILIA A AND B TREATED WITH DIFFERENT VITAMIN K CLOTTING FACTOR CONCENTRATES.

W. Kreuz, G. Aursawad1, M. Roggendorf1, D. Lübbe, M. Funk, T. Göhrdt, T.C. Schwarz1, B. Köhler

The ISTH criteria for the safety of new commercial clotting factor concentrates with respect to hepatitis are evaluated by a determinated set of clinical trials (Miami, FL, 1994). Until now a viral transmission was easily detected by screening tests for HBV or HCV, but hepatitis BV could only be avoided by surrogate tests. Now the possibility to improve a NASVY transmission by detection of anti HCV with the second generation "ortho-HCV" enzyme linked immunosorbent assay (ELISA) developed by Ortho Diagnostik, New Jersey. A further criterion for safety is the inactivation of parovirus B19, because this virus is designated as one of the most thermonassistent viruses in blood products. Anti HCV tests and anti B 19 tests were conducted in three groups of "virgin" patients, who were exclusively treated with defined concentrates:

1) 49 haemophilia A patients, who were treated since 1980 with a pasteurized factor VIII concentrate, tested in aqueous solution at 60 °C for 10 hours (Hemate® HS, Behringwerke Marburg).
2) 5 haemophilia A patients, former high responder patients, who had received high doses of factor VIII concentrates (Hemate® HS) and rape seeded activated prothrombin complex concentrates (Fetia® S-TM, Immuno, Wien) in the course of an inhibitor elimination therapy.
3) 2 haemophilia B patients, who received UpV-prop substituted treated concentrates (PPSB Bioter, Diessel). Group 4) 16 healthy children as a control.

Absence of antibodies to HCV and prevalence of antibodies to parovirus B19 in children with haemophilia A and B treated with different viral clotting factor concentrates. (Supported by the DFG, Pa 263)

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HEMOSTATIC FACTORS AS INDICATORS OF POSTTRAUMATIC ORGAN FAILURE

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Severe polytraumatic events are often deteriorated by multiple organ failure (MOF) in the later posttraumatic course. Therefore, the availability of easily measurable plasma parameters predicting a forthcoming organ complication as early as possible is urgently needed. In this respect we evaluated the predictive value of the hemostatic parameters prothrombin, antithrombin III, plasminogen, D-dimers, antithrombin, protasmin and C1- Inhibitor either separately or summarized in the PFI-index according to AASEN in a prospective study on 69 polytraumized patients (injury severity score > 30 points). In addition, we studied factor VIII antigen as well as tissue plasminogen activator (t-PA), t-PA inhibitor 1 and -dimers. During the observation period (usually 14 days) 11 patients developed lethal MOF, 29 patients overcame reversible organ failure and 29 patients did not show signs of organ dysfunction. At the time of admission to the hospital (mean value: 49 min post trauma) AT III, prothrombin, D-dimers and t-PA indicated the development of later organ failure with specificities and positive predictive values above 60 %. All other parameters did not behave as clinically relevant prognostic factors in this respect. Moreover, the data of AT III and D-dimer at the time of admission allowed a clear differentiation between patients who died and those who overcame the traumatic events. From the 4th posttraumatic day onwards also the PFI-index indicated the later organ dysfunction sensitivity and a specificity value above 60 %.

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