PHARMACOKINETICS AND PHARMACODYNAMICS OF RECOMBINANT HIRUDINS

L. Iyer, M. Kosz, G. Isik, J. Shavit and J. Fareed

The pharmacokinetics and pharmacodynamics of two recombinant hirudin preparations from Knoll AG and Hoechst AG (specific activities of 5485 and 15,000 anti-thrombin units-ATU/mg, respectively) were studied. A single dose (2,500 ATU/kg, i.v.) pharmacokinetic study for anticoagulation was carried out in adult, mongrel dogs (n=4 in each group). The plasma samples obtained at various times were evaluated in a series of global clotting assays and a chromogenic anti-IIa amidolytic assay. Plasma levels of the recombinant hirudins were determined by interpolation from the calibration curves prepared earlier. Pharmacokinetic parameters were obtained using a curve-peeling program ('RESTIP'). The dose-response curves for the tests indicate that the pharmacodynamics of hirudin is assay dependent. Calibration curves for Ca++TT (5 and 10 U/ml) were sigmoidal in shape, indicating dose-dependence and saturation. These two curves were used to determine plasma levels of recombinant hirudin for pharmacokinetic analysis. The elimination half-lives (t1/2, res) were 30.77 minutes and 28.84 minutes for Knoll and Hoechst hirudins, respectively, using the calibration curve for Ca++TT (50 U/ml). Using the calibration curve for Ca++TT (100 U/ml), they were 37.22 minutes and 26.58 minutes for Knoll and Hoechst hirudins, respectively. The results from this study suggest that thrombin titration methods can be optimal to investigate the pharmacodynamics of recombinant hirudins. Furthermore, the pharmacokinetics as determined by the pharmacodynamic parameters, may provide different results depending on the composition of recombinant hirudins.

EXPERIMENTAL STUDIES WITH THE ANTITHROMBOTIC AGENT HIRUDIN WITH SPECIAL REGARD TO ITS INDICATIONS

F. Markwardt

The unique efficacy of the selective binding thrombin inhibitor hirudin justifies pharmacokinetic investigations in relation to its clinical use (F. Markwardt, Hamostasis 1991:21, Suppl. 1). Apart from using hirudin in postoperative venous thrombosis and in DIC, the inhibitor may be suitable for prophylaxis of arterial thrombosis. It might prove very useful in preventing reocclusion after intra-vascular lysis. Furthermore, hirudin seems to be recommendable for the improvement of extracorporeal circulation. Focussing on these possible indications, the antithrombotic action of a recombinant type of hirudin (r-H) was studied.

Under the treatment with r-H, thrombokinase and endotoxin-induced changes in the clotting system which are typical of consumption coagulopathy were less pronounced and the number of fibrin deposits in the organs investigated was significantly diminished. The incidence of thrombotic occlusion caused by extensive damage of the vessel wall as well as reocclusion of the vessels after experimental angioplasty and intra-vascular thrombolysis were r-H dose-dependent, when r-H was given intravenously. Furthermore, r-H dose-dependently reduced, when r-H was used for anticoagulation, the reocclusion time in an arterio-venous shunt model was prolonged. In experimental haemodialysis, the antithrombotic effect of hirudin is assay dependent. Calibration curves for Ca++TT (5 and 10 U/ml), they were 37.22 minutes and 20.55 minutes for recombinant hirudins. This work was in part sponsored by the International Institute of Thrombosis and Vascular Diseases.
5

RECOMBINANT HIRUDIN AS AN ANTICOAGULANT FOR CLINICAL LABORATORY BLOOD SAMPLE COLLECTION

D. Hoepfner, W. Heymann, J.M. Walenga and R.V. Barnes.

Recombinant hirudin (r-H) is a potent anticoagulant which specifically inhibits thrombin (K=10^{-6} M). Unlike heparin, r-H does not require any plasma cofactor for its anticoagulant effect. In contrast to citrate, oxalate, EDTA and heparin, r-H does not alter the electrolyte or protein composition of blood. We have used r-H (10 μg/ml) to prepare whole blood and plasma samples for various clinical laboratory measurements. Plasma samples were not suitable for global clotting tests (PT, APTT, TT); however, molecular markers of hemostatic activation (fibrinogen/fibrin degradation products, enzymes, protease cleavage products, TNF) were measurable. Electrolytes, blood gases, enzymes and proteins were satisfactorily measured as were blood counts and leukocyte differentials. r-H did not interfere in the cell staining process and washed blood cells could be prepared using r-H supplemented buffers for morphologic and functional studies. r-H can also be used as a flush anticoagulant for most automated instruments. Our observations suggest that r-H can be used as a suitable anticoagulant for clinical laboratory blood sampling. Furthermore, the analytes determined in r-H blood may be physiologically more relevant than those determined on blood collected in EDTA, citrate or heparin since blood components other than thrombin are not altered. This work was in part supported by the International Institute of Thrombosis and Vascular Diseases.

Loyola University Medical Center, 2160 S. First Ave., Maywood, IL 60153 USA

6

COMPARATIVE INVESTIGATIONS OF PLATELET ADHESION IN CITRATED AND HIRUDINIZED BLOOD SAMPLES IN PATIENTS TREATED WITH DIFFERENT PLATELET INHIBITORS

M. Basic-Mitic and H. K. Bredinin

Platelet adhesion to siliconized glass was investigated in blood samples anticoagulated with citrate (3.3 g, 1:9 v:v) blood or with a recombinant hirudin (Hoe 023 Hoechst AG, Frankfurt at a final concentration of 10 μg/ml). Using a test described in 1964 (Thrombos. Diathes. Haem. 12:269), results are expressed as index units. Normal values for citrated PRP are indices ranges from 0.8 to 1.5.

In patients receiving a Prostacyclin analogue (lloprost, Schering AG, Berlin) intravenously a marked reduction of platelet adhesion to siliconized glass was detected in both test systems. But the results obtained with hirudin as anticoagulant showed a much stronger inhibition of platelet adhesion. Similarly a stronger reduction in platelet adhesion was observed in hirudinized samples from patients who were treated with intravenous infusion of Pentoxiphyllin.

Our results show that Prostacyclin analogue and Pentoxiphyllin both inhibit platelet adhesion to siliconized glass and the inhibitory effect is more pronounced if hirudin is added. The inhibition of platelet adhesion probably is more important for the inhibition of thrombus formation than the inhibition of platelet aggregation and hirudin seems to be better suited for investigations of platelet adhesion and probably also for other platelet function than citrate as anticoagulant.

Dept. of Internal Medicine, Division of Angiology, J. W. Goethe-University, Theodor-Stern-Kai 7, 6000 Frankfurt/Main, FRG

7

INFLUENCE OF ANTICOAGULANTS ON PLATELET RESPONSES

E. Bruggener and E. Giula

The anticoagulants used for the preparation of platelet-rich plasma (PRP) influence the platelet reactions. In citrated PRP, the calcium ion concentration is diminished whereas the selective thrombin inhibitor hirudin does not change the ionized calcium level. Platelet aggregation, secretion, and thromboxane formation in plasma anticoagulated by citrate, hirudin or hirudin plus citrate were compared. No significant difference was found as to the extent of ADP-induced aggregation in citrated and hirudinized plasma. A biphasic aggregation was induced by adrenaline and PAF in citrated plasma; but the aggregation was reversible and significantly weaker in hirudinized plasma. The secretion of radiolabelled serotonin and the formation of thromboxane were markedly reduced in hirudinized plasma. The collagen-induced aggregation did not differ in both plasma samples, only the formation of thromboxane was less pronounced in hirudinized plasma. The anticoagulant showed a much stronger inhibitory effect in Hirudin anticoagulated platelet concentrates. Thrombin-independent activation of platelets was induced by adrenaline and PAF in citrated PRP anticoagulated by citrate and hirudin. The same platelet response was obtained as in citrated PRP. If calcium chloride was added to PRP anticoagulated by citrate plus hirudin before platelet activation, the aggregation and thromboxane formation were almost the same as in hirudinized PRP. These findings suggest that the antithrombotic agents do not cause any alterations in calcium ion concentration. The calcium ion concentration may account for the different platelet response. Hirudin proved to be a suitable anticoagulant for studying platelet functions at physiological calcium ion concentrations in blood.

Institute of Pharmacology and Toxicology, Medical Academy, D-5010 Erfurt, FRG

8

PROTECTIVE EFFECT OF HIRUDIN ON PLATELET FUNCTION AND ON PRESERVATION OF PLATELET CONCENTRATES

C. Pindur, R. Wenzel, M. Jacob, G. Feld, K. Stocker(*) and U. T. Seyfert

Hirudin as a potent thrombin inhibitor was added to a final concentration of 10 antithrombin units/ml (AT-U) to platelet concentrates prepared by cytophresis using citrate dextrose solution formula A (CD-A). Platelet count and distribution of platelet size (automated haematologic analyzer), platelet aggregation (Born's method), platelet complexes and D-dimers (ELISA) were evaluated. In the presence of hirudin platelet release reaction was significantly lowered, maintenance of platelet aggregability was considerably improved, and morphological alterations of platelets were less pronounced during hirudinized storage in PRP. Hirudin, even in high concentration, without CD-A is not suitable for preservation of platelet concentrates. Thrombin-independent activation steps set in after 60 min, and platelet adhesiveness drops 20% below its initial activity in Hirudin anticoagulated platelet concentrates (up to 100 AT-U) within 5 h. These results show that Hirudin alone (not even in high concentrations) is not suitable for preservation of platelet concentrates, yet, very low Hirudin concentrations (10 AT-U) in CD-A is sufficient to improve the stability of platelet concentrates during the preservation period.

Abteilung für klinische Hämostaseologie und Transfusionsmedizin, Universitätsskliniken, D-6650 Homburg, (*)Pentapharm, CH-Basel
INFLUENCE OF RHIRUDIN ON MICROTHROMBOSIS INDUCED BY RUSSEL'S VIPER VENOM (RVV)

E. Bucha, G. Nowak, and J. Meier

Russell's viper venom leads to defibrination, spontaneous haemorrhage, shock, and renal failure in man. The pathogenic reactions of this intoxication were studied in an animal model. The administration of small amounts of RVV (10-25 μg/kg x h) was followed by formation of microthrombi in rat lungs. Previous application of 125I-fibrinogen (3 MBq, 1-2 h before the experiment) and \( ^{3}H \)-labelled platelets (1 MBq, 2-4 h before the experiment) served to measure the microthrombosis continuously by means of a gamma-scintillation probe. Much lower venom doses were required for the induction of platelet deposition than for fibrin deposition in rat lungs. The administration of rhirudin before the application of venom prevented the platelet accumulation and the deposition of fibrinogen, resp. Already very low doses of hirudin were able to prevent fibrin deposition whereas much higher doses were necessary to inhibit platelet deposition (200/1000 μg/kg s.c.).

Preliminary LD50 experiments with and without rhirudin pretreatment resulted in the following findings: hirudin proved to have a beneficial effect on the animal blood coagulation system by reducing the lethality caused by RVV (4.3 fold lower LD50 values after pretreatment with rhirudin).

Institute of Pharmacology and Toxicology, Medical Academy Erfurt, Nordhäuser Str. 74, D-5010 Erfurt, FRG; *Pentapharm AG Ltd, Basel, Switzerland

INTERACTIONS OF APROSULATE SODIUM WITH THE BLOOD COAGULATION SYSTEM

R. J. Klauser and W. Ranke

Aprosulate sodium (LM 1000/2) is a chemically defined polymer with the molecular weight of 2300 dalton. This agent exhibits potent antithrombotic effects in several animal models. The interactions with the blood coagulation system were studied in global and specific assays.

In the APTT, aprosulate exhibited moderate activity while the activity in amidolytic and clotting anti-factor Xa assays was very low. The agent was virtually inactive in an amidolytic anti-factor IIa assay. However, using thrombin time measurements a low but definite inhibition of thrombin could be demonstrated. Here, a marked difference between the potencies of aprosulate to inhibit human or bovine thrombin was observed. Human thrombin was much more effectively inhibited. The anticoagulant activity in the APTT was independent of the presence of AT III. In an amidolytic assay it could be demonstrated that aprosulate inhibits thrombin by interacting with heparin cofactor II.

The prothrombin time was prolonged by aprosulate only at very high concentrations indicating that the extrinsic system is not influenced by this agent. In contrast, it could be shown in an amidolytic factor Xa generation assay that the intrinsic system was inhibited by aprosulate.

Thus, aprosulate sodium exhibits a multimodal interaction with the blood clotting system. However, the mode of action is different from heparin or low molecular weight heparins.

Department of Chemistry and Pharmacology Luitpold-Werk, Chemisch-pharmazeutische Fabrik, Eisl- stattstraße 9, D-8000 München 70, FRG

MODULATION OF THE FIBRINOLYTIC SYSTEM AND THE CONTACT PHASE BY LACTOBIOSIC ACID

W. Heller, H.P. Wendel

The aim of this study is an in vitro investigation of the systems of the contact phase of the coagulation system and of the cellular components, with lactobiocic acid (LBA) in a circuited HLM-system. In order to be able to compare the initial values to the next values and to determine changing reactions, we must eliminate the influence of the hemodilution.

Following parameters were determined: aPTT, PK, KK, KKI, F XII-inhibition, F XII, TAT, AT III, FFA, PF4, PMN-elastase. The two applied lactobionic acid dosages under discussion are 50 μg/ml and 25 μg/ml. In this study they will be referred to as LBA 50 and LBA 25. The other two dosages of 100 μg/ml and 75 μg/ml which we also used in the recirculation model are not a subject of discussion. In the model a reduction to 10 μg/ml proved to be no longer feasible. For the LBA a dosage of 50 μg/ml proves the most favourable for attaining a total coagulation inhibition. LBA shows no interaction with AT III, neither in the initial nor in the recirculation phase which is not the case with the investigated proteins. An essential difference exists between LBA and heparins in the initial phase. With the applied LBA dosages in the recirculation phase an insignificant release of PF4, whereas with the use of heparins a significant release of this parameter takes place. In this study the formation of TAT complexes lies higher significantly lower than the heparin groups. In the recirculation phase the hemolysis level is comparable for LBA and heparins.

Abt. für Thorax- und Gefäßchirurgie der Universität Tübingen, Calwerstr. 7, D-7400 Tübingen

BIOCHEMICAL AND PHARMACOLOGICAL PROFILE OF SYNTHETIC ANALOGUES OF HEPARIN

W. Jeske and J. Paredes

We have used a chemically synthesized pentasaccharide analogue of the heparin binding region to AT III and a sulphated lactobionic acid amide derivative, namely LM 10082, a specific activator of HC II along with heparin to determine the relative contribution of AT III and HC II in the inhibition of protease activation in plasma. In addition, we have used two defined biochemical systems to determine the modulatory effect of these two heparin analogues. In the plasma studies, heparin exhibited strong anticoagulant actions in the assays based on thrombin and factor Xa inhibition. Pentasaccharide only exhibited inhibitory actions towards Xa based assays, whereas LM 10082 exhibited activities in thrombin based assays. On a solar basis heparin was found to be much more active than the other agents. In defined biochemical systems using purified enzymes, heparin exhibited both the anti-Xa and anti-IIa activities in AT III supplemented systems. Pentasaccharide only exhibited anti-Xa activities and LM 10082 did not exhibit any significant activities. Thus, LM 10082 is devoid of any interaction with AT III. In the HC II assays for the evaluation of thrombin inhibition, heparin and LM 10082 exhibited profound inhibitory effects, whereas pentasaccharide was not active. These studies suggest that specific synthetic analogues of heparin such as the pentasaccharide and LM 10082 can be used to study the relative contributions of AT III and HC II in the control of protease activation during thrombogenesis. This work was in part sponsored by the International Institut of Thrombosis and Vascular diseases.

Loyola University Medical Center, 2160 S. First Ave., Maywood, Illinois 60153 USA
In some clinical trials the effect of subcutaneously administered LMW heparin in deep venous thromboses had been studied. After treatment of one week only a slight regression of thrombosis was detected. The aim of our pilot trial was to study if a LMW heparin (Sandoz, MW 5000 - 7000 daltons) given intravenously by continuous infusions leads to a sizeable reopening of occluded veins.

Dose finding and development of a monitoring regiment of this LMW heparin were additional aims of this trial. The study included 21 patients with deep vein thrombosis not older than 3 weeks verified by phlebography. Phlebography was performed before, weekly during and at the end of LMW heparin treatment. The medication was superintended all 3-4 days by checking the typical clinical signs: Anti-Xa-activity, aPTT U/kg/h LMW heparin Sandoz and was monitored by aPTT anti Xa activity and heptest. A marked increase of tPA antigen was detected in 20 patients after the third day of treatment with a minimum of 30 mg/ml and a maximum of 74 mg/ml. Regression of thrombosis of more than 30 % was found in 67 % of the patients. Reopening of occluded veins more than 70 % were observed in 9/21 patients.

In 7 of these patients phlebography (evaluation according to the Marder score) showed total recanalisation. Increase of thrombosis was not detected in any patient. This study suggest that intravenous administration of this LMW may be effective in the treatment of recent venous thrombosis. A randomized multicenter trial comparing the treatment of an LMW with unfractionated heparin is in the planning phase.

PREVENTION OF DEEP VEIN THROMBOSIS (DVT) IN PREGNANCY, AFTER CAESAREAN SECTION AND AFTER GYNAECOLOGICAL ABDOMINAL SURGERY, A COMPARISON BETWEEN LOW MOLECULAR WEIGHT HEPARIN (LMWH) AND STANDARD HEPARIN (UFH).

U.Dittmer, W.Rath, J.Schrader, C.Züchner, F.Schaler, W.Kuhn

The efficacy and the safety of the LMW Heparin Fragmin was compared to a low dose standard heparin in a prospective, randomized trial of 230 patients. The study included 30 pregnant women (group I), 100 patients with caesarean section (group II) and 100 patients with other gynaecological abdominal surgery (group III). All women at "low-risk" to develop a DVT (received 2500 anti-Xa units LMW heparin once daily or 5000 units UFH twice a day). The dosage of LMW for the "high-risk" patients was 5000 anti-Xa units once daily and for the UFH 5000 units three times a day administered subcutaneously. In group I prophylaxis was started with the immobilisation of the patient and in the operative group (UII) 500 ml dextrose were applied intravenously followed by the first Fragmin injection 6 hours later. The medication was superintended in the 3.4 days by checking the typical clinical signs of thrombosis. Before and during and the treatment the following blood parameters were analysed: Anti-Xa activity, ATIII, PTT, TT, Hb, Htc, thrombocytes, white blood cells, GOT, GPT, AP and yGT.

Only one patient in group III treated with UFH developed a DVT. No pulmonary embolism was observed. There were no significant differences between the heparin groups with regard to intensity of pain and frequency of burning during and after the subcutaneous injections. The bleedings from the injection sites after Fragmin prophylaxis showed a significant lower frequency (p<0.01). Two patients in group II reported allergic reactions against UFH in prehistory. After LMW heparin there occurred no complications. In this heparin group the average size of haematoma was significantly smaller compared to patients after UFH prophylaxis (p<0.01). For the postoperative blood loss no differences were observed. In the UFH-group three patients showed postoperative wound haematoma and two patients had problems with wound healing. In the Fragmin-group no postoperative wound haematoma were found and two patients had deflections of the sutures.

With respect to our results the LMW is as effective as the UFH in preventing DVT. The advantages of Fragmin prophylaxis are: better acceptance because of one dose regimen, lower frequency of bleedings from the injection sites associated with smaller haematoma in these areas.

PREVENTION OF DEEP-VEIN THROMBOSIS WITH LMW HEPARIN (CY 216) IN PATIENTS WITH ELECTIVE TOTAL HIP ARTHROPLASTY

H.-D. Breyer, J. van de Loo, K. Koppenhagen, J. Poister, V.W. Ruhfe, M.-D. Vandenbroeck, M. Biegholdt (for the GHAT Group)

In a double-blind randomised multicentre trial efficacy and safety of LMWHe in a fixed dose of 48 mg (10 000 anti-Xa U) were compared to unfractionated heparin (UF heparin, 5000 IU t.i.d.). 341 patients undergoing elective total hip replacement were included. 169 receiving LMWH, 172 UF heparin. DVT assessments were done by phlebography in 45 of 136 patients (33.1 %) in the LMWH group and in 47 of 137 patients (34.3 %) in the UF heparin group. The incidence of proximal DVT was 10.3 % (14/136 patients) in the LMWH group and 19 % (36/137 patients) in the UF heparin group. Pulmonary embolism (PE) occurred only in 2 of 167 evaluable patients (1.2 %) in the LMWH group and in 6 of 168 patients (3.6 %) in the UF heparin group (P=0.044, two sided).

Intra- and postoperative blood loss, transfusion requirements, haemoglobin drop, major bleeding, and frequency of wound haematoma did not differ in both treatment groups.

Intra- and postoperative blood loss, transfusion requirements, haemoglobin drop, major bleeding, and frequency of wound haematoma did not differ in both treatment groups. Prophylaxis of deep-vein thromboembolism in hip surgery with one daily dose of LMWH CY 216 (48 mg) is as effective and safe as prevention with UF heparin (5000 IU t.i.d.). There is a tendency for reduced rates of PE and proximal DVT in favour of LMWH CY 216.

"The German Hip Arthroplasty Trial Group, c/o J. van de Loo, Medizinische Universitätsklinik, Abt. Innere Medizin A, Albert-Schweitzer-Str. 33, D-4400 Münster"
THROMBOEMBOLIC COMPLICATIONS IN ORTHOPEDIC SURGERY
A PROSPECTIVE STUDY OF 598 PATIENTS
M. Krüger-Franke, F. Weis, E. Kalugina, W. Weber

From 1.10. - 31.12.1989 598 patients, who underwent orthopedic surgery in the Staatliche Orthopädische Klinik were interrogated by a pre-, intra- and postoperative questionnaire up to 3 months from operation date.

In contrary to other studies concerning thromboembolic complications in orthopedic surgery (Haas 1987, Scherer 1989, Reilman 1988) the number of patients was divided into 4 groups corresponding to the expected difficulty and duration of the operation.

46 patients in group I, who underwent minor surgery obtained 3x5000 I.E. Heparin subcutaneously, 243 patients in group II obtained apart from 3x5000 I.E. Heparin 500 ml 6% dextrane 60 preoperatively and 500 ml 10% dextrane 60 postoperatively. They underwent operations of greater intensity and duration on the upper and lower extremities. 302 patients of group III obtained 3x5000 I.E. Heparin and 500 ml 6% dextrane 60 preoperatively because of severe operations (pelvis, hip). 7 high-risk patients obtained apart from 3x5000 I.E. Heparin subcutaneously 500 ml 6% dextrane 60 preoperatively and 500 ml 10% dextrans 60 postoperatively for 5 days.

We saw in group I 2,2% thromboembolic complications while in group II 1,7% and in group III 3,3% complications with thrombosis and pulmonary embolism occurred. No such complications occurred in group IV.

The total incidence of deep vein thrombosis was 2,0% and of pulmonary embolism 0,5%. These results show the importance of preoperative risk-check and individual prophylaxis of thromboembolic complications in orthopedic and traumatologic surgery.

Staatliche Orthopädische Klinik München, Harlachingerstraße 51, 8000 München 90

IS THE APPLICATION OF Enoxaparin SUFFICIENT TO PREVENT DVT IN MAJOR ORTHOPEDIC SURGERY? DOUBLE CONTROL BY DUPLEX AND PHLEBOGRAPHY.

N. Papaioannou, G. Babis, K. Kavadias, Th. Pantza-

moupolou, K. Papadakis, M. Souelem

This prospective study (since Jan. 1990) reports on the prophylactic application of a low molecular heparin in major operations on hip and knee. To 108 patients were given 12 hrs. preoperative 40mg Enoxaparin and postoperative 40mg daily, till the full mobilisation of the patient. According to the protocol, each patient should be pre- and postoperatively controlled by Duplex, and finally by a phlebography. From the 108 patients in 3 cases a DVT and in 2 cases a non-fatal pulmonary embolism were clinically manifested. In all these cases, the clinical symptoms were in agreement with the Duplex and phlebographic findings. The only exception was a pulmonary embolism. In our patients the proximal DVT incidence was 13,9%, whereby the diagnosis was made by the Duplex technique and phlebography.

The results of the study have so far shown that:

1) The application of Enoxaparin leads to a reduction in the incidence of thrombosis and pulmonary embolism. 2) Amongst clinically symptomatic and asymptomatic patients the Duplex technique has proven itself to be an extremely reliable method for diagnosing proximal DVT. 3) In the diagnosis of proximal DVT, the Duplex technique has a sensibility and specificity of approximately 98%.

Athens Univ. Orthopedic Clinic # Dept. of Vascular Surgery # * X-Ray Dept. # ++ KAT Hospital. Correspondence to: Dr. N. Papaioannou, Kerameos st. 6, Athens 115-28, Greece.
THE CONTROL OF PLASMINOGEN ACTIVATION BY VITRONECTIN AT THE VESSEL WALL
K.T. Preisner, I. Leppandt and J. Grulich-Hann

Vitronectin (VN) functions as multivariant extracellular matrix associated component, thereby inducing endothelial cell attachment and spreading but also interacting with glycosaminoglycans and proteinase inhibitors. In particular, binding of VN to plasminogen activator inhibitor-1 (PAI-1) leads to stabilization of the inhibitor, such that VN-PAI-1 complexes are abundant in the subendothelium as well as during platelet release reactions. VN associated with the matrix of cultured endothelial cells (HUVEC) was found to be exclusively derived from the culture medium, since no de novo biosynthesis of VN could be detected in these cells. In contrast, cultured human ormental mesothelial cells (HOMC) which are in many respects similar to HUVEC, were found to accumulate in the culture medium about 10 ng VN/106 cells over a period of 48 hours. Secretion of endogenous VN in HOMC was increased about three-fold in the presence of high concentrations of heparin, while cycloheximide completely inhibited this effect. In contrast to HUVEC, HOMC did not secrete appreciable quantities of PAI-1 into the culture medium. These findings indicate that vascular endothelial cells may rely on VN derived from the circulation, whereas mesothelial cells are dependent on de novo biosynthesis of the adhesive protein. Moreover, the amount of PAI-1 deposited into the matrix of HUVEC correlated with the amount of VN found at that site, such that a VN-deficient matrix was not able to harbour PAI-1. Since binding of PAI-1 as well as plasminogen relies exclusively on the presence of matrix associated VN, this adhesive component appears to be a crucial regulatory component for the control of the initial phase of blood coagulation at the vessel wall. Whether VN has also a major influence on later events related to thrombosis remains to be established.

Abteilung Hämostaseologie und Transfusionsmedizin, Kerckhoff-Klinik, Max-Planck-Gesellschaft, Sprudelhof 11, D-6350 Bad Nauheim.

PROCAGULATORY EFFECTS OF PLASMIN: FACTOR-X- AND PROTHROMBIN-ACTIVATION
K. Henkel-van Beber, R.E. Zämmersann

Participation of plasmin in the fibrin-formation during thrombolytic therapy was examined by measuring the amidolytic activity of plasmin-derived factor X- and prothrombin-degradation-products on chromogemic substrates. The participation of plasmin in the fibrin-formation during fibrinolysis caused by a plasmin concentration of 142 nmol/l and reaches the maximum of thrombin-like enzyme activity is 0.45 % of the thrombin activity detectable by activation with Echis carinatus venome. According to our results, 45 nmol/l plasmin produced 0.06 % F Xe-like activity in comparison to the activation rate of Russell’s viper venom. The observations suggest that a surplus of free plasmin may contribute to a fibrin-formation during fibrinolytic therapy, especially when performed by fibrinogen-independent plasminogen-activation with streptokinase.

Physiologisches Institut der Westfälischen Wilhelms-Universität Münster, Robert-Koch-Straße 28, 4400 Münster, FRG

ROLE FOR OXIDIZABLE AMINO ACID RESIDUES IN THE INTERACTION BETWEEN T-PA AND FIBRIN
T.W. Stief, E. Martin, M. Vinuesa, J.M. Rodriguez

Evidence has recently been presented that activated phosphatases (Mø) express both urinary (u-PA) and tissue type (t-PA) plasminogen activator. Major cell products of Mø and polymorphonuclear neutrophils (PMN) are reactive oxidants of the human or canine type. Since PMN and Mø are involved in inflammatory and fibrinolytic processes, we were interested in the interaction of u-PA, t-PA, and plasmin with oxidants of the leukocyte type. The enzymes were treated with chloramine-1, which at pH 8.5 is a selective oxidant for methionine residues. Oxidation by chloramine-TH of u-PA abolishes about 60 % of both streptokinase-inhibitor-1, a major influence on later events related to thrombolysis remains to be established.

Department of Hematology, Univ. Hospital “Virgen del Rocío”, E-41013 Seville, Spain

MOLECULAR INTERACTION BETWEEN PLASMINOGEN ACTIVATOR INHIBITOR 1 (PAI-1) AND FIBRIN
Hartmut J. Ehrlich, Raymond Klein-Gebbink, Klaus T. Preisner, J. Peper, J.A. Heijl, and Haus-Preissner, Hartmut J. Ehrlich, Raymond Klein-Gebbink, Klaus T. Preisner, J. Peper, J. A. Heijl, and Haus-Preissner

We have recently shown that the protease specificity of PAI-1 towards thrombin is greatly enhanced by the PAI-1 binding protein vitronectin (Ehrlich et al., J Biol Chem 265, 13029, 1990). In order to identify additional cofactors for PAI-1 specificity, we investigated whether this serpin would also interact with fibrin. PAI-1 binds to fibrin-Sepharose and can be eluted with increasing [NaCl]. Binding of PAI-1 to hepatic-Sepharose can be efficiently competed with solution-phase heparin (HC~o:7uM). Whereas heparin does not interfere with the rate of t-PA inhibition by PAI-1, it accelerates the inhibition of thrombin by PAI-1 and the formation of SDS-stable complexes between PAI-1 and thrombin, and thus leads to a mutual neutralization of both PAI-1 and thrombin.

In an attempt to localize the heparin binding site in PAI-1, we utilized recombinant DNA-technology to construct 10 single point mutants of PAI-1, replacing individual positively charged amino acid residues by alanine. Recombinant wild-type and mutants, purified from lysates of transformed E. coli, were then assayed for inhibition of and complex formation with thrombin in the presence of heparin. We will show that specific arg- and lys-residues in the helix D subdomain mediate the effect of heparin on PAI-1 and relate this finding to natural mutants of other serpins with a decreased affinity for heparin.

Dept. Molecular Biology, CB of the Netherlands Red Cross Blood Transfusion Service, NL-1066 CX Amsterdam; present address: Division of Hemostasis and Transfusion Medicine, Kerckhoff-Klinik, MPG, Sprudelhof 11, D-6350 Bad Nauheim
EFFECT OF DIPYRIDAMOLE IN VIVO AND EX VIVO ON THE FIBRINOLYTIC PARAMETERS TISSUE-PLASMINOGEN-ACTIVATOR (t-PA) AND PLASMINOGEN-ACTIVATOR-INHIBITOR (PAI-1)

K. Huber, J. Wojta, M. Jörg, T. Rimplf, I. Lang, and B.R. Binder

We investigated the effect of different concentrations of dipyridamole (DIP), a platelet inhibiting and vasodilating substance, on t-PA and PAI-1 in plasma samples of patients with coronary artery disease and on t-PA and PAI-1 expression in human umbilical vein endothelial cell cultures (HUVEC) measuring t-PA antigen (ELISA) as well as PAI-1 antigen (ELISA) and activity (functional lattion assay).

PAI-1 activity and t-PA antigen levels in blood samples taken one hour after infusion of a diagnostic dose (0.7 mg/kg) of DIP exhibited significantly decreased values as compared to the pre-infusion levels (t-PA-difference: 0.76 ± 0.21 ng/ml; p < 0.001; PAI-1-difference: 0.77 ± 0.33, p < 0.05). However, addition of comparable concentrations of DIP to blood samples ex vivo had no effect. In HUVEC cultures, however, both PAI-1 antigen and t-PA antigen expression were stimulated by addition of increasing concentrations of DIP up to a cytotoxic concentration.

Decrease of t-PA and PAI-1 concentrations in vivo after DIP of diagnostic reasons might be explained by an increased liver clearance of DIP (t-PA) and due to the DIP induced increase of heart rate while DIP does not seem to be effective on fibrinolytic parameters in platelet-free plasma.

Department of Cardiology and Lab. Clin.-Exp. Physiology, University of Vienna, Austria

SHORT-TERM ULTRAHIGH STREPTOKINASE (UHSK) TREATMENT IN DEEP VENOUS THROMBOSIS

M. Martin and D.J.O. Fiebach

The present study reports on 176 deep vein occlusions which were treated by streptokinase. The UHSK scheme consisted of one or multiple I.V. infusion courses with an influx rate of 1.5 million units per hour (one course = 9 million I.U. SK over 6 hours). The number of UHSK courses varied one to four, but in most cases a 2-course scheme was administered. Without exception one UHSK course was given on one day, hence the number of UHSK courses was identical with the number of treatment days. Of the 176 deep vein occlusions the most proximal locations were: One calf vein, 109 femoral veins, 45 iliac veins, and two subclavian veins. The therapeutic result was as follows: Total clearance 74/176 = 42.0 %, partial clearance 65/176 = 38.3 %, no clearance 38/176 = 21.6 %. The occlusion history was an important factor for venous thrombosis removal. Thrombi older than 14 days did seldom respond to lytic treatment. Thrombus length was also a factor correlated with lytic success in that the shorter the occlusion was the more frequent their removal rate became. The simplicity of the UHSK technique and the low rate of bleeding complications indicate that this new SK regimen is a valuable modality in the treatment of venous occlusions.

From the Geriatric Hospital (Head: Prof. M. Martin) and the Radiology Department (Head: Prof. Dr. B.J.O. Fiebach & Prof. Dr. L. Magnus), Städtische Kliniken Duisburg, W. Germany

DOSE-RANGING STUDY OF THE NOVEL RECOMBINANT PLASMINOGEN ACTIVATOR BM 06.022 IN HEALTHY VOLUNTEERS

U. Martin, E. von Möllendorff, W. Akpan and G. Neugebauer

The recombinant plasminogen activator (rPA) BM 06.022 consists of the kringle 2- and pro-enzyme domains of human t-PA and is unglycosylated due to its expression in E. coli cells. The tolerability, the hemostatic effects, and the pharmacokinetic properties of rPA were investigated in 18 male healthy human volunteers following i.v. bolus injection over 2 min. Cohorts of 3 volunteers received successively 0.1125, 0.55, 2.2, 3.3, 4.4, and 5.5 MU rPA which was well tolerated.

The area under the activity-concentration vs. time curve (AUC) increased dose-dependently and linearly. At 5.5 MU rPA the AUC of 3132.47 IU·h·ml⁻¹ was about equivalent to that expected to represent half the AUC of the effective dose in myocardial infarction patients. Systemic clearance at 5.5 MU rPA was 306 ml/min. Residual fibrinogen, plasminogen, and α2-antiplasmin were 100 ± 3 %, 87 ± 3 %, and 79 ± 3 %, respectively. 2-h postinjection of 5.5 MU rPA. Fibrin D-dimers increased dose-dependently and were highest with 1147±380 mg/ml at 5.5 MU rPA. Clotting times remained unchanged. Platelet count did not decrease. We conclude that rPA was well tolerated after i.v. bolus injection and appeared to be a fibrin-selective fibrinolytic agent.

Dept. of Pharmacology, Boehringer Mannheim GmbH, Sandhofer Straße 116, D-6800 Mannheim 31

ROLE OF CYCLIC NUCLEOTIDES IN THROMBIN INDUCED PAF SYNTHESIS BY ENDOTHELIUM

R. Heller, P. Hussolino*, D. Ghigo*, A. Bosia*, and U. Tili

Agonist-induced synthesis of platelet-activating factor (PAF) by human endothelial cells (EC) might alter their non-thrombogenic properties as PAF is thought to mediate blood cell adhesion to EC. Thus, reduction of PAF synthesis in EC could be important for prevention of thrombus formation. We investigated the effect of agents that increase intracellular levels of cAMP ( forskolin, a direct activator of adenylyl cyclase; iloprost, a stable analog of PGI2) and of cGMP (sodium nitroprusside, a nitrovasodilator) on thrombin-induced PAF synthesis in EC. 10−50 μM forskolin caused a 2-10-fold increase of cAMP and a dose-dependent inhibition of PAF production which was complete at highest cAMP levels. Similar changes were seen with iloprost (1 μM: cAMP level 2fold, PAF synthesis inhibition 90%). The increase of cGMP by sodium nitroprusside (1 μM: cGMP level 1.5-3fold) was accompanied by a dose-dependent inhibition of PAF production up to 65%. Enhancement of intracellular cAMP or cGMP caused inhibition of thrombin-induced rise of intracellular Ca²⁺ and of Ca²⁺-dependent activation of lyso-PAF acetyltransferase (key enzyme of PAF synthesis). This points to calcium mobilization block as the major cause for the described effects on PAF production. cAMP/cGMP act synergistically in inhibiting PAF synthesis. The results suggest that endogenously generated mediators which (increase PGI2/PGI2-like effects) may modulate the synthesis of PAF thus participating in maintaining the thromboresistance of EC.

Institut für Pathol.Biochemie der Medizin.Akademie Erfurt. Nordhäuser Str.74, D-0-5010 Erfurt, *Dipartimento di Genetica, Universita di Torino, Via S. Bisia, 1-10126 Torino.
Transiently reduced platelet α-granule secretion after cytotoxic chemotherapy

A. Wehmeier, R. Spickmann, W. Schneider

Chemotherapy-induced tumor lysis may cause activation of coagulation and fibrinolysis, and may result in clinically overt disseminated intravascular coagulation if untreated. Besides procoagulant activity, platelet-activating factors can be released by malignant tumors and may lead to increased platelet consumption. In a prospective study on 19 previously untreated patients with solid tumors and malignant lymphomas, we determined changes in platelet secretion and platelet aggregation in whole blood and platelet-rich plasma (PRP) after cytotoxic chemotherapy. Intravascular thrombin activity was monitored by plasma levels of fibrinopeptide A and thrombin-antithrombin complex (TAT). Patients were investigated before, and 4, 10, and 23 days after initiation of chemotherapy.

In contrast to our expectations, B-thromboglobulin plasma levels were slightly (not statistically significant) increased on day 10 and also returned to baseline on day 23. This was accompanied by a 14% reduction of platelet a-granule secretion in whole blood but not in PRP. TAT plasma levels were slightly (not significantly) increased on day 10 and also returned to baseline on day 23.

In conclusion, platelet α-granule secretion is transiently reduced after cytotoxic chemotherapy, and platelet function may also be slightly impaired. This is not paralleled by reduced TAT levels and is thus caused by an effect of chemotherapy on megakaryopoiesis.

Department of Hematology, Oncology, and Clinical Immunology, Heinrich-Heine-Universität, Düsseldorf, FRG

RELEASE OF THE EXTRINSIC PATHWAY INHIBITOR INTO POST-HEPARIN PLASMA

J. Harenberg, M. Schäffer, G. Stehle, C.E. Dempfle and D.J. Heene

The antithrombotic properties of glycosaminoglycans can be explained only partially by their anticoagulant, profibrinolytic, antiplatelet and haemorrhhoetological effects. The release of other plasma proteins, particularly of the extrinsic pathway inhibitor (EPI), may be biologically relevant for the mechanism of action of heparins and heparin derivatives in the prophylaxis of thromboembolism.

Serial determinations of the EPI were performed in a set of ten healthy persons each after intravenous or subcutaneous administration of 5000 anti-factor Xa units of an unfractonated (UF) or a low molecular weight (LMW) heparin preparation. After intravenous injection no differences were seen between the kinetics of the EPI in post heparin plasma of the volunteers, who received UF or LMW heparin, respectively. The release of EPI was significantly higher and lasted for a longer period of time in post LMW heparin plasma after subcutaneous administration of LMW heparin.

It is suggested that the higher release of EPI may contribute to the improved biological properties of LMW heparins.

I. Medizinische Klinik, Fakultät für Klinische Medizin Mannheim der Universität Heidelberg, Theodor Kutzer Ufer, 6800 Mannheim
Anti-insulin antibodies can be detected in the serum of most type I (insulin-dependent) and type II (noninsulin-dependent) diabetic patients treated for several years with bovine or porcine insulin and even in some patients treated with human insulin. It has been shown that circulating insulin immune complexes (IIC) are present in these patients.

In cultured human monocytes/macrophages, surface expression of thromboplastin (TP) was induced by IIC in a dose- and time-dependent manner. Maximum TP (5-fold increase) was measured after 24 h of culture with IIC at a corresponding dose of 2 μg insulin/ml. No TP production was seen either by induction with insulin or anti-insulin antibody alone. These results support the link between coagulation and inflammation in the atherogenesis of diabetic patients.

Division of Cardiology, Medical School, D-3000 Hannover, and Department of Internal Medicine, University of Göttingen, Federal Republic of Germany

### Possible Role of Extracellularly Released Phagocyte Proteinases in the Coagulation Disorder During Liver Transplantation

F. Rees, M. Jochem, W. Machleidt, G. Himmelreich, R. Rosolak and G. Blumberg

Graft perfusion in orthotopic liver transplantation (OLT) is frequently associated with a DIC-like state, that may be important for the development of bleeding complications, influencing the outcome of the procedure. In 10 consecutive patients undergoing OLT, we studied two phagocyte proteinases of different origin in graft liver perfusate and in systemic blood during OLT as well as their effects on hemostasis.

As compared with plasma samples highly significant increases of cathepsin B and thrombin-antithrombin III-complexes (TAT), as well as highly significant decreases in AT III, protein C and C3-inhibitor were observed in the perfusate. VWF and fibrinogen were slightly decreased, whereas the elastase-alpha1-proteinase-inhibitor-complexes (EPI) were elevated. In plasma the activity of cathepsin B remained unchanged during the pre-reperfusion phases of OLT, but after revascularisation of the graft this cysteine proteinase excessively increased. The EPI showed a gradual increase in plasma until reperfusion when a more pronounced increase occurred. In parallel with the rise in these two proteinases after reperfusion TAT increased (p<0.02) and the activities of antithrombin III and C1-inhibitor in plasma decreased (p<0.05). Twelve hours after revascularisation plasma levels of TAT, antithrombin III and C1-inhibitor had returned to the pre-reperfusion ranges, whereas cathepsin B and EPI were significantly elevated above the baseline levels.

For the first time we could prove the occurrence of phagocyte proteinases in graft liver perfusate. The observations are consistent with our hypothesis, that extracellularly released lysosomal proteinases may play a role in the development of a DIC-like constellation including thrombin formation after revascularisation of the liver graft.

Universitätsklinikum Rudolf Virchow, Spandauer Damm 130, D-1000 Berlin 19 and Universität München, Stadtmobastenstr. 20, D-8000 München 2

### Haemostaseological Effects of Heparin-Induced LDL-Precipitation (HELP) in Patients with Hypercholesterolemia and Coronary Heart Disease

P. Schuff-Werner, E. Schütz, S. Schulz, T. Eisenhauer and V.W. Armstrong

Heparin-induced extracorporeal LDL-precipitation (HELP) is now established in the treatment of severe hypercholesterolemia refractory to conventional therapy. The procedure is based on the heparin-induced precipitation of a limited number of proteins at acidic pH. Several coagulation factors including fibrinogen bind to heparin and are precipitated in the extracorporeal circuit. The acidic pH of 5.12 might also cause acidic hydrolysis of other coagulatory proteins. In 18 patients undergoing regular HELP-treatment at weekly intervals we investigated the qualitative and as far as was possible the quantitative loss of coagulatory proteins during passage through the extracorporeal circuit. We also compared haemostaseological parameters before and after a treatment of around 3000 ml plasma. The global coagulation activity as measured by thromboplastin time (TTP) decreased to around 50%. Further analysis showed an approximately 60-70% reduction of fibrinogen, factor II, V, and VIII, whereas the factors IX, X, XI and XII decreased to around 50% of their pre-apheresis activity. In contrast, the factors VII and XIII were only reduced by 25-35%. Fibrinogen (50% decrease) and the coagulation inhibitors AT III (30% decrease), C3-inhibitor (70% decrease) and protein C (50% decrease) and S (50% decrease) were also eliminated. The rebound kinetics after apheresis show that with the exception of fibrinogen all coagulation factors are restored within 24-48 hours after apheresis. Regular treatment at weekly intervals does not lead to any coagulatory deficiency as shown by the normal haemostatic parameters measured after one or two years.

Zentrum Innere Medizin, Universitätsklinikum, Robert-Koch-Str. 40, D-3400 Göttingen

### The Significance of Lupus Anticoagulants in Childhood

A.-M. Mingers*, A.H. Sutor**, H.W. Kreth*

In children, the clinical significance of anticoagulants (LA) seems to be different as compared to adult patients. Of 39 children with LA published so far, 17 (45%) had a bleeding tendency and only 6 (15%) thrombosis. With one exception, all patients with thrombosis were over 10 years of age. (This was a 5 year old Chinese girl with systemic lupus erythematosus (SLE).)

Ten children with LA had a diagnosis of SLE (5 with a bleeding tendency, 4 with thrombosis, and 1 without haemostaseological disorders). In patients with a bleeding tendency, the inhibitor had not only blocked the intrinsic pathway but also prothrombin. Clinically relevant thrombocytopenia was only observed once. LA were recognized by chance in children without haemostaseological disorders (mostly at the time of coagulation analysis before adenotonsillectomy). In most cases LA were present for only a short time. There is evidence for a familial disposition. It is suggested that the temporary presence of LA is a frequent finding in children. However, in the young child, these antibodies are rarely associated with clinical bleeding tendencies or thrombosis.

* Univ.-Kinderklinik, Josef-Schneider-Str. 2, W-8700 Würzburg
** Univ.-Kinderklinik, Mathildenstr. 1, W-7800 Freiburg
WHAT IS THE INDEX OF HYPERCOAGULABILITY IN THE CORD-VENOUS-BLOOD IN THE CASES OF NEONATAL ASPHYXIA ?

S. Tsubuki, H. Matsuda

Although we can prove the hypercoagulability with TEG in almost all cases in cordovenous-blood, at the same time the decrease in each of the coagulation-factors was also recognized. In order to approach a resolution of these pathological contradiction, we have tried to explore the thrombin-reacting substance (APTT, TTP) and the presence or absence of fetal fibrinogen.

Methods
1) 48 cases of cordovenous-blood were divided by Apgar score, asphyxia (Apgar 7) and normal (Apgar 8).

2) The in vitro Bleeding Time of cord-venous-blood was markedly more prolonged. Particularly in the case of asphyxia, the Bleeding Time was markedly more prolonged.

College of Medical Technology, Hokkaido University N-12, W-5 Sapporo, 060 Japan

HEMORRHAGIC DIATHESIS AND THERAPY IN PREMATURE AND MATURE INFANTS

S. Popov-Covic, H-J. Herfelde, R. Esser

44 infants with bleeding tendency were examined. 37 of them were born before (median 33.5 week of pregnancy) and 7 in term. Their median age was 5.5 days, and body weight 2330 g, of which was an average of bleeding tendency was caused by the premature rather than the premature infants. Singular patients showed multiple petechiae or pulmonary bleeding. Gastro-intestinal tract, kidney or intracerebral hemorrhage. 50 % of the babies developed symptoms due to suspected sepsis, furthermore some suffered from congenital heart failure, diseases accompanied by shock, extracorporeal etc. Blood samples of all the infants were examined for platelet count, resonance thrombography (RTG), in singular cases bleeding was caused by coagulation-factor levels < 30%.

Blood samples of all the infants were examined for platelet count, resonance thrombography (RTG), in singular cases bleeding was caused by coagulation-factor levels < 30%.

The development of IgG antibodies against FVIII:C remains one of the most severe complications of haemophila treatment. The frequency is reported to be 5-25%. FVIII antibodies usually occur during growth and are associated with a high risk of spontaneous bleeding episodes. Rapid inhibitor elimination, before the occurrence of severe bleedings with possible muscle and joint impairment, is intended. Since 1979 we have conducted inhibitor elimination by inducing immunotolerance. From 1981 to 1986 we successfully performed continuous treatment with high doses of factor VIII concentrate in 14 children. Treatment schedule: Initially factor VIII was given twice daily at a dosage of 50-500 U/kg bw/day (Hemophiloid). In high responders this therapy was supplemented by activated prothrombin complex concentrate (100-200 U/kg bw-day Feiba, Immuno Wien). After inhibitor elimination doses of factor VIII and Feiba were gradually reduced and the latter discontinued after normalization of F VIII recovery. We then continuously reduced factor VIII until prophylactic dosage was achieved. Low responders received 50-60 U/kg F VIII concentrate every second or third day. Results: Elimination of FVIII inhibitors was successful in 10/14 patients. 4/14 have not yet concluded therapy. In high responders we have received FVIII concentrate as a prophylactic treatment without reappearance of inhibitor (follow up period after elimination 2-10 years). The children did not develop impairment of joint or muscle function. Conclusion: For successful and rapid elimination of FVIII inhibitors it is important to start continuous administration of high dose factor VIII concentrate and Feiba early in the course of the disease. This can ensure both: early treatment and normalization of hemostasis and FVIII inhibitors.

Inst. 1 Exp. Hämatologie und Transfusionsmedizin der Universität Bonn, Sigmund-Freud-Straße 24, 5300 Bonn 1

Calibration of the VDGH Reference Plasma for Standardization of the Normal Value of Prothrombin Time H. Lang; R. Spathes

The Prothrombin Time (PT), obtained from a fresh normal plasma pool, is the basic PT value both for the 100% of normal and for the calculation of the INR. The problematic nature of that standardization is generally known.

Now lyophilized normal plasma pools are used successfully as reference point for the assessment of precicifity studies. A lyophilized normal plasma of the Committee of Hematology of the West German Association of Diagonistics End Diagnostic Instruments Manufacturers (VDGH) was calibrated against fresh plasma pools in an international study.

A basic calibration was done by using the same certified BCR thromboplastin (BCT/099). The endpoint was determined manually and by using the coagulometer Scholinger-Gross. An additional testing where each participant used his own routine thromboplastins and methods was performed. The VDGH Reference Plasma shows a deviation from the average fresh plasma pool of 1.0 both with the BCT/099 and with the whole of the thromboplastins (PT Ref.pl. (sec)/PT fresh pl.pool (sec)). But there are obtained some statistical differences between the thromboplastins. Moreover there is a difference between plain and combined thromboplastins. But there is no statistical difference in endpoint establishment between the used methods.

Inspite of these statistical deviations the VDGH Reference Plasma seems to be suitable for the standardization of the PT-normal value.

IMMUNO AG, Industriestr. 67, A-1220 Wien; *Baxter Deutschland GmbH, Edisonstr. 3, D-8044 Unterschleissheim

FACTOR VIII INHIBITORS IN CHILDREN WITH HAEMOPHILIA

W. Kratz, S. Eloranta, R. Esser

The development of IgG antibodies against FVIII:C remains one of the most severe complications of haemophila treatment. The frequency is reported to be 5-25%. FVIII antibodies usually occur during growth and are associated with a high risk of spontaneous bleeding episodes. Rapid inhibitor elimination, before the occurrence of severe bleedings with possible muscle and joint impairment, is intended. Since 1979 we have conducted inhibitor elimination by inducing immunotolerance. From 1981 to 1986 we successfully performed continuous treatment with high doses of factor VIII concentrate in 14 children. Treatment schedule: Initially factor VIII was given twice daily at a dosage of 50-500 U/kg bw/day (Hemophiloid). In high responders this therapy was supplemented by activated prothrombin complex concentrate (100-200 U/kg bw-day Feiba, Immuno Wien). After inhibitor elimination doses of factor VIII and Feiba were gradually reduced and the latter discontinued after normalization of F VIII recovery. We then continuously reduced factor VIII until prophylactic dosage was achieved. Low responders received 50-60 U/kg F VIII concentrate every second or third day. Results: Elimination of FVIII inhibitors was successful in 10/14 patients. 4/14 have not yet concluded therapy. In high responders we have received FVIII concentrate as a prophylactic treatment without reappearance of inhibitor (follow up period after elimination 2-10 years). The children did not develop impairment of joint or muscle function. Conclusion: For successful and rapid elimination of FVIII inhibitors it is important to start continuous administration of high dose factor VIII concentrate and Feiba early in the course of the disease. This can ensure both: early treatment and normalization of hemostasis and FVIII inhibitors.

Center of Pediatrics and Internal Medicine*, University Hospital, Frankfurt/M
Mesenteric vein thrombosis due to protein C deficiency

J. Kilbmann, G. J. Wedel and M. Hoffmeister

About 10% of patients presenting with intestinal ischemia a mesenteric vein thrombosis is present. It can occur in association with polycthemia, portal venous stasis, tumor infiltration, sepsis, trauma or a hypercoaguable state. Clinically most important are deficiencies for Protein C, Antithrombin III and Protein S.

In 4 patients with the thrombosis of the mesenteric vein a Protein C deficiency could be detected. All patients had a history of recurrent deep venous thrombosis, but Protein C deficiency was known before only in one of them. Three patients developed abdominal symptoms within 1-6 days prior to admission. The patient who was known to have Protein C deficiency and therefore received long term Coumarin-therapy was admitted with upper gastrointestinal bleeding. In hospital the patient developed septicemia, followed by increasing abdominal symptoms. All patients had to undergo surgery for acute abdomen within 12 hrs. after being transferred to our surgical unit. All patients had a segmental gangrene of the bowel due to mesenteric vein thrombosis requiring bowel resection. All patients had Protein C levels below 45%, while Protein S and Antithrombin III were within the normal range. Three patients recovered and were discharged on long term Coumarin-treatment. Patient number 4 died postoperatively due to intractable septic shock.

If patients with a history of venous thrombosis develop abdominal symptoms one should always think of mesenteric vein thrombosis due to a possibly as yet undiscovered Hypercoagulability, especially if no other reason for their complaint can be identified. A thorough coagulation work-up in such a case, e.g. Protein C - Protein S or Antithrombin III deficiency can be found, long term anticoagulation is necessary to prevent further venous thrombosis.

Department of Surgery, Philipps-University of Marburg, Baldinger Straße, 3550 Marburg

Fibrinmonomer test identifies nonresponders in postoperative thrombosis prophylaxis

G. Vogel¹ and E. Spanuth²

Intravascular activation of the coagulation system produces disseminated intravascular coagulation or deep vein thrombosis (DVT) and is characterized by circulating soluble fibrin monomer complexes (FM) in plasma. In order to evaluate the prognostic and diagnostic value in 156 patients with fractures of femur, neck of the femur, tibia, fibula and with knee joint or elective hip surgery receiving low dose heparin prophylaxis (B ± 5000 U s.c./24 h) soluble FM have been determined using the FM-Test Boehringer Mannheim (erytbrocyte aggl. test acc. to Largo Largo). Plasma samples were taken prior to and on the 1st, 2nd, 3rd, 5th, 7th and 9th day following the operation. Diagnosis of DVT was carried out by 125I-labelled fibrinogen test in parallel. Positive results were checked by phlebography on the 7th or 9th postoperative day. In patients without DVT positive FM results were obtained only in 26%.

The present study aimed at the determination of a correlation between the kallikrein-kinin and coagulation systems and the effectiveness of low-dose heparin prophylaxis. 42 patients with fractures of femur, neck of the femur, tibia, fibula and with knee joint or elective hip surgery receiving low dose heparin prophylaxis (B ± 5000 U s.c./24 h) soluble FM have been determined using the FM-Test Boehringer Mannheim (erytbrocyte aggl. test acc. to Largo Largo). Plasma samples were taken prior to and on the 1st, 2nd, 3rd, 5th, 7th and 9th day following the operation. Diagnosis of DVT was carried out by 125I-labelled fibrinogen test in parallel. Positive results were checked by phlebography on the 7th or 9th postoperative day. In patients without DVT positive FM results were obtained only in 26%.

The present study aimed at the determination of a correlation between the kallikrein-kinin and coagulation systems and the effectiveness of low-dose heparin prophylaxis. 42 patients with fractures of femur, neck of the femur, tibia, fibula and with knee joint or elective hip surgery receiving low dose heparin prophylaxis (B ± 5000 U s.c./24 h) soluble FM have been determined using the FM-Test Boehringer Mannheim (erytbrocyte aggl. test acc. to Largo Largo). Plasma samples were taken prior to and on the 1st, 2nd, 3rd, 5th, 7th and 9th day following the operation. Diagnosis of DVT was carried out by 125I-labelled fibrinogen test in parallel. Positive results were checked by phlebography on the 7th or 9th postoperative day. In patients without DVT positive FM results were obtained only in 26%.

The present study aimed at the determination of a correlation between the kallikrein-kinin and coagulation systems and the effectiveness of low-dose heparin prophylaxis. 42 patients with fractures of femur, neck of the femur, tibia, fibula and with knee joint or elective hip surgery receiving low dose heparin prophylaxis (B ± 5000 U s.c./24 h) soluble FM have been determined using the FM-Test Boehringer Mannheim (erytbrocyte aggl. test acc. to Largo Largo). Plasma samples were taken prior to and on the 1st, 2nd, 3rd, 5th, 7th and 9th day following the operation. Diagnosis of DVT was carried out by 125I-labelled fibrinogen test in parallel. Positive results were checked by phlebography on the 7th or 9th postoperative day. In patients without DVT positive FM results were obtained only in 26%.

The present study aimed at the determination of a correlation between the kallikrein-kinin and coagulation systems and the effectiveness of low-dose heparin prophylaxis. 42 patients with fractures of femur, neck of the femur, tibia, fibula and with knee joint or elective hip surgery receiving low dose heparin prophylaxis (B ± 5000 U s.c./24 h) soluble FM have been determined using the FM-Test Boehringer Mannheim (erytbrocyte aggl. test acc. to Largo Largo). Plasma samples were taken prior to and on the 1st, 2nd, 3rd, 5th, 7th and 9th day following the operation. Diagnosis of DVT was carried out by 125I-labelled fibrinogen test in parallel. Positive results were checked by phlebography on the 7th or 9th postoperative day. In patients without DVT positive FM results were obtained only in 26%.

The present study aimed at the determination of a correlation between the kallikrein-kinin and coagulation systems and the effectiveness of low-dose heparin prophylaxis. 42 patients with fractures of femur, neck of the femur, tibia, fibula and with knee joint or elective hip surgery receiving low dose heparin prophylaxis (B ± 5000 U s.c./24 h) soluble FM have been determined using the FM-Test Boehringer Mannheim (erytbrocyte aggl. test acc. to Largo Largo). Plasma samples were taken prior to and on the 1st, 2nd, 3rd, 5th, 7th and 9th day following the operation. Diagnosis of DVT was carried out by 125I-labelled fibrinogen test in parallel. Positive results were checked by phlebography on the 7th or 9th postoperative day. In patients without DVT positive FM results were obtained only in 26%.
Adsorption of thrombin to expanded polytetrafluoroethylene (PTFE) was studied by the use of powdered biomaterial and determination of the adsorbed proteases with a fluorogenic substrate. Surface-bound thrombin activity was not observed unless more than 0.1 units per ml had been applied. Albumin, however, considerably improved binding with maximal effect at 0.1% albumin and declining at higher concentrations. Albumin also protected soluble thrombin from inactivation by PTFE and other hydrophobic surfaces. If PTFE was incubated with plasma, negligible thrombin activity was retained. However, after repeated treatment of PTFE with fresh plasma samples adsorbed activity rose with each step. Most likely, this activity originated from a pool of thrombin reversibly blocked by antithrombin III. It is suggested that thrombin activity also will accumulate if blood is streaming along a PTFE-surface.

Adsorbed thrombin improved the subsequent binding of fibrinogen as determined by a monoclonal antifibrinogen antibody combined with an enzyme-mediated colour reaction. In a slow reaction the fibrinopeptides A, AP and AY were released indicating fibrinogen-fibrin conversion. Fibrinopeptide B appeared very slowly.

Max Planck-Institut für Biochemie, Am Klopferspitze, D-8033 Martinsried bei München.

The adsorption of fibrinogen and other model proteins on modified agarose surfaces

H.P. Jennissen and A. Demiroglou

In principle, there is no reason to believe that blood proteins adsorbing to hydrogels or solid surfaces employed in biomedical devices will behave fundamentally different from proteins adsorbing to substituted agarose surfaces. Therefore we are applying this model system to blood proteins.

The binding of the two Ca++-binding proteins fibrinogen (human, bovine) and calmodulin, was studied on uncharged alkyl-N-(carbamate linked) and alkyl-S-Sepharoses (2) (thioether linked) by a column method. In both cases adosorption strongly depends on the degree of substitution (i.e. surface concentration) and the length of the immobilized alkyl residue. Binding is of much higher affinity on the thioether than the carbamate linked agarose derivatives. Non-adsorbing gels for fibrinogen are obtained on ethyl- and propyl-S-Sepharose at a degree of substitution of 2 µmol/ml packed gel. Increasing the chain length from ethyl-S- to butyl-S-agarose or increasing the degree of substitution from 2 to 8 µmol/ml packed gel leads to a strong adsorption of fibrinogen (ca. 0.3 mg/ml packed gel). The experiments show that agarose can be substituted with alkyl residues in such a way as to yield either adsorbing or non-adsorbing surfaces for fibrinogen and other proteins.

In parallel to the study of protein adsorption on particulate alkyl agaroses experiments are underway to apply the technique of total internal reflection fluorescence (TIRF) to the analysis of conformational changes of model proteins from blood and tissues during the adsorption and desorption cycle on alkyl modified silica surfaces.

[1] Jennissen, H.P. (1988) Makromol. Chem., Macromol. Symp. 17, 111-134

[2] Demiroglou, A. and Jennissen, H.P. (1990) J. Chromatogr., 521, 1-17

Institut für Physiologische Chemie, Universität-GHS-Essen, Universitätsstr. 55, 4300 Essen 1

Surface modification of vascular prosthesis by Endothelial Seeding.
S.B. Kövecser, R. Rösing1, B. Greger, H. Brugger2, E. Frick J. Fingerle2

The replacement of small caliber arteries with synthetic material is associated with a high risk of thrombosis. Efforts to induce antithrombotic properties of the grafts by means of Endothelial Seeding have been successful in animal experiments. However, this technology is so far not compatible with clinical situations.

It is the aim of our project to improve adhesion and proliferation of endothelial cells seeded on biomaterials. Experiments with an in vitro perfusion circuit and with mini-pigs have shown that the physico-chemical properties of the biomaterial as well as the type of preparation with glycoproteins of the extracellular matrix are important determinants of the endothelial cell seeding result. Hydrophobic grafts as Polyurethan demonstrated improved endothelial cell adhesion and retention compared to hydrophobic PTFE materials. Coating of graft material with glycoproteins had beneficial effects on endothelial cell adhesion. Fibronektin and Vitronektin (25 µg/ml cell culture medium coating) resulted in a cell adhesion rate of 60-80%. Similar pretreated prostheses implanted in "Göttinger mini-pigs" were completely endothelialized after 4 weeks. Further experiments will focus on the acceleration of endothelialization and on functional assessment of seeded endothelium on foreign surfaces.

Dep. of Surgery and Div. of Physiology, University of Tübingen, Germany, 7400 Tübingen. Unit of Haemostasis Research Karolinska-Klinik Bad Nauheim
**RHEOLOGICAL STABILITY OF ENDOTHELIAL MONOLAYERS SEEDED ON UNMODIFIED AND MODIFIED VASCULAR GRAFT MATERIAL**

D. Drenckhahn, H. Schnittler, P. Franke

Lining of synthetic vascular grafts with autologous endothelial cells has been shown to improve the patency of small caliber grafts in animals. In the present study we were interested in developing procedures to grow monolayers of vascular endothelial cells on artificial graft material. For that purpose glass cover slips and plane sheets of expanded Polytetrafluoroethylene (ePTFE, 30 μm fibre length) were coated with polyornithine (PORN) or with PORN and a second layer of laminin. Laminin, a naturally occurring protein of the subendothelial matrix, has been recently shown to be important for cellular adhesion. Both PORN and PORN/laminin allowed human umbilical cord endothelial cells to form a stable monolayer on the surface. Exposure of the monolayers to arterial levels of fluid shear stress (3.5 dynes/cm²) revealed considerable differences in the shear stress resistance. The cells grown on PORN alone were sheared off within the first 10 min of shear stress exposure, whereas the monolayers grown on PORN/laminin remained intact over full length of the experiment (3 h). We are currently trying to improve shear stress stability of the monolayers by coating the graft material with combinations of various extracellular matrix proteins, synthetic peptides containing adhesion sequences and polar lipids.

Anatomisches Institut der Universität Würzburg, Koellikerstr. 6, D-8700 Würzburg

---

**Intimal Hyperplasia in expanded Tetrafluoroethylene (ePTFE) Grafts implanted in the abdominal Rat Aorta**

J. Fügnerl, V. Hermann*, E. Frick and G. Kövér

MNF-Physiologie I, Ob dem Himmelreich 7 and *Chirurgische Klinik, Auf dem Schmarrenberg, 74 Tübingen

Healing of artificial vascular grafts is accompanied by intimal hyperplasia, consisting mostly of multilayered smooth muscle cells located mainly at sites of anastomosis. This process leads to human narrowing and hence facilitates thrombotic failure of the grafts. Especially in small caliber substitutes this phenomenon is the main cause of graft failure. We studied the kinetics of intimal hyperplasia in expanded Polytetrafluoroethylene (ePTFE)-grafts implanted into the abdominal aorta of the rat. During the healing process endothelial cells enter the inner graft surface via pannus ingrowth from the adjacent native vessel, which is thought to be beneficial by exerting antithrombotic effects. We were interested whether endothelial cells would also inhibit intimal proliferation and asked the question whether removal of endothelial cells (using an emboleetomy catheter) from the aorta prior to grafting would enhance intimal hyperplasia. By incorporation of Bromodeoxyuridine (BrdU) into DNA of cells in S-phase, which can be detected using a monoclonal anti-BrdU-antibody, we determined rates of proliferation in intimal cells. Additionally we measured the thickness of the neointima morphometrically. One week after implantation we found high proliferative indices (84%, n=5) which remained high even after eight weeks (11%, n=5). The size of the neointima in the grafts was not increased after endothelial cells were deliberately removed from the aorta. This was surprising since the aorta itself a strong intimal reaction was evident. The inhibition of endothelial cell regrowth obviously did not enhance graft neointimal growth. Neointima cells could be identified immunohistochemically as smooth muscle cells using anti-alpha-actin-antibody. Endothelial cells could be detected (using anti-von Willebrand factor-antibody) only on grafts implanted into undenuded aortae. These results suggest that the lack of endothelial regrowth on vascular grafts does not lead to enhanced neointimal thickening.
Danazol Induced Alterations Of Coagulation In Patients Treated For Endometriosis

U. Cirkel, K.W. Schweppe, Ch. Ebert and H.P.G. Schneider

Over a six-month period 62 women suffering from histologically proven endometriosis had been treated with 800 mg per day of the ethinyl oestradiol derivative danazol. Mean age of the patients was 30 + 8 years. Before, every 4 weeks during therapy and 4 weeks after danazol treatment blood count and parameters of the coagulation system had been checked. There was a significant increase (p < 0.05) in hemoglobin and hematocrit. No changes were found in the number of erythrocytes, leucocytes and thrombocytes, the mean cellular erythrocyte volume, thrombin time, and concerning the factors VII, VIII, XII, and -entrypsin. Antithrombin III levels increased, while -macroglubulin values decreased. Only the delay of fibrinogen, to a minimum of 151 ng/dl, and the increase of plasminogen, to a maximum of 16,14 mg/dl, was statistically significant (p < 0.01). These in part contradictory changes suggest that hypercoagulability may occur under danazol therapy. However, its clinical relevance remains unclear.

Universitäts-Frauenklinik, 4400 Münster/Westf.
Albert-Schweitzer-Str. 33

Danazol Induced Alterations Of Coagulation In Patients Treated For Endometriosis

U. Cirkel, K.W. Schweppe, Ch. Ebert and H.P.G. Schneider

Over a six-month period 62 women suffering from histologically proven endometriosis had been treated with 800 mg per day of the ethinyl oestradiol derivative danazol. Mean age of the patients was 30 + 8 years. Before, every 4 weeks during therapy and 4 weeks after danazol treatment blood count and parameters of the coagulation system had been checked. There was a significant increase (p < 0.05) in hemoglobin and hematocrit. No changes were found in the number of erythrocytes, leucocytes and thrombocytes, the mean cellular erythrocyte volume, thrombin time, and concerning the factors VII, VIII, XII, and -entrypsin. Antithrombin III levels increased, while -macroglubulin values decreased. Only the delay of fibrinogen, to a minimum of 151 ng/dl, and the increase of plasminogen, to a maximum of 16,14 mg/dl, was statistically significant (p < 0.01). These in part contradictory changes suggest that hypercoagulability may occur under danazol therapy. However, its clinical relevance remains unclear.

Universitäts-Frauenklinik, 4400 Münster/Westf.
Albert-Schweitzer-Str. 33
Thrombolytic therapy has proved effective for massive pulmonary embolism (PE), but clinical experience during pregnancy is very limited. We report a case of successful thrombolytic therapy of PE, clinical stage III, in early pregnancy with rt-PA (Actilyse®). A 30 year-old woman, gravida 3, para 2 in the 11th week of pregnancy presented with severe right pleurisy, dyspnea, tachycardia (HR 160/min.) and hypotension (RR 90/50). Further investigation showed: SI QIII type in ECG, right ventricular dilatation and reduced function by degradation product, increased pulmonary artery pressure (PAP), (systolic 38, diastolic 21, mean 20 mmHg) and poor arterial blood gas analysis (pH 7.45, pO2, 28, pCO2, 65 mmHg, BE, -2.1). Because of the patient's life-threatening condition, thrombolytic therapy was initiated with rt-PA, being chosen for its fibrin specificity. A total dose of 60 mg rt-PA over 8 hours was administered, given in three fractions of 20 mg as short infusions, to prevent haemorrhage from the placental site. The patient received additional 30,000 IE Heparin/24 hrs. Masked symptomatic and haemodynamic improvement with intact ventricular function and elevated PAP was noted. No adverse effects occurred. For further prophylaxis of thromboembolism the patient was anticoagulated with low molecular weight heparin up to normal delivery. This case provides evidence for the efficacy and relative safety of rt-PA in the treatment of PE in pregnancy.

Abbildungen Innere Medizin III und IV, Medizinische Universitätsklinik und Poliklinik, Robert-Koch-Str. 8, D-7990 Ulm

Clinical and Prognostic Relevance of Tumor-Associated Proteases in Gynecologic Oncology

The capacity of solid tumors to invade the surrounding tissue and to metastasize is correlated with the formation and degradation of structural elements of the extracellular matrix of the tumor cells. Substances with both procoagulant activity and fibrinolytic activity are important factors in the formation or degradation of the "fibronectin-fibbin-gel matrix". This gel is subsequently transformed into the extracellular matrix which together with the tumor stroma forms the tumor stroma. When analyzing tumor stroma degradation activity, it is obvious that the protease plasmin catalyzes the disintegration of fibrin and fibronectin. Additional compounds of the tumor stroma and of the basement membrane are also, at least in part, cleaved by plasmin or other proteases such as collagenase IV and collagenase D.

The plasminogen activator, urokinase (uPA), seems to play a central role as it was shown that elevated content of uPA is correlated with a high risk for early relapse and shorter overall survival at least in breast cancer. It is worth mentioning that by means of quantifying uPA, patients with a relative high or low risk can even be selected within the classical risk groups which so far were defined by clinical or locoregional extension of the tumor and the hormone receptor status only. Evidently, as uPA content in human breast cancer tissue is an independent prognostic factor, one may speculate that those experimental or clinical data, which correlated increase in uPA-synthesis with malignancy, may be of direct relevance for the tumor biology of the human being. Moreover, due to these recent observations on the prognostic significance of tumor-associated proteases, prospective for the selection of risk collectives within the node-negative breast cancer patients for adjuvant therapy to be considered. It may well be possible that one may affect tumor invasion and metastasis by inhibiting proteolytic activity of solid tumors by disturbing the binding of proteases to tumor cell surface receptors. As it is only a quantitative aspect which separates benign physiologically from tumor cell pathophysiology, experimental evidence suggests that thereby less drastic forms of palliative therapy can be found.

Cell biology of the platelet functions: adhesion, retraction and secretion.

E. Morgenstern

Platelet and mobile cells of connective or inflammatory tissue possess similar functions. Both of them are able to react with other cells or with extracellular material and are equipped with a contractile cytoskeleton (CC) that enables the cells to retract networks of fibrils. Cell-activating, transient contacts are initiated by binding of ligands (adhesive glycoproteins) to certain transmembrane receptors (cytadhesins, e.g. integrin/ GP1b/IIIa). The contact is mediated by the formation of localized, intramembranous patches composed of the ligand-receptor-complex, GPIb-IX-V and actin-binding proteins (talin, vinculin, -actinin). Filaments of the actin-bundles and filamentous adhesions are demonstrated to be attached coaxially to the complex. Thus, the traction of the CC in stimulated cell is transmitted by the linkage between the cytoskeleton and the extracellular substrates (surface of other cells, collagen-, fibronectin-fibres, extracellular matrix). Internalization causes retraction of fibers whereas spreading of such cells results from the interaction with solid and plane surfaces. On the other hand, resting secretory cells and platelets are provided with a submembranous cytoskeleton (SMC) that is linked to certain transmembrane receptor molecules (in platelets: thrombin-receptor GP1b) and serves as a fibrinogen filaments and actin-binding proteins. The assembly and fusion of the plasmemnal and/or the organelle membrane can occur only after SMC rearrangement. Thus, the SMC is thought to act as a barrier preventing the exocytosis in resting cells. In platelets the molecular and morphological reactions depending on the cytoskeleton (CC and SMC) do not differ from those observed in other cells, which perform adhesion, spreading or retraction and secretion.

Medizinische Biologie, Universität des Saarlandes, D-66050 Homburg/Saar

MODULATION OF PLATELET ACTIVATION BY TRANSMEMBRANE MOVEMENTS OF H+, NA+, AND CA2+ IONS

N. Giffert

Ca++-ions: Since the introduction of fluorescent indicators for cytosolic free Ca++ a lot of work has been devoted to characterization of Ca++ fluxes occurring during platelet stimulation. Ca++ is increased via influx across the plasma membrane and some part stems from intracellular Ins 1,4,5-P3-sensitive pools. H+ - ions: Changes in intracellular pH were monitored using the fluorescent dye BCECF which monitors cytosolic pH and is stained by a variety of platelet agonists such as thrombin, ADP, and platelet-activating factor rapidly increases pH via Na+/H+ exchange. Most likely, the activity of this ion transport is enhanced by phosphorylation of a protein kinase C-dependent reaction. Inhibition of Na+/H+ exchange can negatively modulate a variety of platelet responses including the release of arachidonic acid, Ca++ mobilisation, aggregation, secretion and aggregation. Na+ -ions: Using the Na+-sensitive fluorescent sodium-binding benzofuran isophtalate (SBFI) we could recently show that thrombin raises cytosolic free Na+ from 22 to approx. 60 mmol/L without a subsequent decrease for 20 min. despite intact Na+/K+ ATPase. This may indicate that thrombin inhibits this enzyme via a hitherto unknown mechanism. The rise in Na+ may affect the activity of G-proteins which are known to be negatively modulated by Na+ in the range of 20 to 80 mmol/L.
Platelet derived soluble factors such as PAF or PGD₂ have been suggested to affect the activity of neutrophils (PMNL) in mixed cell suspensions or whole blood. However, the role of contacts between both cell types for their functional interaction has not yet been defined. In an attempt to investigate whether PMNL can respond to platelets in a contact-dependent manner, a method was applied which rendered it possible to monitor the activity of both cell types concomitantly in the same sample. Aggregation as a phenomenon of platelet activation and the luminol-amplified chemiluminescence (CL) as a measure of the oxidative burst in PMNL were recorded with a lumin-aggregometer simultaneously. Both resting platelets and platelets stimulated with thrombin, the TXA₂ mimetic U 46619, or ADP induced the CL of PMNL in mixed suspensions with physiological platelet and PMNL concentrations. Platelet homogenates and supernatants from resting or stimulated platelets were ineffective on PMNL. No difference was found between experiments with platelets which were aspirated and those with platelets not treated with aspirin. In heparinized whole blood, aggregation and CL required platelet stimulation with U 46619 or ADP. Intercellular collisions and bivalent cations were necessary for the platelet-dependent CL since no-stirring and EDTA strongly reduced the number of platelet-PMNL contacts and the CL response. A similar inhibitory effect was elicited by red cell ghosts. Those CL responses triggered by platelet stimuli were also inhibited by roprost, RGDS peptide, and - in the case of ADP or U 46619 - the absence of exogenous fibrinogen, i.e. conditions which inhibit platelet aggregation. Exogenous fibrinogen could not substitute for fibrinogen. Immunogold-labeling revealed fibrinogen in the contact spaces between stimulated platelets and PMNL. In contrast, fibrinogen was not detected in the intimate contact-regions between resting platelets and PMNL. These observations indicate that platelets provide a strong stimulus for PMNL releasing intercellular contact and - in the case of stimulated platelets - binding of fibrinogen to its integrin receptors. (Supp. by the DFG, Pa. 263.)

Institut für Klinische Chemie, Klinikum Mannheim der Universität Heidelberg, D-6800 Mannheim.

PLATELET-NEUTROPHIL-INTERACTIONS: THE ROLE OF CYTOSKELETAL CHANGES ON THE INTERACTION BETWEEN RESTING PLATELETS AND STIMULATED NEUTROPHILS

A. Ruf and H. Patscheke

Abstract:
Platelet-neutrophil interactions are an important process for the production of inflammation and for the resolution of infection. Under normal circumstances, neutrophils adhere to and aggregate with platelets, forming a stable platelet-leukocyte aggregate. The formation of such aggregates is mediated by specific interactions between leukocyte-derived integrins and platelet-derived ligands, such as fibrinogen and von Willebrand factor. However, in certain pathological conditions, such as infections, inflammatory diseases, or cancer, these interactions may be altered, leading to abnormal platelet-neutrophil interactions and to the development of thrombosis or bleeding disorders.

DISTURBED PLATELET-FIBRINOGEN INTERACTION CAUSED BY A STRUCTURALLY ABNORMAL MEMBRANE GLYCOPROTEIN (GP) IIb-IIIa COMPLEX

M. Meyer, C.M. Kirchmaier, B. Jablonka, M. Just, A. Schirmers, H.K. Breddin

Two related patients (brother and sister) have been suffering from moderate bleeding complications since early childhood. Bleeding time was prolonged and platelet count normal or subnormal. Patient's platelets were functionally characterized by a decreased platelet adhesion and spreading, strongly inhibited ADP- or collagen-induced aggregation and normal ristocetin-induced agglutination. Further biochemical analysis of this thrombasthenia-like bleeding disorder revealed a half normal amount of GP IIb-IIIa complex. Specific fibrinogen binding to ADP-stimulated platelets was found to be only 7 - 10 % of the normal amount. After Triton-solubilization GP IIb-IIIa complex interacted normally with fibrinogen. It may be assumed that a structural alteration of the GP IIb-IIIa complex interferes with the expression of fibrinogen binding sites after platelet stimulation.

Abteilung Medizinische Genetik der Medizinischen Akademie Erfurt, Arnstädt Str. 34, D-0-5082 Erfurt

DIAGNOSIS OF HEPARIN ASSOCIATED THROMBOCYTOPENIA (HAT) AND SELECTION OF A COMPATIBLE HEPARIN BY A RAPID AND SENSITIVE HEPARIN INDUCED PLATELET ACTIVATION (HIP-) TEST

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

HAT is a serious complication of heparin therapy. Confirmation of the diagnosis is important for further treatment. In the HIP-test a washed platelet suspension of a normal donor is incubated with inactivated patient serum and low (0.2 U/ml) and high (100 U/ml) heparin concentrations in a microtiter tray on a magnetic stirrer for 45 min, results are read visually. 34 sera from patients with suspected HAT were assessed with platelets of 4 donors using the aggregometer test (AT), the serotonin release assay (SRA) and the HIP-test, simultaneously. HIP-test proved to be as sensitive and specific as SRA, which is time consuming and requires radioactive substances. The AT missed 25% of positive sera. 20 sera of patients with confirmed HAT were tested simultaneously in the SRA and HIP-test for crossreactivity with standard heparin, the LMW-heparins Fragmin and Fraxiparin, the heparinoid pentosan polysulfate and the LMW heparinoid Org 10172. Both tests gave identical results. All sera showed crossreactivity in vitro with all types of heparin, except Org 10172 which showed crossreactivity in only 1 serum. A of these patients in whom further anticoagulation was mandatory were treated successfully with Org 10172. The HIP-test is a useful tool for diagnosing HAT, and for selecting an appropriate heparin for further treatment.

Institut für Klinische Immunologie und Transfusionsmedizin am Klinikum der Justus-Liebig-Universität Gießen, Langenhanstraße 7, 6300 Gießen
REGULATION OF THE FREE ARACHIDONATE CONCENTRATION IN HOMOGENATES OF HUMAN PLATELETS.

L. Pausch and H. Patscheke
Freie arachidonate (AA) is the precursor for prostaglandins, thromboxanes A2, and 12-HETE and is liberated from phospholipids upon platelet stimulation. The concentration of AA depends on the activities of the deacylating and reacylating enzymes of the LANDS cycle, namely phospholipase A2 (PLA2), arachidonoyl-CoA-synthetase (AS) and lysophospholipid-acyltransferase (LAT). It has not been shown to which extent an activation of PLA2 and/or an inhibition of AS or LAT contribute to AA liberation in intact platelets. Therefore, we investigated the activities of PLA2, AS, and LAT in sonicated platelets in order to approach conditions within an intact platelet. ASA-treated washed platelets were homogenized by sonication and the eicosanoids and phospholipids were extracted after various time intervals. Platelets prelabelled with [14C]AA were used for the determination of AA liberation from pre-existing phospholipids, whereas unlabeled otherwise similar platelets served for the assays of AA and LAT activities measured as the formation of [14C]labelled phospholipids from [14C]AA or [14C]AA-CA, respectively. [14C]Labelled phospholipids, AA, 12-HETE were analyzed by radio TLC and a linear analog (Bertold LB 2842). [14C]AA-prelabelled platelets released no [14C]eicosanoids prior to sonication but their homogenates continuously formed [14C]AA and [14C]12-HETE that reached 4.7 % of the total radioactivity during 40 min at 37°C. Inhibition by apyrase 4 U/ml of the AA-PLA2 depending AS increased the [14C]eicosanoid production to 12.5%. On the other hand, Mg-EGTA 3 mM reduced the [14C]eicosanoids to 2.4 % during 40 min incubation. Homogenates of unlabelled platelets incorporated [14C]AA 0.1 µM into phospholipids (63.2 %) and formed 12-HETE (41.7 %) within 20 minutes at 37°C. Apyrase 4 U/ml prevented the incorporation into the phospholipids and caused a slight almost exhausted production of [14C]12-HETE. Probably because of this mechanism it potentiated thrombotic effect of fibrinolytic enzymes including streptokinase and tissue plasminogen activator. A study is in progress on treatment of diabetic angiopathy, retinopathy and nephropathy.

State University of New York at Buffalo, Roswell Park Cancer Institute, 666 Elm St., Buffalo, NY 14263 USA

Submitted

PLATELET AGGREGATION IN DISEASE CONDITIONS; EFFECT OF VARIOUS AGGREGATION INHIBITORS.

J. L. Ambrus, C. M. Ambrus, H. Gastpar, S. Stadler, S. Sayrid.

Methods were developed to study platelet aggregation in vivo in monkeys (Macaca arctoides) using an implanted screen filtration device and in vitro using measurement of circulating platelet aggregates. In vitro and ex vivo platelet aggregation was studied using a platelet aggregometer. Morphologic evaluation was by scanning electronmicroscopy. Increased platelet aggregability was found in diabetes mellitus types I and II, SS-genotype sickle cell disease, adult respiratory distress syndrome (ARDS), chronic arteriosclerotic cardiovascular disease (COAD) and metastatic malignant melanoma (MM). Normal red blood cells which lost much of their ATP content during storage in blood banks tended to induce platelet aggregation. Several groups of chemicals were found to reversibly inhibit platelet aggregation in vitro and in vivo. This included methylxanthines (MX), pyrimido- pyridazines, and lipidasozinanolides. Irreversible inhibitors included acetylsalicylic acid. The (MX) pentoxifylline was selected for clinical studies. It inhibited ex vivo pathologic platelet aggregation in patients with (COAD), ARDS, and MM. "It's mechanism of action appears to be based in part on release of prostaglandin I2 and tissue plasminogen activators from the endothelium. Probably because of this mechanism it potentiated thrombotic effect of fibrinolytic enzymes including streptokinase and tissue plasminogen activator. A study is in progress on treatment of diabetic angiopathy, retinopathy and nephropathy.

Zweijährige Erfahrungen mit zwei verschieden rekombinannten Faktor VIII-Konzentraten

H.-H. Brackmann,* E. Aygören,* I. Scharrer, J. Oldenburg und U. Hammerstein

Institut für Expt. Hämostaseologie und Transfusionsmedizin der Univ. Bonn,
*Zentrum der Inneren Medizin, Universitätsklinikum Frankfurt/Main

Im Zusammenhang mit der Übertragung von Infektionskrankheiten bei Anwendung von Plasmakonzentraten, die auch durch entsprechende Virusinaktivierung eine 100 %ige Virussicherheit nicht gewährleisten können, hat die Herstellung rekombinanter Gerinnungskonzentraten besondere Bedeutung erlangt. Derzeit stehen 2 verschiedene rekombinannter Faktor VIII-Konzentraten der Firmen Baxter und Cutter in klinischer Erprobung. Über die bisherigen Erfahrungen der beiden beteiligten Hämostase-Zentren Bonn und Frankfurt/Main, wird berichtet:

1. Anzahl der Patienten:
   Baxter Cutter
   Bonn 100 (1 Pat.) 100 (1 Pat.)
   Frankfurt 75 75

2. Beobachtungszeitraum:
   (Monate) Bonn 20,9 20,9
   Frankfurt 16,2 16,2
   (15,5-17) (15,5-17) (20-18)

3. Konzentratverbrauch:
   (Units, Gesamt)
   Bonn 1.306.322 1.042.390
   Frankfurt 664.650 556.300

4. Blutungen:
   Bonn 36 100 (1 Pat.)
   Frankfurt 75 41

Die klinische Effizienz insbesondere der Behandlung von Blutungsereignissen zeigte eine aus Plasmakonzentraten verlässliche Effizienz. Bisherige laborchemische Untersuchungen zeigten keine Veränderungen. Allergische Reaktionen sowie das Auftreten von Hemmernkörpern konnten nicht festgestellt werden. Weitere Patienten werden in die Studie aufgenommen werden müssen um die bisherigen zufrieden-stellenden Ergebnisse zu bestätigen.
PURITY CHARACTERISTICS OF THE NEW GENERATION OF FACTOR VIII CONCENTRATES.

H. Beiser and Th. Wüst

Institute of Transfusion Medicine, University of Freiburg, Germany

New purification procedures including biotechnology and more effective techniques for virus inactivation without harming the factor VIIIIC activity resulted in a new generation of therapeutic products with extremely high specific activities. These products not only differ in their content of other plasma proteins but especially in the content, reduction or absence of VWF activity, and/or VWF antigen and the multimeric pattern of VWF. With in our regular quality control analysis of in-vitro characteristics of therapeutic clotting factor concentrates we investigated the actual factor VIII concentrates including two recombinant products by an extended program.

VIIC-activity tested with one-stage-, two-stage- and chromogenic methods in parallel does not differ off the known limits from the labelled activity in most of the products. For the recombinant VIIIC concentrates there are distinct differences of the VIIIC content calculated from the results of the three methods. Most of the other proteins, especially immunoglobulins and fibronectin are only present in trace amounts.

The albumin part of the total protein in most of the concentrates is considerably to extremely high, so that there is only theoretically a chance to calculate the correct specific VIIIC activity from the difference of the amount of total protein minus the albumin content.

PERSONAL EXPERIENCES WITH VIRUS-INACTIVATED FACTOR VIII/FACTOR IX CONCENTRATES IN CLINICAL VIRUS-SAFETY STUDIES

KL. Schimpf

Clinical retrospective and prospective multicenter virus-safety studies, in which we participated and which were carried out according to the study protocols recommended by ESC of ITPH, showed the following results:

A dry-heated factor VIII concentrate (60°C/72 hrs.) still carried infectious hepatitis NAB viruses. 83% of first-treatment patients developed hepatitis NAB.

After treating 26 patients with hemophilia A or von Willebrand’s disease for the first time with 32 lots of pasteurized factor VIII concentrate (Haemate) none of these patients developed hepatitis. In another study of 29 patients who were treated for the first time with 13 lots of pasteurized factor VIII concentrate (Beriate) also none developed hepatitis.

Furthermore, 29 patients who were first-treated with vapor-heated blood coagulation factor concentrates (27 with factor VIII concentrate S-TIM 3 and 2 with factor IX concentrate S-TIM 4) also did not develop post-infusion hepatitis.

Also, clinical retrospective multicenter studies showed, after treating patients with the pasteurized Haemate (155 patients) or vapor-heated factor VIII S-TIM 3 and factor IX S-TIM 4 (60 patients), no anti-HIV seroconversion.

Rehabilitation Hospital and Hemophilia Center, Stiftung Rehabilitation, Bonn-Biesarstrasse, D-6900 Heidelberg, Germany

SOME ADDITIONAL METHODS TO DIFFERENTIATE CRITICAL ISCHEMIA

H. Rieger

For better differentiation of critical circulatory disturbances in the area of the lower extremities those methods should be used which have proved effective attempts to identify critical ischemia not by measuring macrocirculatory values, but rather locally, by examining the cutaneous microcirculation. At the present time transcutaneous oxygen partial pressure measurement (tcpO2), fluorescence perfusography (FA) and Laser Doppler Fluxmetry are available and more or less. tcpO2-measurements are of additional use in the estimation of an advanced ischemia, or a prognosis of the healing of an ischemic wound or of the level of an anamputation. Fluorescence angiographic signals mainly correlate with tcpO2 measurements but are more sensitive above all within the very low flow range because the oxygen electrode needs the remaining oxygen for its own function.

By means of Laser Doppler Fluxmetry it is possible to detect red blood cell movements in cutaneous vessels and to record local fluctuations in vasoconstriction over a long period. The different vasoemotion patterns can determine wether these reflect healthy or insufficient microcirculation and critical ischemia respectively.

The most reliable informations will be obtained where the methods are used to complement one another. The weak point of the individual method can be compensated and overall errors minimised.

Aggertalklinik, hospital for vascular diseases, 5250 Engelskirchen/Cologne
CORONARY REOCCLUSION - WHY THEY OCCUR AND HOW TO PREVENT THEM

Dietrich C. Gulba

Thrombolytic therapy of acute myocardial infarction stands today in an unchallenged position. After successful thrombolysis, however, the reopened coronary vessels are threatened by abrupt early reclosure, occurring in some 20 to 40 % of the patients. Four independent risk factors for reocclusion have been isolated so far: 1) The severity of the residual stenosis, 2) the state of the coronary blood flow, and 3) the presence of any visible thrombus in the coronary angiogram. Furthermore, 4) the state of activation of the coagulation cascade - which may be further enhanced by thrombolytic drugs - has been recognized as major promoter for coronary reocclusion. Anticoagulation with heparin and coumadins is the basis of all therapeutic approaches for reocclusion prevention. Additional balloon angioplasty was another promising approach which, however, failed to improve the prognosis of the patients (TIMI, TAMI, GESG-trials). The results of the HART-study demonstrated two different phases after reperfusion. In the early phase, heparin performs better while aspirin is superior for the prevention of late reocclusions. In our own experience, by the addition of aspirin to IV heparin, in-hospital reocclusion rates were cut down by half to some 10 %. From the lessons we have learned while trying to prevent reocclusions, it is our opinion, that in the near future more specific antithrombotic agents like hirudin or GP IIb/IIIa-receptor antibodies will progressively gain importance.

Med. Hochschule Hannover, Abt. Kardiologie, Konstanty-Gutschow-Str. 8, D-3000 Hannover 61
PLATELET-INDUCED THROMBIN GENERATION TIME (PITT), A NEW SENSITIVE TEST SYSTEM TO DETECT PATIENTS AT RISK FOR VENOUS OR ARTERIAL THROMBOSIS?

H. K. Breddin and M. Basic-Micic

No single test exists so far to predict thrombosis in single patients at risk as in the postoperative period or in patients with peripheral occlusive disease. Numerous trials in this field have been reported with controversial results.

We have developed a new system in which the time of aggregation and coagulation - platelet induced thrombin-generation time (PITT) - in platelet rich plasma (PRP) is registered. PRP is obtained from blood samples which are partially anticoagulated with hirudin or a low molecular weight heparin. Thrombin is generated at platelet surfaces and leads to aggregation (Tagg) and subsequent coagulation.

30 patients with different fresh thrombotic venous or arterial episodes have been included in a pilot study. In 5 out of 7 of these patients the time until aggregation (Tagg) as well as the coagulation time (Tc) were markedly shorter than in normal volunteers (n= 32, Tc was 8.7 min. ± 2.7) for samples anticoagulated with 5 pg Fraxiparin. It seems possible that this new test principle in its present or in a more developed form can be used to identify patients at risk for arterial or venous thromboses.

The binding of affinity-purified anticardiolipin antibodies (ACA) to liposomes that contained cardiolipin or phosphatidylserine was investigated. Binding of ACA to liposomes only occurred in the presence of human (or bovine) plasma (or serum) indicating the involvement of a plasma component. This component was purified and identified as 82-glycoprotein I (82-GP-I), also referred to as apolipoprotein H.

Purified 82-GP-I was essential for ACA to bind to liposomes that contained cardiolipin or phosphatidylserine, but not to other plates; 82-GP-I is a single chain polypeptide (50 KD) with a selective avidity for negatively charged phospholipid surfaces. Binding of 82-GP-I interferes with the factor Xa/Va catalysed activation of prothrombin which takes place at the phospholipid surface. 82-GP-I can therefore be considered as a natural circulating anticoagulant.

1Dept. of Biochemistry, Cardiovascular Research Institute Maastricht, the Netherlands 2Divisione d Ematologia, Ospedali Riuniti, Bergamo, Italy

The lupus inhibitor-study of the GTH

I. Scharrer, E. Kaib, V. Hach-Wunderle and the GTH-inhibitor-studygroup

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

Up to now we have knowledge of 218 patients with lupus inhibitors from 13 hospitals. They are on average 39.2 years of age. The rate of fetal losses was 20% (n=17/135 women); in 10 women repeated abortions had occurred.

Concerning the underlying diseases, in 26% of the patients SLE was established. In 32% of the patients thrombotic disorders were reported.

Concerning the laboratory tests, the aPTT was used most often, in 96% of all cases. Anticardiolipin antibodies were studied in 92 patients and turned out to be positive in 72. Coagulation factors were measured in 181 patients; out of these 41 patients showed a reduction of several factors. A deficiency of factor II was observed in 31% of the patients (n=37/119), of factor V in 28% (n=5/18), of factor VII in 38% (n=9/19), of factor VIII:C in 39% (n=21/54), of factor IX in 85% (n=30/35), of factor X in 24% (n=4/17), of factor XI in 57% (n=24/42) and of factor VII in 82% (n=80/98).

These preliminary data point out the high frequency of lupus inhibitors, as well as the great variability concerning the clinical features and the diagnostic approaches. We hope to gain further information on the basis of an international questionnaire, which will be demonstrated.
ANTICARDIOLIPIN ANTIBODIES
S. Kaprio
During the last years much attention has been focused on so called antiphospholipid antibodies (like the lupus anticoagulant and anticardiolipin antibodies) as acquired laboratory abnormalities associated with a number of disease states. Anticardiolipin antibodies (ACA) have been reported in patients with various underlying diseases (especially autoimmune disorders). (A)ngiobraiic data of the literature the prevalence of ACA in systemic lupus erythematosus (SLE) is 44 %. In patients with SLE a significant correlation between ACA and thrombosis, neurologic disorders, or thrombocytopenia exists. ACA are also prevalent in various non-SLE disorders (e.g. rheumatoid arthritis, acute and chronic infections), although in these groups of patients none of the above mentioned associations have been shown conclusively.
ACA were also detected in patients without evidence of SLE who suffered from arterial or venous thrombosis and in women with recurrent fetal loss. We studied 266 patients with venous thrombosis and 49 women with recurrent abortions. About 7 % of patients with venous thrombosis and 10 % of women with recurrent abortions had elevated levels of ACA IgG and/or IgM. Out of these ACA positive patients 40 % of the venous thrombosis patients had positive titers of antinuclear antibodies (ANA) and 25 % of women with recurrent abortions had ANA subsets, assuming the presence of subclinical autoimmune phenomena in these patients. In patients with venous thrombosis there were no differences with respect to clinical (e.g. no higher incidence of arterial thrombosis in ACA positive patients) and laboratory (e.g. no difference in the prevalence of protein C-, protein S- or antithrombin III - deficiency) features between patients with normal and elevated ACA levels. The clinical significance of elevated ACA levels in venous thrombosis remains therefore uncertain.
First Medical Department, Div.of Hematology and Hemostasisology, Univ.of Vienna; A-1090 Vienna; Austria

ACQUIRED DEFICIENCIES OF COAGULATION INHIBITORS
B. Kerker-Matthes
Coagulation inhibitors may be altered in:
I. Physiological alterations: a) At birth and during the first years of life, diminutions of pro- and anticoagulatory active coagulation factors are known.
   b) During pregnancy, a diminution of total and free protein S levels occurs, and in the newborn, a parallel increase in free protein C levels. The lowest levels were measured at the time of delivery, one for the elevated risk to suffer from thromboembolic events during pregnancy and parturition.
II. Pathological alterations: a) Lowered synthesis: In patients suffering from acute and chronic liver diseases, a diminution of all plasma proteins synthesized by the liver is known, particularly AT III and protein C. A lowered synthesis of protein C and S also occurs in cases of vitamin K deficiency.b) Enhanced consumption: In disseminated intravascular coagulation, a diminution of all coagulation proteins occurs, due to increased turnover. AT III infusion is useful for interruption of this condition. An enhanced urinary loss of coagulation inhibitors can be demonstrated in patients with nephrotic syndrome, and recently in patients suffering from advanced malignancies. c) Acute phase response: In disease states leading to an acute phase reaction, such as malignant tumors and inflammatory diseases, an increase of C4b-BP-bound protein S is observed. This results in decreased free protein S levels in patients suffering from advanced malignancies. d) Drugs: Oral contraceptives lead to diminutions of free protein S levels. In the initial phase of oral anticoagulant therapy, protein C decreases faster than other anticoagulation proteins, leading to a thrombophilic state, which might cause coumarin necrosis.
Zentrum Innere Medizin der Universität Gießen
MOLECULAR MECHANISM OF NEW THROMBOLYTIC DRUGS

R. B. Binder

Tissue plasminogen activator (t-PA) has solved major problems associated with first generation thrombolytic agents. This is due to specific domains like the kringle domains and the finger domain added as modules to the simple serine protease domain catalyzing activation of plasminogen. Therefore, a more fibrin-specific plasminogen activation leads to more fibrin-specific dissolution of thrombi and less severe side effects. However, recent large clinical trials showed that differences between cheap streptokinase and expensive tissue plasminogen activators are not remarkable and reocclusion and stroke may be more often associated with t-PA treatment. Therefore, there might be a need to further improve thrombolytic drugs which should be made possible by knowledge of molecular mechanisms involved in fibrinolysis. Minimizing costs can be achieved by either a longer half life of the drug in vivo, e.g. by interference with the clearance mechanisms or by a combination of thrombolytic agents leading to the same effectiveness but lower total costs. In order to optimize thrombolysis, prehospital administration as a bolus should be possible which in turn implies that the drug has to be more specific for occluding vs. reparative thrombi. Targeting of a fibrinolytic agent to an occluding thrombus might be achieved by using other clot components as compared to fibrin. Arterial occluding thrombi are especially rich in platelets and therefore targeting of a plasminogen activator to a platelet-rich thrombus might be preferable. Platelets and thrombi are thought to be involved in the process of reocclusion; therefore a combination of plasminogen activators together with anti-platelet and/or anti-thrombin drugs should be promoted. Such plasminogen activators might be achieved by either altering parts of the molecule itself like adding or deleting specific modules or by combining desired properties of different molecules by hybridization of those molecules or by simply combining different drugs or in a new regimen. However, it remains questionable whether evolution can be improved by engineering.

Clin. Exp. Physiology, Dept. Med. Physiology, Univ. Vienna, Schwarzenbergstr. 17, A-1090 Vienna, Austria

THE DEVELOPMENT OF THROMBOLYTIC THERAPY IN ACUTE MYOCARDIAL INFARCTION

R. Schmutzler

Thrombolytic therapy in acute myocardial infarction with streptokinase (SK) started in USA in 1958. Between 1960 and 1975 many controlled, randomized studies of patients treated with SK intravenously respecting mortality statistically have been published mainly in Europe. In 1976 Chazov with fibrolysin and 1978 Rentrop with SK were the first to introduce angiographically controlled intracoronary thrombolysis in patients. There is agreement that the time interval between the event of infarction, starting treatment and early reperfusion of the occluded artery is the most important point to minimize infarct size and saving myocardial function. Up till now a lot of experience from many multicenter studies with the plasminogen activators SK, UK, APSAC, rt-PA and scu-PA given intravenously as soon as possible has been published. The mean patency rate undulates between 75% and 90% depending on the choice of activator and the dosage regimen. I shall touch actual problems, different meanings, and unanswered questions. I am still under discussion if one of the different activators is more effective in patients than another one. How are the optimized dosage strategies, e.g. front loading, or in the combination of two activators to improve the benefit/risk ratio, minimising the bleeding tendency and reducing the reocclusion rate. It is to mention the strategies of the attending antithrombotic therapy such as heparin, aspirin or other drugs. The early and late mortality rate can be improved by immediate or delayed invasive PCI or bypass surgery.

Am Freudenberg 83, D-5600 Wuppertal 1

STREPTOKINASE, UROKINASE, AND rt-PA IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION - CLINICAL STUDIES

K.-L. Neuhäus

Streptokinase has been the first thrombolytic agent which was shown to reduce in-hospital mortality of patients with acute myocardial infarction. Mortality was reduced by about 25 to 30% when intravenous Streptokinase was given within six hours from symptom onset with a further reduction of 25% by adding aspirin as demonstrated in the ISIS II trial. There is still some controversy about the efficacy of thrombolytic treatment after more than six or even twelve hours. No subset of patients revealed detrimental effects of thrombolysis, although intracranial bleeding occurs in about 0.5% with or without heparin or aspirin. With Urokinase, no mortality trial has been conducted. Even though it is widely used because of the lack of side effects and antigenicity. With rt-PA, a reduction in-hospital mortality similar to Streptokinase has been demonstrated both in the ASSENT and in the comparative ISIS-2 trial. The latter has been criticized because heparin was given late and only subcutaneously. Furthermore, the so called front-loaded application of rt-PA seems to be significantly more effective than the standard regimen with respect to early reperfusion, and may further improve early survival. The question which thrombolytic agent and which regimen is optimal in the treatment of acute myocardial infarction still waits for an answer.

Med. Klinik II, Städtische Kliniken, Mönchebergstr. 43, D-3500 Kassel

HEPARIN THERAPY IN ACUTE MYOCARDIAL INFARCTION

H.D. Bruhn

It has always been appreciated that left ventricular mural thrombus formation may follow anterolateral and inferior myocardial infarction which has involved endocardial injury. Clinical and post-mortem studies have suggested a systemic embolism rate that approaches 9% of all myocardial infarctions. In recent studies it was shown that subcutaneously administered heparin reduced the incidence of left ventricular mural thrombi significantly. In addition heparin was essential also after thrombolytic therapy by increasing patency rates in comparison to placebo patients. The incidence of venous thrombo and pulmonary emboli is decreased in myocardial infarctions by heparin therapy, too. In further own investigations we could demonstrate that heparin therapy administered in usual dosage does not inhibit fibroblast proliferation so that ruptures of the myocardium should not occur more frequently under heparin therapy.

I. Medizinische Universitätsklinik Kiel, Schittenhelmstr. 12, 2300 Kiel 1
INDIKATIONEN UNG ERGEBNISSE VON PTCA UND BYPASSOPERATION BEIM AKUTEN MYOKARDINFARKT

R. Uebis

Nach ersten vielversprechenden Ergebnissen einer kombinierten Therapie mit Fibrinolyse mit Streptokinase und perkutaner transluminaler Koronarangioplastie (PTCA) des Infarktgefässes an ausgewählten Patienten haben drei große randomisierte Untersuchungen zwar bestätigt, daß die koronarangioplastischen Resultate einer solchen kombinierten Behandlung günstig sind, doch hat jedoch das perkutane transluminale Angioplastiekonzept aufgrund der angiographisch dokumentierten Mehrgefäßerkranckung und rezidivierende Ischämie in Frage. In offenen Studien haben diese Patienten, wenn sie frühzeitig nach der effektiven Reperfusion operiert werden, eine sehr günstige Prognose mit einer 5-Jahres-Uberlebensrate von 90%.

Medizinische Klinik II
Medizinische Fakultät der RWTH
Pauwelsstraße 30, D-5140 Aachen

92

LONG-TERM ANTITHROMBOTIC THERAPY AFTER MYOCARDIAL INFARCTION

J. Kienast and J. van de Loo

The long-term antithrombotic approach to patients with myocardial infarction after hospital discharge aims at preventing 1. late recurrences of coronary thrombotic events and 2. left ventricular thrombosis with its risk of systemic embolization. As shown in a recent meta-analysis of several randomized placebo controlled trials (Antiplatelet Trialists' Collaboration, 1988) platelet inhibitor therapy in patients after myocardial infarction reduces cardiovascular mortality by 13%, nonfatal reinfarction by 31% and nonfatal stroke by 42%. In this regard, aspirin appears to be the most thoroughly studied drug and is currently recommended as the routine antithrombotic treatment in the early and late postinfarction periods at a dose of 160 to 325 mg daily (ACC/AHA Guidelines, Circulation 1990). A recently published trial supports previous evidence that long-term oral anticoagulation may provide similar benefits (Smith et al., NEJM 1990). Its general use, however, is not recommended because of the greater risk of hemorrhage and the need for costly and inconvenient laboratory monitoring of therapy. Oral anticoagulant therapy for the first 3 months or even beyond this period appears to be required in patients at a particular risk of left ventricular mural thrombosis and systemic thromboembolism (e.g. in patients with large anterior transmural infarction, with ventricular aneurysms, large akinetic region, atrial fibrillation or diffusely dilated and poorly contracting left ventricle).

Medizinische Klinik der Westfälischen Wilhelms-Universität. Albert-Schweitzer-Str.33, 4400 Münster

93

EFFECT OF AUTOLOGOUS BLOOD PRIME VOLUME AND AUTOLOGOUS PLATELET TRANSFUSION ON PLATELET FUNCTION DURING CARDIOPULMONAL BYPASS

P. Franke, H. Neuhold, G. Boeden and M.A.A. Kratzer

The negative effect of cardiopulmonary bypass on platelet function is well known. In order to improve thrombocyte function during these situations we examined two modifications of the method generally used. a) The prime volume of the heart-lung machine for the test group was filled with 2000 ml physiological saline (NaCl) and 600 ml autologous heparinised whole blood as compared to 2600 ml of NaCl for the control group. b) Four units of platelet rich plasma (500 ml) was prepared from the patient's blood (cell separation: Haemonetics V 50) just before the operation and reinfused postoperatively. Measurement of platelet function during and after the operation was performed with a global method (bleeding time ex vivo, Kratzer & Born, Thrombostat 4000, VDG-von der Glotz, Seesen).

Our data show a sudden rise in ex vivo bleeding time (BT > 400) and volume (BV > 800 μl) one minute after start of the extracorporeal circulation (n = 24, reference values: BT = 120-150 sec., BV = 200 μl). These changes persisted for the duration of the extracorporeal circulation. Furthermore modification a) had no effect on ex vivo BT and BV, but the modification b) resulted in significantly lower BT and BV values during the whole procedure. Infusion of platelets at the end of the operation improved the platelet function.

Institute of Clinical Chemistry, University, 8 Munich 70, Marchlominstr. 15, Germany.

94

MODEL STUDIES ON THE HEMOCOMPATIBILITY OF SOME BIOMATERIALS ESPECIALLY HOLLOW FIBER AND SILICONE MEMBRANES

W. Heller, H.P. Wendel, R. Klaffschenkel, H.-E. Hoffmeister

For preclinical evaluation of improvements in oxygenators an ex vivo heart lung machine model to simulate extracorporeal circulation has been developed. Three different membrane oxygenators from Germany, Italy and the USA (A, B, C) were tested in the model. Oxygenator A had a siliconised, unporous diffusion membrane with plates totalling 0.7m² gas exchange surface. Oxygenator B had a hollow fibre membrane of 0.4m² and oxygenator C a flat membrane also of 0.4m². 500 ml volumes of heparinised recalificed RCD blood (max. 48h old) were recirculated for 90 minutes in a short-circuited system at 28°C. Hemodilution and gas flow were similar to operating conditions. Eight blood samples were taken from each run. With regard to corpuscular alterations no significant differences between A, B and C were seen which refer to the kalikrein-kinin and fibrinolytic systems the course of activation and inhibition was very similar. Investigations with sensitive immunological methods revealed significant differences between the three oxygenators.

Zentrum für Thorax-, Herz- und Gefäßchirurgie der Universität Tübingen, Calwerstr. 7, D-7400 Tübingen
InVESTIGATIONS ON THE HEMOCOMPATIBILITY OF TWO BUBBLE-OXYGENATORS IN AORTOCORONARY BYPASS-OPERATIONS

W. Heller, H.P. Wendel, H.-E. Hoffmeister

After major surgery, elevated WBC or temperature, leads to activation of several hemostaseological systems. The oxygenator of the heart lung machine is regarded as the main component for this activation. In a randomised study two bubble oxygenators (A and B) were tested under identical conditions in two groups of 22 patients who underwent cardiopulmonary bypass (CPB). Blood samples were taken before and after CPB and were investigated for activation of the coagulation, Kallikrein-kinin and fibrinolytic systems and for damage of blood corpuscular components. Contact system activation was less with oxygenator A and reduced platelet damage was seen (lower levels of platelet factor 4). With both oxygenators PMN-elastase levels increased but no significant difference between the two groups was found. Because of the risk of lung complications following contact system activation it would appear that oxygenator A would be better than oxygenator B.

Abt. für Thorax-, Herz- und Gefäßchirurgie der Universität Tübingen, Calwerstr. 7, D-7400 Tübingen

FIBRINOLYSIS PARAMETERS AFTER OPENHEART SURGERY

H. Kempter and M. M. Kratzer

50 to 70% decreases of PM, AP, AT, MG, KK and C1IN during CPB were observed. Within two postoperative days the recovery of PM was 70%, of AP, AT, MG and C1IN 100%, while MG and KK only increased 10%. TAT (2.4 μg/ml, median) during CPB slightly increased to 8 μg/ml. After neutralization of heparin by protamine chloride at the end of CPB TAT distinctly decreased to 20 μg/ml and slowly normalized within two days. Non-croslinked TDP and FDP during CPB increased 1.2-1.4 fold, FDP 2.5 fold and were found to be normal at the first postoperative day. DD at end of CPB increased 2.3 fold, however, DD median did not exceed normal ranges. EL and LPM were found to be elevated at the first and second postoperative day.

Increase of TAT, TDP, FDP and FDP indicated activation of coagulation and fibrinolysis during CPB. Formation of croslinked fibrin at that time was obviously inhibited, but was increased after CPB, as indicated by increase of DD. PO. increase of LPM and EL might indicate either nonspecific activation of granulocyte proteases or liberation of lysosomal enzymes from granulocytes by the usual transphys of whole blood after CPB.

Institut f. Exp. Hämatologie und Transfusionsmedizin der Universität Bonn, Sigmund-Freud-Straße 26, 5300 Bonn 1

HEMOSTATIC PARAMETERS OF TWO GROUPS OF CHILDREN WITH CONGENITAL HEART FAILURES

H.-J. Herrfelder, S. Popov-Cenic, M. Hötzle, A. Urban, A.M. Brecher

The examinations indicated no chronic alterations of haemostasis due to CHD in children with or without DIC. The TGA group exhibited fibrinolytic activations with or without DIC.

Institut f. Exp. Hämatologie und Transfusionsmedizin der Universität Bonn, Sigmund-Freud-Straße 25, 5300 Bonn 1
HEMORHEOLOGICAL CHANGES DURING AORTOCORONARY BYPASS OPERATION USING DIFFERENT DRUGS
U.T. Seyfert, P. Feindt*, I. Volkmer*, E. Wenzel

A prospective randomized double blind study including 4 groups of 10 patients treated with heparin (HEP), prostacyclin (PGI2), aprotinin (APO)/HEP, APO/PGI2 was performed to investigate changes in the course of rheological parameters during the course of aortocoronary bypass operation. Patients in this study were operated without the use of a cell saver.

Results:
1. No significant changes in fibrinogen, erythrocyte and hemoglobin, serum and plasma viscosity levels during the course of extracorporeal circulation and in the postoperative period.
2. Increased levels of fibrinogen/fibrin degradation products (Fibrinostika - Organon, Technika) in the control- and PGI2 groups vs. APO (p <0.001). Exclusion of ex vivo, in vitro effects induced by APO.

Conclusion: Treatment with high doses of aprotinin is safe and leads in our laboratory panel to no negative effects on rheological parameters despite of differences in fibrinogen/fibrin turnover.

Abt. f. Klin. Hämostaseologie und Transfusionsmedizin und Thoraxchirurgie* der Universitätskliniken, D-6650 Homburg/Saar

ADSORPTION OF PLASMA COMPONENTS TO PTFE (POLYTETRAFLUORETHYLENE) AND ITS EFFECT UPON ADHESION OF ENDOTHELIAL CELLS AND FIBROBLASTS
E. Schlosser, B. C. Adelmann-Grill, and H. Hörmann

Optimal function of artificial vascular grafts requires controlled ingrowth of host cells. Whereas a homogenous layer of endothelial cells is probably desirable at the luminal face of the graft fibroblast hyperplasia at the sites of anastomoses is clearly to be avoided. Blood components adsorbed to the graft are likely of crucial importance for attachment of cells to the prothesis. The aim of the present project is to unravel basic mechanisms of interaction between graft material, blood components, and cells so as to provide rational way to facilitate or prevent attachment of specific cell types to the graft material.

PTFE beads were coated with fibrinogen, fibronectin, and thrombin either separately or in combinations. There were significant synergistic effects in the adsorption characteristics of these proteins. To simulate the in vivo situation PTFE was exposed repeatedly to fresh whole plasma. This resulted in progressive accumulation of thrombin, fibrinogen, fibronectin, and factor XIII on the polymer. Cells did not adhere to untreated PTFE. Efficient attachment of endothelial cells could be accomplished by exposing the material to fresh plasma first, and repeated coating with fresh plasma over a period of eight days resulted in increased cell adhesion proportional to the number of coats. Fibroblasts responded similarly but they attached more slowly and in smaller numbers.

These data show that proteins that are normally present in plasma in very low concentrations can specifically accumulate on the prothesis material and thus regulate ingrowth of cells.

Max-Planck-Institut für Biochemie, D(W)-8033 Martinsried

HAEMOSTASIS AND BLOOD BIOCOMPATIBILITY DURING AORTOCORONARY BYPASS OPERATION USING PROPHYLACTIC APROTININ TREATMENT
U.T. Seyfert, P. Feindt*, I. Volkmer*, H. Haack, E. Wenzel

Substantial bleeding after aortocoronary bypass operation complicates postoperative patient care and increases risk of transfusion related infections.

Patients and methods. 4 groups of 10 patients (control group-heparin, prostacyclin group-PGI2, aprotinin group-APO, PGI2/APO-group) who have to undergo aortocoronary venous bypass operations (without use of cell saver).

Results: 1. No significant changes in platelet count, fibrinogen levels, fibrin monomers using the different drug regimes. 2. Decreased exliberation of β-TG and PF 4 in the aprotinin groups during the course of bypass operation compared to heparin and PGI2 (p<0.01).
3. Decreased levels of dimers and TAT in the APO and APO/PGI2 groups (p<0.001). 4. PAI activity increased in APO vs. PGI2 and heparin (p < 0.001), PAI-Antigen decreased (p<0.001).
5. Increase of elastase levels using all substances. 6. Reduction in postoperative blood demand in the APO group (p<0.001). 7. No negative influences of APO on urinary production and renal function compared to heparin and PGI2.

Conclusion: Administration of aprotinin using our dose regime demonstrates good compatibility and safety compared to other drugs used during heart surgery.

Abt. f. Klin. Hämostaseologie und Transfusionsmedizin, *Abt. f. Herz- und Thoraxchirurgie der Universitätskliniken, D-6590 Homburg/Saar
PARTITIONING BY CHROMATOGRAPHY OF HIV-1 DURING MANUFACTURE OF A HIGHLY PURIFIED F IX CONCENTRATE (IMMUNINE®)

G. Wöber, M. Sasagary and Y. Linnau

Both with classically and monoclonal-antibody-purified coagulation factor concentrates the absence of infectious agents cannot be guaranteed. Therefore, it is desirable, beyond efficient virus inactivation procedures, to further extend the safety margin of such preparations.

Apart from vapour heat treatment for virus inactivation, the manufacture of IMMUNINE, a highly purified F IX concentrate, includes ion exchange and hydrophobic interaction chromatography. We were interested in the partitioning of HIV between the F IX-containing and the waste effluent fraction during column chromatography. A high-titre suspension of HIV-1 was deliberately added to the column feed, and the virus content was determined in virus fractions.

We found that with both chromatographic steps the virus titre of respective F IX-containing fractions was reduced by approx. 2 orders of magnitude compared with the column input. These results show that purification of factor concentrates may contribute to virus safety, albeit to a limited extent. An efficacious virus inactivating step, clinically proven according to SBC regulations, with the column input was reduced by approx. 2 orders of magnitude compared with the column input. These results show that purification of factor concentrates may contribute to virus safety, albeit to a limited extent. An efficacious virus inactivating step, clinically proven according to SBC regulations, remains indispensable.

The solvent detergent (80) tri-(N-butyl)phosphate is used in combination with Tween 80 to inactivate viruses. The efficacy of this method was checked for the Biostep factor VIII preparations "antihemophilic globulin A human" (AHG) and "Octa-VI. Factor VIII concentrate human (Octa VI.)" for FVIII, FVIII-1, FVIII and Sendai virus. The inactivation for FVIII was tested in two chimpanzees. The factor VIII solution was contaminated with the titrated NAB virus (Hatchinson strain). Aliquot portions were frozen at -80 °C and stored for the challenge experiment. The remainder of virus-containing factor VIII solution was treated with 0.5 % TNBP and the plasma was thawed for eight hours at 25 °C and afterwards frozen at -80 °C. Two chimpanzees received intravenous injections of 10 ml TNBP/Tween-treated samples. The inoculated animals were monitored for six months. Neither animal developed FVIII infection.

The rates of FVIII and VWF inactivation were determined in cell cultures. The Sendai virus titration was carried out in hen's eggs.

Virus inactivation for the Biostep factor VIII concentrate was examined. By application of 3 x 1000 IE FEIBA per day a rise of FVIII activity within 72 hours to 76 % and an inhibitor capacity was determined by F VIII inhibitor assay via serial dilutions of NP with either buffer or PP. A weak inhibitor capacity of 1.7 Bethesda U was evaluated. The inhibitor capacity was determined by F VIII inhibitor assay via serial dilutions of NP with either buffer or PP. A weak inhibitor capacity of 1.7 Bethesda U was evaluated.

The initial therapeutical application of FVIII concentrate was not successful. FVIII activity did not increase and bleeding tendency persisted. Thereafter use of FEIBA concentrate (factor eight inhibitor bypass activity) was examined. By application of 3 x 1000 IE FEIBA per day a rise of FVIII activity within 72 hours to 76 % and after 96 hours to 158 % was achieved. F XI (55 %) and F XII (20 %) as well as F IX (90 %) also slowly increased. Bleeding tendency disappeared.

Virus inactivation for the Biostep factor VIII concentrate was examined. By application of 3 x 1000 IE FEIBA per day a rise of FVIII activity within 72 hours to 76 % and after 96 hours to 158 % was achieved. F XI (55 %) and F XII (20 %) as well as F IX (90 %) also slowly increased. Bleeding tendency disappeared.

The nature of the inhibitor at present is under investigation.
ACUTE HIV-1 INFECTION AFTER TREATMENT OF HEMOPHILIA B WITH A BETA-PROPiolACTONE-IV/VIRUS-INACTIVATED PSPS-CONCENTRATE

J. Ch. SORHT, M. WALKA, M. GAFR

The 22-YEAR-OLDMED HEMOPHILIA B PATIENT HAD BEEN ON IRREGULAR FACTOR-REPLACEMENT THERAPY WITH A PROTHROMBIN-COMPLEX-CONCENTRATE (PPBS BIOTEST), 4000 I.U. daily dose, the factor level reached a maximum of 12 %, the inhibitor was 0 to 2 B.U. Increasing intervals between infusions lowered factor-levels to less than 1 %, with rising inhibitor titres. Treatment was changed to 2000 I.U.FEIBA 2-daily. The total doses were 1.050.000 I.U. of Factor VIII, and 650.000 I.U. FEIBA. 279 days after the start of treatment a significant elevation of liver enzymes was noted. The levels increased for 6 weeks, then slowly returned to normal. A anti-HCV-titre (hepatitis non-A-non-B-marker) could be demonstrated, a stored serum dating from 7 months after start of treatment was shown to be anti-HCV negative. Other viral studies, including HIV-1-Antibodies remained negative.

CONCLUSION: Advanced methods of virus inactivation have shown to be highly effective. The risk of viral transmission, however, is not completely eliminated.

Univ.-Kinderklinik, Robert-Koch Str.40, D-3400 Göttingen
CONVENTIONAL DETECTION OF HAEOMOPHILIA-B CARRIERS
G. Lutze, H.-J. Presser and H. Urbahn

65 obligatory and possible female carriers of haemophilia B were several times haemostaseologically investigated. Obligatory carriers were subdivided in hypo-IX-aemia and dys-IX-aemia (Normodys-IX-aemia or Hypodys-IX-aemia). Criterion of carrier state was the quotient IX:C/IX:Ag. About 80 percent of obligatory carriers have shown the following results:

IX:C/IX:Ag
1. Hypo-IX-aemia about 1 (0.8 - 1.2)
2.1. Normodys-IX-aemia about 0.9 (0.2 - 0.6)
2.2. Hypodys-IX-aemia corresponding to 1 or 2.1.

Possible carriers of haemophilia B were classified in comparing their results with those of obligatory carriers. The haemostaseological examination should be used as basis for genomic diagnostics. The methods are quickly and easily to perform and allow in several cases of carriers to avoid genomic diagnostics.

Institut für Klinische Chemie und Laboratoriumsdiagnostik und Klinik für Innere Medizin der Medizinischen Akademie, Leipzig Str. 44, 0-3690 Magdeburg

SUBCLASSIFICATION OF HAEOMOPHILIA B
G. Lutze, H.-J. Presser and H. Urbahn

Concerning the molecular defects on gene or gene products the haemophilia B is of different appearance. Subclassification of haemophilia B is an essential requirement to estimate the defects in factor-IX-molecule or in factor-IX-gene in cases of conventional diagnostics of female carriers. The present classification of haemophilia B is insufficient in this way.

Based on examination of 54 haemophilia-B patients the following subclassiﬁcation is recommended:

1. Hypo-IX-aemia (proportional reduction of IX:C and IX:Ag)
2. Dys-IX-aemia (disproportional reduction of IX:C)
   2.1. Normodys-IX-aemia (IX:C < 1 % - 35 %; IX:Ag > 65 %)
   2.2. Hypodys-IX-aemia (IX:C < 1 % - 35 %; IX:Ag < 65 %)

Every subclass is further divided with regard to degree of severity (BRINKHOU). The recommended subclasses are reproducible confirmed by diagnostic controls.

Institut für Klinische Chemie und Laboratoriumsdiagnostik und Klinik für Innere Medizin der Medizinischen Akademie, Leipzig Str. 44, 0-3690 Magdeburg

SUBSTITUTION OF FACTOR XIII IN EXACERBATED CHRONIC INFLAMMATORY BOWEL DISEASE (CIBD)
C.E. Dempfle, R. Gladisch, A. Röckel, J. Magez, D.L. Heene

Inflammation of the colon in patients with exacerbated chronic inflammatory bowel disease (CIBD) is often accompanied by intestinal bleeding, especially in patients with ulcerative colitis. Since factor XIII is required for haemostasis as well as cellular invasion and acts by covalently crosslinking fibrin and by attaching proteins such as fibronectin to clots, healing of bleeding intestinal lesions in patients with CIBD was thought to be influenced by factor XIII. Measurement of plasma factor XIII activity in 22 patients with exacerbated ulcerative colitis and 45 patients with exacerbated Crohn's disease revealed levels of 44 ± 28 % and 51 ± 22 %, respectively, upon admission. Similar levels were detected upon measurement of factor XIII A-antigen, whereas immunological levels of factor XIII B, the carrier subunit of plasma factor XIII, were within the normal range. Patients with remission stage ulcerative colitis and Crohn's disease display normal levels of factor XIII activity and antigen, the factor XIII activity values being 69 ± 21 % (n = 7) and 94 ± 21 % (n = 16), respectively. Plasma levels of factor XIII activity were found to be closely related to intestinal inflammatory activity in the patients examined, the numerical coefficients of correlation with C-reactive protein (CRP) being R = 0.87 and R = 0.71 in patients with ulcerative colitis and Crohn's disease, respectively. Upon intravenous treatment with 1250 U of placental factor XIII concentrate (Fibrogammin®, Behringwerke, Marburg), plasma levels of factor XIII activity were found to increase by 34 ± 9 % after 30 minutes, and by 23 ± 11 % after 24 hours (n = 15). Making preliminary conclusions from the experiences made upon treatment of several patients with exacerbated CIBD it appears as if adjuvant treatment with placental factor XIII concentrate considerably accelerates cessation of intestinal bleeding in patients with CIBD.

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

Longterm Intervall F XIII substitution in congenital (homozygote) F XIII deficiency. F XIII recovery studies in 3 homozygote patients after substitution with F XIII concentrate.
H. G. Emrich, R. Egbring, R. Seitz, L. Lerch

Since 1971 one patient with severe F XIII deficiency was treated with F XIII concentrate in long term intervals of 4-6 weeks beginning with a dosage of 250 units every 4 weeks. The dosage was increased to 1250 units during the patients growth.

After substitution of 1155 unites of F XIII concentrate HS in 1982 we found an increase of F XIII level to 28%, recovery of 71% and a half life of 4.9 days. 1990 the FXIII level increased only up to nearly 20% of normal measuring with C14 incorporation assay as well as with a new photometric assay for F XIII of Behring company developed by Dr. Fickemacher.

The increase after F XIII substitution was measured by both methods in 3 other patients with congenital severe deficiency.

Additionally six relatively exhibited comparable diminished F XIII levels in both test systems and could be identified as Heterozygot.

20 years long term-intervall-treatment had been performed without HIV infection.

Dept. of Intern. medicine University of Marburg.
DYSFIBRINOGENEMIA IN TWO FAMILIES: STRUCTURAL ABNORMALITIES IN THE BB CHAIN
M. Meyer, G. Lütze, E. Gronnica-Ihle
Laboratory coagulation tests indicated a dysfunctional fibrinogen in two families: decreased Quick value, prolonged thrombin and reptilase times, normal fibrinogen values when measured by heat precipitation, Biuret method or immunological methods but decreased values with coagulation-dependent methods. Further studies on purified fibrinogen revealed in one family (3 patients) a normal fibrinopeptide (FP) release. Electrophoretic analysis demonstrated normal A chains and chains but B chains with an increased apparent Mr, a more acidic pi range and a higher glycosylation than normal (fibrinogen Berlin II).
In the second family (1 patient) FP release was slightly delayed. Abnormal 80 chains with increased apparent Mr, more basic pi range and normal glycosylation were detected (fibrinogen Magdeburg II). In both cases the molecular abnormalities reside in the B portion of the 80 chains. All dysfibrinogenemic patients of the two families are heterozygous as concluded from the presence of about 50% normal 80 chains.
Abteilung Medizinische Genetik der Medizinischen Akademie Erfurt, Arnstädter Str. 34, D-0-5082 Erfurt

DIFFERENT APROTININ APPLICATIONS INFLUENCING HEMOSTATIC CHANGES IN ORTHOTOPIC LIVER TRANSPLANTATION (OLT)
G. Himmelreich, M. Muser, R. Steffen, W.G. Bechstein, H. Riess
The effect of different aprotinin applications on hemostatic changes and blood product requirements in orthotopic liver transplantation (OLT) were investigated in a prospective and randomized study. 13 patients received aprotinin as a bolus (3x0.5 Mill.KIU) whereas 10 patients were treated with continuous aprotinin infusion of 0.1 to 0.4 Mill.KIU per hour. Signs of hyperfibrinolysis especially before and after reperfusion of the graft liver measured by thromboelastography were significantly lower in the infusion group. Tissue plasminogen activator (t-PA) activity increased during the anhepatic phase to a significant lower extent in the infusion group. Blood product requirements during OLT were higher in the bolus group. All 23 patients are still alive and only two women of the bolus group needed retransplantation.
Furthermore, we investigated the early perfusate of the graft liver in both groups and detected signs of a decreased t-PA release in the infusion group. This may be attributable to a protective effect of aprotinin to endothelial cells. Our results demonstrated an advantage of aprotinin infusion even as continuous infusion compared with bolus application in OLT.
Universitätsklinikum Rudolf Virchow, Abt. f. Innere Medizin und Chirurgie, Spandauer Damm 130, D-1000 Berlin 19

THROMBIN ANTIHYBRID III (TAT) COMPLEXES, PROTHROMBIN-FRAGMENTS (F1+2), ELASTASE MARKERS, WILLEBRAND-FAC TOR AND F XIII DEFICIENCY IN SYSTEMIC NEUTROBLASTIC VASCULITIS (SNV)
K. Andressy, R. Egbring, R. Seitz, W.B. Schwerk, J. Herburg*
Vasculitis is a common vessel reaction of different stimuli in a variety of disorders (Wegener's Granulomatosis, Wegener's Schönmann Henoeh, Morbus Schönlaun Henoch (MSH) and some infections. While in WG circulating antibodies against cyttoplasmat constituents of PMN's (ANACA's) are found to be important for pathogenesis, stimulation of PMNL's with protease release and activation of coagulation (DIC) and fibrinolysis as well as F XIII deficiency additionally may be responsible for haemostatic abnormalities. Ten patients with WG, four patients with MSH and some with sepsis were investigated for ANCA test, ESR, CRP, v.Wf, TAT, Prothrombin-fragments (F1+2) EIPx, TAT, and Elastase related Fp. B6 30-43 (Instit. for Forens. Medicine, Uppsala, Sverige, Head.: Prof. T. Saldeen).
Results: 9 of 10 patients with WG, all with MSH and sepsis exhibited high values of TAT complexes during active phase of diseases. In WG high ANCA-titer correlate with TAT and F 1+2 fragm., and with increased F XIII/v.vWf levels, indicating endothelial cell damage. FpBB 30-43 and ELF levels were found only moderate but highly elevated in bacterial super infection. In patients with MSH factor XIII deficiency was responsible for severe bleeding complications as intranbral haematomata of the duodenum and skin haematomata. Bleeding complications due to factor XIII deficiency < 20% were successfully treated with F XIII concentrate. Various forms of vasculitis exhibited different haemostatic disturbances shown by comparison of two forms of SNV (WG and MSH). But in WG and related forms of vasculitis increased Thrombin generation is observed in acute phase of disease by measuring TAT complexes and F1+2 fragments.
Dept. of Internal Medicine, University of Heidelberg and Marburg,*Behringwerke

EFFECT OF RETRANSFUSION OF AUTOLOGOUS DEEP FRESH FROZEN PLASMA (DFFP) ON HEMOSTASIS
H. Neumeier and J.U. Wieding
In an earlier study 3 plasmapheresis machines ("PCS"/ HAEMONETICS, "Autopheresis-C"/ BAXTER, "Plasmapur-Monitor"/ ORGANON-TEKNIKA) were compared with the conventional manual bloodbag-centrifugation method. We detected a measurable activation of the clotting system especially when performing the traditional manual method, but also, although less pronounced, when performing plasmapheresis with any of the machines. In order to learn more about possible effects on recipients of DFFP we now performed plasmapheresis on 10 voluntary healthy blood donors, once with a machine and once using the traditional method. The obtained autologous DFFPs were retransfused 7 to 10 days later. Samples were taken from the DFFPs and from the volunteers before and after retransfusion in intervals of 15, 30, 60, 120, 240, and 300 minutes, and were tested for activation of factors of the clotting system.
Thrombin-antithrombin III-complexes (TAT), prothrombin-fragments (F1+2), fibrinopeptid A (FPA) and soluble fibrin (SF) as markers of activation of the clotting system rose within the first hour after DFFP retransfusion. TAT, PTF and SF returned to their starting levels after 2 - 3 hours, FPA a little later. The rise of TAT and PTF indicates an increased production of thrombin, the rise of FPA and SF indicates an increased turnover of fibrinogen-fibrin caused by thrombin action on fibrinogen. The level of fibrinogen degradation products is a product generated later within fibrinogen. After retransfusion the TAT levels found after 5 hours in the recipients' blood were nearly 2 fold elevated as compared to the initial level. They ranged clearly above the standard value (marginally pathological). Because it is essential for patients who need DFFP, not to undergo any activation of their haemostatic system, the question of the clinical relevance of this activation detected with sensitive laboratory methods remains unresolved and requires further investigations.
Abtlg. f. Transfusionsmedizin der Universität, D-3400 Göttingen
RECURRENT THROMBOSIS AND LUNG EMBOLISM WITH DIMINISHED FACTOR VII AND AT III ACTIVITY, Cysteine, Thionine 8 SYNTHASE DEFICIENCY AND HOMOCYSTINURIA. SUCCESSFUL TREATMENT WITH ANTITHROMBIN III CONCENTRATES AND VITAMIN B6.

H.W. Schinelle, R. Ebbing, R. Seitz, U. Willenbockel and K. Weigand

Our report concerns a 24 year old male with homocystinuria, high levels of methionin (meth), homocystin (hc) ~ homocystin (HCs) and ristocetin cofactor (Rcf), factor VII (20%), and antithrombin III (AT III = 40%), deficiency. He suffered from recurrent thrombosis such as sinus sag. sup. thrombosis, left popliteal-, lower leg vein thrombosis VII (20%), and antithrombin III (AT III = 40%), deficiency. The vitamin B6 regimen may activate the synthesis of proteins such as F VII may be enhanced by heparin treatment since May 1989 ceased thromboembolic complications so far. The vitamin B6 regimen may activate available traces of cystathionine β synthease (CBS). HCs and serin are condensed by this enzyme to cystathionin, a precursor of e.g. cystein. The Hc-induced encephalopathy and the activation of coagulation could be improved, as shown by Rcf decrease and AT III increase. Furthermore, the synthesis of proteins such as F VII may be enhanced by vitamin B6 activation of CBS.

Familial examination exhibited a sister with homocystinuria as well, but without thromboembolic complications. The father and the mother are heterozygotes of the disorder.

Experiences with Densitometric Scan of von-Willebrand Factor-Multimers Analysis

Th. Eller, P. Brauer, J. Albert, F. Koller
Zentrallabor der Med. Universitätsklinik Würzburg, FRG

The analysis of vWF-multimers with SDS agarosegel electrophoresis and direct immunostaining is a valid method to precisely subtype von-Willebrand-disease. Densitometric scans of the gels were performed in order to get more information of multimer composition in vWF compared by visual estimation. The scans are done at 600nm. Resulting peaks are separated into 3 groups representing the small, medium and large multimers (Tatewaki W. et al. (1989) Thromb. Res. 56, 191-195). Area under the slope and relative percentage was calculated for each group. Thus we analysed plasma samples of 27 patients for multimer composition of vWF which visually appeared to be normal.

In most cases (91%) 16 multimeric bands were found in plasma while 18 bands could be detected in platelet lysates. The median of relative percentage was 11.5% in group I (range 8.4-19.3%), 35.5% in group II (21.7-43.5%) and 53.9% (41-66.6%) in group III respectively. 90% of around 1 ranged from 9-16% for group II from 26-42% and 10 group III from 45-57%. These ranges could be interpreted as "normal ranges" for each multimer group. Patients suffering from vWF type Ila or Iib showed a significant increase of multimers of group I and II and a decrease of group III. In three patients with a bleeding history a moderate increase was detected in group I and II and a decrease in group III. Two cases demonstrated low multimers of group I and II and high concentrations in group III, a situation which was described as supranormal structure by Mannucci et al. (1989) Blood 74, 978-983.

Multimers analysis using densitometric scans improves the diagnosis of vWD because slight variations of the structure of multimers are detectable in contrast to visual examination of gels.

Dipl.-Chem. Th. Eller, Zentrallabor der Med. Universitätsklinik, Josef-Schneider-Str.2, 8700 Würzburg

RAPID AND DEADSIMPLE TECHNIQUE FOR THE ANALYSIS OF PLATELET GP CLEFTOPROTEIN DEFICIENCIES

B. Kehrel, G. Wiehe, U. Breite

Glanzmann's thrombasthenia is associated with the absence or a qualitative defect in GPIlla/Illa complex. Another disorder, the Bernard-Souiller syndrome is associated with the absence of GPIb/IX complex and GPV. The deficiency of GPV was published by us and others to be associated with disturbed platelet-collagen interaction. For screening purpose, especially with blood from small children, we developed a rapid and very simple assay for the analysis of platelet GP deficiencies, which needs less than 1 ml of blood.

Mouse monoclonal anti-platelet membrane GP antibodies were bound to magnetobeads coated with sheep anti-mouse antibodies and the beads washed thoroughly by using their magnetic properties. 20 μl of platelet-rich plasma from patients with glycoprotein deficiencies and from healthy volunteers were then incubated with antibody-coated magnetobeads. The mixtures were then examined under a phase contrast light microscope. Only if antigen was present on the platelet surface the platelets were bounded to the beads building large agglutinates and rosettes.

Using several different monoclonals against different epitopes of the examined glycoprotein it is possible to screen for glycoprotein deficiencies. With monoclonals directed against function-dependent epitopes it is even possible to gain a rough idea on the function of the examined platelets.

Universitätskliniken, Innere Medizin A, Exp. Hämostaseoforschung, Domagkstr.3, D-4400 Münster

DIAGNOSIS AND THERAPY OF FIBRINOMETHOSIS FOR PREVENTION OF THROMBOEMBOLISM

K. Miller-Weisheiss

Efficiency control in the prevention of thrombosis is usually done by registration of clinical manifestation of thrombocytopenia, i.e. by failing the ria of therapy. Many clinical trials demonstrate a resulting risk for postoperative thrombosis (up to 25%), laboratory testing (PTT or FII) can facilitate the resulting risk only indirectly because they simply register the defect of the ability of heparinization - for low dose heparin no monitoring is recommended. Laboratorycontrol by parameters of activation however demonstrates the kinetic of heparinization and enables a disting of diagnosis and therapeutic monitoring into the state of prethrombosis (S.Fugel and W. Spanuth, Klin. Woch. 66, 1020, 1988) or even thrombosis (K. Moway et al., Klin. Invest. 80, 1935, 1987). Thus instead of only diagnosing thrombosis it is possible to prevent it by employing therapy, among the many measurable activation products of the hemostatic system only few are simple enough to allow full time regular monitoring in cases of emergency, mostly applicators are e.g. the fibrinogen (FII) test according to large and D-Dimer test (Int. Co.) To us the 24 hours a day routine of FII and D D in diagnosis and therapeutic monitoring appeared logical and consistent. Theoretical approaches following pathophysiologi- cal lines (normal - normal, normal - (pre)thrombosis - co-factorially ineffective anticoagulation - with monitoring results from FII norm, D D norm, FII norm, D D high to FII norm, D D subnormal) respectively yielded 3 years of valuable clinical experience. This may be proved by few concrete descriptions. A clinical trial for postoperative prevention of thrombosis has been started. Multicentric controlled clinical studies would be useful and desirable. In conclusion we consider the regular monitoring of thrombosis prevention therapy by activation parameters of heparinis not only recommendable but essential for any hospital laboratory. If the resulting risk of thromboembolic diseases is to be diminished and not only be regarded as inevitable. Unfortunately along with hemostology these requirements tend to be cornered as if neart for devotees and enthusiasts only.

Institut für Klinische Chemie und Laboratoriodiagnostik Bürgerhospital, Tannroder Str. 14-16, D-7000 Stuttgart 1
PROTHROMBIN FRAGMENT 1+2 (F1+2): MULTICENTER EVALUATION OF A NEW ENZYME IMMUNOASSAY

H.D. Bruhn¹, R. Egbring², H. Pelzer³, R. Seitz³, C. Wagner³

Determination of prothrombin activation fragment 1+2 (F1+2) in plasma enables an estimation of the in vivo generated thrombin concentration. A recently developed enzyme immunoassay for the quantification of F1+2 was evaluated in a multicenter study in different clinical situations.

The assay is based on the sandwich principle utilizing two different antibodies directed against human F1+2 and human prothrombin respectively. For F1+2 concentrations between 0.09 and 4.3 nM/L intrasay coefficients of variation (CV) between 4 and 12 % and intersay CVs between 5 and 12 % were determined. The reference range for F1+2 concentrations in plasma samples of healthy individuals (n = 265) was found to be 0.4 to 1.1 nM/L, (median: 0.58 nM/L). These values are in excellent agreement with prior published data (1). Patients with thrombo-embolic disorders exhibited increased F1+2 concentrations (> 1.5 nM/L) before therapy; under standard heparin therapy F1+2 levels were significantly reduced. Similar effects were detected in patients under low dose heparin therapy. In patients with acute inflammatory infection a significant increase of F1+2 levels was observed between 30 and 90 min after start of therapy; levels of thrombin-antithrombin II·complex (TAT) exhibited a similar time course. Patients under stable oral anticoagulant therapy (n = 60) exhibited significantly decreased F1+2 levels, ranging from 0.1 to 0.4 nM/L. During the first two days of therapy increased F1+2 concentrations were detected which decreased significantly to reduced levels on days 3 to 6; in the stable phase of therapy (INR 2.0 - 4.0) F1+2 concentrations of 0.4 nM/L were measured. The results of the present study indicate that F1+2 is a suitable parameter for characterization of the activation of the coagulation system. Furthermore, determination of F1+2 can be of value in detecting both hypercoagulable as well as hypocoagulable states.

¹University of Kiel, Germany; ²University of Marburg, Germany; ³Behringwerke AG, Marburg, Germany.

(1) Pelzer, H.; Schwarz, A.; Stüber, W.: Determination of human prothrombin activation fragment 1+2 in plasma with an antibody against a synthetic peptide. Accepted in Thromb. Haemostasis.
EVALUATION OF A NEW IMMUNOCHEMICAL ASSAY FOR ANTITHROMBIN III
E.M. Solleder, F. Keller, B. Schmidt, D. Steuer and M. Lammers

A new immunochemical assay for the determination of antithrombin III (AT III) concentration was evaluated. It utilizes a precalibrated Turbiquant reagent and is performed on the Turbimune System (TTS) of Behringwerke AG, Marburg. The measuring range of the assay is 0.035 to 0.5 g/l. The measuring time is approx. 30 seconds per determination, making the assay suited for emergency applications. Its precision is characterized by coefficients of variation of 1.1 to 3.0 % within run (n=20), and 1.8 to 5.1 % from day to day (n=10). The high precision allows all determinations to be done in singlicate. Lipaemic, icteric or haemolytic samples or the presence of heparin did not interfere with the assay. Comparison to other immunochemical methods for AT III determination revealed a good agreement of results: y (TTS) = 0.94x (immunonephelometry) -0.00; r=0.953, n=103, and y (TTS) = 0.94x (radial immunodiffusion) + 0.02; r=0.988, n=100. AT III concentrations determined with the TTS were also highly correlated with the functional activity as determined with a chromogenic method: r=0.921, n=109. The AT III reference range for 114 apparently healthy persons was 0.21 to 0.35 g/l (2.5th to 97.5th percentile) with no difference between males and females.

Medizinische Universitaetsklinik, Zentrallabor, Josef-Schneider-Straße 2, 6700 Würzburg, Germany

SIMPLE, MONOTEST ASSAYS FOR ANTITHROMBIN AND HEPARIN BASED ON FACTOR Xa INHIBITION AND CHROMOGENIC PEPTIDE SUBSTRATES
P. Finberg and C. Isacson

Antithrombin activity can be determined by adding 400 µl of diluted plasma (50µl + 5 ml buffer with heparin) to a lyophilized mixture of Factor Xa and a suitable chromogenic substrate for this enzyme in a disposable photometer cuvette. After a rapid dissolution the enzyme is inhibited by antithrombin in the sample. The remaining intact enzyme splits off the chromophor from the substrate. The reactions are stopped by adding 400 µl of 5% acetic acid. The colour development is inversely proportional to the amount of antithrombin.

The assay of heparin is built on the same principle. The buffer contains no heparin but dextran sulfate 4000 to quench heparin antagonists (FP4) usually released during sample preparation. The plasma is diluted by adding 200 µl + 3 ml buffer and 400 µl of this is added to the cuvette.

After measuring the amount of free chromophor the amount of analyte, antithrombin and heparin, respectively, is read in batch specific diagrams. By using this single stage procedure with unsaturated substrate kinetics the temperature dependence is low, making it possible to perform the assays at room temperature. These assays are suitable for both acute situations and in laboratories where only one or a few assays are performed on each occasion. Standardization of AT III heparin was fully acceptable for ordinary heparin as all preparations tested gave approximately the same activity but for certain LMW heparin caution must be taken to avoid mistreatment of patients.

Kabi Diagnostica AB, Studsvik, S-611 82 Nyköping, Sweden

A NEW SENSITIVE GLOBAL ASSAY FOR PLATELET AND COAGULATION DEFECTS. PRINCIPLE AND FIRST CLINICAL RESULTS IN MILD HEMORRHAGIC DISORDERS.
M. Basic-Micic, E. Kling, H. K. Breddin

We have recently developed a new very sensitive test system, Platelet-Induced thrombin-generation time (PITT), in which thrombin formation due to platelet activation leads to aggregation and subsequent coagulation of platelet rich plasma (PRP). The blood samples are anti-coagulated with low concentrations of a low molecular weight heparin - Fraxiparin (Fx), r-hirudin (H), pentasaccharide or other direct or indirect thrombin-inhibitors. Platelet rich plasma (PRP) obtained from these samples is stored at room temperature until tested. 0.6 ml FRP are placed in a disc-shaped cuvette, and rotation is started 30 min. after blood sampling. Rotation temperature is 37° C. The time from the start of rotation (20 rpm) until aggregation (Tagg) and clotting (Tc) is registered. In healthy persons coagulation time (Tc) was 6.7 ± 2.7 min., n = 31 (Fx-PRP 5 µg/ml).

A group of 5 patients with mild v. Willebrand-Syndrom (vWF > 50 % PTT normal or only slightly prolonged) has been investigated, Tc in these patients was 14.0 ± 5.83 min. In Fraxiparin PRP. Patients with different discrete coagulation or platelet function defects showed a marked prolongation of PITT, whereas conventional tests as aPTT did not detect any abnormality or only slightly differed from normal values.

In 5 patients with mild haemophilia A (F. VIII values 10 - 50 %) PITT was markedly prolonged (Tc in HIR-PRP 19.4 ± 1.3 min and in Fx-PRP 14.0 ± 8.3 min.).

Dept. of Internal Medicine, Division of Angiology, J. W. Goethe-University, Theodor-Stern-Kai 7, 6000 Frankfurt/Main, FRG

INFLUENCE OF PRE-ANALYTIC FACTORS ON LABORATORY PARAMETERS OF HEMOSTASIS
U. Funke, G. Töpfer, M. Schulze, A. Seifert and G. Lütz

The validity and reliability of laboratory tests is not only influenced by the specific test and its performance but also by a variety of determinants effecting the sample before its analysis. These so-called pre-analytic conditions can have a substantial effect on the final result particularly of tests for analysis of patient's hemostasis, in order to evaluate the influence and relevance of several parameters in hemostatic analysis; this effect is often underestimated but has an important relevance on the validity and reliability of results.

Zentrallabor des Kreiskrankenhauses, D-08800 Zittau
An immunodepleted as well as a congenital deficient plasma of high quality has to meet the following requirements:

- Linearity of the calibration curve from 1 to 100%
- Steep slope of the calibration curve
- Remaining activity of the deficient factor 1%
- Other clotting factors in the normal range: 50 to 150%
- No contact activation during production.

Problems and examples concerning these points were discussed.

IMMUNO AG, Smolagasse/Einfahrt Lange Allee, A-1220 Wien

**Problems Concerning the Production of Immunodepleted Deficient Plasmas**

E. Moritz, H. Lang

Nonradical excited oxygen species (NEOS), i.e. agents released by reactive oxidants of the chloramine/HCl type, are major leukocyte tools in inflammatory processes and have been shown to alter enzyme/inhibitor balances. Thus, a regulation of PAI activities in the local microenvironment of activated leukocytes by NEOS would be of physiological importance. Therefore, this study was undertaken to determine the effects of oxidants of the leukocyte type on PAIs in human plasma. The assay system used seems to imitate certain aspects of the oxidative ambiance of activated phagocytes. PAI-2 in human plasma is severalfold more resistant against oxidative attack by chloramine-T than PAI-1. PAI-2 in human plasma is severalfold more resistant against oxidative attack by chloramine-T than PAI-1. PAI-2 in human plasma is severalfold more resistant against oxidative attack by chloramine-T than PAI-1. PAI-2 in human plasma is severalfold more resistant against oxidative attack by chloramine-T than PAI-1.

**Lupus Anticoagulant Not Related to Platelet and Red Cell Autoantibodies in a Patient with SLE and Evans' Syndrome**

D. Söngen, A. Wehmeier, L. Rahmer, W. Schneider

Lupus anticoagulant (LA) activity is mediated by autoantibodies against anionic phospholipids resulting in a prolongation of clotting in phospholipid-dependent assays (aPTT, Kaolin clotting time, KCT). Patients with systemic lupus erythematosus (SLE) have a three times higher incidence of thrombocytopenia than LA-negative SLE-patients. The association between LA activity and other autoimmune phenomena has not been conclusively demonstrated. We investigated a 34-year old woman with SLE (4 ARA criteria), severe thrombocytopenia (56 x 10^9/l), and absent lupus anticoagulant. LA was closely related to disease activity and the presence of anticardiolipin antibodies but not to impaired platelet aggregation. In conclusion, 22% of unselected SLE patients in our series were LA-positive. LA was closely related to disease activity and the presence of anticardiolipin antibodies but not to impaired platelet aggregation.
The kaolin clotting time (KCT) is a sensitive screening test for the detection of a lupus anticoagulant (LA). Mixing studies of the KCT result in characteristic pattern allowing a differentiation between acquired inhibitors and simple factor deficiencies, but there are no data available concerning false positive results in the presence of heparin or cumarine [D.A. Trippelt & J.T. Brandt, Bio/Data Corp. 9/88/5K: 7-13].

We investigated 50 consecutive plasma samples from patients either undergoing high dose heparin therapy or receiving cumarine. To our surprise about half of the samples were found to be positive for anti-cardiolipin antibodies (ACLA) of the classes IgG or IgM. We excluded these samples from further investigation because they are suspicious for having a LA. For evaluation of the mixing studies we calculated a numerical index for circulating anticoagulant activity (ICA) [E. Rosner et al., Thromb Haemostas, 57/2 1987: 144-147]. An ICA greater or equal 15 was considered to be positive.

Plasma samples from patients in the stable phase of oral anticoagulation (N=11) demonstrated a median ICA of 5.5 without any false positive result. Plasma samples from patients receiving high dose heparin therapy (N = 13) revealed a median ICA of 18.4 and a rate of 67 % false positive results.

Our results about the specificity of the KCT running in mixing studies are compatible to those from other investigators concerning the aPTT.

Zentrallabor der Medizinischen Universitätsklinik, Josef-Schneider-Str. 2, W-8700 Würzburg

For evaluation of the tissue thromboplastin inhibition Test (TTI) most investigators calculate a ratio of the diluted prothrombin time (DPT) of the patients plasma sample and the DPT of the plasma sample from a healthy volunteer. Unfortunately not only patients dealing with a lupus anticoagulant (LA) but also patients with other coagulation disorders - particularly those receiving heparin or cumarine - exceeded the proposed cutoff value of 1.3. Therefore the TTI is widely accepted as a sensitive but nonspecific test for the detection of a LA [M.H. Rosove et al., Blood 68/2 1987: 472-478].

We investigated anti-cardiolipin negative plasma samples from patients receiving either cumarine (N=11) or high dose heparin therapy (N=13). Concerning the old TTI-ratio all patients receiving cumarine and 31 % of the patients receiving heparin revealed false positive results. Additionally we calculated a new TTI-index which also includes the baseline prothrombin time [E. Rosner et al., Thromb Haemostas, 57/2 1987: 144-147]. Concerning the new TTI-index no patient receiving cumarine revealed a false positive result and only one patient receiving heparin resulted borderline false positive. The results are discussed in detail.

The new TTI-index significantly increases the specificity of the TTI. The sensitivity does not seem to be diminished [P. Brauer et al., J. Clin. Chem. Clin. Biochem. 28/10 1990: 701].

Zentrallabor der Medizinischen Universitätsklinik, Josef-Schneider-Str. 2, W-8700 Würzburg

The ability of the coagulation laboratory to diagnose LA has become more critical as the association of LA with thrombotic disorders, spontaneous abortion and immune diseases is well documented. Modified aPTT tests using different phospholipid preparations and concentrations were compared in 62 well characterized SLE patients with (n=21) and without thrombotic events (n=41). A group of 29 patients with a history of deep vein thrombosis as well as 50 patients suffering from ARC/AIDS (20 hemophiliacs, 30 non-hemophiliacs) served as controls. We have detected LA activity in 23 of 25 SLE patients with thromboembolic events using human platelet, soybean or pig brain phospholipids in concentrations resulting in a normal range between 50 and 70 seconds. None of the thrombosis patients without SLE had functional LA. Only 5 % of plasma samples obtained from ARC/AIDS patients showed LA activity. Best sensitivity was obtained by simultaneous determination of coagulation times in patients plasma (A), platelet free human normal plasma (B), 1:1 mixture of A+B (C) and subsequent calculation of results according to Rosner (Thromb. Haemost. 57 (1987) 144) and graphical evaluation.

In conclusion functional detection of LA can be performed using modified aPTT assay systems, whereby optimized preparations and concentrations of phospholipids and simultaneous determination of a mixture with platelet free human normal plasma have to be used. Furthermore suitable evaluation procedures should be applied.

*Medizinische Klinik der Universität München, Klinikum Innenstadt, Ziemssenstr.1, 8000 München 2, FRG
**Immuno AG, Vienna, Austria

For evaluation of the tissue thromboplastin inhibition Test (TTI) most investigators calculate a ratio of the diluted prothrombin time (DPT) of the patients plasma sample and the DPT of the plasma sample from a healthy volunteer. Unfortunately not only patients dealing with a lupus anticoagulant (LA) but also patients with other coagulation disorders - particularly those receiving heparin or cumarine - exceeded the proposed cutoff value of 1.3. Therefore the TTI is widely accepted as a sensitive but nonspecific test for the detection of a LA [M.H. Rosove et al., Blood 68/2 1987: 472-478].

We investigated anti-cardiolipin negative plasma samples from patients receiving either cumarine (N=11) or high dose heparin therapy (N=13). Concerning the old TTI-ratio all patients receiving cumarine and 31 % of the patients receiving heparin revealed false positive results. Additionally we calculated a new TTI-index which also includes the baseline prothrombin time [E. Rosner et al., Thromb Haemostas, 57/2 1987: 144-147]. Concerning the new TTI-index no patient receiving cumarine revealed a false positive result and only one patient receiving heparin resulted borderline false positive. The results are discussed in detail.

The new TTI-index significantly increases the specificity of the TTI. The sensitivity does not seem to be diminished [P. Brauer et al., J. Clin. Chem. Clin. Biochem. 28/10 1990: 701].

Zentrallabor der Medizinischen Universitätsklinik, Josef-Schneider-Str. 2, W-8700 Würzburg

To determine the diagnostic efficacy of various commercially available methods for PrC, we carried out a study using a chromogenic substrate based (Diade, Miami, FL), a clot based (Activol®, American Diagnostics, Greenwich, CT) and an rocket electrophoresis based (Helena Laboratories, Beaumont, TX) assay. The three assays compared well when the calibration curves with normal human plasma were prepared in a five point assay (r>0.95). However, when the plasma samples from patients with a functional deficiency of PrC were analyzed, the results showed marked discrepancies. In another separate study the chromogenic substrate based methods failed to identify a functional deficiency of PrC (n=50). However, the clot based method provided falsely high values in patients on heparin therapy and with a high FDP titre. The immunoassay only recognized the effect of hemodilution and absolute decrease in the PrC antigen. In patients treated with prothrombin complex concentrates, the three assays showed markedly different results. The chromogenic substrate method consistently quantitated false high PrC levels and failed to provide a reliable level of PrC. Several factors influenced both the chromogenic substrate and clot based assays resulting in false PrC levels. High factor VIII Levels and hypofibrinogenemia resulted in falsely high results in the clot based assays. We have carried out studies on the validity of PrC assays in patients with hypercoagulable state and obtained similar results. Our observation suggests that the currently available PrC methods are not reliable and the results from these assays should be carefully assessed. This work was in part sponsored by the Int'l Inst. of Thrombosis and Vascular Diseases.
IN VIVO RECOVERY OF AN ANTITHROMBIN III CONCENTRATE IN CONGENITAL AND ACQUIRED ANTITHROMBIN III DEFICIENCY

Ch. Peclhaner, R. Erhart, B. Blauhut, M. Fischer, F. Kunz, W.-D. Sweirzina, A. Arnold

Different batches of Antithrombin-III-concentrate from the CRTS-Lille/Biotest (France) have been examined in clinical practice and in vitro.

The preparation was found to have a 114-fold concentration over normal human plasma. In a heparin cofactor assay 90 % of the Antithrombin III was biologically active. Specific activity was 1600 U/g protein.

In 11 patients with disseminated intravascular coagulation and an antithrombin III plasma activity of less than 60 % (range 21-58) the mean in vivo recovery was 75 % (range 51-101). In 11 patients suffering from acute thromboembolic or hepatic disease with acquired antithrombin III deficiency (mean 48 %, range 35-61) the recovery ranged from 55 to 100 % (mean 70). In a patient with congenital antithrombin III deficiency type I (activity and antigen 50 %) recovery was measured with 3 different lots and was in the range of 64 to 120 %.

The unexpected pharmakokinetic behaviour of Antithrombin III in long term substitution therapy has been described by other authors and will be discussed.

No adverse side effects were noted. Neither were coagulation laboratory tests changed after substitution.

GerinnungsLabor der Univ. Klinik für Innere Medizin, Anichstr. 35, A-6020 Innsbruck
DIFFERENCES OF PAI-1 LEVELS IN BLOOD SAMPLES TAKEN SIMULTANEOUSLY FROM DIFFERENT ARTERIAL AND VENOUS SITES

K. Huber, R. Beckmann, F. Weidinger, P. Probst, F. Kaindl, and B.R. Binder

To investigate possible differences in plasminogen-activator-inhibitor-1 (PAI-1) plasma levels in venous or arterial blood samples we collected blood simultaneously from femoral artery, femoral vein, and pulmonary artery during 14 routine cardiac catheterizations into plastic tubes prepared with EDTA (final concentration 5.10⁻²M).

By measuring PAI-1 activity (U/ml; functional titration assay) and PAI-1 antigen (ng/ml; ELISA) in these blood samples we could demonstrate an increase of PAI-1 antigen levels in pulmonary artery as compared to femoral vein (PAI-1 activity: 1.2±0.5; PAI-1 antigen: 0.37±3.6) and increased PAI-1 levels in femoral artery as compared to femoral vein (PAI-1 activity: 1.2±0.5; PAI-1 antigen: 3.6±6). Furthermore, PAI-1 activity levels were comparable in pulmonary artery and femoral artery.

We conclude that increased PAI-1 levels in central venous blood as compared to peripheral venous blood could originate from venous blood supply from the liver and/or coronary sinus.

Department of Cardiology and Lab. Clin.-Expt. Physiology, University of Vienna, Austria
DOSE ADJUSTMENT OF RECOMBINANT TISSUE TYPE PLASMINOGEN ACTIVATOR—A NEW THERAPEUTIC ATTEMPT IN CHILDREN SUFFERING FROM VENOUS AND ARTERIAL THROMBOSIS

W. Kreuz, U. Nowak-Oblotzky, D. Schwaibl, R. Linde, B. Konnubier

Since 1980 in the university children’s hospital Frankfurt/Main, 104 children and adolescents suffered from venous or arterial thrombosis. Thrombolytic therapy has now been used as a therapeutic regimen in pediatric patients. We report our experience with rt-PA (Activase®/Behring-Thomaes) in 15 children suffering from arterial (n=3) or venous thrombosis (n=12) within the following underlying diseases: rhabdomyosarcoma, acute lymphoblastic leukemia, chronic myeloblastosis, sickle-cell anemia, post thrombotic syndrome, aplasia, and central arterial respectively venous catheters. Thrombotic events were diagnosed with ultrasound, computed tomography, angiography and plethysmography and then thrombolytic therapy with rt-PA immediately started. rt-PA dose was 0.2 mg/kg bw/d to 2 mg/kg bw/d for 7 to 14 days, low dose heparin was added in parallel. Systemic lysis was carried out in 8 patients, local lytic therapy in 7. When thrombolysis was terminated we administered heparin (70U to 400U/kg bw/d) for 7 to 14 days to prevent recclusion, followed by long-term prophylaxis with coumarine for venous thrombosis and antiplatelet agents for arterial occlusions. No patients exhibited a decrease in both fibrinogen and plasminogen during rt-PA thrombolysis. Complete reperfusion was seen in 11 children and partial reperfusion in 3. Thrombolytic therapy had to be stopped in one child due to bleeding from a punctured central vein. Further investigations and clinical experience are necessary to establish an appropriate dose adjustment of rt-PA in children.

Center of Pediatrics, University Hospital Frankfurt am Main, Germany

ACUTE t-PA RELEASE BY DEFIBROTIDE

H.-P. Klöcking and A. Hoffmann

Among other characteristics, defibrotide—a polydesoxyribonucleotide isolated from mammalian (porcine) tissue—proves to be an active profibrinolytic agent. This action is attributed both to the inactivation of inhibitors of fibrinolysis and to the production of higher concentrations of tissue-type plasminogen activator (t-PA) (review: O.N. Ulatin, Semin. Thromb. and Haemost., Suppl. 14:58, 1988). Previous investigations were supplemented by studies in the acute t-PA release by defibrotide in the isolated perfused pig ear and in whole animals (rats).

Within a concentration range of 10^-e to 10^-4 mol/l, defibrotide caused an increase in t-PA release of 10^- in in vitro systems. In further studies, the acute t-PA releasing effect of defibrotide was studied in rats after i.v. administration. The results are summarized as a dose dependent increase in IU of t-PA/m1 plasma. Compared to the control (saline), defibrotide induced a dose-dependent increase in acute t-PA release: 3.6 ± 1.6 IU/ml (10 mg/kg i.v.); 6.5 ± 3.5 IU/ml (30 mg/kg i.v.); 10.8 ± 4.8 IU/ml (50 mg/kg i.v.). The administration of 50 mg/kg i.v. suppressed also the activity of the t-PA inhibitor (PAI-1).

Medical Academy Erfurt, Nordhäuser Str. 74, Erfurt D-5010 FRG

ALTERATION OF THE FIBRINOLYTIC NETWORK IN PATIENTS WITH TOTAL HIP REPLACEMENT: LABORATORY AND CLINICAL FINDINGS

P. Bacher, B. Horst*, H.-G. Breyer*, J.M. Walenga, D. Hoppensteadt and J. Fareed

The surgical intervention of patients undergoing total hip replacement leads to changes in the coagulation and fibrinolytic system. The aim of this investigation was to find alterations in this network influenced by different antithrombotic drugs and the occurrence of DVT. Seventy-one patients with concomitant undergoing total hip replacement were included in a randomized clinical double-blind study. Thirty-six patients received 5000 IU T.I.D. UF-heparin and 35 patients were treated with one injection of 48 mg Fraxiparin for thromboysis prophylaxis. Clinical parameters such as age, sex, obesity, smoking habits and previous diseases were recorded. Blood was drawn preoperatively and on the 3rd and 10th postoperative day. The following parameters were analyzed: (clot-based): APTT, PT, 5UTT, Heptest, Heptest-B, Factor VII: c, Factor VIII: c, (amidolytic-based): AT III, anti-IIa, anti-Xa; (ELISA-based): t-PA, u-PA, PAI, protein C, D-dimer, FDPD, FDP, TAT, prothrombin fragment F 1+2, Factor VII: ag, Factor VIII: ag. Bilateral ascending phlebography was performed two weeks after surgery. Over the 10 day period all patients showed an increase in t-PA, D-dimer, FDP, TAT, and PAI-antigen and a decrease in PAI-activity. In addition the twenty patients with DVT showed slightly higher D-dimer, FDP and PAI-activity levels which could be related to higher fibrinolytic activity. Only t-PA was different on day 10 due to the heparin prophylaxis. However, the LMW-heparin in a prophylactic dosage did not increases the fibrinolytic activity. This work was in part sponsored by the International Institut of Thrombosis and Vascular Diseases.

Loyola Univ. Med. Ctr., 2160 S. First Ave., Maywood, IL 60153 USA and *Freie Univ. Berlin, Dept. of Traumatology, Berlin, Germany

THE INFLUENCE OF CLINICAL CONDITIONS ON THE CLOTTING AND FIBRINOLYTIC SYSTEM

P. Bacher, B. Horst*, H.-G. Breyer*, J.M. Walenga, D. Hoppensteadt and J. Fareed

In a randomized double-blind study, 71 patients with concomitant undergoing elective total hip replacement, thirty-six patients received 5000 IU T.I.D. UF-heparin and 35 patients were treated with one injection of 48 mg Fraxiparin plus two placebo injections for thromboysis prophylaxis. Clinical parameters such as age, sex, obesity, smoking-habits, previous diseases and the occurrence of DVT and bleeding complications were recorded. Blood was drawn preoperatively and on the 3rd and 10th postoperative day. The following parameters were analyzed: (clot-based): APTT, PT, 5UTT, Heptest, Heptest-B, Factor VII: c, Factor VIII: c, (amidolytic-based): AT III, anti-IIa, anti-Xa; (ELISA-based): t-PA, u-PA, PAI, protein C, D-dimer, FDP, FDP, TAT, prothrombin fragment F 1+2, Factor VII: ag, Factor VIII: ag. Bilateral ascending phlebography was performed two weeks after surgery. Statistical significancies were calculated and a regression analysis was performed to rule out cross-influences. Significant correlations were found between age and D-dimer, t-PA and APTT. In addition, D-dimer, t-PA and Heptest were significantly influenced by sex. Smoking had an impact on anti-Xa, Heptest and TAT. Significantly higher D-dimer, FDP, PAI:ag and TAT levels were found patients with postoperative wound hematomas. Obese patients had a slightly higher incidence of venous thrombosis. Based on this data and that of other studies, it is our aim to determine certain clinical and laboratory patterns associated with the hypercoagulable state. This work was in part sponsored by the International Institut of Thrombosis and Vascular Diseases.

Loyola University Medical Center, 2160 S. First Ave., Maywood, IL 60153 USA and *Freie Universität Berlin, Dept. of Traumatology, Berlin, Germany
THE EFFECT OF STREPTOKINASE ON THE WILLEBRAND FACTOR LEVEL IN VIVO AND IN ENDOTHELIAL CELL CULTURE

I. Altorjéy, J. Hársfalvi, M. Udvardy and K. Rak

Rise in vWF:Ag and platelet agglutinating activity after a single Streptokinase dose was first reported by Hamilton et al., in 1985. The exact mechanism is still unclear. Plasma vWF levels and ristomycin cofactor activity were measured during thrombolytic therapy carried out in acute myocardial infarction. A considerable increase was seen in both factors. Plasma di-gestion of vWF could not be proved by SDS electrophoresis, however there was a slight increase in the electrophoretic mobility of vWF by two-dimension immuno-electrophoresis. When whole blood or plasma was treated with therapeutic concentration of SK, no change was observed in vWF:Ag level, Ristomycin cofactor activity and multimeric pattern. Thereafter the effect of SK on vWF release from cultured human umbilical cord vein endothelial cells was studied. No increase in the vWF level of culture medium could be detected at any time of incubation up to 24 hours. It seems that the increase of vWF level during SK treatment is not the result of a direct effect of SK on ECs. The role of intermediate components is suggested.

2nd Dept. Med., Univ. Med. School, Debrecen Hungary

AZELAIC ACID DECREASES THE FIBRINOLYTIC POTENTI-AL OF HUMAN MELANOMA CELL LINES IN VITRO

H. Plendl, J. Hedderich*, W. Grote

In summary, AZA decreases the fibrinolytic potential of 4 human melanoma cell lines in vitro. This decrease may be operative in the mechanisms by which AZA affects the behavior of transformed melanocytes in vivo.

1 Lab. Clin. Exp. Physiology, Univ. Vienna, Schwarzwannerstr. 17, A-1090 Vienna, Austria
2 1. Dept. Dermatology, Univ. Vienna, Austria

COMPUTER AIDED DIAGNOSIS OF HEMOSTATIC DISORDERS

H. Plendl, J. Hedderich, W. Grote

In situations where a (human) coagulation expert is not available, a computer based consultation system might prove useful. We developed an expert consultation system that supports the diagnosis of haemostatic disorders. Prototyping was done in an object oriented programming system (OOPS; Smalltalk/V, Digialtalk Inc.) on a PC. In this particular OOPS, inferencing mechanisms are implemented for both forward and backward chaining. After different experiments with our prototype implementation which proved the feasibility of using expert system techniques in the diagnosis of haemostatic disorders, a knowledge base was created in ESE (Expert System Environment for MVG and VM, IBM).

Abt. Humangenetik und *Abt. f. Med. Statistik u. Dokumentation im Klinikum der Universität, Schwanenweg 24, 2300 Kiel, FRG

FIBRONECTIN DECREASES THE STIMULATORY EFFECT OF FIBRIN AND FIBRINOGEN FRAGMENT FCB-2 ON PLASMIN FORMATION BY t-PA

R. Beckermaier1, M. Geiger1, C. de Vries2, H. Pannekoek2, B.R. Binder1

Fibronecin is an adhesive, dimeric glycoprotein (Mr ~ 440,000) which is involved in many biological processes. During blood coagulation it is bound and crosslinked to fibrin. Fibrin binding is achieved by structures (type I repeats) which are homologous to the "finger" domain of t-PA. t-PA also binds to fibrin via the "finger" domain and additionally via the "kringle 2" domain. Fibrin binding of t-PA results in stimulation of its activity and plays a crucial role in fibrino-lysis. Since fibronecin might interfere with this binding, we studied the effect of fibronecin on plasmin formation by t-PA. In the absence of fibrin, fibronecin had no effect on plasminogen activation. In the presence of stimulating fibrinogen fragment FCB-2, fibronecin increased the duration of the initial lag phase (= time period until maximally stimulated plasmin formation occurs) and decreased the rate of maximal plasmin formation which occurs after that lag phase mainly by increasing the Michaelis constant (Km). These effects of fibronecin were dose-dependent and were similar with single- and two-chain t-PA. They were also observed with plasminogen treated FCB-2. An apparent K_i of 43mg/ml was calculated for the inhibitory effect of fibronecin, when plasminogen activation by recombinant single-chain t-PA was studied in the presence of 10mg/ml FCB-2. When a recombinant t-PA mutant lacking the finger-domain was used in a system containing FCB-2, no effect of fibronecin was seen, indicating that the inhibitory effect of fibronecin might in fact be due to competition of fibronecin and t-PA for binding to fibrinogen via the finger domain.

1 Lab. Clin. Exp. Physiology, Univ. Vienna, Schwarzwannerstr. 17, A-1090 Vienna, Austria, and 2 Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands
The pharmacokinetics of urokinase (two-chain urokinase-type plasminogen activator, tcu-PA) and single-chain urokinase-type plasminogen activator (scu-PA) were studied in 20 patients with acute myocardial infarction (AMI). Ten consecutive patients received 2.5 million units tcu-PA by bolus injection within 5 min during the first 6 h after AMI (group I). Ten further consecutive patients became 200,000 U tcu-PA within 5 min, followed by 4.5 million U scu-PA by intravenous infusion over 1 h (group II). An enzyme immunoassay was developed for urokinase antigen (UK:Ag) determinations, and a fibrinolytic assay for determinations of fibrinolytic activity was applied. Calculations of half-life and area under the curve (AUC) were performed with "Topfit", and median values are given in the text. Using a 3-compartment model, in group I, 98% of urokinase antigen was cleared with a half-life of 60.6 min. After scu-PA, UK:Ag was cleared with half-life (AUC in parentheses) of 6.9 min (74.8%), 29.5 min (23.6%), and 359.7% (2.2%). The half-disappearance times of fibrinolytic activity were 18 and 6 min in group I and II, respectively. There was a significant correlation between antigen and activity determinations after tcu-PA (r = 0.435) and after scu-PA (r = 0.88). A more pronounced decrease of plasminogen was observed after tcu-PA. These results stress the need of different routes of administration and help explain different rates of recocclusion, when patients with AMI are treated with different fibrinolytic agents.

Department of Clinical Haemostaseology and Transfusion Medicine, Department of Internal Medicine III and Department of Biochemistry, University of Saarland, D-6850 Homburg/Saar, F.R.G.
Unter niedrig dosierter Streptokinasegabe beobachteten wir keinen signifikanten Unterschied errechnen ließ. Auch unter rt-PA konnten wir bestimmten, was durch die erhöhte Plasminaktivität von r-tPA. Compared with baseline values the control values 3 hours after start of thrombolysis increased significantly (TAT: 18.8 ± 6.6 / 29.7 ± 8.8, p=0.05; F1+2: 4.9± 0.9 / 9.8 ± 10.6, p<0.0005).

In vitro after addition of streptokinase, urokinase and tissue plasminogen activator to normal plasma, a dose-dependent increase of both TAT and F1+2 could be demonstrated, which was inhibited by the low molecular weight proteinase inhibitor galexate mesilate (FOY) in concentrations above 5 x 10^-6M. FOY did not inhibit plasminogen activation as assessed by a chromogenic substrate assay.

Thus it may be concluded that treatment with thrombolytic agents both in vivo and in vitro leads to an activation of coagulation. The increase of the F1+2 fragment levels indicates that actually thrombin is activated during thrombolysis. The mechanism remains to be elucidated. Further clinical studies should be addressed to the question whether this effect is involved in early recollection of infarct vessels.

Zentrum Innere Medizin, Abteilung Hämatologie / Onkologie, Klinikum der Philipps-Universität, Baldingerstr., D - 3550 Marburg.

**ACTIVATION OF PROTHROMBIN IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION (AMI) AND IN VITRO BY THROMBOLYTIC AGENTS**

R. Seitz, H. Pelzer und R. Egbring

As already shown by several groups during thrombolytic therapy, fibrinopeptide A and thrombin-antithrombin III (TAT) complexes increase in the patient's plasma. Since it was not clear whether preformed thrombin is washed out from thrombus or necrotic tissue, or whether actually prothrombin is activated, we determined the plasma levels of the prothrombin activation product, F1+2 fragment, in 11 AMI patients undergoing thrombolytic therapy with 1.5 MU streptokinase. Compared with baseline values the control values 3 hours after start of thrombolysis increased significantly (TAT: 18.8 ± 6.6 / 29.7 ± 8.8, p=0.05; F1+2: 4.9± 0.9 / 9.8 ± 10.6, p<0.0005).

In vitro after addition of streptokinase, urokinase and tissue plasminogen activator to normal plasma, a dose-dependent increase of both TAT and F1+2 could be demonstrated, which was inhibited by the low molecular weight proteinase inhibitor galexate mesilate (FOY) in concentrations above 5 x 10^-6M. FOY did not inhibit plasminogen activation as assessed by a chromogenic substrate assay.

Thus it may be concluded that treatment with thrombolytic agents both in vivo and in vitro leads to an activation of coagulation. The increase of the F1+2 fragment levels indicates that actually prothrombin is activated during thrombolysis. The mechanism remains to be elucidated. Further clinical studies should be addressed to the question whether this effect is involved in early recollection of infarct vessels.

Zentrum Innere Medizin, Abteilung Hämatologie / Onkologie, Klinikum der Philipps-Universität, Baldingerstr., D - 3550 Marburg.

**LASSEN SICH BEI DER NIEDRIG DOSTEREN LOKALEN LYSE MIT r-tPA SYSTEMISCHE VERÄNDERUNGEN IM GE-

R. Weichenheim, H. Stiegler, E. Standl, H. Mehner

Unter niedrig dosierter Streptokinasegabe beobachteten wir systemische Gerinnungsveränderungen, die auf fehlenden Fibrinselektivität zurückzuführen waren. Um mögliche Alterationen der Gerinnung auch unter r-TPA nachzuweisen, bestimmten wir neben den Routineparametern Quick,PTT, Thrombinzeit und Fibrinogen, das Plasminogen, das F2- Antitplasin, die Thrombinaktivität, den Plasminogen-Aktivator sowie die Konzentration des D-Dimer- und der Fibrin/Fibrinogenspaltprodukte vor, unmittelbar nach, sowie 3, 6 und 24h nach dem Eingriff. 43 Patienten wurden mit einer mittleren rt-PA-Dosis von 5,5mg je Eingriff behandelt, wobei sich die Aktivitäten im Bereich der foemellen Unterschiede ergeben. Während sich die Werte von Plasminogen durch die Lyse nicht wesentlich änderten, fiel das F2- Antitplasin unmittelbar nach der Lyse signifikant ab. Ab dem ersten Tag zeigten die Indikatoren der Fibrinolyse ein signifikantes Ansteigen, während das Plasminogen konstant blieb. Die genannten Veränderungen erwiesen sich als rasch reversibel und waren nach 24h nicht mehr nachweisbar. Wie für die systemischen Veränderungen zu verschiedenen Zeitpunkten angegeben, läßt sich sagen, daß tatsächlich eine Fibrinolyse auftritt, die von der fibrinolytischen Substanz abhängig ist.

**COMPARISON OF THROMBOLYSIS WITH STREPTOKINASE AND UK IN DEEP VEIN THROMBOSIS**

P. Peters. C.-J. Schuster, H. Geiling

Longterm fibrinolysis is an established therapy in deep vein thrombosis in order to avoid a postthrombotic syndrome. Discussion about the fact that thrombolytic agents are still ongoing. In a retrospective study we compared streptokinase (SK) and urokinase (UK) in 58 patients with deep vein thrombosis (age 39±14 years). A phlebographic was done before and after lyse. RESULTS:

| n (patients) | SK | UK |
|-------------|----|----|
| duration of symptoms | 153±169 | 165±236 |
| duration of lysis | 99±48 | 204±107 |
| success rate (%) | 65 | 77 |
| complications (%) | 21 | 23 |
| - interruption | 50 | 77 |
| - transfusions | 26 | 17 |
| - allergy | 13 | 9 |

Success rate under streptokinase and urokinase is identical in thrombosis with short duration of symptoms. In patients with a prolonged history of symptoms urokinase is the more potent thrombolytic substance while complication rate is comparable.

Medizinische Klinik St. Antonius Hospital, Dechant-Deckers-Str.8, D-5184 Eschweiler
FACTOR IX, ANTITHROMBIN- AND TAT-COMPLEXES IN LIVER DISEASES AND CARCINOMA PATIENTS

B. Kenkes-Matthes, H. Breyll, K.J. Matthes

Introduction: Intravascular activation of the coagulation cascade is discussed in those suffering from liver diseases and from malignancies. Patients with malignant diseases are known to have an elevated risk of thromboembolic events, while liver patients often have bleeding complications. To demonstrate activation of the coagulation cascade at different steps, IX, TAT- and TAT-complexes were measured as well as factors IX, X, protein C and S. Patients: 1. Normals, n=13; 2. Chronic aggressive hepatitis (CAH), n=17; 3. Liver cirrhosis (CT), n=15; 4. Pulmonary carcinoma (P.ca.), n=36; 5. Gastrointestinal carcinoma (G.ca.), n=36.

Methods: Two-side immunoassays were developed for TAT- as well as for IXAT-complexes. Monoclonal antibodies against thrombin (resp. F. IX) were covalently attached to polystyrene tubes, antibody against antithrombin was radiolabeled enzymatically. Results: 1. In all patient groups, IXAT-complexes were increased to about the same extent: CAH: 2089±1396; CT: 2105±2456; P.ca.: 2378±2575; G.ca.: 2761±2862 (normals: 673±410 UI/ml). 2. In contrast, liver patients showed only a moderate increase in TAT-complexes, while patients suffering from malignant disease presented extremely high levels: CAH: 193±158; CT: 295±92; P.ca.: 139±3096; G.ca.: 4371±5080 (normals: 53±44 UI/ml). Conclusions: 1. An activation of the coagulation cascade was demonstrated in liver diseases and malignancies. 2. Relatively low IXAT-complexes in liver diseases and high TAT-complexes in patients suffering from malignant diseases reflect the thrombosis risk of the latter group in comparison to liver patients.

Univ. Zentrum Innere Medizin, D-6300 Gießen.

PARTIAL CHARACTERISATION OF TISSUE FACTOR CLONED FROM MOUSE METH-A SARKOMA CELLS

Y. Zhang, A. Bierhaus, J. Lin, P. Schumacher, R. Bravo, R. Ziegler, P. Nawroth. Univ. Heidelberg

Meth-A sarcomas respond to TNF in vivo. An important feature of the TNF effect is thrombus formation. For the study of TNF induced changes in mouse tissue we cloned mouse tissue factor from Meth-A cells. A cDNA library was prepared by ligating tumor cDNA into Uni-Zap arms and packed into "GIGA-Pack II Gold". 200,000 phages were screened with a tissue factor probe cloned from fibroblasts. 4 positive clones were isolated. To compare the sequence of tissue factor derived from malignant tissue with the sequence from tissue factor cloned from fibroblasts, we converted the positive phages into pBluescript by in vivo excision and sequenced. The sequence showed large homology with the published tissue factor sequence, however several heterogeneities were observed. The physiological significance of these heterogeneities within the 3'end of mouse tissue factor remains unknown. When polyA RNA was isolated from Meth-A sarcoma cells and hybridized with specific DNA probes for mouse tissue factor, several bands could be demonstrated (1, 2.4 kb).

These studies demonstrate that tissue factor is present in TNF responsive tumor cells and that sequence differences between tissue factor from malignant and non malignant cells can be observed.

Tumor Necrosis Factor/Cachectin enhances transcription of the preproendothelin and tissue factor gene in cultured endothelial cells.

A. Bierhaus, S. Stilgenbauer, P. Schumacher, M. Yagisawa*, T. Masaki*, R.E. Lang, R. Ziegler, P.P. Nawroth. Univ. Heidelberg, BRD; *Univ. Tsukuba, Japan.

Tumor Necrosis Factor (TNF) induced tumor necrosis involves occlusion of the tumor vasculature. We tested if TNF could increase in addition to tissue factor also the expression of the potent vasoconstrictor endothelin in cultured endothelial cells. As probe we used the 496 bp Sac fragment of preproendothelin cDNA. When endothelial cells were incubated with TNF a time dependent increase in endothelin mRNA could be observed. Induction of mRNA was independent of protein synthesis. Dose dependence showed half maximal response at about 300pM, corresponding to the binding of TNF to endothelial cells. The mechanism of TNF dependent endothelin induction was investigated by nuclear run on assays. Nuclear run on assays showing newly in vitro transcribed RNA, demonstrated enhanced transcription of RNA binding to preproendothelin cDNA after induction with TNF. The TNF induction of the endothelin gene is under control of protein kinase C and cAMP.

In conclusion: TNF induces enhanced transcription of the potent vasoconstrictor endothelin in addition to tissue factor in a time and dose dependent manner. This might play a role in TNF induced changes in tumor vasculature in vivo.

THE EFFECT OF HEMOGLOBIN ON PLASMA CLOT FORMATION

P. Hopfner, W. Kolesch, E. Bajam

Fibrin clots formed in plasma exhibit a ultrastructure, which is different from the ultrastructure of fibrin clots formed in purified systems, and various plasma proteins are known to affect the clot formation process. We were interested to examine the effect of hemoglobin in this respect. Human hemoglobin was purified from an EDTA whole blood pool (Hb A > 96 %). For the experiments, purified hemoglobin, a citrated normal plasma pool and crude bovine thrombin were used. Dilutions were done using barbital buffer pH 7.6 without or with protease (25 IB/ml). For a typical series of experiments, the hemoglobin concentrations ranged from 0.0 - 50 mg/ml, fibrinogen was 2.0 mg/ml and thrombin was 0.25 U/ml. In all samples the presence of hemoglobin caused a concentration-dependent shortening of the thrombin clotting time. This effect was also noted at hemoglobin concentrations which can be found in plasma during haemolytic episodes (> 50 mg/ml). In contrast, albumin up to 50 mg/ml had no appreciable effect. Further, clots formed in the presence of hemoglobin exhibited a tendency to absorb less thrombin than those which were formed in its absence.

In summary, our experiments indicate that hemoglobin concentrations > 50 mg/ml do have a procoagulant effect in plasma. Such concentrations will be common in the microenvironment of decaying erythrocytes and can be frequently observed in total plasma during haemolytic episodes.

Zentrallaboratorium, Krankenanstalt Rudolfstiftung, Juchgasse 25, 1030 Vienna, Austria.

THE EFFECT OF HEMOGLOBIN ON PLASMA CLOT FORMATION

P. Hopfner, W. Kolesch, E. Bajam

Fibrin clots formed in plasma exhibit an ultrastructure, which is different from the ultrastructure of fibrin clots formed in purified systems, and various plasma proteins are known to affect the clot formation process. We were interested to examine the effect of hemoglobin in this respect. Human hemoglobin was purified from an EDTA whole blood pool (Hb A > 96 %). For the experiments, purified hemoglobin, a citrated normal plasma pool and crude bovine thrombin were used. Dilutions were done using barbital buffer pH 7.6 without or with protease (25 IB/ml). For a typical series of experiments, the hemoglobin concentrations ranged from 0.0 - 50 mg/ml, fibrinogen was 2.0 mg/ml and thrombin was 0.25 U/ml. In all samples the presence of hemoglobin caused a concentration-dependent shortening of the thrombin clotting time. This effect was also noted at hemoglobin concentrations which can be found in plasma during haemolytic episodes (> 50 mg/ml). In contrast, albumin up to 50 mg/ml had no appreciable effect. Further, clots formed in the presence of hemoglobin exhibited a tendency to absorb less thrombin than those which were formed in its absence.

In summary, our experiments indicate that hemoglobin concentrations > 50 mg/ml do have a procoagulant effect in plasma. Such concentrations will be common in the microenvironment of decaying erythrocytes and can be frequently observed in total plasma during haemolytic episodes.

Zentrallaboratorium, Krankenanstalt Rudolfstiftung, Juchgasse 25, 1030 Vienna, Austria.
169

INTERACTION OF PLATELET AGGREGATION AND GRANULOCYTE FUNCTION.
E. Jakob, A. H. Sutor

Platelets are not only effective in hemostasis but contribute also to the defense of infectious agents, and vice versa granulocytes react not only with bacteria but also play an important role in hemostasis.

To study the interaction of platelets and granulocytes we used a whole blood aggregometer (Chronolog). By observing simultaneously the aggregation of platelets and the generation of oxygen radicals from granulocytes with a luminol-dependent chemoluminescence system in 12 normal persons the following results were found: During the process of platelet aggregation (18 ± 5 Ohm) induced by collagen or ADP stimulation of granulocytes was observed about 60-90 sec later by an increase of the luminol-dependent chemoluminescence. This effect was present in whole blood (1,5±0.7 arbitrary units) as well as in a system, where only platelets and granulocytes were present (7±3 arbitrary units). When platelets were aggregating without granulocytes in PRP an increase of luminol-dependent chemoluminescence was not observed; neither did isolated granulocytes show an increase of luminol-dependent chemoluminescence, after ADP had been added.

From these data we conclude, that platelet aggregation stimulated by ADP or collagen induces the release of oxygen radicals from granulocytes.

Universitäts-Kinderklinik, Mathildenstr. 1, 7800 Freiburg

170

ANALYSIS OF BLOOD-COAGULATION IN REGIONAL HYPERTHERMIC EXTREMITIES PERFUSION (RHEP) WITH CYTOPSYTICS
S. Birkner, U. Semmelroggen, H. Kostering

RHEP takes place in therapy of malignant melanoma of the Extremities. There were several cases reported with thromboembolic and bleeding complications in RHEP. Therefore we made 2 studies with 13 pigs and 31 patients to analyse the alterations in blood coagulation and fibrinolytic system.

During the operation and anticoagulation with Heparin (300 IE/kg bodyweight) were prothrombin time and thrombin time at most prolonged. The partial thrombin time (Quick) and fibrinogen were lowered. Factor XIII was not measurable low, the thrombocyte count decreased. Further we found decreasing of plasminogen, alpha-2-antiplasmin and antithrombin III.

After the operation and antagonizing with protamin and 48 h later most of the parameters of coagulation system were normalised.

Our studies show that alterations in blood coagulation system can not be the only reason for the complications of RHEP.

According to Virchow's Triad changes at the endothelial variations of blood stream could have at least the same value as plasmatic coagulation alterations.

Medizinische Klinik der Universität Göttingen, Robert Koch Str., D - 3400 Göttingen

171

Plasminogen activator inhibitor 2 (PAI-2): No tumor marker in B-HCG-positive testicular cancer and malignant histiocytoma
C. Salat, B. Reinhardt, C. Clemm, E. Hiller

PAI-2 is found in the trophoblastic epithelium of human placenta or in the plasma of pregnant women reaching levels of about 300 ng/ml at term. It also occurs in malignant cells as in fibrosarcoma cell lines or in the human histiocytic lymphoma cell line u-937.

To investigate the possible significance of PAI-2 as a tumor marker we evaluated PAI-2 antigen levels in normal controls (group A, n=10), patients with B-HCG positive testicular cancer (group B, n=12), malignant histiocytoma (group C, n=10) and pregnant women in the last trimester (group D, n=10).

Materials and methods: Citrated blood samples were taken in the morning and immediately centrifuged. The plasma was stored at -70°C until assayed. PAI-2 antigen was determined by an enzyme immuno assay (TintElize PAI-2, Paesel und Lorei, Frankfurt, West Germany).

Statistical analysis was performed by Mann-Whitney-test.

Results (mean values +/− SEM): Group A 8,43 (+/−2,38) ng/ml; group B 7,47 (+/−0,56) ng/ml; group C 8,57 (+/−2,26) ng/ml; group D 215 (+/−15,46) ng/ml.

PAI-2 antigen found in pregnant women correlated well with the levels described by others. There was no significant difference between normal controls and patients with B-HCG positive testicular cancer or with malignant histiocytoma. So PAI-2 is not useful as a tumor marker in these patients.

Medizinische Klinik III, Klinikum Großhadern der Ludwig-Maximilians-Universität München

172

PANCREATIC CARCINOMA CELL ADHESION TO PLATELETS IS MEDIATED BY GP1b/IIIa AND VLA6 ON PLATELETS
R.J. Weinel, S. Santos, E. Heimüller, J. Kuhnen and M. Rothmund

Platelet-tumor cell interaction is thought to play an important role in tumor invasion and metastasis. The contact between tumor cells and platelets is presumably facilitated through receptors for adhesive proteins on platelets and the tumor cell surface. To identify the receptors for adhesive proteins on platelets, which might be responsible for tumor cell-platelet interaction, we studied the adhesion of human pancreatic carcinoma cells (cell lines No. 2, 3, 44) to platelets. A panel of monoclonal antibodies against receptors for adhesive proteins on platelets (GPIb against GP1b/IIIa, GII4 against VLA2, GPIa against VLA6) were used in addition with platelet function were used. In addition we investigated the effect of RGD, a peptide representing the cell binding sequence of fibronectin and a variety of other adhesive proteins, on tumor cell adhesion to platelets. Washed human platelets were immobilized on microtiter-plates and preincubated with monoclonal antibodies or RGD, thereafter tumor cells were added. At the end of the incubation period non-adherent tumor cells were removed and the number of tumor cells adhering to the platelets determined.

G15, recognizing GPIb/IIIa on platelets, inhibited the adhesion of all tumor cell lines to platelets by 80-85 %, G16, recognizing the platelet collagen receptor, had no inhibition of tumor cell adhesion to platelets. G15, recognizing the platelet laminin receptor, inhibited tumor cell adhesion to platelets by 55-75 %, RGD inhibitor tumor cell adhesion to platelets by 60-75 %, while a controlled peptide in which 1 aminoacid was replaced, had no effect on tumor cell adhesion to platelets.

We conclude that GPIb/IIIa and VLA6 are involved in the adhesion of pancreatic carcinoma cells to platelets. This interaction is least partly mediated through adhesive proteins possessing the RGD cell binding sequence. The platelet collagen receptor VLA2 seems to be not involved in tumor cell-platelet interaction.

Department of Surgery, Philippie-University of Marburg, Baldinger Straße, 3550 Marburg
MECHANISM OF FACTOR X DEFICIENCY IN AL-AMYLOIDOSIS

J. Lin, A. Bierhaus, M. Kraft-Czepa, R. Waldherr, M. Roth, H. Böhrer, W. Kissel, K. Andrassy, E. Ritz, P.F. Nawroth. Univ. Heidelberg: EMBL, Univ. of New Mexico

Factor X deficiency in amyloidosis is thought to be dependent on factor X binding to amyloid. A patient with AL-amyloidosis, kidney failure and a rapidly decreasing PT levels (under 10%) is presented. The coagulation abnormality was due to a shortened half of factor X (6 minutes). Protein C was normal. Splenectomy was performed to reduce the mass of amyloid. Preoperatively, the patient needed 24000 units PPS (Prothrombin complex 8-7, Immuno) per hour. After ligation of the splenic artery, the substitution with factor X could be reduced to 8000 U/h. Under hemostaseologic surveillance no adverse effect of such high PPSB administration could be observed in this patient. Postoperative PT was 48%, decreasing in the following 2 weeks to 20% and remaining constant since 6 weeks without further substitution. The slow decrease from 40% to 20% indicates saturability of factor X binding to amyloid. Immunofluorescence could demonstrate colocalisation of factor X with AL-amyloid in the patients spleen, but not in other tissue without severe amyloidosis. The association of factor X with the spleen containing amyloid could also be demonstrated in Western blots. This report demonstrates the safety of splenectomy under substitution of Vitamin K dependent coagulation factors and that splenectomy is a therapeutic option for these patients. However long term follow up is needed to evaluate the benefit of this procedure.

EFFECT OF DDAVP IN PATIENTS WITH GLANZMANN’S THROMbasthenia and with Hermansky-Pudlak syndrome

G. Töpfer, U. Funke, M. Schulze, A. Seifert and J.U. Wieding*

We studied the influence of DDAVP (0.3-0.4 μg/kg) in 2 patients with Glanzmann’s thrombasthenia and in one patient with Hermansky-Pudlak syndrome.

In patients with Glanzmann’s thrombasthenia DDAVP-infusion did not change the following parameters: Bleeding time (> 15 min), platelet aggregation using collagen and ADP in whole blood and in PRP (no aggregation). Interestingly enough, ristocetin aggregation was also abnormal, showing complete desaggregation after a partial aggregation of 40% in PRP and a repeated aggregation and desaggregation in whole blood. After DDAVP only the ristocetin aggregation in PRP became normal. In the patient with Hermansky-Pudlak syndrome DDAVP-infusion shortened the bleeding time (from 14.5 to 6 min) and increased platelet aggregation in PRP (collagen: from 16 to 40%) and in whole blood (collagen: from 26 to 48 Ohm, ristocetin: from 4 to 12 Ohm). In addition, the reaction times for platelet aggregation shortened after DDAVP. The ristocetin cofactor increased five-fold. These effects lasted at least 4 hours. From our data, we conclude that DDAVP does not alterate bleeding time and platelet aggregation with the exception of ristocetin-induced-aggregation in patients with Glanzmann’s thrombasthenia but improves these parameters in the Hermansky-Pudlak syndrome. From our data we cannot conclude, that these findings indicate clinical improvement as the studies were done while the patients were not bleeding.

Universitäts-Kinderklinik, Mathildenstr.1, 7800 Freiburg
TRYPsinemia and Coagulation Disturbances in Acute PancrEatitiss
S. Männlinghoff, J.U. Wieding and H. Köstering

Trypsin is said to play a central role in the pathogenesis of acute pancreatitis and its complications. Until now, however, it was impossible to measure tryptase activity in plasma because of high concentrations of antiproteases. A new assay employs the chromogenic substrate Cbo-Val-Gly-Arg-pNA and is said to be specific and sufficiently sensitive for tryptase in plasma. 24 patients with acute pancreatitis were examined. Beside tryptase activity and concentration (RIA), amylase and lipase activity were evaluated. Routine coagulation tests (PT, PTT, TT, TAT, FDP-LAT), turbidimetric measurement of soluble fibrin (SF), and TAT-ELISA were performed as well as D-dimer and FDP-LAT, photometric ATIII and protein C determination and platelet counts.

To render the course observations comparable (n=15), the moment of highest tryptase activity was defined as day 1.0. In this way, coagulation disturbances could be demonstrated more impressively than by any other method. Mean tryptase activity rose from 4.2±0.6 to 10.1±1.0 U/l and correlated quite well with the severity of the disease.

Coagulation tests demonstrated a disseminated intravascular coagulation with beginning consumption coagulopathy and reactive fibrinolysis in all patients. While PT, PTT and TT showed no significant alterations, fibrinogen rose continuously, acting as an acute phase protein. SF and TAT were elevated already at patients' admission. D-dimer-LAT showed earlier positive results than the FDP-assay. The very early peak of D-dimers may be a sign of dysfibrinogenemia in acute pancreatitis.

Though its mean correlations were still rather weak (0.2 < r < 0.5), tryptase activity correlated far better with blood coagulation disturbances than any other parameter. In single courses even excellent correlations (r > 0.9) with TAT, SF and especially D-dimer concentrations could be seen. These observations stress the central (but not sole) importance of tryptase in the pathogenesis of coagulation disorders in acute pancreatitis.

Haematologica, Universitätsklinikum, 3400 Göttingen / FRG

HAMOSTASEOLOGICAL AND HAMOSTASEOLOGICAL EFFECTS OF LDL-APHERESIS USING CASCADE FILTRATION
E. Schütz, P. Schuff-Werner, A. Hagenmuth, S. Schule, T. Eisenheuter and V. W. Armstrong

Low-density-lipoprotein-(LDL)-apheresis is indicated in patients with severe hypercholesterolaemia not sufficiently treatable by conventional means. At present, four different LDL-apheresis techniques are available: immunoadsorption; dextrane-sulfate-adsorption; heparin-induced LDL-precipitation and cascade filtration, which operates by molecular sieving with a cut-off of 700 kD. We have investigated this technique with regard to its specificity concerning coagulatory proteins and haemostasologically relevant plasma proteins in 4 patients. After a single treatment of 3000 ml plasma, fibrinogen is lowered to 40 % of the pre-apheresis value. The activities of the coagulation factors II, VII and X decrease to around 50 % of their original values. Factor VIII activity is chiefly affected by cascade filtration and decreases to around 6 to 10 %. Plasminogen plasma levels are lowered to 72 % and the activities of haemostasologically relevant inhibitors such as C1q-inhibitor and protein C are lowered to 70 % and 75 %, respectively. Protein S concentrations as measured by the Laurell-technique decreased to 50 % of the pre-apheresis value. Plasma viscosity and erythrocyte aggregation improve because of the lowering of large plasma proteins such as fibrinogen, IgM and α-macroglobulin; whereas whole blood viscosity remains unchanged because of haemodilution due to albumin loss. In summary, cascade filtration leads to considerable changes in coagulatory parameters, comparable to other semi-selective apheresis techniques. Haemostasologically positive effects of this method are unfortunately counteracted by an increase in haemorheology.

Zentrum Innere Medizin, Universitätsklinikum, Robert-Koch-Str. 40, D-3400 Göttingen, FRG
MONOCYTE FUNCTIONS IN HYPERCHOLESTEROLEMIA

W. Loeche, S. Krause, A. Pohl, C. Pohl, I. Schauer, A. Liebrenz, and U. Till

Adhesion of blood monocytes to endothelium and emigration into the intimal layer is a sign of an early atherosclerotic lesion. In order to get more insight into the atherogenic potential of blood monocytes we studied functions of these cells obtained from 20 patients (10 males and 10 females, mean age 44.1 ± 6.7 years) and compared the results with those obtained in 19 age and sex matched controls. Mononuclear cells (MNC) were prepared from venous blood by centrifugation on Ficoll-Paque and used for the various tests. Compared to controls adhesion to plastic surface and phagocytic activity measured as ingestion of zymosan particles was increased by 57% (p<0.005) and 19% (p<0.0002), respectively. Measurements of cell motility using a Boyden chamber without and with 10^-8 M of the chemotactic peptide FMLP in the lower compartment indicated that spontaneous motility (no FMLP) was also significantly increased by 50% (p<0.002) in patients when compared to controls. In controls FMLP-induced chemotaxis was higher than spontaneous motility. However, in patients FMLP caused a marked reduction of the motility by about 47% (p<0.002).

The changes in adhesion, phagocytic activity and spontaneous motility of MNC observed in our patients indicate that the atherogenic potential of blood monocytes may be increased in hypercholesterolemia, which could be a direct consequence of elevated plasma cholesterol level. The inhibition of motility induced by FMLP we observed in the patients cannot be explained yet.

Institut für Pathologische Biochemie der Medizinischen Akademie Erfurt. Nordhäuser Str. 74, D-0-5010 Erfurt.

HEMMUNG DER THROMBOZYTENREAKTIVITÄT EX VIVO NACH 4 WÖCHIGER EINNABME EINER KOMBINATION MEHRFACH UNTERSUCHTES FETTSAUREN (MAXGLANDIN®)

Chr. Seymansk, R. Ohrrogge, H. Ebroch & K. Schöhr

Bei 10 gesunden männlichen Nichtrauchern wurde vor, während (2. Woche, 4. Woche) und nach einer vierwöchigen Einnahme von 1.5 g/d Öl der achharzigen Johannishaere (3x Kapseln MAXGLANDIN® Linolsäure (60%), Alpha-(14%) und Gammalinolensäure (19%)) die Thrombozytenfunktion ex vivo untersucht. Nach Stimulation mit Kollagen 0.6 µg/ml zeigte sich eine Hemmung der Thrombosenenaggregation, ATP-Sekretion und Thromboxan-Freisetzung:

| Aggregation | ATP-Freisetzung | Thromboxan-Freisetzung |
|-------------|-----------------|------------------------|
| vor 60 ± 10 | 53 ± 17         | 18.9 ± 4.1             |
| 2.W. 10 ± 5*| 13 ± 9*         | 6.1 ± 1.3*             |
| 4.W. 10 ± 5*| 18 ± 11         | 6.7 ± 1.3*             |
| nach 50 ± 10| 55 ± 16         | 16.2 ± 2.7             |

(* p < 0.05 vs. VK/NK; x ± SEM)

Ähnliche Befunde ergeben sich auch bei ADP (0.3-2 µg/ml) und Kollagen (0.3 und 1.2 µg/ml). Die Sensitivität der Thrombocyte wurde erhöht. Die Untersuchung zeigt, daß das Fettsguregemisch in der verwendeten Dosierung zu signifikanten Veränderungen von Eicosanoidstoffwechsel und Thrombosenenaggregation hält.

Institut für Pharmakologie, Heinrich-Heine-Universität, Düsseldorf, Moorstr. 5, W-4000 Düsseldorf 1

IDENTIFICATION OF ENDOTHELIAL AND MESOTHELIAL CELLS IN HUMAN OMENTAL TISSUE AND IN OMENTUM-DERIVED CELLS BY VON WILLEBRAND FACTOR AND OTHER SPECIFIC CELL MARKERS

B. Pötzsch, J. Grulich-Henn, R. Rössing, D. Wille, G. Müller-Berghaus

Human omental tissue has been used as a source for the isolation and cultivation of microvascular endothelial cells, but also for mesothelial cells. Since both cell types have several morphological and functional features in common, concerns were raised whether endothelial cells can be separated from mesothelial cells by the previously described methods. In the present study, endothelial cells were identified in the capillaries of native human omentum by several endothelial-cell specific markers, such as von Willebrand factor (VWF), lectin-specific ligand Ulex europaeus I (UEA-I), and the endothelial cell-specific antibody PAL-E. These markers which were not found positive with mesothelial cells were applied to characterize omentum-derived cells previously claimed to be microvascular endothelial cells. These cultured cells proved to be negative for VWF, UEA-I-ligand and PAL-E epitope. In contrast to this, the cultured cells stained positive for cytokeratin and vimentin. Furthermore, immunoprecipitation studies showed that omentum-derived cells did not synthesize or store VWF, indicating the nonendothelial nature of these cells. Finally, electron microscopy demonstrated microvilli on the surface of omentum-derived cells, indicative for their mesothelial origin. The data presented demonstrate that the cells obtained using the previously described methods for the isolation and cultivation of "microvascular endothelial cells" from omental tissue were not endothelial cells, but essentially mesothelial cells. Therefore, these data suggest that the use of these markers is not appropriate to identify microvascular endothelial cells exclusively.

Hemostasis Research Unit, Karolinska-Klinik, Sprudelhof 11, 6850 Bad Nauheim.

INTRAALVEOLAR IMPLANTATION OF HOMOLOGICAL COLLAGEN PADS FOLLOWING ORAL SURGERY IN PATIENTS WITH A HIGH RISK OF POSTOPERATIVE BLEEDING

H.-A. Herten, J.-F. Höning, F. Halling, K.G. Wiese und H. Köstering

Within the prophylactic therapy of postoperative bleeding in patients with a high risk of bleeding after dental surgical treatment the intralveolar implantation of resorbable hemostytic agents as xenogenous collagen comcibed with fibrin glue can be recommended. The xenogenous collagen pads may cause immunological complications if applied to humans at several times. The purpose of this study is to investigate the efficiency of a new collagen pad made from human placenta preventing postoperative bleeding complications and stimulate accelerated wound healing in patients with hemorrhagic diathesis.

154 anticoagulated patients underwent 883 oral surgical procedures (mainly tooth extraction). Because human collagen in combination with human fibrin glue was applied to the alveolar socket as hemostyptic agent the oral anticoagulatory therapy could be maintained. In 12 cases minimal postoperative bleeding was observed that could be managed by local therapy (i.e. intraoral wound dressing). In all cases primary wound healing and excellent tissue compatibility was observed. The results demonstrated that the combination of human collagen pads with fibrin glue is sufficient in local hemostatic therapy of patients with hemorrhagic diathesis.

1) Abt. Kiefer- und Gesichtschirurgie des Zentrums Zahn-, Mund- und Kieferheilkunde der Universitätsgesellschaft Göttingen
2) Blutgerinnungslabor des Zentrums Innere Medizin der Universitätsgesellschaft Göttingen
A NEW METHOD FOR THE ASSESSMENT OF BLEEDING TIME AS AN INDICATOR OF ANTICOAGULANT ACTIVITY

B. Vollmayer, K. S. Herrmann and H. Kreuzer

Standard template techniques to measure bleeding time (BT) cause mixed arterial-venous bleeding and assess primarily platelet function. Transsection-BT of the rat tail is sensitive to coagulation defects but intravascular controls are not possible. We propose a new method for the assessment of anticoagulant activity in vivo.

Hamster cheek pouch venules (diameter 50 ± 10 μm (mean ± SD)) demonstrating good flow were transsected with a single incision under microscopic control. BT was not sex dependent. Neither 50 mg/100 g acetylsalicylic acid (ASA) i.v. nor 1 mg/100 g indomethacin p.o. had an influence on the BT: control 43 ± 27 sec (n=11); ASA 48 ± 25 sec (n=36); indomethacin 59 ± 32 sec (n=40). However 40 U/100 g heparin i.v., 9 mg/100 g phenprocoumon p.o. and 0.1 U/100 g ancrod i.a. significantly prolonged the BT: heparin 70 ± 61 sec vs. intravascular control 34 ± 18 sec (n=8); ancrod 134 ± 55 sec vs. intravascular control 39 ± 20 sec (n=7); phenprocoumon (n=46) 75 ± 31 sec vs. control (n=58) 36 ± 20 sec.

In our opinion the BT in the presented model is correlated with coagulation activity but not with platelet function. The method provides reproducible results and allows repetitive measurements with intravascular control.

Dept. of Cardiology and Pulmonology, University of Göttingen, Robert-Koch-Str. 40, 3400 Göttingen

SYNERGISTIC EFFECTS OF ANTIAGLOTIC DRUGS IN DIFFERENT THROMBOSIS MODELS

D. Seifig, A. Klee, E. Kremer and K.U. Weidmann

Thrombosis represents a number of diseases with high complexity and justifies multiple therapy for a multifactorial process. Thrombosis and hemostasis are controlled by several independent mechanisms. Thus, the concerted action of drugs along different biochemical pathways may represent a promising therapeutic approach in thrombosis (G. de Gaetano et al., Drugs 33:517, 1986).

The antiaglotic potential of a combination of a cyclooxygenase inhibitor and a cAMP phosphodiesterase inhibitor may be greater than the individual agents. However, when studying the antiaglotic effects of pentoxylol (POF) or dipyridamole (DIPY) and acetylsalicylic acid (ASA) in healthy or spontaneously diseased rats or rabbits, we found an obvious dependence on time interval between the application of the drugs. Consecutive administration of first POF followed 30 to 90 minutes later by ASA (principle of HWA 5112) resulted in supraadditive antiaglotic effects. A similar mode of action could be observed after administration of DIPY and ASA. The most effective dose relation for the sequential administration of POF and ASA comes to 5 or 10 to 1, but amounts for DIPY and ASA 1 to 1. Regarding the undesirable side effects of ASA, a low dose equivalent of ASA in such combination might be of great benefit.

Such drug combinations have been investigated in different models of thrombosis: induced microembolisation in rat lung, arteriolar or venular thrombus formation by argon laser injury or by photochemical reaction (D. Seifig and E. Kremer, Thromb. Res. 42:331, 1986).
INFLUENCE OF NOCLOPROST ON HUMAN PLATELET FUNCTION
E. Gluss, I. Amon and K. Jacobsen

Nocloprost, the 38-Choro,16,16-dimethyl-protaglandin E1, may be used in the therapy of gastric ulceration since it has potent gastric antisecretory properties and provides cytoprotection of the gastric mucosa. The influence of nocloprost on platelet aggregation and thromboxane A2 formation was studied.

In platelet-rich citrated plasma (PRP), nocloprost alone caused aggregation of platelets which was biphasic in some cases. Aggregation by nocloprost (1 μmol/l) corresponded to ADP (6 μmol/l)-induced aggregation and was accompanied by thromboxane A2 formation. Nocloprost-induced aggregation occurred also in hirudinized PRP. Comparable aggregation of washed platelets in Tyrode's solution was induced by nocloprost (1 μmol/l) and thrombin (0.1 IU/ml). Nocloprost was able to potentiate the aggregation induced by low concentrations of ADP or adrenaline. The nocloprost-induced aggregation at concentrations > 1 μmol/l, however, was completely inhibited by iloprost (10 nmol/l), while sulotroban or indomethacin (50 μmol/l each) inhibited the aggregation by 83% and 72%, respectively.

In platelet-rich citrated plasma (PRP), the aggregation was inhibited by low concentrations of ADP or adrenaline. The nocloprost-induced aggregation at concentrations > 1 μmol/l, however, was completely inhibited by iloprost (10 nmol/l), while sulotroban or indomethacin (50 μmol/l each) inhibited the aggregation by 83% and 72%, respectively.

In platelet-rich citrated plasma (PRP), nocloprost alone caused aggregation of platelets which was biphasic in some cases. Aggregation by nocloprost (1 μmol/l) corresponded to ADP (6 μmol/l)-induced aggregation and was accompanied by thromboxane A2 formation. Nocloprost-induced aggregation occurred also in hirudinized PRP. Comparable aggregation of washed platelets in Tyrode's solution was induced by nocloprost (1 μmol/l) and thrombin (0.1 IU/ml). Nocloprost was able to potentiate the aggregation induced by low concentrations of ADP or adrenaline. The nocloprost-induced aggregation at concentrations > 1 μmol/l, however, was completely inhibited by iloprost (10 nmol/l), while sulotroban or indomethacin (50 μmol/l each) inhibited the aggregation by 83% and 72%, respectively.

Hypertension occurs in about 10% of all gravidities. According to Hentemann, W. Schmidt, E. Wenzel, H. Kiesewetter, A. Hettenbach, F. Jung, P. Scher, B. Hummel, M. Zimmermann, S. Robert-Koch-Straße 28, 4400 Münster, and M. Kandt, J. Schrader, U. Neu, G. Schoel, C. Bellingrath, Robert-Koch-Str. 40, 3400 Goettingen, FRG.

The therapeutic use of nocloprost as anti-ulcer agent requires a much lower plasma level than necessary for the induction of aggregation.

I Institute of Pharmacoogy and Toxicology, Medical Academy Erfurt, Nordhäuser Str., Nordhäuser Str. 74, 0-5010 Erfurt and 2Asche AG, Hamburg Fischers Allee 49, 2000 Hamburg 50, FRG

ON THROMBOCYTE FUNCTION IN CASE OF GRAVIDITY HYPERTENSION
H. Kniesewetter, A. Hettenbach, F. Jung, P. Schäfer, B. Hummel, M. Hentemann, W. Schmidt, E. Wenzel

Hypertension occurs in about 10% of all gravidities. According to recent investigations it seems that the proportion of thromboxane (TXA2) to prostacycline (PGI2) is shifted in favour of thromboxane. The elevated thromboxane level leads to an increased spontaneous thromboocyte aggregation. Thromboocyte aggregation was measured according to the method of 1.) Broddin (PAT III) and 2.) Grotmeyer (platelet reactivity: PRI). The data of six female patients with gestosis are given below.

Age Height Weight Gravidity Blood Pressure Platelet PRI
years cm kg week mmHg I/Au degree
30 163 113 36 135/80 217.000 0 1.01
35 163 113 36 160/110 129.000 1 0.99
24 156 65 35 160/110 93.000 12 1.17
38 128 12 38 164/100 75.000 0 1.14
28 165 78 32 140/95 54.000 0 1.10
26 173 68 22 180/130 32.000 0 1.14

Four out of the six gravidae showed a pathologically increased platelet reactivity by applying the test according to Grotmeyer. In only one patient with a thromboxane count of 93.000 [l/Au] the PAT III according to Broddin also revealed a pathological result.

Abt. für Klinische Hämostaseologie und Transfusionsmedizin, Universität des Saarlandes, D-66600 Homburg-Saar
INHIBITION OF PLATELET PAI-I RELEASE WITH THE NITRIC OXIDE DONOR SIN-1 BUT NOT WITH ORGANIC NITRATES
Spannagl M, Drummer C, Lüdtke S, Geiger R, Schramm W
Med. Klinik, Klinikum Innenstadt, Universität München, FRG

A dose-dependent increase of plasminogen activator inhibitor (PAI-1) release is observed in platelet rich plasma (PRP) following stimulation with either ADP, platelet activating factor (PAF) or collagen. This may reflect an important mechanism involved in fibrinolysis also in vivo. In the present work we have conducted experiments to determine the effects of several nitrovasodilatory compounds on the stimulated release of platelet PAI-1 during platelet aggregation. In addition to organic nitrates (Glyceroltrinitrate, Isosorbide-dinitrate, molsidomine) and its bioactive hepatic metabolite SIN-1 was investigated. PRP, adjusted to 250 000 platelets/microliter was preincubated for three minutes with increasing concentrations of the respective nitro-compound. Aggregation (Born-method) was induced with either 4 µM ADP, 1600 nM PAF or 2 µM collagen. PAI-1 activity (KabiVitrum, München) was determined from supernatant of PRP.

Results: Maximal inhibition (75% reduction compared with control) of platelet PAI-1 release was obtained with 10 µM SIN-1. 0.1 µM SIN-1 already reduced PAI-1 release by 25%. In contrast, the parent compound molsidomine as well as the organic nitrates only affected platelet PAI-1 release in the millimolar range. The maximal inhibition of PAI-1 release that was obtained with 1 mM molsidomine or 1 mM organic nitrates equalled the 25% reductions that were observed with 0.1 µM SIN-1. The effects of SIN-1 and the other nitro-compounds on PAI-1 release were consistently observed, independent of whether ADP, PAF or collagen was applied to stimulate platelets.

Conclusion: The present data demonstrate that the nitric oxide donor SIN-1, but not organic nitrates, strongly inhibits platelet PAI-1 release. Furthermore, the data suggest that the endogenous nitric oxide (endothelium derived relaxing factor) also might influence fibrinolysis by reducing platelet PAI-1 release in vivo.

LEUCOCYTE DEPLETION OF PLATELET CONCENTRATES USING TWO DIFFERENT FILTERS: EFFECT ON PLATELETS
E. Biesch, N. Gindi, V. Weihsbach, S. Serke, N. Schney, R. Eckstein

Filtration of platelet concentrates (PCs) to reduce the white blood cell contamination (WBCC) is a method to avoid alloimmunization and subsequent refractoriness to transfused platelets (plts). We studied plt loss, leucocyte depletion (LD), lymphocyte subsets (LS), plt volume distribution (PVD), plt morphology (PM) and plt function before and after filtration through two different polyester filters as well as the posttransfusional plt increment (PTI) 1 h after transfusion. Eight "oversized" single-donor PCs (median 490g; range 400-550g) with a median of 3.7 (2.6-5.2) x 10^11 plts were prepared by cytopsisis. After 6 h storage a control sample was taken (C) and the PCs were equally divided in two parts which were subsequently filtered through two different polyester filters as well as the posttransfusional plt increment (PTI) 1 h after transfusion. Eight "oversized" single-donor PCs (median 490g; range 400-550g) with a median of 3.7 (2.6-5.2) x 10^11 plts were prepared by cytopsisis. After 6 h storage a control sample was taken (C) and the PCs were equally divided in two parts which were subsequently filtered through two different polyester filters (PL-100, Pall, Dreieich [A]; Soporcell PL-SA, Diamond, Köln [B]). Samples for in vitro investigations were taken and tested in parallel with C. Medians for plt volume distribution (PVD), plt morphology (PM) and plt function before and after filtration through two different polyester filters as well as the posttransfusional plt increment (PTI) 1 h after transfusion.

RISK FACTORS OF THROMBOSIS IN ORTHOPEDICS AND TRAUMATOLOGY
W. Heller, Z. Engel

The patients were divided into five groups. In group 1 the patients had to undergo a removal of metallic implants. The patients in group 2 showed only slight woundings, such as broken ankle joints. In group 3 seriously injured patients with polytraumata were to be found. In group 4 fractures in patients with general diseases had been operatively treated. In group 5 all the operated patients suffered from a malignant basic disease. Following proteins of the coagulation and fibrinolytic were determined from the first preoperative up to the eighth postoperative day: fibrinogen, anti thrombin III, plasminogen, α₁-antitrypsin, α₂-macroglobulin and Cl-inactivator. Groups 4 and 5 showed reduced concentrations of fibrinogen, anti thrombin III and plasminogen. In these groups the preoperative values were already 25% lower than in groups 1 and 2, to which they were compared. Whereas in group 4 variations of at most 1 8% of the initial level could be established, the concentration of fibrinogen, anti thrombin III and plasminogen on the first postoperative day was in the tumor group respectively 50%, and for the last two substances 65% under the standard limit of tolerance. The investigations revealed group 3 as a conditional endangered group: the preoperative values between 6 and 13% below the normal range, were reduced during the operation at about 25%. The concentrations of Cl-inactivator and α₁-antitrypsin were typically high.

Abt. für Thorax-, Herz- und Gefäßchirurgie der Universität Tübingen, Calwerstr. 7, D-7400 Tübingen

MODEL STUDIES ON THE HEMocompatibility of SOME BIOMATERIALS ESPECIALLY HOLLOWFIBER- AND SILICONE MEMBRANES
W. Heller, H.P. Wendel, R. Klaffschenkel, H.-E. Hoffmeister

For preclinical evaluation of further technical improvements in two oxygenator types an extra-corporeal circulation was here simulated in a HLM ex vivo model. Three different membrane oxygenators produced in Germany, Italy and in the USA (A, B, C) were thereby tested. The difference between them was that unlike B and C, oxygenator A was provided with a siliconised, unporous diffusion membrane with plates of altogether 0,7m² gas exchange surface. Oxygenator B had a hollow fibre membrane of 0,4m² and oxygenator C a flat membrane of also 0,4m². The filling volume was in A and B 120 ml each, in C 300 ml. Test conditions: 500 ml heparinised, recalculated ACD whole blood (max. 48h old) were recirculated for 90 minutes in an ashort-circuited system at 28°C. All in all 8 blood samples were taken. Hemodilution and gas flow occured according to op-conditions. The evaluation of the blood samples resulted, with regard to corpscular alterations, in no significant difference between the three oxygenators. With respect to the luekin-kinin and fibrinolytic systems the course of activation, resp. inhibition, was almost identical. Only the investigation with very sensitive immunological methods on platelet activation allowed the determination of considerable differences between the oxygenators.

Abt. für Thorax-, Herz- und Gefäßchirurgie der Universität Tübingen, Calwerstr. 7, D-7400 Tübingen
Sulfated lactohionic acid amides represent a new group of antithrombotic compounds. Four synthetic compounds constitute a homologous group; they differ in the length of the alkyl chains. The anticoagulant as well as the antithrombotic effect, however, is dependent on the molecular weight. The substance with the code number LW 10082 \( \text{[Hexadesaccharide trihexenylphosphoryl-(4-0-6-D-galactopyranosyl-} \) \( \text{D-glucosaminylectose octasulfate]} \) is the most active compound in this group. The compound is a polyanion with a molecular weight of 2388 daltons and is characterized by defined structure. As this substance is chemically easily available and as stability studies have shown satisfactory results, LW 10082 was selected for further development. The WHO has given LW 10082 the INN "Aprosulate sodium".

In a concentration of 2.5 - 50 \( \mu \text{g/kg} \) slight effect was observed on APTT and Heptest. The AT III depletion of plasma did not change the results of the assays, suggesting that AT III is not required for the anticoagulant activity.

Aprosulate sodium showed antithrombotic activity in the PCC/RFF induced thrombosis in rabbits after i.v. (ED \( \text{\text{EM}} \leq 100 \) - 200 \( \mu \text{g/kg} \)) and s.c. (ED \( \text{\text{EM}} \leq 2-4 \mu \text{g/kg} \)) administration as well as in the in vitro test, the laser induced thrombosis model of Brededin and a rat jugular vein haemostasis model.

The anticoagulant and antithrombotic activity was readily neutralized by protamine. In equimolar doses protamine neutralized 70% of the thrombosis in the rat jugular vein model and the thrombin time. If the protamine dose was increased to 2.5 times the dosage of LW 10082, a complete antagonism took place to neutralize the anticoagulant and antithrombotic effects of Aprosulate sodium.

Aprosulate sodium is an innovative compound; for the first time, a homogeneous chemical agent with a defined molecular weight is available as antithrombotic agent.

The Department of Pharmacology and Chemistry, Luitpold-Company Munich, FRG.

---

**Activations of Factor X by Platelets in Patients with Chronic Megakaryocytic Leukemia (CMF)**

A. Geha, R. Krause, E. Seifried

Platelets promote the catalysis of two sequential reactions in the coagulation cascade: the activation of Factor X (FX) to F Xa by a complex of FXa and F VIII, and the conversion of F Xa and F Va to an enzyme complex of F Xa and F V. Direct platelet FX activation activity (FX-A), independent of FX activation via the intrinsic or extrinsic pathway has been shown by Bencard et al. The evaluation of FX-A, determined by coagulase assay, in patients with CMF and bleeding complications has been demonstrated by Cortella et al. We describe a specific test, using chromogenic substrate assay for platelet FX-A measurement. Promotion of FX-A, indicated by hydrolysis of S 2337 (Kabi Vitrum) was preformed by incubation of gel filtered platelets, 0.1 ml CaCl\(_2\) (0.05 M) and 0.2 ml Tyrode solution with 0.05 ml protamin complex concentrate (Fc) 1 U/ml or purified F X 1 U/ml. 25 patients with C-MF were studied. Mean extinction (± SD) of FX-A in the patients group was 0.095 ± 0.063 and 0.147 ± 0.025 (ns) in the volunteers group (n = 10). 6 of 25 patients with bleeding complications showed significant (p < 0.01) reduced FX-A in comparison to asymptomatic patients. No significant differences between symptomatic and asymptomatic patients were found using chromagenic assay with PC or the clotting assay, described by Cortella. These results support evidence of FX-A as diagnostic criteria in the evaluation of bleeding complications in patients with CMF.

Abteilung Innere Medizin III, Medizinische Universitätsklinik und Poliklinik, Fehl-Koch-Str. 8, 7900 Ulm
Acute leukemias in childhood may be complicated by bleeding and/or thrombotic events. Among risk factors for hemostatic complications low platelet count, pro- and anticoagulant factors of blood cells, leukostasis, and therapy with L-Asparaginase (ASP) have been listed. To study the influence of ASP therapy on the hemostatic system, we examined pro- and anticoagulant factors in 6 children treated according to the ALL/NHL-BFM 90 protocol, whereby ASP therapy is started on day 12 during induction therapy with prednisone, vincristine, and daunorubicin.

Following ASP therapy the following factors decreased markedly (lowest values in brackets): fibrinogen (25 mg/dl), AT III (20%), plasminogen (22%), protein C (65%), total protein G (45%). After cessation of ASP therapy all these factors increased to normal values. None of the 6 patients was substituted with pro- or anticoagulant factors, none of the patients had a bleeding episode or a thrombosis. In one other patient with ALL the decline of F i, AT III, and Plasminogen was reversed when Escherichia-ASP was replaced by Erwinia-ASP. From these data we conclude that ASP causes a marked decrease of pro- and anticoagulant factors. However, we cannot conclude whether these laboratory data indicate a risk for bleeding and/or thrombosis, and whether prophylaxis is advisable. These questions can only be answered by a larger clinical study.

Universitäts-Kinderklinik, Mathildenhöhe 1, 7800 Freiburg

DECREASED PLATELET AGGREGATION AFTER REPERFUSION IN ORTHOTOPIC LIVER TRANSPLANTATION (OLT)

G. Himmelreich, H. Kuntz, P. Neuhaus, G. Blumhardt, H. Riss

Increased blood loss is a major contributory factor to postoperative short- and long-time outcome in orthotopic liver transplantation (OLT). Hyperfibrinolysis has been identified as a paralleling increased bleeding tendency. However, other factors may contribute as well. We investigated for the first time platelet aggregation during the course of 10 OLT and found a marked reduction of adenosine diphosphate-, collagen-, and ristocetin-induced platelet aggregation immediately after reperfusion. Since this time period is well known for increased bleeding tendency, it became evident that apart from hyperfibrinolysis an intermediate dysfunction of platelet aggregation may be of causative importance for intraoperative bleeding. It is postulated that graft liver perfusate is reducing platelet aggregation.

Universitätsklinikum Rudolf Virchow, Abt. f. Innere Medizin und Chirurgie, Spandauer Damm 130, D-1000 Berlin 19

PREPARATION OF HIGH MOLECULAR WEIGHT, POLYVALENT LIGANDS OF THE TXA₂/PGH₂ RECEPTOR AND THEIR EFFECTS ON HUMAN PLATELETS

M. Müller and H. Patscheke

In order to develop non-competitive TXA₂/PGH₂ receptor antagonists, we synthesized polyvalent ligands with the capability to simultaneously bind to two or several TXA₂/PGH₂ receptor sites. These polyvalent antagonists can be expected to bind with extremely high affinity because of cooperative binding and should produce an irreversible, although non-covalent receptor blockade. The competitive TXA₂/PGH₂ receptor antagonist sulotroban was chemically linked to poly(2-hydroxyethyl)-starch (HES) or poly-L-lysines (PL) with molecular weights of 450 and 200 kD or 322, 249, and 40 kD, respectively. The carboxyl group of sulotroban was activated with N, N'-carbonyldimidazole prior to the formation of its esters or amides with HES or PL respectively. The products (Sulo-HES and Sulo-PL) were purified by GFC and their degree of derivatization characterized by spectrophotometry and sulfur content analysis. Sulo-HES inhibited the U 46619-induced platelet shape change if ≥ 0.5 % of the hydroxyl groups were derivatized with sulotroban, whereas Sulo-PL required a derivatization of at least 50 % of its ε-amino groups in order to overcome the platelet-agglutinating activity of the matrix. Platelet agglutination was due to the polycationic properties of the PL matrix and could be inhibited by neutralization with heparin. If 52, 56 or 61 % of the ε-amino groups were acylated with sulotroban, Sulo-PL 249 inhibited the shape change induced by the TXA₂ mimetic U 46619. Neither Sulo-PL nor Sulo-HES were effective on the platelet shape change induced by ADP or PAF. At a comparable degree of derivatization in various Sulo-PL (55 %) and various Sulo-HES (2.5 %) preparations, the IC₅₀ decreased with the increasing molecular weight: 25 nM for Sulo-PL 249, 6.5 nM for Sulo-PL 322 and 2.7 μM in Sulo-HES 40, 0.26 nM in Sulo-HES 200, and 0.82 μM in Sulo-HES 450. These observations show that the inhibitory effects of sulotroban derivatives of HES (Sulo-HES) and PL (Sulo-PL) on U 46619 induced platelet activation strongly depend on the molecular weight of the polymer matrix and the degree of its derivatization with sulotroban. (Supported by the Deutsche Forschungsgemeinschaft, Pa 263)

Institut für Klinische Chemie, Klinikum Mannheim der Universität Heidelberg, Postfach 10 00 23, 6800 Mannheim
SURFACE ANALYTICAL INVESTIGATIONS ON THE HEMO-
COMPATIBILITY OF BIOMATERIALS
W. Kerfin, C. Plog and R. Karsch

Surface analytical studies were carried out by Static Secondary Ion
Mass Spectrometry (SSIMS) and Scanning Electron Microscopy
(SEM) with Energy Dispersive X-Ray Analysis (EDX) on artificial
bloodvessels made of e-PTFE, carbonized e-PTFE, polyurethane,
dacron and plasmadeposited PTFE on dacron. Spectra of positively
and negatively charged secondary ions have evaluated semi-quantiti-
tatively to get information on surface chemistry of the materials
after different incubation tests and after explantation, compared to
unused vessels.

It could be shown, that
(1) UC-ePTFE is not completely carbonized at the surface and the
fibres are damaged by the modification procedure.
(2) Adsorption of human serum albumine and human immunoglobuli-
ne is much higher on UC-ePTFE than on e-PTFE.
(3) There is no layer of pure PTFE on dacron in the case of plas-
ma-deposited PTFE, but a layer of a copolymer consisting of tetra-
fluoroethylene and ethylene.
(4) The SSIMS-method makes it possible to detect even very low
quantities of surface contaminations, as could be shown in a case,
where only monolayers of polydimethylsiloxane were present at the
surface of a polyurethane vessel.
(5) SEM studies on Sepharose 2B, 4B and 6B showed that the gel
particles not only consist of pores, whose size is in agreement with
chromatographic measurements, but also of non-porous dense areas.

Studies are continued to develop a more quantitative description of
protein adsorption on surfaces. Investigations on a more detailed
description of surface chemistry of modified Sepharose and glass-
slides are in progress in order to get a better understanding of
their binding properties of blood proteins.

Dornier GmbH, Postbox 1420, D-7990 Friedrichshafen

INTERACTION OF PLATELET AGGREGATION AND GRANULO-
CYTE FUNCTION.
E. Jakob, A. H. Sutor

Platelets are not only effective in hemostasis but contribute
also to the defense of infectious agents, and vice versa
granulocytes react not only with bacteria but also play an
important role in hemostasis.

To study the interaction of platelets and granulocytes we used
a whole blood aggregometer (Chronolog). By observing
simultaneously the aggregation of platelets and the generation
of oxygen radicals from granulocytes with a luminol-dependent
chemoluminescence system in 12 normal persons the follow-
ing results were found: During the process of platelet aggrega-
tion (18 + 5 Ohm) induced by collagen or ADP stimulation
of granulocytes was observed about 60-90 sec later by an
increase of the luminol-dependent chemoluminescence. This
effect was present in whole blood (1.5 ± 0.7 arbitrary units) as
well as in a system, where only platelets and granulocytes
were present (7 ± 0.3 arbitrary units). When platelets were
aggregating without granulocytes in PRP an increase of
luminol-dependent chemoluminescence was not observed;
nor did isolated granulocytes show an increase of luminol-
dependent chemoluminescence, after ADP had been added.

From these data we conclude, that platelet aggregation
stimulated by ADP or collagen induces the release of oxygen
radicals from granulocytes.

Universitäts-Kinderklinik, Mathildenstr. 1, 7800 Freiburg