

**PP5 Vascular Biology****PP5.1 Animal and Experimental Models****PP5.1-1****Clopidogrel improves endothelial function and NO bioavailability in congestive heart failure**

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Background: Treatment of patients with coronary artery disease with clopidogrel not only inhibits platelet activation, but also improves endothelial function and NO bioavailability. Similar to coronary artery disease, patients with chronic congestive heart failure (CHF) display endothelial dysfunction and increased platelet activation. Using the experimental CHF model following chronic myocardial infarction (MI) in rats we investigated, whether treatment with clopidogrel positively modifies endothelial function in CHF.

Methods: MI was induced in healthy male Wistar rats by ligation of the left anterior descending coronary artery. After 8 weeks, animals were randomized to placebo or the platelet P2Y₁₂ receptor antagonist clopidogrel (5 mg/kg twice daily, given

by gavage) for another 2 weeks. Afterwards, endothelial function was assessed in isolated aortic rings in organ bath experiments.

Results: Endothelium-dependent, acetylcholine-induced vasorelaxation was significantly attenuated in CHF rats (EC50: 179.5±19.9 nmol/l, Rmax: 63.5±5.5%) compared to sham-operated animals (EC50: 51.5±19.5 nmol/l, Rmax: 93.4±2.6%, p<0.001), which was significantly improved by treatment with clopidogrel (EC50: 40.4±4.6 nmol/l, p<0.001; Rmax: 99.5±0.3%, p<0.001). Basal NO bioavailability, which was determined indirectly by additional vasoconstriction of phenylephrine-precontracted aortic rings following addition of the NO synthase-inhibitor N^o-Nitro-L-arginine, was significantly impaired in CHF rats (54.7±3.2% of maximum phenylephrine contraction, p<0.001) compared to sham-operated animals (103.9±8.3% of maximum phenylephrine contraction) and was improved by clopidogrel (76.7±2.7% of maximum phenylephrine contraction, p<0.001).

Conclusion: Clopidogrel improved endothelial function and NO bioavailability in rats with CHF. This mechanism might at least partially contribute to the vasoprotective effects of clopidogrel.

PP5.1-2

New insight into the function of VKORC1L1

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Objectives: VKORC1L1 is a member of enzymes that are present in vertebrates, plants and bacteria. Furthermore, it is the isozyme of VKORC1 that is known to reduce vitamin-K-epoxide to vitamin-K-quinone in the vitamin K cycle. Herein, we investigated the role of VKORC1L1 in intracellular antioxidation.

Design and Methods: We measured VKOR enzymatic activity and corresponding expression of VKORC1L1 mRNA from hydrogen peroxide treated HEK cells. Activity was assayed by determination of vitamin-K1-epoxide to quinone reduction by HPLC. Transcription rate of VKORC1L1 was analysed by real-time PCR with specific probes in reference to PBGD as housekeeping gene. Localisation was performed by co-expression of c-terminal eGFP-tagged VKORC1L1 and commercial fluorescent vectors for different subcellular components by fluorescence microscopy.

Results: Examination of VKORC1L1 response to peroxide treatment revealed a five-fold increased mRNA-level with a maximum after 40 minutes. As expected, VKOR activity was enhanced after induction, too. Furthermore, about 250% rise in activity sustained at 120 minutes corresponds well to the increase of expression after exposure to hydrogen peroxide. Co-expression of labelled VKORC1L1 shows a localisation of VKORC1L1 in the same cellular component like VKORC1, the endoplasmatic reticulum.

Conclusion: Rising transcription rate of VKORC1L1 and postponed increased VKOR activity as reaction to oxidative stress indicates a connection to cellular defence against free radicals. The localisation of VKORC1L1 in the endoplasmatic reticulum suggests a radical scavenge mechanism to protect this cellular component against oxidative damage caused by oxygen radicals. These underline results our hypothesis of an antioxidant function of VKORC1L1.

PP5.1-3

Fractalkine promotes platelet activation and vascular dysfunction in chronic heart failure: a link to impaired Clopidogrel responsiveness

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Background: Endothelial dysfunction and enhanced platelet reactivity in chronic heart failure (CHF) contribute to reduced prognosis. CHF patients display an impaired response to clopidogrel. We investigated whether fractalkine is linked to impaired clopidogrel responsiveness in patients with CHF. In CHF rats, the influence of fractalkine on endothelial function and platelet reactivity were analyzed.

Methods: Fractalkine levels were determined by ELISA. Platelet surface expression of P-selectin and the fractalkine receptor (CX3CR1) were analyzed by flow-cytometry. Vascular function of isolated aortic rings was assessed in organ bath studies.

Results: Fractalkine serum levels were increased in CHF patients (CHF: 1548±196pg/mL; Control: 968±191pg/mL, p<0.05), and high fractalkine levels were found in patients with impaired clopidogrel responsiveness measured by the P2Y₁₂-specific platelet-reactivity-index (PRI) (PRI>50%: 1526±98pg/mL; PRI<50%: 744±137pg/mL, p<0.01). In CHF rats following coronary ligation, serum and urine levels of fractalkine were significantly increased compared to control (serum: CHF: 1509±168pg/mL; Control: 1181±58pg/mL, p<0.05). CHF-rats displayed impaired response to clopidogrel (PRI: CHF64%; Sham: 30%, p<0.05). Fractalkine significantly attenuated endothelial function in CHF rats and augmented P-selectin expression on platelets from CHF rats. Expression of CX3CR1 on the platelet surface was increased in CHF rats (CX3CR1 mean fluorescence intensity:

CHF45; Sham: 34, p<0.01). Furthermore, fractalkine desensitized platelets to the endogenous platelet inhibitors prostacyclin and nitric oxide, whose bioactivity was already impaired in CHF rats.

Conclusion: We observed increased serum fractalkine levels and platelet expression of the fractalkine receptor CX3CR1 in heart failure. Fractalkine desensitizes platelets to endogenous platelet inhibitors and contributes to impaired clopidogrel responsiveness in CHF.

PP5.1-4

Plasma ADAMTS13 activities in non-human primates and rabbits do not change upon administration of human recombinant VWF

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Objectives: Von Willebrand factor (VWF) is composed of a series of multimers, the sizes of which are regulated by the plasma metalloprotease ADAMTS13. Transient increases in VWF levels, triggered for instance by DDAVP treatment, were reported to result in a decrease in ADAMTS13 activity, possibly due to its exhaustion upon exposure to an excess of substrate. Thus, treating von Willebrand disease type 3 patients with recombinant human von Willebrand factor (rVWF) might cause a drop in ADAMTS13 levels.

Design and Methods: The effect of administration of high doses of rVWF on endogenous ADAMTS13 levels was tested in animal models. Various doses of human rVWF (300, 600, and 1200 RCo IU/kg BW) were injected into rabbits and cynomolgus monkeys, plasma samples were collected at a range of time points, and ADAMTS13 activity (FRET assay) and VWF:Ag were determined. ADAMTS13-specific cleavage products were detected by immunoblot analysis.

Results: VWF antigen rose sharply in a dose-dependent manner (~25 IU/ml VWF:Ag for the highest dose, 15 min after injection) and then declined gradually (~7 IU/ml VWF:Ag for the highest dose, 18 hours after injection). By contrast, the ADAMTS13 activity did not show relevant changes throughout the entire test period in the rabbit or in the monkey samples. Both rabbit and cynomolgus ADAMTS13 recognized human rVWF as the specific cleavage products were detectable at all doses administered.

Conclusions: The animal studies clearly indicate that an excess of intravenously administered rVWF leading to supraphysiological levels does not exhaust ADAMTS13.

PP5.1-5

Measurement of von Willebrand Factor (VWF) function under physiological flow conditions

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Objectives: VWF is essential for primary haemostasis. Binding of VWF to exposed extracellular matrix components in injured vessel walls leads to platelet tethering, activation and adhesion. At low shear conditions, platelets are able to bind directly to collagen, but under high flow present in human arterial circulation, the presence of plasmatic VWF is essential to achieve stable platelet adhesion. We evaluated the binding of VWF to immobilized collagen I or III at various shear rates, qualified the collagen-bound VWF and analyzed VWF-mediated platelet binding using an in vitro flow-chamber model.

Design and Methods: Experiments were performed using physiological concentrations of VWF, erythrocytes and platelets in a flow-chamber (μ-slide VI, ibidi®, Germany) coated with collagen I or III. Binding of Wilate® (Octapharma PPGmbH, Austria) was tested applying physiological low to high shear rates. Collagen-bound VWF was qualified via antibody detection and multimer analysis. Adhesion of fluorescence labelled platelets was determined using time-lapse microscopy and computer based software analysis.

Results: All VWF multimers, i.e. from low to high molecular weight, bound to both, collagen I and III at low to high shear rates. Wilate® mediated stable platelet adhesion to collagen at high shear rates applying physiological concentrations of 1 IU/mL VWF:Ag.

Conclusion: The established in vitro flow-chamber model was successfully used for the determination of VWF activity under defined shear rate conditions in real time. Our system represents a promising device for the investigation of the impact of VWF nativity, multimer size and triplet structure on VWF function under flow.



PP5.1-6

Anti-inflammatory and antinociceptive effects of the direct thrombin inhibitor hirudin

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Objectives: Increasing evidence points to extensive cross-talk between inflammation and coagulation, whereby inflammation leads not only to activation of coagulation, but coagulation also considerably affects inflammatory activity. The purpose of this study was to investigate the effect of the thrombin inhibitor hirudin on an acute inflammatory process in rats.

Design and Methods: Acute inflammation was produced by subplantar injection of 0.1 ml of 2% carrageenan in a hind paw of male Sprague-Dawley rats. Paw volume was measured before and at different time intervals after carrageenan injection. In non-inflamed animals was injected saline instead carrageenan. Hirudin was administered subcutaneously 30 minutes before carrageenan injection. Hotplate latency test was used to quantify antinociception. Leukocyte count was determined with an automated counter. C Reactive Protein (CRP) levels were measured with a sandwich enzyme-immunoassay. Fibrinogen levels were determined by a modification of the method of Ratnoff and Menzie (1954).

Results: Injection of carrageenan in the rat paw induced an edema reaching its maximum after 4 h and decreasing over 96 h. Leukocyte count, CRP and fibrinogen levels reached a maximum 12h, 8h and 36h after carrageenan injection, respectively. Hirudin showed a significant inhibition of carrageenan-induced edema and increased the latency period in the hotplate test. Leukocytes count and CRP levels were significantly decreased in hirudin treated rats. High fibrinogen levels present in inflamed rats were not affected by hirudin treatment.

Conclusions: These results suggest that a single application of hirudin prior to carrageenan-induced inflammation has significant anti-inflammatory and antinociceptive effects.

PP5.1-7

Severe arteriolar necrosis with renal thrombotic microangiopathy in a septic porcine 2-hit model

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The balance between VWF and ADAMTS13 is assumed to be involved in the development of sepsis-associated thrombotic microangiopathy (TMA). We hypothesized that a decreased ADAMTS13-activity in a porcine 2-hit model of hemorrhage and sepsis is associated with laboratory and morphological findings of TMA. Animals (n=21, BW 24 kg) were subjected to hemorrhagic shock (45 min). After re-transfusion, sepsis was induced via intraperitoneal implantation of fibrin clot to restrain continuous release of 109 vital E.coli. Blood samples for determination of ADAMTS13-activity, VWF:Ag and platelet count, creatinine and hematocrit for normalization were drawn. Directly postmortem we examined renal TMA development (CD61 staining) and ischemic acute tubular necrosis (epithelial brush border staining). ADAMTS13 activity remained stable after hemorrhage, however, declined stepwise during sepsis accompanied by a strong drop off in platelet count (356 to 142 GptL-1). Moderate increase in VWF:Ag was found. Development of renal dysfunction was indicated by a slight, but significant increase in plasma creatinine concentration (107.0 vs. 141.0 μmolL-1, p<0.05). Characteristic findings for TMA with glomerular microthrombi in kidneys, fibrinoid necrosis of preglomerular arterioles and interlobular arteries with interstitial bleeding were observed. Glomerular luminal platelet activation and ischemic acute tubular necrosis were most prominent in septic animals in comparison to sham-operated ones. Similar to patients with severe sepsis a decline in ADAMTS13 activity in association with platelet consumption was observed in a septic porcine model whereas renal TMA was present in one animal. Therefore, we present an animal model to perform functional studies focusing on pathophysiological mechanisms of sepsis-associated TMA.

PP5.2 Atherosclerosis

PP5.2-1

YKL-40, a player in extracellular matrix remodeling, is increased in morbid obesity but declines with weight loss

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Objectives: YKL-40 is a protein involved in extracellular matrix remodeling. Elevated YKL-40 was found in advanced stages of atherosclerosis/coronary heart disease. Morbid Obesity (MO) is associated with high cardiovascular disease (CVD)

morbidity and mortality. Thus, we asked, whether YKL-40 is elevated in MO patients and might contribute to the increased CVD event rate in MO.

Design and Methods: 17 patients with MO were included, who were studied before (BMI:47±6kg/m²) and after weight loss by gastric bypass surgery (BMI:34±6kg/m²). Observation period: 17.4±2.2months. Patients were compared to 20 age/sex-matched controls (CO;BMI:22±4kg/m²). We determined fasting Monocyte-chemoattractant-Protein-1 (MCP-1), glucose, insulin and lipids and insulin and glucose obtained in a two hour glucose tolerance test, and HOMA-Insulin Resistance. All tests were performed before and after bariatric surgery.

Results: YKL-40 values were tripled (123±61ng/ml) in MO vs. CO. After a weight loss of 40±18kg, YKL-40 levels were reduced to 85±42ng/ml, however, were still doubled vs. CO. Delta YKL-40 (Preoperative minus postoperative) were related to deltas of fasting insulin (R=0.754,p=0.005), HOMA-IR (R=0.789,p=0.002) and MCP-1 (R=0.676,p=0.016). Multivariate regression demonstrated that preoperative MCP-1 had the strongest independent association with preoperative YKL-40 (beta=0.805,p=0.01), as had the deltaHOMA-IR with deltaYKL-40 (beta=0.789,p=0.002).

Conclusions: The novelty of this study is the significant elevation of YKL-40 in MO and that these elevated levels can be lowered by weight loss. The significant association with MCP-1 levels is tempting and invites to speculate that MCP-1 attracting monocytes to plaque site and YKL-40 being involved in plaque rupture might together be responsible for the increased CVD risk.

PP5.2-2

Effect of anti-glycemic control on diminished endothelial progenitor cells in type 1 diabetic children

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Objectives: The risk of cardiovascular death before the age of 40 is 20-fold increased in patients with type 1 diabetes mellitus (T1DM) compared with nondiabetics. The mechanisms mediating this risk are not completely understood. Since endothelial progenitor cells (EPC) predict cardiovascular morbidity and mortality in nondiabetics, we performed a longitudinal study in T1DM children, enumerated EPC and studied their associations.

Design and Methods: 120 children, 90 T1DM and 30 controls (CO) were included and matched for age, gender and BMI. EPC (CD34+/CD133+/CD309+) were enumerated by flow cytometry at the beginning and one year thereafter, when the patients were scheduled for their routine follow up examination. In order to analyse changes of variables during the one year observation time, delta values were calculated.

Results: EPC were reduced in T1DM children vs. CO: 607±368vs.1037±504,p<0.001. Multivariate regression revealed that HbA1c was the strongest independent predictor of EPC (Beta=-0.319,p=0.003). Overall glycemic control at the beginning and end of study did not differ (7.8±1.2vs.7.8±1.2rel.%,p=ns). However, we observed HbA1c changes during the study in the individual patient of -4.30/+3.10 rel. %. Calculating the mean change of EPC per mean change of HbA1, resulted in an increase of 93 EPC per each 0.1rel. % reduction of HbA1c and vice versa.

Conclusions: In conclusion, this is the first study demonstrating diminished EPC in children with T1DM. The observed increase of EPC in children with improved diabetic control within one year suggest that optimization of glycemic control could be relevant in reducing the high cardiovascular disease burden in T1DM patients.

PP5.2-3

Factor Seven Activating Protease: a link between inflammation and coagulation in coronary artery disease

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Introduction and hypothesis: FSAP may be involved in the progression of atherosclerosis and the development of associated clinical events. It is present in unstable lesions and its plasma level and activity are increased in patients with coronary artery disease (CAD). The molecular mechanism, however, by which circulating FSAP influences the progression of CAD is not yet entirely understood. The present study was performed to examine the relation between FSAP and the pro-inflammatory activation of macrophages.

Methods: The influence of FSAP on the activation of transcription factors of the NFκappaB family and cAMP response element-binding protein (CREB) was assessed by electrophoretic mobility shift assays. Degradation and phosphorylation of the inhibitor protein IκBα, ICAM-1, IL-6, and TF mRNA were analysed.

Results: FSAP treatment (20 µg/ml) induces IkappaB-dependent NF-kappaB activation in freshly isolated human monocytes in a time-dependent fashion. It induces the phosphorylation and proteolytic degradation of the inhibitor protein IkappaB α . The phosphorylation of p65 was induced by FSAP, which is known to contribute to the enhancement of DNA-binding activity of NF-kappaB. In parallel, FSAP induced the expression of ICAM, IL-6, and TF, genes known to be under the control of NF-kappaB. Consistent with this, aprotinin, a pharmacological inhibitor of FSAP, blocks FSAP-induced gene expression. In contrast, CREB phosphorylation and activation was not detected in FSAP-treated monocytes.

Conclusions: Biological functions of FSAP in macrophages extend beyond its role in promoting thrombosis/fibrinolysis. Thus, FSAP may play an important role in atherosclerosis by enhancing the inflammatory response of human macrophages as a novel activator of NF-kappaB.

PP5.2-4

Vascular (coronary/cerebral) events following retinal vein occlusion: A 8-yr follow up

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We have followed-up for 8 yrs (mean, 7.9 \pm 2.7 yrs) 117 consecutive patients (61 M, 56 F; mean age 54 \pm 13; age at the event 51 \pm 13) referred to our Centre for fluorangiographically documented retinal vein occlusion (RVO; 62 central; 48 branch, BRVO; 7 both types in different eyes). As many as 202 matched apparently healthy individuals (105 M, 97 F; mean age 52 \pm 12) from the same geographic area served as controls. At the time of the RVO, hypertension (64.6% vs. 28.4%; OR 4.6 CI 2.8–7.5; $p < 0.0001$) and diabetes mellitus (17.8% vs. 8.1%; OR 2.5 CI 1.1–5.5; $p < 0.05$) had a higher prevalence in patients than in controls. No other cardiovascular risk factor nor inherited/acquired thrombophilic abnormalities significantly differed in controls and patients. In contrast, BRVO differed from CRVO as to age and prevalence of diabetes mellitus, overweight and hypertension (55 vs. 47 yrs; $p < 0.0022$; 9.7% vs. 8.7%; 83.9% vs. 57.8%; 78.7% vs. 55.9% respectively; p always < 0.05). Eight yrs after the RVO, 90/117 patients (77%) gave their consent to undergo a follow-up visit. Fifty-eight of them (64%) had experienced new vascular events: coronary/cerebral non-fatal ischemic events ($n=38$); retinal events ($n=10$), venous thrombosis ($n=10$). In 22/90 patients (24%) in whom antiplatelet agents were employed throughout the follow-up, a lower prevalence of overall vascular recurrence (45.4%[10/22] vs. 70.6%[48/68], $p=0.06$) was found. These data are consistent with RVO as being an atherosclerosis-related event, and support the possibility that the correction of hypertension and/or diabetes mellitus is a major direction to be pursued to prevent RVO.

PP5.2-5

Cholesterol-induced apoptosis is associated with downregulation of the activated leukocyte cell adhesion molecule (ALCAM) in human monocytic cells

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Migration of monocytes plays an important role in development and progression of atherosclerosis. The activated leukocyte cell adhesion molecule (ALCAM/CD166) is important for cell migration and leukocyte invasion. The present study investigates the impact of cholesterol-enrichment on the expression of ALCAM in the human monocytic cell line U937. Monocytic U937 cells were enriched with cholesterol by incubation with methyl-beta-cyclodextrin (M β CD)-cholesterol-complex. Expression of adhesion molecules was determined by flow cytometry; apoptosis by Annexin-V FITC/PI (propidium iodide) double-label cytometry. Migration of calcein-AM-loaded U937 cells was quantified in 3µm-chemotaxis chambers by fluorescence of transmigrated cells. Incubation of monocytes cells with cholesterol (10 - 100 µg/mL) for 18 h induced a concentration-dependent increase in early and late phase apoptosis, while M β CD alone had no effect. Increased apoptosis rate was associated with a reduction of ALCAM expression by $>50\%$. In contrast, expression of VCAM-1 (vascular cell adhesion molecule-1) was strongly increased and ICAM-1 (intercellular cell adhesion molecule-1) levels were not affected by cholesterol loading. Pretreatment with the nonselective caspase/apoptosis inhibitor Q-VD-OPh (100µmol/L) partially prevented cholesterol-induced alteration of adhesion molecule expression. Cholesterol loading was also associated with an 80% reduction of cell migration towards 10% serum. Q-VD-OPh partially rescued migration capacity in cholesterol-rich monocytes. This effect was prevented by addition of ALCAM-neutralizing antibodies (10 µg/mL), but not an isotype-matched IgG. Cholesterol-induced apoptosis in monocytic cells is accompanied by reduced expression of ALCAM. Loss of ALCAM under conditions of cholesterol-loading and apoptosis attenuates monocyte migration, a mechanism which may expedite vascular lesion formation.

PP5.3 Cellular Activation and Signaling

PP5.3-1

Inhibition of platelet alpha-granule release strongly attenuates granulocyte adhesion and activation on decellularized porcine heart valve tissue

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Objectives: This in-vitro study was designed to investigate the impact of platelet adhesion on attraction and activation of polymorphonuclear leukocytes (PMN) by decellularized xenogeneic tissue.

Methods: Cryostat sections of decellularized porcine heart valves were sequentially incubated with platelet-rich plasma (PRP) and isolated, autologous PMN. In further experiments, platelets were pre-incubated with either Cytochalasin D (CytD) or iso-butyl-methyl-xanthine (IBMX) to block platelet alpha-granule release. To investigate the involvement of the complement system, specimens were exposed to PRP that had been pre-incubated with 10 mM EDTA. At the end of the incubations, sections were washed and fluorescently stained for CD 41, thrombospondin-1 (TSP-1), CD45, CD11b, and the complement factor iC3b.

Results: Laser scanning microscopic examination revealed the binding of multiple activated platelets to the decellularized porcine tissue. Platelet adhesion was associated with upregulated expression and secretion of TSP-1. Pre-treatment of tissue specimens with PRP induced a strongly enhanced binding and activation of subsequently added PMN. Blockade of platelet alpha-granule release by either CytD or IBMX markedly reduced the deposition of TSP-1 and significantly decreased the adhesion of PMN. Although inhibition of complement activation by addition of EDTA to the PRP preparations inhibited iC3b deposition it failed to prevent PMN binding.

Conclusion: Platelet adhesion to acellular porcine heart valve tissue induces TSP-1 secretion with subsequent PMN binding and expression of the activation marker CD11b. The interaction of platelets with the decellularized matrix seems therefore to play a key-role in the early non-specific inflammatory response toward acellular xenogeneic implants independent from complement activation.

PP5.3-2

The extracellular adherence protein (Eap) of staphylococcus aureus activates platelets via the ATP-gated P2X1 cation channel and Fcgamma RIIA

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Objectives: The extracellular adherence protein (Eap), secreted by the major pathogen *Staphylococcus aureus*, is known to influence human immunity. The known direct interaction with fibrinogen and some integrins prompted us to study the effect of Eap on platelets.

Methods: Platelet activation was studied by aggregometry and flow cytometry.

Results: Eap (1–10 µg/ml) activated human platelets in a dose, time, and temperature dependent manner. It induced fibrinogen and surprisingly von Willebrand factor binding as well as platelet aggregation. Activated platelets bound thrombospondin-1 as well as the coagulation factors VIIa, VIII and XIII and annexin V. Eap induced the secretion of the granules and the formation of platelet-leukocyte associates. Eap treatment lowered the number of free SH-groups on the platelet surface significantly and Eap action on platelets was blocked by SH-reactive DTNB, PAO, pCMBS, gliotoxin and the protein disulfate isomerase inhibitor bacitracin. Fab fragments of the Fcgamma RIIa inhibitory antibody IV.3 (2µg/ml) blocked Eap action on platelets and the known inhibitor of Fcgamma RIIa signal transduction, the tyrosine kinase inhibitor Piceatannol, inhibited as well. NF449, which selectively blocks P2X1 receptors, blocked platelet activation by Eap.

Conclusions: The extracellular adherence protein (Eap) of *Staphylococcus aureus* activates platelets via the ATP gated cation channel P2X1 and Fcgamma RIIA (CD32). P2X1 cation channel and Fcgamma RIIa are sensitive to SH-Blockers. This might explain why Eap induced cell activation was blocked by thiol reactive tools. Whether the found signalling way for Eap is general for other blood and vascular cells has to be verified.



PP5.3-3

CD11B-positive microparticles are an independent predictor of cardiovascular events in patients with coronary artery disease

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Many cells of the vascular compartment play a key role in the pathogenesis of coronary artery disease (CAD) and its complications. Accurate determination of the activation status of these various cell types might thus be helpful to identify coronary patients at high risk for future cardiovascular events (CVE). Microparticles (MP) are membrane fragments known to be reliable markers of cell apoptosis and activation.

Objectives: To determine if MP levels from different cell types were associated with the severity of atherosclerotic lesions and the occurrence of future CVE in patients with CAD.

Design and Methods: Baseline plasma MP levels were measured by flow cytometry in 172 patients undergoing coronary angiography at the Timone Hospital (Marseille, France). 77 patients underwent stent implantation and were followed-up for 1 month.

Results: 12 CVE were recorded during the follow-up period. In the entire cohort of patients, levels of leukocyte-derived MP (CD11b+MP) were found strongly but negatively related to the number of occluded coronary arteries ($P = 0.005$) and were significantly higher in patients under statin therapy ($P = 0.001$). In the followed-up population, CD11b+MP were lower in patients with CVE ($P = 0.0001$). This difference remained significant after adjustment on the number of occluded arteries and on the statin therapy status ($P = 0.004$). Interestingly, monocyte-derived MP (CD14+MP) were more elevated in patients with future CVE ($P = 0.0005$).

Conclusions: Leukocyte-derived MP provide a useful tool in identifying patients at high risk of recurrence independently of the severity of atherosclerotic lesions.

PP5.3-4

Phospholipids in misguided thrombus resolution after splenectomy

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Purpose: Splenectomy is associated with an increased risk of chronic thromboembolic pulmonary hypertension (CTEPH). CTEPH is a life-threatening condition characterized by single or recurrent pulmonary thromboemboli that obstruct or obliterate the pulmonary vascular bed. The aim of our study was to investigate the role of phospholipids in the pathogenesis of altered thrombus resolution after splenectomy.

Methods: We utilized a mouse model of stagnant flow venous thrombosis to characterize venous thrombus resolution. Vena cava ligation was performed one month after splenectomy. At days 3, 7, 14 and 28 after vena cava ligation thrombi were harvested for histology and electrospray ionization - mass spectrometry analysis. Blood samples were collected for FACS.

Results: Thrombus areas of splenectomized mice were significantly larger than those of controls at all time points (ANOVA, $n=8$, $p<0.03$). The composition of phospholipids enclosed in the thrombus was significantly different between thrombi of splenectomized mice and thrombi of control mice. In parallel, whole blood FACS revealed higher counts of CD41-platelet microparticles (day 14: 3216 versus 927 cells/ μ l, $p<0.05$) and leukocyte/platelet aggregates (day 14: CD11b/CD41, 56.4 versus 38.7%, $p<0.05$).

Conclusion: We suggest that an altered phospholipid profile in thrombus may derive from platelets and leukocytes after splenectomy. The loss of mechanical filtering function of the spleen permitting the accumulation of phospholipids in the peripheral circulation is a key mechanism of thrombus persistence.

PP5.3-5

Measurement of procoagulant potential of blood microparticles carrying Tissue Factor

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Blood microparticles (MPs) have high diagnostic and prognostic interest, not only for circulatory diseases, but also for inflammatory, malignant or infectious pathologies. These MPs objectivate blood cell activation, but they also contribute to the disease course worsening through their procoagulant effect. A special focus concerns MPs which expose Tissue Factor (TF), involved in thrombotic diseases. From the studies of JM Freyssinet's group, we adapted a new method which specifically measures the procoagulant activity of blood MPs carrying Tissue Factor (MP-TF). Plasma is obtained with the specific cautions required for avoiding ex-vivo cell

(mainly platelets) activation, using a double centrifugation at Room Temperature. This plasma is then diluted and introduced into a microwell coated with a specific Anti-TF MoAb. Microparticles exposing TF are captured, and their activity is measured through Factor Xa generation. The revelation mixture contains Factor VII or (better) VIIa in a constant and in excess concentration, Factor X and calcium. There is a direct dose response relationship between MP-TF concentration and Factor Xa generated. This Factor Xa is then measured by its activity on a specific chromogenic substrate and colour development is measured at 405 nm. The assay is calibrated with recombinant TF, at a known concentration, which is relipidated at a well defined Phospholipids concentration (12.5 % PS) using liposomes, and spiked into normal plasma. The assay has a dynamic range from 20 pg/ml to 1,000 pg/ml TF, and is performed within less than 2 hours. Tested plasma can be used diluted 1:2 or at higher dilutions according to the expected MP-TF concentration in the assayed specimen. This assay introduces a new analytical tool for measuring an emerging marker of blood activation in circulatory or malignant diseases. The diagnostic and prognostic value of this marker is amplified by the nature of MP-TF, which are an indicator, but also a trigger for thrombotic pathologies. It offers promising perspectives for the early diagnosis of thrombotic or malignant diseases.

PP5.4 Endothelial Cells, Extracellular Matrix and Angiogenesis

PP5.4-1

Regulation of mouse embryonic stem cell commitment into the endothelial lineage by p38 mitogen-activated protein kinase

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Embryonic stem (ES) cells are able to give rise, in vivo, to all of the three germ layers and, in vitro, to differentiate into a broad variety of cell lineages which give us large perspectives in regenerative medicine. This process is strictly controlled by the potent morphogen retinoic acid (RA), when added from day 3 to day 5, it induces ES cell differentiation into neurons, and, conversely, inhibits cardiomyogenesis, skeletal myogenesis and endothelial cell lineage. We found that p38MAPK activity peaked spontaneously between day 3 and day 5, during mouse ES cell differentiation and that RA completely inhibited this peak of activity. At the opposite of wild type cells, p38 α -/- ES cells differentiated spontaneously with or without RA treatment into neurons, and did not form cardiomyocytes, myocytes or endothelial cells. Similar results were obtained by treating wt ES cells with a p38MAPK-specific chemical inhibitor (PD169316). By genetic and biochemical approaches, we demonstrate that the control of p38MAPK activity constitutes an early switch, committing ES cell into either neurogenesis (p38 off) or several mesodermal lineages (p38 on). Furthermore, our results suggest that Flk-1+ cells could constitute the common mesodermal progenitors targeted by p38MAPK in this process. Finally, after stable transfection of an expression vector, we were able to express an activated form of p38 α in wtES cells as well as in p38 α -/- ES. Analysis of the differentiation capacities of these cells will be presented.

PP5.4-2

Endothelial progenitor cells, insulin resistance, inflammation, and their associations in morbidly obese patients undergoing massive weight loss induced by bariatric surgery

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Objectives: Morbid Obesity (MO) is associated with high cardiovascular disease (CVD) morbidity and mortality. Traditional risk factors only partly explain why life is diminished by 12 years. Since endothelial progenitor cells (EPC) predict CVD events and death, low EPC in MO patients may contribute to the increased mortality. Thus, we investigated EPC in MO patients before and after weight loss.

Design and Methods: 109 patients with MO (BMI:46 \pm 7kg/m²) were compared with 64 controls (CO) (BMI:23 \pm 2kg/m²) and 95 patients with massive weight loss 1.6 \pm 0.4 years after bariatric surgery (BMI:33 \pm 6kg/m²). Circulating progenitor cells (CPC, CD34+/133+), EPC (CD34+/133+/309+) and activated EPC (actEPC, CD34+/133+/309+/31+) were enumerated by FACS. EPC/CPC ratio, actEPC/EPC ratio and concentrations of fasting MCP-1, IL-18, hsCRP were determined.

Results: CPC, EPC and actEPC were drastically reduced in MO patients compared to CO. CPC, EPC and actEPC were significantly higher after a mean weight loss of 39 \pm 19 kg, but did not reach the numbers of CO. Reduction of EPC and actEPC were significantly associated with BMI, insulin resistance (fasting-insulin/glucose, 2-hour-insulin/glucose, HOMA-IR), and markers of inflammation (hsCRP, MCP-1, IL-18).

Conclusions: The novel finding of this study is a significant impairment of EPC in MO patients and that high levels of adipocyte-derived IL-18 suppress activation of

EPC. Marked weight loss by bariatric surgery resulted in an increase of CPC, EPC, actEPC and respective ratios. It is tempting to speculate that the reduced CVD mortality of MO patients after weight loss could partly be explained by increase of EPC/act-EPC, both assumed to have a critical role in vascular repair mechanism.

PP5.4-3

The gut microbiota triggers Tissue Factor-dependent angiogenesis

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Objectives: Germ-free (GF) mice exhibit arrested formation of capillaries in the small intestinal villi compared with ex-GF mice that were colonized with normal gut microbes (CONV-D) or conventional-raised (CONV-R) animals. The mechanisms underlying these phenotypic differences are unknown. In the present study we identify angiogenic factors responsible for microbially-induced capillary network formation and assess whether microvascular angiogenesis in the gut is dependent on the signaling function of Tissue Factor (TF).

Design and Methods: To elucidate the potential role of TF, ex-GF mice were injected intraperitoneally with an anti-TF antibody or an isotype control antibody prior to a 14 day colonization with a cecal microbiota harvested from CONV-R mice that stimulates capillary network formation. The vascular marker CD31 (PECAM-1) was used to quantify villus vascularization by immunohistochemistry and qRT-PCR. To uncover proangiogenic genes upregulated in response to microbial colonization qRT-PCR-analyses were performed on small intestinal tissues collected from GF, CONV-R and CONV-D mice that were treated with either anti-TF or control antibody.

Results: We found that microbial colonization of the intestine leads to formation of an intricate vascular network. This process was efficiently blocked by administration of anti-TF antibody prior to microbial colonization of the animals. A distinct set of proangiogenic genes was identified to be upregulated in a TF-dependent manner following microbial colonization.

Conclusion: Our results suggest that the signaling function of TF is crucial for microbiota-induced formation of capillary networks in small intestinal villi. This mechanism is potentially relevant in inflammatory bowel disease (IBD), where mucosal neovascularization occurs.

PP5.4-4

Acute pro-coagulatory response of the endothelium to melanoma cells

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Objective: Tumor cell spreading is accompanied by an intrinsic pro-coagulatory activity and by inflammatory conditions. Endothelial von Willebrand factor (VWF) is emerging as a key protein in both these processes.

Methods: Supernatants (SN) obtained from invasive melanoma cell lines MV3 or WM9 were tested for human umbilical vein endothelial cells (EC) activation either by VWF release using ELISA and perfusion experiments or NFkappaB activation analysed by EMSA. Expression of NFkappaB-dependent genes was assayed by PCR and protein content by ELISA or FACS. Melanoma-mediated thrombin generation and activity was measured using chromogenic substrate S-2238.

Results: MV3 cells induce an instantaneous, massive release and immobilization of ultra-large VWF (ULVWF) at the luminal endothelial membrane via EC PAR-1 activation initiating tumor cell adhesion. WM9 cells, in contrast, activate the NFkappaB pathway in EC followed by an up-regulation of IL-6 and TF expression. Moreover, both melanoma cell lines express TF which contributes to generation of thrombin in blood plasma.

Conclusion: Melanoma cells can directly (via secreted mmPs) or indirectly (via TF-mediated thrombin generation) activate PAR-1 on EC leading to an acute luminal ULVWF release. Binding of melanoma cells to ULVWF supports adhesion and facilitates reciprocal communication such as activation of the NFkappaB pathway. Thus, ULVWF release and stabilisation may be enhanced by up-regulation of TF or production of IL-6 which diminishes the activity of VWF-degrading protease ADAMTS13. We hypothesize that the switch from an anti- to a pro-inflammatory and pro-coagulatory surface of EC plays a pivotal role in melanoma cell extravasation and spreading.

PP5.4-5

Regulation of endothelial protein C receptor and thrombomodulin shedding in human endothelial cells

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Objectives: Endothelial protein C receptor (EPCR) and thrombomodulin (TM) play pivotal roles in coagulation, cell proliferation, and inflammation. However,

their activities are changed by ectodomain cleavage and release as soluble forms (sEPCR and sTM). In the current study we analysed the mechanisms involved in the regulation of EPCR and TM shedding.

Methods: The activations of ERK-1/2, p38 MAPK, and c-Jun N-terminal kinase (JNK) were studied by cell-based ELISA and the levels of sEPCR and sTM by specific ELISA kits.

Results: IL-1 β and TNF- α , but not IFN- γ or IL-6 induced a rapid sEPCR release in human umbilical vein endothelial cells (HUVEC) correlating with the activation of p38 MAPK and JNK. The cleavage of EPCR was also up-regulated by thrombin, anisomycin, a JNK agonist, PMA, calcium-ionophore, thiol alkylators and oxidants, as well as by lipid raft disruptors. Both basal and induced shedding was prevented by metalloproteinase inhibitor, TAPI-0, and by radical scavenger, N-acetylcysteine (NAC). However, the intracellular generation of radical oxidant species did not correlate with the EPCR release and antioxidants failed to block the EPCR shedding. Further results suggest that a direct targeting of thiol groups involved in the control of metalloproteinase activities is responsible for the observed effects of NAC on EPCR shedding. Similar to EPCR the release of TM in HUVECs is regulated by TAPI-0 sensitive mechanisms, but NAC profoundly increased the cleavage and release of sTM.

Conclusions: The shedding of EPCR and TM is strongly regulated by different cell signaling pathways and thiol-oxidizing or reducing agents.

PP5.4-6

Detecting heparanase inhibitors with a novel heparanase activity assay fit for routine

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Human heparanase overexpression promotes tumour metastasis, angiogenesis and is associated with poor prognosis. Therefore, heparanase inhibitors (HI) are considered as promising candidates for tumour therapy. So far, no simple, rapid assay is available for testing potential HI. The aim of this study was to develop an assay based on the finding that fondaparinux represents a substrate for heparanase and on the idea to quantify the degradation of fondaparinux by means of its anti-factor Xa (aXa)-activity. Structurally defined semi-synthetic glucan sulfates (GS) were examined for their heparanase inhibitory activity. The assay was established as a two step micro-plate-assay. First, heparanase is incubated with the substrate fondaparinux and different concentrations of potential HI. After stopping the reaction by freezing, the remaining substrate is quantified by its aXa-activity in a chromogenic substrate assay (bovine FXa, antithrombin, S2222). Initially, the following assay parameters were evaluated and optimized: incubation time, concentrations of substrate and heparanase, heparanase stability and inactivation method, as well as aXa-assay conditions. The GS structure-dependently inhibited heparanase with IC50 values ranging between 0.5ng/mL and 5000ng/mL. Their activity increased with increasing degree of sulfation (DS) and decreasing molecular weight (MW). Beta-1,3-GS were about 10 times more active than alpha-1,4/1,6-GS with similar DS and MW. In conclusion, a novel chromogenic heparanase activity micro-plate-assay suitable for high-throughput screening has been developed. By studies on structure-activity relationships using this assay, beta-1,3-GS were identified as potent HI, which may contribute to their proven antimetastatic activity.

PP5.4-7

Aortic valve stenosis progression parallels changes in extracellular matrix gene expression and angiogenesis

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Background: Aortic valve disease is the most frequent native valve disease in Europe, and the third most frequent cause of cardiovascular death. The biological mechanisms underlying aortic valve degeneration, ultimately resulting in calcific aortic stenosis show similarities to atherosclerosis. We investigated extracellular matrix (ECM) molecules and angiogenesis as important components of atherosclerosis in aortic stenosis.

Methods: Aortic leaflets from valve replacement surgeries and heart transplants were collected in the operating room after a careful echocardiographic validation of mean valve gradients (mvg) within 7 days of surgery. RNA expression profiles of healthy (control), as well as sclerotic (mvg=6 \pm 2mmHg, mean \pm SD), mildly (mvg=22 \pm 12mmHg), moderately (mvg=49 \pm 16mmHg) and severely stenotic (mvg=63 \pm 15mmHg) aortic valve leaflets (n=5 per group) were analyzed using an Affymetrix Human Gene 1.0 ST Array. Genes with an absolute fold change of \pm 2.0 in at least 4 samples per group were selected for further analysis.

Results: Collagen types I, III, V, XIV, XV and XXI as well as cathepsin S, B, D and K and perlecan showed a valve gradient-dependent increase of expression. Expressions of collagens type Ia1, Ia2, IIIa1 and Va1 and cathepsins and D



showed a significant correlation with stenosis progression (14.5 (9–17) m/sec/year, median(range), $p < 0.05$). The antiangiogenic ECM molecule chondromodulin was at a low level across the spectrum of mvgs, while VEGF decreased in parallel to increasing mvg.

Conclusion: The data demonstrate an increase in ECM and suppression of angiogenesis in direct correlation with mvg and aortic stenosis progression.

PP5.4-8

Correlation of different circulating endothelial progenitor cells to stages of diabetic retinopathy – first in vivo data

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Objectives: To investigate vasculogenic circulating progenitor cells (CPC), endothelial progenitor cells (EPC), and activated EPC in patients with type 1 diabetes mellitus (T1DM) with or without diabetic retinopathy (DR).

Design and Methods: A case-control study comparing 90 patients with T1DM with and without DR was performed. Patients were studied and staged for retinopathy according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) classification. 90 patients were included: 30 without DR (CO), 30 with mild non-proliferative DR (mNPDR), 10 with moderate-severe NPDR (msNPDR), 10 with mild-moderate proliferative diabetic retinopathy (mmPDR) and 10 with high-risk PDR (hrPDR). CPC (CD34/CD133), EPC (CD34/CD133/CD309) and mature actEPC (CD34/CD133/CD309/CD31) were enumerated by flow cytometry.

Results: EPC were reduced in mNPDR (114 ± 66 ; $p < 0.001$) and msNPDR (77 ± 40 ; $p = 0.042$) vs CO (244 ± 115). In contrast, EPC were unchanged in mmPDR (248 ± 155) vs CO. Strikingly, EPC were augmented in hrPDR (389 ± 124) vs all other stages. Numbers of undifferentiated progenitor cells (CPC) did not differ between CO, mmPDR and hrPDR. A three times augmentation of actmature EPC in hrPDR (325 ± 118 ; $p < 0.001$) vs CO (100 ± 49), but also against all other stages of DR was observed. The percentage of actmature EPC/EPC was augmented in an ETDRS classification dependent way.

Conclusions: In type 1 diabetic patients with diabetic retinopathy, circulating progenitor cells (CPC) show a stage-related regulation. In non-proliferative retinopathy a reduction of CPC, in proliferative retinopathy an dramatic increase of mature reactivated endothelial progenitor cells was observed.

PP5.5 Inflammation, Cytokines and Auto-Immunity

PP5.5-1

A role for Flk-1 and PECAM-1 in monocyte recruitment into resolving thrombi

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Background: Angiogenesis and leukocyte recruitment are key components of thrombus resolution. Platelet endothelial cell adhesion molecule-1 (PECAM-1 or CD31) plays diverse roles in vascular biology including monocyte transmigration, angiogenesis, platelet function, and thrombosis. Thus, PECAM-1 represents an important link between leukocyte migration and angiogenesis. In the present study we investigated the effect of an endothelial cell-specific deletion of VEGF-R2/flk-1, an important regulator of angiogenesis, and deletion of PECAM-1 in a murine model of stagnant flow venous thrombosis.

Methods: Thrombosis was induced in the infrarenal vena cava of Tie2/Cre Flk-1 flox/flox mice on a C57/BL6 background and PECAM-1^{-/-} on a FVB/n background by creating a venous stenosis with a silk suture. Thrombi were harvested on days 3, 7, 10, 14 and 28 after surgery (n=8 per time point). Non-transgenic siblings served as controls. Trichrome and immunohistochemical staining were performed.

Results: Thrombus cross-sectional area analysis over time demonstrated a significant increase in thrombus area by day 7 after surgery in flk-1^{-/-} and by day 3 in PECAM-1^{-/-} animals compared with controls. Immunohistochemical staining using an antibody against antigen F4/80 for detecting macrophages in the thrombi revealed a decreased number of monocytes in flk-1^{-/-} animals on day 7 and at all time points in PECAM-1^{-/-} animals.

Conclusion: Cell-specific deletion of VEGF-R2/flk-1 and deletion of PECAM-1 lead decreased monocyte numbers in thrombi and result in misguided thrombus resolution. The data demonstrate that both endothelial Flk-1 and PECAM-1 might play a role in monocyte recruitment into resolving thrombi.

PP5.5-2

ACE inhibition reduces monocyte MFG-E8 and MCP-1 expression associated with increased serum fractalkine levels in rats with chronic heart failure

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Introduction: Chronic heart failure (CHF) still remains a cause of high morbidity and mortality. Immune activation and inflammation influence CHF pathogenesis. The pro-inflammatory chemokine fractalkine induces monocyte activation. Fractalkine promotes mfg-e8 expression on macrophages. We investigated whether (a) Fractalkine serum levels are significantly enhanced in CHF, (b) high fractalkine levels influence mfg-e8 and MCP-1 expression on peripheral blood monocytes, and (c) these proinflammatory markers can be positively modulated by a standard CHF treatment (ramipril: 1mg/kg/d).

Methods: In rats with CHF following chronic coronary ligation for 10 weeks, fractalkine levels were determined in serum and urine using ELISA. Peripheral blood monocytes were isolated via Histopaque gradient. Mfg-e8 and MCP-1 protein expression on these monocytes were assessed by Western Blotting and expressed as mean±S.E.M. Analysis was done via unpaired Student's t-test. P values<0.05 were considered statistically significant.

Results: Fractalkine serum and urine levels were significantly higher in CHF compared to sham-operated rats (Serum: CHF: 1509 ± 168 pg/mL; Sham: 1181 ± 58 pg/mL, $p < 0.05$). Mfg-e8 and MCP-1 expression on peripheral blood monocytes were significantly enhanced in CHF animals (mfg-e8: CHF: 1.36 ± 0.36 arbitrary units (au); Sham: 0.79 ± 0.08 au; MCP-1: CHF: 1.79 ± 0.29 au; Sham: 1.01 ± 0.17 au, $p < 0.05$) and attenuated by ramipril therapy.

Conclusion: Fractalkine serum levels and monocyte expression of its downstream target mfg-e8 were significantly increased in experimental CHF as was the burden of MCP-1. Ramipril reversed the pro-inflammatory changes in CHF monocytes, indicating that ACE inhibition might beneficially modulate chemotactic/atherogenic signalling in CHF.

PP5.5-3

Bivalirudin reduces platelet and monocyte activation after elective percutaneous coronary intervention

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Concomitant antithrombotic therapy is essential in prevention of ischemic events in percutaneous coronary intervention (PCI) and stenting. With new anticoagulant medications being developed and applied in PCI, this raises the question of possible interactions with platelet and leukocyte activation. We, therefore, sought to investigate the influence of bivalirudin and heparin in platelet and leukocyte activation in patients undergoing elective PCI.

Methods and Results: 46 Patients were recruited consecutively in the setting of the Intracoronary Stenting and Antithrombotic Regimen-Rapid Early Action for Coronary Treatment (ISAR-REACT)-3 trial and randomly assigned to receive either unfractionated heparin or bivalirudin during elective PCI. Surface expression of CD62P (P-Selectin), CD42b (GPIb), CD40L, PAC-1 on circulating platelets and CD11b, CD14 and CD15 on circulating leukocytes were evaluated by flow cytometry. Cytokine levels of IL-12p70, Tumor necrosis factor (TNF), IL-8, IL-6, IL-1 and IL-10 were determined by cytometric bead array. Platelet surface expression of PAC-1, P-Selectin and GPIb was significantly reduced after PCI in patients receiving bivalirudin as compared to heparin. Similarly CD11b expression on CD14+ monocytes was diminished after bivalirudin. Yet, no differences in cytokine levels between the bivalirudin and the heparin group before or after PCI were observed.

Conclusion: Our data suggest that bivalirudin may reduce platelet and monocyte activation in patients undergoing elective PCI. Thereby, bivalirudin might reduce periinterventional thrombotic complications.

PP5.5-4

Effect of Drotregocin alpha (activated) of sRAGE levels during the time course of patients with severe sepsis

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Background: The receptor for advanced glycation end products (RAGE) is a member of the Ig superfamily and a multiligand receptor interacting with a diverse class of ligands. RAGE has secretory isoforms referred to as soluble RAGE (sRAGE), which comprise the extracellular ligand-binding domain but are lacking the cytosolic and transmembrane domains. In humans, endogenous sRAGE is produced by alternative splicing of RAGE mRNA. We measured levels of sRAGE in septic patients treated with and without Drotregocin alpha (activated) in septic patients.



Patients and Methods: Blood samples were obtained from septic patients (n=8; control-group) and septic patients under treatment with Drotrecogin alpha (activated) (n=8) on days 1, 3 and 5 of severe sepsis. sRAGE levels were measured by ELISA-methods. Unpaired and paired Student's t-test and Spearman correlation were used to compare results.

Results: sRAGE levels decrease from day 1 (baseline) (Mean= 1,61 ng/ml +/- SEM=0,50) to day 5 (Mean = 0,29 ng/ml +/- SEM=0,09) in septic patients treated with Drotrecogin alpha (activated) and were significantly lower on day 5 as compared to day 1 (p<0,05). In septic patients not treated with Drotrecogin alpha (activated) (control-group) there was no significantly decrease of sRAGE levels from day 1 (Mean=0,38 ng/ml +/- SEM=0,01) up to day 5 of sepsis (Mean=0,26 ng/ml +/- SEM=0,05).

Conclusion: We can show that Drotrecogin alpha (activated) significantly decreases sRAGE serum levels during the time course of septic patients. This observation strongly suggests that Drotrecogin alpha (activated) plays an important role in regulating the cellular damage during sepsis.

PP5.5-5

Inflammation and endothelial dysfunction in children with type 1 diabetes mellitus

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Objectives: Despite dramatic improvements in diabetes management, the macrovascular disease (MVD) morbidity and mortality in type 1 diabetes mellitus (T1DM) stayed elevated resulting in a loss of life of up to 20 years. Since the mechanisms are not completely understood, we investigated inflammation and endothelial dysfunction in children with T1DM in comparison to healthy controls (CO).

Design and Methods: We included 175 children, 140 T1DM, 35 CO. As a general marker of systemic vascular inflammation, we used C-reactive protein (CRP), as early marker of endothelial dysfunction, endothelial progenitor cells (EPC). EPC circulate in the peripheral blood, are involved in neovascularisation, endothelial dysfunction and predict MVD morbidity and mortality. EPC (CD34+/CD133+/CD309+) were enumerated by direct immunostaining technique and recorded on a flow cytometer.

Results: Patients (T1DM) and controls (CO) were matched for age and gender. EPC were significantly impaired in children with T1DM vs. CO (609±359vs.1137±504,p<0.001). Other differences were found in systolic blood pressure (115±15vs.101±8 mmHg,p<0.001), LDL-cholesterol (90±28vs.83±15mg/dl,p=0.03), glucose (154±83vs.90±8mg/dl,p<0.001), CRP (112±14vs.101±8mg/dl,p<0.001), each T1DMvs.CO. Multivariate regression revealed that HbA1c was the strongest predictor of EPC in our cohort (beta=-0.355,p<0.001). Remarkably, EPC and CPR were not associated with one another.

Conclusions: In conclusion, this is the first study investigating both inflammation and endothelial dysfunction (by EPC measurements) in children with T1DM: EPC are significantly reduced and CRP levels are significantly elevated. The missing association of CRP and EPC in our study cohort, suggests a from inflammation independent demetrial effect on EPC in children with T1DM.