Abstracts

14th Conference of the European Society for Clinical Hemorheology and Microcirculation

27 – 30 June 2007

Dresden, Germany

Contrast induced Nephropathy using Sodium Bicarbonate or Sodium Chloride and Isosmolar Iodixanol

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Background: For prevention of contrast induced nephropathy (CIN) after coronary angiography, hydration with sodium chloride solution is recommended. A recent study showed a benefit of sodium bicarbonate hydration versus sodium chloride when using the non-ionic monomeric low-osmolar contrast medium iopamidol (Merten GJ. et al, JAMA 2004). The aim of this study was to determine the effects of sodium bicarbonate versus sodium chloride on CIN rates in a cohort with chronical nephropathy using the non-ionic dimeric isosmolar contrast medium iodixanol.
Methods: The study was a prospective, randomized, single-center, double-blind trial including 135 patients (age: 71.4+7,6 yrs, 30f/105m) with elevated baseline serum creatinine levels (SCr) (mean SCr 138.06+38.9 µmol/l). Eligible patients were randomized to receive either a 154-mEq/l infusion of sodium bicarbonate (n=66, group A) or one of sodium chloride (n=69, group B) as a bolus of 2 ml/kg per hour for 2 hour before and as an infusion of 1 ml/kg per hour for 6 hours between and after the angiography with administration of iodixanol. The primary end point of this study was a SCr increase of 25% or 44 µmol/l on the first or second day following diagnostic contrast medium application. SCr, serum cystatine C (CysC), plasma viscosity (PV) and urinary enzymes (alaninaminopeptidase (AAP), N-acetyl-ß-D-glucosaminidase (NAG) and α1-microglobuline (α1M)as indicators of early tubular impairment were measured at baseline and day 1 and 2 after contrast medium administration.
Results: The total CIN rate was 4.4 % (n=5) and similar in both groups (group A: 4,5%, n=3, group B: 2.9%, n=2). All patients with CIM showed a decline of their SCr values 10 to 14 days after angiography, no patient required any additional therapy. The first day after application of iodixanol there was a significant increased of SCr, Cyst C, AAP, NAG, α1M and PV in both groups. On the second day after contrast medium application Cyst C, AAP, NAG and PV were still significantly increased compared with the initial values. Comparing the treatment groups we observed a significant higher increase of AAP, NAG and α1M in the sodium bicarbonate group at the first day after application of contrast medium.
Conclusion: There is a low incidence of CIN after administration of contrast medium iodixanol in patients with chronic nephropathy if combined with a sufficient sodium chloride hydration. The control measurements 10 to 14 days after the diagnostic procedure showed prediagnostic values in all CIN patients. No additional benefit by using sodium bicarbonate was found in this study.

Effects of heavy-resistance training on platelet indices and fibrinogen concentration

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Previous studies demonstrated that strength training reduces the risks for coronary heart disease (Goldberg, 1989). Most of the resistance training studies have focused on the effects of resistance training on hormones, muscular strength, and neuromuscular system and no information is available regarding the chronic effects of this exercise modality on platelet indices. Therefore, the present investigation was designed to examine the effects of 8 weeks of resistance training on these blood parameters.

Fifteen collegiate untrained healthy male subjects were randomly divided in control (N=6; age, 23±1 y) and experiment (N=9; age, 22±1.5 y) groups. Training group performed a resistance-training programme three days a week for 8 weeks, while the control group participated in no resistance training. The resistance exercise protocol included the performance of 3 sets of 8-10 repetitions of six exercises. Since the subjects were untrained the resistance exercise intensity for the first two weeks of training was 70% of 1RM and for the second two weeks was increased to 80% of 1RM. Four resting blood samples were taken before training, after 4 and 8 weeks of training and after 4 days of recovery. The samples were analysed for measurements of fibrinogen concentration, platelet count, plateletcrit (percent of platelets), mean platelet volume (MPV), and platelet distribution width (PDW).

No significant difference between pre and post-exercise values was observed in the control group for all measured platelet variables. Statistical analysis of 1RM data for training group revealed a significant increase (between 20% to 35%) in muscular strength following 8 weeks of training. A significant main training effect was found for platelet count and plateletcrit ($F_{3,21}=9$, and $F_{3,34}=9.4$, respectively, $p<0.001$). Post-hoc analyses revealed a significant increase in platelet count and plateletcrit during first 4 weeks of training with no significant changes in second 4 weeks (between week 5 to week 8). However, MPV, PDW, and fibrinogen data did not change significantly in response to 8 weeks of resistance training.

Rises in platelet count and plateletcrit following resistance training in the present study, could be due to a fresh release of young large platelets during first period of training (first 4 weeks), particularly from the splenic pool, into the circulation. Probably the amount of increase in plasma volume during second 4 weeks of training exceeds that of platelet count and masks the increases in platelet count. Therefore, it could be concluded that resistance training induces thrombocytosis in peripheral blood and that the mechanism responsible for this might be the fresh release of platelets under physical stress.

Responses of red blood cell indices to heavy-resistance training in men

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Physical training triggers a series of changes in the erythrocytic system of the peripheral blood that results in the elevation of the oxygen-carrying capacity of blood. Effects of endurance training and low intensity resistance training on red blood cell (RBC) indices have been investigated. However, the effect of high intensity resistance training on these variables has not been determined yet and the present study was designed to examine this.
Fifteen collegiate untrained healthy male subjects (22.6 ± 1.5 years) were randomly allocated to either a sedentary control (N = 6) or resistance training (N = 9) group. The training group undertook a strength-training programme three days a week for 8 weeks, while the control group had no physical activity during this period. The training protocol included the performance of 3 sets of 8-10 repetitions of six exercises at 80% of 1RM. Body mass, baseline strength (1 RM), body fat percent and blood samples were taken before training, after 4 and 8 weeks of training and after 4 days of detraining. Resting blood samples were analysed for RBC count, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red blood cell distribution width (RDW).
No significant difference between pre and post training values was observed in the control group for all measured variables. Statistical analysis revealed a significant main effect of training on RBC count ($F_{3,21}$=15.4, P<0.001), haemoglobin concentration ($F_{3,21}$=16.9, P<0.001) and haematocrit ($F_{3,21}$=14.3, P <0.001). Post-hoc analyses indicated that RBC count, haemoglobin, and haematocrit decreased significantly during the first 4 weeks of training and increased between weeks 5 to 8. However, the other RBC indices including, MCV, MCH, MCHC, and RDW did not change significantly (P>0.05) in response to resistance training.
Although some of the RBC parameters demonstrated significant changes after 4 weeks of training and during the second period of training (week 5 to 8), there were no differences between baseline and post-training data. Decreases in RBC counts, haemoglobin concentration and haematocrit during the first 4 weeks of training could be attributed to an expansion of plasma volume or intensified haemolysis of erythrocytes during this period which is a generally accepted result of strenuous exercise and weight lifting. It was concluded that eight weeks of resistance training does not induce significant changes in the RBC parameters in healthy male subjects.

Association of Lewis Null Blood Group with Proatherogenic Inflammatory Markers in Healthy Subjects

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The NHLBI Family Heart Study and Copenhagen Male Study found that the Lewis blood group null (negative) phenotype, Le(a-b-) was associated with an approximately 2-fold increase in the prevalence of ischemic heart disease (IHD) and the risk of an incident ischemic event compared to subjects with other Lewis types. The effect of Le(a-b-) was independent of "conventional" risk factors such hypertension, elevated triglycerides, high LDL, low HDL, obesity, etc. The mechanism is unknown. A structural similarity between sialyated Lewis antigens and the selectin ligand sialyl Lewis X suggested a possible link with inflammation.
To test this hypothesis, we studied 225 healthy subjects (70 male, 155 female, mean age: 64±10 years) enrolled in an ongoing cardiovascular disease study. The study subjects were screened for any history of atherosclerotic disease on entry to the study, demographic data and „conventional‰ laboratory risk factors for atherosclerosis were measured, together with five non-specific markers of inflammation: ESR, CRP, fibrinogen, RBC aggregation (RCA) and plasma viscosity (PV). The baseline carotid intima-media thickness (IMT) was measured via ultrasound, and the Lewis red blood cell phenotype was determined by serology.
Forty five of the 225 subjects (20%) had the Le(a-b-) phenotype. Each of the inflammatory markers was increased in Le(a-b-) relative to the other Lewis groups: PV (1.65 cP vs. 1.57 cP, $p<0.001$), fibrinogen (4.20 vs. 3.66 g/L, $p<.05$), CRP (3.27 vs. 2.16 mg/L, <0.05), RCA was increased by 27% ($p<0.001$), and ESR by 91% (29.3 vs. 15.5, $p<0.001$). In 8 of 16 Le(a-b-) subjects the ESR exceeded 30mm/hr, compared to only 3 of 53 with Lewis(a-b+) subjects, and in 10 of 21 Le(a-b-) subjects CRP was above 3 mg/L compared to 5 of 53 with Le(a-b+) subjects.
The large increase in ESR strongly supports the hypothesis that Le(a-b-) is a pro-inflammatory phenotype, and the elevation of fibrinogen and CRP are consistent with proatherogenic inflammation. Since the Lewis type is genetically determined, and can be identified long before the development of atherosclerosis, it could be useful as a risk marker for primary and secondary prevention of cardiovascular disease.

Age dependent changes of arterial wall viscoelasticity

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Viscoelastic characteristics (VEC) of old rat aorta (Wistar, 10 months) were obtained by the method of sinusoidal excitation of intraluminal pressure (p) in the cylindrical arterial preparations. The frequency of pressure excitation (fexc) was swept in the range 3 - 30 Hz up and down while response volume oscillations were recorded. The resonance curves were plotted by volume response oscillations versus excitation frequency at several constant values of p. Natural frequency (f0), dynamic modulus of elasticity (E') and coefficient of viscosity (beta) were estimated from each resonance curve and the graphics of VEC versus p were drawn.

The results showed that f0 for old rat aorta decreased linearly with p whereas our previous data for young rat aorta (Wistar, 4 months) showed independence of f0 on p. E' for old rat aorta increased nonlinearly with p with the values being higher in comparison to young rat aorta. This means stiffening of rat aorta with age, which is in accordance with the known literature data. The beta-values increased linearly with p being higher in comparison to young rat aorta, which means raised intrinsic friction. VEC values were higher at decreasing fexc, which suggests that the direction of the excitation sweeping is also means of biomechanical behaviour of the arterial wall.

It could be concluded that VEC worsen with age, which endangers the arterial wall integrity, especially at higher intraluminal pressure and in the presence of atheromatous plaques or aneurysm.

Time Dependent Variation of Human Blood Conductivity as an Estimation of RBC Aggrgation

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The time dependent changes of blood conductivity at different regimes of shear rates were investigated. Human blood conductivity was measured in a Couette viscometric flow. Contraves Low Shear 30 rotational viscometer as a base unit and a concurrent measuring system, including a device, developed by the conductometric method and a software for measurement of conductivity of biological fluids and natural biological mixtures (Data acquisition system), previously described [1,2] were used for the experiments.

Normal human conservated with CPD-A_1 blood (450 ml blood/63 ml CPD-A_1) and human blood from patients with different pathologies were investigated. The time variation of blood conductivity at different flow regimes – rectangular and trapezium-shaped and the dependences of the apparent whole blood viscosity on time were experimentally investigated under electric field of 2 kHz. The kinetics of conductivity signals were recorded both under flow conditions and after the complete stoppage of shearing at the above shear rate changes from 0.94 to 94.5 s^{-1} .

The experimental relationships between the blood conductivity, apparent blood viscosity, shear rates and time at rectangular changes of shear rates from 94,5 s^{-1} to 0,945 s^{-1} at T=37 ^{0}C show that the human blood conductivity is time and shear rate dependent under transient flow. It is established that the blood conductivity is dependent on the regime and time of the applied shear rates in the Couette viscometric flow. The results show that valuable information could be received about the mechanical properties of blood, in particular about the kinetics of “rouleaux formation”. The time dependences of the blood conductivity follow the morphological transformations of RBC aggregates during the aggregation-disaggregation processes. These results suggest that this technique may be used to clarify the mechanism of dynamics of RBC aggregates. Thus a method, based on dielectric properties of dispersed systems in Couette viscometric blood flow could be applied to investigate the kinetics of RBC aggregation and the break-up of the aggregates.

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Hemorheological Changes and Characteristic Parameters Derived from Whole Blood Viscometry in Chronic Heroin Addicts

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A group of 15 chronic opioid addicts under methadone maintenance therapy with mean age 26.5 ± 7.3 years was studied. Whole blood viscosity was measured using a rotational viscometer Contraves Low Shear 30 (Zurich, Switzerland) with measuring system MS 1/1 at a steady flow over a shear rate range of 0.0237 s^{-1} to 128.5 s^{-1} at temperature 37 ^{0}C. The results have been compared with a control group of 19 healthy subjects (9 female and 10 male; mean age 34.84 ± 4.06 years). It was found that the mean whole blood viscosity values of the investigated group of heroin abusers (n=15) were elevated compared to that of healthy persons (n=19) over the whole shear rate range and fell by more than ten orders of magnitude. These results show deviations too wide for diagnostic purposes. The present investigation uses the coefficients of the power law, which itself incorporate whole blood viscosity data in the entire shear rate range. Linear correlation shows that whole blood viscosity in intravenous drug users correlate positively ($p<0.05$) only with the changes in the hematometric indices of erythrocytes - RBC, HGB and HCT. the red blood cell count (RBC), mean erythrocyte volume (MCV), hemoglobin (HGB), hematocrit (HCT), mean hemoglobin content of erythrocytes (MCH), HGB/HCT values (MCHC), red blood cell distribution width (RDW), and thrombocyte count (PLT) as well, were especially analyzed in chronic opioid users. Correlations of the power law coefficients have been searched with the red blood cell count (RBC), mean erythrocyte volume (MCV), hemoglobin (HGB), hematocrit (HCT), mean hemoglobin content of erythrocytes (MCH), HGB/HCT values (MCHC), red blood cell distribution width (RDW), and thrombocyte count (PLT) too. Whole blood viscosity at low shear rates can be described accurately by the power law and the coefficients of the model are useful for the estimation of the changes between the investigated groups and relationships with the hematometric indices.

Reperfusion injury and inflammatory responses following limb revascularization surgery

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After revascularization of an acute occlusion on the infrarenal aorta or on peripherial vessels the development of a serious ischaemic-reperfusion injury is a menacing challenge and a hard task in peripherial vascular surgery. A whale of evidences point to oxidative stress, as an important trigger in the complex chain of events leading to reperfusion injury following peripherial vascular surgery interventions. In the present study the authors aimed to examine the oxidative stress parameters, the antioxidant-prooxidant state and the expression of leukocyte adhesion molecules (CD11a and CD18) following the revascularisation surgery of the lower limb.
9 patients were examined in the prospective randomised study. Lower limb embolism, thrombosis and abdominal aorta aneurism were the indications of the vascular-surgical intervention. Peripherial blood sample collection was before the operation (ischaemic period), and after the reperfusion in the 2nd and 24th hours, and on the 7th day. We measured the rate and the speed of the free radical production of the leukocytes, and detected the activity of superoxide-dismutase (SOD) and the concentration of reduced glutathion (GSH). The degree of lipidperoxidation was marked with the quantity of malondialdehyde (MDA). The expressions of the adhesion molecules were measured with flowcytometry.
Our results showed, that the speed and rate of free radical production significantly increased in the early reperfusion ($p<0,05$). The level of the antioxidant enzymes decreased after the revascularisation. The CD11a and CD18 expression of the granulocytes significantly ($p<0,05$) decreased right after the revascularisation, but with a gradual elevation, until the 7th day they exceed the ischaemic value.
Our results showed the turnover of the sensitive antioxidant-prooxidant balance after revascularisation operation. Thus, we suggest the preoperative monitoring and possible therapeutic supplementation of the antioxidant state in patients going for vascular surgery intervention.

Effects of CO2-gas on microcirculation of the skin in patients with chronic venous insufficiency

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Introduction: In patients with CVI there is a venous congestion which leads to venous hypertension in the affected extremity. By walking the muscle pump puts pressure on the venous system and this pressure again leads to hypertension, the so called ambulatory venous hypertension. The loss of function of the venous valvules allows the pressure to be carried on backwards into the capillary net. This results in a damage of the capillaries in the skin with pathomorphologic changes such as dilatation, torquation, branching, formation of glomerulum -like shaped capillaries and rarefication of the capillary bed. These changes in the appearance of the capillaries are accompanied by a disturbed metabolism of the tissue, the O2 supply decreases. Correlating clinical signs for the drop of metabolic supply are the trophic changes typically seen in patients with CVI like hyper- and hypopigmentation, induration, capillaritis alba and in the end ulceration. The low tissue supply can also be expressed by a lowered tcpO2 in the affected tissue LDF allows a statement on the tissue perfusion due to an optic laser shift on light reflected by moving blood cells. Depending on the positioning of the laser fibers the depth of the recorded signal ranges between 1 to 7 mm, which pictures the thermoregulative (85%) and the nutritive (15%) capillaries in the tissue. The light of the laser transmitted to the tissue undergoes a change in its wavelength when hitting moving blood cells which is called the Doppler shift. The magnitude and the frequency of the wavelength change are related to the amount and the velocity of the hidden blood cells and therefore represent the perfusion of the investigated tissue.
Methods: The skin perfusion on the distal lower leg was measured in 19 patients with advanced chronic venous insufficiency (CEAP stage 4 and 5) under treatment with a CO2-releasing wound dressing (= verum) in comparision to the treatment with a regular wound dressing of the same type (control group = placebo). The trials were an intraindividual controlled comparison of the application of verum and placebo wound dressing which was applicated and measured at two different times of investigation. Atrophy of the skin was found in 89.47% of the test persons, hyperpigmentation in all while hypopigmentation was found in 52.63% additionally. Capillaritis alba occured only in 21.05% of the test persons, induration was asessed in 42.11% and in 5.26% in terms of dermatosclerosis. 31.58% had had one or more (by example reoccurring) ulcers before (CEAP stage 5 or Widmer stage 3a) of whom 15.79% were triggered by a trauma. Patients with an active ulcer did not paticipate.
Results: Laser Doppler Flux (LDF) as a gauge of skin perfusion increased under the application of the CO2-releasing wound dressing significantly not so the under the application of the placebo. The LDF-signal can be split into different frequency bands which represent the neurogenic and metabolic energy, the heart, the breathing and the vasomotion (myogenic energy). By Wavelet analysis the vasomotion frequency band of the obtained LDF-signal was examined for differences in the two groups and it was shown that under the influence of CO2-gas the wave of vasomotion was flattened.
Conclusion: In this controlled study the hypothesis was verified that CO2 gas (released out of the prototype of a wound dressing) would lead to a distinct increase of LDF in patients with advanced chronic venous insufficiency as a gauge for skin perfusion. By wavelet analysis it could be shown, that the vasomotion was less under the influence of CO2 which leads to the conclusion that CO2-gas causes a higer skin perfusion by dilatation of the capillaries in patients with advanced chronic venous insufficiency.

Leukocyte rolling and recruitment by endothelial cells: hemorheological experiments and numerical simulations

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The recruitment of leukocytes from the blood stream and their subsequent adhesion to endothelial walls are essential stages to the immune response system during inflammation. The precise dynamic mechanisms by which molecular mediators facilitate leukocyte arrest are still unknown. In this study combined experimental results and computer simulations are used to investigate localized hydrodynamics of individual and collective behaviour of clusters of leukocytes. Leukocyte-endothelial cell interactions in post-capillary venules of Wistar rats’ cremaster muscle were monitorized by intravital microscopy. From these experiments the haemorheologic and haemodynamical measured parameters were used in time dependent three-dimensional computer simulations, using a mesoscopic lattice Boltzmann solver for shear thinning fluids. The dynamics of leukocyte clusters under non-Newtonian blood flow with shear thinning viscosity was computed and discussed. In this paper we present quantified distri butions of velocity and shear stress on the surface of leukocytes and near vessel wall attachment points. We have also observed one region of maximum shear stress and two regions of minimum shear stress on the surface of leukocytes close to the endothelial wall. We verified that the collective hydrodynamic behaviour of the cluster of recruited leukocytes establishes a strong motive for additional leukocyte recruitment. It was found that the lattice Boltzmann solver used here is fully adaptive to the measured experimental parameters. This study suggests that the influence of the leukocytes rolling on the increase of the endothelial wall shear stress may support the activation of more signalling mediators during inflammation.

Influence of contrast media on Red Blood Cell Deformability

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Introduction: Iodinated contrast media, (CM), are widely used either to visualize blood vessels during angiography or to enhance the density of the parenchyma in different organs. Great amounts of contrast media are infused intra-arterial during vessel desocclusion intra-vascular procedures. During these procedures, protection of microvascular bed is of great importance. Iodinated CM, both ionic and non-ionic induce various effects on blood components which are attributed to the chemical nature of contrast media, their electrical charge and the osmolality of the solution in which they are administrated. The haematological effects of iodinated CM are on red blood cell, (RBC) –morphology, aggregation and rheology [1]. Purpose of our work was to assess any changes of mechanical and rheological properties of RBCs by measuring its deformability using a filtration method. To our knowledge there is no study addressing the changes of RBCs deformability during angiography.

Methods: Deterioration of blood perfusion through the microvascular bed can be affected by the decrease of RBCs deformability. Blood sample from 31 patients (21 male) who underwent Intra Venous angiography were used. Samples were taken before infusion of contrast media as well as 5, 30 and 60 minutes after the infusion in a concentration of 300 mg/ml of CMs. Contrast media that was used was Iodixanol (18 patients), Iopentol (4 atients) and Iopromide (9 patients). The filtration measurements were made by using the initial flow rate method as described elsewhere [1]. In this method the measured time lapse t_s of the filtered RBCs suspension is compared with the corresponding time of buffer filtered, t_b. The difference of t_s and t_b depend on the rheological properties of the RBCs membrane. Between the time lapses t_s and t_b, the following relationship is valid:

$$IR = \frac{(t_s - t_b) \times 100}{t_b \times H_{ct}} \quad [2]$$

Where, IR, is the Index of Rigidity and Hct the haematocrit of the RBC suspension. High IR values indicate low RBCM deformability.

Results and Discussion: Results showed an increase of 59% of IR in all the 31 patients treated with the CM during the angiography. Particularly in the 4 patients treated with Iopentol (IMAGOPAQUE) the IR had an increase of 47%, ($P < 0.001$) in the 9 patients treated with Iopromide (ULTRAVIST) the IR had an increase of 84% ($P<0.001$) and the 18 patients treated with Iodixanol (VISIPAQUE) the IR had an increase of 49% ($P<0.001$) in comparison to their initial, before the infusion of CM, drawing blood sample which they were considered as controls (paired t-test).

The decreased RBC deformability may be result of the osmotic shrinkage induced by all the CMs and hence increased viscosity of the hemoglobin solution [3].

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Impaired Erythrocytes Deformability in H2O2-Induced Ooxydative Stress: Protective Effect of L-Carnosine

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Impaired red blood cell deformability is a hemorheological perturbation induced by many kinds of diseases.An increase in free radicals causes a reduction in erythrocyte flexibility and deformability.Carnosine is a dipeptide abundant in skeletal muscle and brain of humans.One of the main function of carnosine is its antioxidant and free-radical scavenger effect. In this study our aim is to investigate the protective effect of L-carnosine on RBCs in H2O2-induced oxidative stress in vitro conditions.
Twenty male wistar albino rats, 10 were 3 months old, 10 were 12 months old used.The blood from each rat were divided into ten tubes and these blood samples divided into two groups. The first tube of the first group was the control and the rest 4 tubes were treated with different concentrations of L-carnosine.All tubes in the second group were incubated with H2O2 additively.The deformability indexes of the erythrocytes were measured by a laser diffractometer (Myrenne Rheodyne SSD). L-carnosine has improved the RBC deformability significantly which is impaired by H2O2 treatment ($p<0.05$).Increase in deformability is more significant in young rat group when compared to old rat group. L-carnosine, as an antioxidant molecules, has a dose dependent positive effect on RBC deformability and has improved or protect the deformability of erythrocytes, especially in young rat group which impaired by H2O2-induced oxidative stress in vitro conditions. The results of this study first suggest that L-carnosine supplemention can be used to improve the RBC quality or to protect them from oxidative damage in survival of RBC in the circulation.

Effects of endothelial and erectile dysfunction on microcirculation in the corpus cavernosum

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Erectile function is critically dependent upon the activation of the endothelial nitric oxide synthase (eNOS) in the corpus cavernosum cells of the penile tissue. Nebivolol is a ß1-selective ß-adrenoceptor blocker (ß-ARB) with additional vasodilating properties, which have been attributed to an eNOS-activation. The present study investigated whether nebivolol is able to increase eNOS-activity in erectile tissue.
Murine penile tissue was incubated in an organ bath under control conditions, as well as in the presence of nebivolol or metoprolol (each 10 µM). Immunfluorescent staining was performed using specific antibodies against eNOS-translocation or eNOS-serine 1177 phosphorylation. In addition, slices of murine and human erectile tissue were incubated with diaminofluorescein, a specific fluoresence marker of NO-liberation.
Under control conditions, we observed a small eNOS-translocation and serine 1177-phosphorylation. After application of metoprolol, eNOS-translocation and serine 1177-phosphorylation was slghtly decreased. Only in the presence of nebivolol, a significant increase in eNOS-translocation and serine 1177-phosphorylation of eNOS were observed. These alterations of the eNOS protein induced after application of nebivolol went along with an increased DAF-fluoresence in both murine and human erectile tissue.
Conclusions: ß-adrenoceptor blockers differentially influence erectile tissue. Since cardiovascular diseases are often going along with the development of erectile dysfunction, the nebivolol-induced eNOS-activation in corpus cavernosum may be beneficial when treating patients suffering from cardiovascular disease.

Oxidative Stress in Juvenile Myocardial Infarction: Evaluation at the Initial Stage and after 12 Months

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Few are the information about the role of oxidative stress in acute myocardial infarction (AMI) and even less in juvenile AMI.
We enrolled 105 subjects (97 men and 8 women) aged < 46 years (mean age 39.6 ± 5.5 years), with recent AMI (T1). As parameters of oxidative status we determined the thiobarbituric acid reactive substances (TBARS) and total antioxidant status (TAS). The NO production was evaluated by measuring the concentration of the end products of NO in vivo: nitrite and nitrate (NOx). We subdivided AMI patients according to the presence or absence of the main risk factors (family history, smoking, hypercholesterolemia, diabetes mellitus, essential hypertension), according to the number of risk factors and to the extent of coronary lesions. We repeated the evaluation after 12 months (T2). At T1 in AMI subjects TBARS and NOx were significantly increased and TAS was significantly decreased in comparison with control subjects. As regards the single risk factors, only NO metabolites were significantly lower in non-smoker than in smoker AMI subjects. Subdividing AMI subjects according to the number of risk factors, there were no significant differences between the subgroups. Similarly, subdividing AMI subjects according to the number of stenosed coronary vessels, there were no significant differences between the subgroups. In the whole group of AMI subjects the ejection fraction was not related to the oxidative parameters or NO metabolites. Nor in the subgroups according to the number of risk factors nor in the subgroups according to the extent of coronary lesions was the ejection fraction related to the oxidative parameters or NO metabolites. At T2, TBARS and NOx were decreased and TAS was increased compared to T1, but all parameters were still altered in comparison with control subjects.
The persistence of an impaired oxidative stress 12 months after AMI requires two different considerations: first of all, the use of antioxidant therapy after AMI may be advisable; moreover, the altered oxidative status observed so much time after AMI may be present also before AMI. Regarding this latter aspect, our project of re-evaluating the same AMI subjects two years or even later after AMI may give further information.

Distribution of Proteolytic, Apoptotic, and Angiogenic Factors in Atherosclerotic Lesions in Relation to the Direction of Blood Flow

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Rupture of thin-fibrous cap plaques occurs most frequently at the upstream shoulder of lesions, where shear stress gradient is steepest and wall shear stress is increased. In accordance with this, a higher frequency of inflammatory cells, chemokines, and chemokine receptors was detected in upstream plaque shoulders. To investigate whether the mechanisms leading to plaque destabilization and rupture upstream are related to increased proteinase activity, apoptosis, and enhanced neovascularization which provides entry points for inflammatory cells, we characterized proteolytic, apoptotic, and angiogenic protein expression along the atherosclerotic lesions.
Serial longitudinal sections of 30 human carotid specimens (AHA classification IV-VI) were immuno-histochemically analyzed for the expression of mast cell chymase, cathepsin L, and Bax using specific monoclonal antibodies. Antibody against von Willebrand factor (vWF) was used to detect vasa vasorum, and VEGF and CTGF were stained as pro-angiogenic growth factors. Immunoreactive cells were digitally counted in upstream and downstream regions of the plaques and the differences between the regions were analyzed statistically using paired t-test.
Immunohistochemical analyses of the longitudinal sections of carotid plaques showed that the mean numbers of chymase-positive cells were significantly higher upstream as compared with downstream shoulder of the atherosclerotic lesions (34.7±3.8 upstream versus 12.8±3.5 downstream, $p<0.001$, $n=30$). Similarly, the expression of cathepsin L was significantly increased in the upstream shoulder. In this region, induced expression of Bax, a pro-apoptotic protein, was detected in smooth muscle cells of the fibrous cap, as well as in foam cells at the border of lipid core. With regard to angiogenic growth factors, CTGF and VEGF immunoreactivity was significantly higher upstream as compared with downstream region of the atherosclerotic lesions, and was often associated with occurrence of intimal neovascularization.
This study demonstrated that plaque instability at upstream region is related to increased expression of proteases, increased apoptosis of smooth muscle cells, and enhanced neovascularization. These findings underscore the close association of local hemodynamic forces with endothelium-mediated changes in plaque composition and destabilization processes.

Endothelial Dysfunction and Monocyte Recruitment in Cells Exposed to Non-uniform Shear Stress

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Atherosclerosis, the major cause of death in the world, results from a combination of local blood flow patterns and systemic risk factors. We investigated whether non-uniform shear stress at bifurcations induces pro-atherogenic endothelial dysfunction and monocyte recruitment.
Bifurcating flow-through cell culture slides were used to expose human umbilical vein endothelial cells (HUVECs) to laminar or non-uniform shear stress for 18 h at 10 dyne/cm2. For the adhesion assay, HUVECs were subsequently perfused with medium containing THP-1 monocytic cells for 1h. Protein expression was determined by immunofluorescence, and quantified using MetaVue software.
In areas exposed to chronic laminar shear stress, endothelial cell elongation and alignment with the direction of flow was accompanied by the reduction in F-actin stress fibers. On the contrary, cells exposed to non-uniform shear stress near the outer walls of bifurcations were characterized by irregular, unaligned shape and disorganized F-actin fibers. These phenotypic alterations were accompanied by significant changes in protein expression: Laminar shear stress resulted in a significant 5-fold induction of eNOS ($P<0.01$) as compared with static conditions. On the contrary, the protein levels of endothelin-1 and connective tissue growth factor (CTGF) were strongly decreased under laminar shear stress. In the areas of non-uniform shear stress, eNOS was dramatically reduced, whereas endothelin-1 was upregulated ($p<0.05$). Moreover, non-uniform shear stress induced the expression of CTGF, and this induction was RhoA-dependent, because it was almost completely inhibited in cells transfected with dominant negative RhoA-N19, and when cells were treated with simvastatin (1 μmol/L) during flow. In accordance with in vivo studies, no massive upregulation, but rather moderate induction of VCAM-1, ICAM-1, and selectin expression in single endothelial cells was observed in areas of non-uniform shear stress. In agreement with this, monocyte recruitment, which was nearly undetectable under laminar shear stress, was slightly induced by non-uniform shear stress.
Inhibition of antioxidative mechanisms and overexpression of atherogenic proteins appears to be the first step in non-uniform shear stress-induced endothelial dysfunction. It creates a pro-inflammatory milieu, which in the presence of circulating cytokines may drive the full endothelial response, and lead to enhanced monocyte recruitment into the vessel wall.

Contrast-Enhanced Ultrasound in comparison to MS-CT in blunt abdominal trauma

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To evaluate the effectiveness of Contrast-Enhanced Ultrasound (CEUS) in diagnostic and characterizing hepatic, renal and splenetic traumatic injuries compared to conventional Ultrasound (US) and Multislice Computed Tomography (MSCT).
Between January 2005 and January 2007, 78 patients (48 males, 30 females, mean age 56 years) with blunt abdominal trauma were examinated by conventional US, CEUS and MSCT. CEUS was examined with low MI-technique using 1,6 to 2,4 ml intravenous injection of SonoVue (Bracco, Italy) and using a multifrequency transducer 2-4 MHz (Siemens, Sequoia, Acuson). CT examinations were performed by 64 detector CT scanner (Somatom Sensation 64, Siemens Medical Systems, Forchheim, Germany) before and after intravenous contrast agent administration. Hepatic, renal and splenetic injuries were then analyzed and the conspicuity findings have been compared.
In 15 of 78 patients conventional US identified solid organs injuries: 8 hepatic, 2 renal and 5 splenetic injuries. CEUS identified 3 more injuries (2 hepatic and 1 splenetic) which have been missed by conventional US. CEUS identified traumatic lesions in 18/78 patients. In one of the 18 Patients even active bleeding could be identified. In CEUS solid organ injuries appeared hypoechoic. MSCT identified 18 solid organs injuries in 78 patients, according to the CEUS results.
CEUS greatly increases visualization and characterization of hepatic, renal and splenetic injuries compared with conventional ultrasound and correlated well with MSCT. The imaging technique shows even small blood flow and vascular structures can be depicted in detail. In our institution it is an additional examination to MSCT in unclear cases. Especially in patients with contraindication for contrast agents for example because of renal failure or allergy and in hemodynamically compromised patients, due to its bedside availability CEUS provides a good alternative to MSCT.

Contrast enhanced Ultrasound versus CT-angiography in classification of abdominal aortic Endoleak between Typ II and III after endovascular repair

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To assess the effectiveness of Contrast enhanced Ultrasound (CEUS) in identification and classification of endoleaks between Typ II and Typ III after endovascular repair of abdominal aortic aneurysm (EVAR) in comparison with CT-angiography (CTA).
Between January 2005 and January 2007, 58 patients (37 males, 21 females, mean age 65 years) were examinated by CEUS and MSCT. CEUS was examined with low MI-technique using 0,9 to 1,6 ml intravenous injection of SonoVue (Bracco, Italy) and using a multifrequency transducer 2-4 MHz (Siemens, Sequoia, Acuson). Biphasic CTA examinations were performed by 64 detector CT scanner (Somatom Sensation 64, Siemens Medical Systems, Forchheim, Germany) at the same intervals during post-EVAR follow-up. In conflicting cases, digital subtraction angiography (DSA) or follow up with CEUS and CTA was performed.
With contrast enhanced Ultrasound 17 endoleaks (14 Typ II and 3 Typ III) after endovascular repair of abdominal aortic aneurysm could be detected. Whereas CTA depicted 16 endoleaks. 1 endoleak was missed with CTA. In contrast to CEUS 9 Typ II and 7 Typ III endoleaks were depicted with CTA. CEUS agreed with the CTA-findings in 9 Typ II and 3 Typ III endoelaks. In all cases, DSA or the follow up confirmed the CEUS classification.
In our study CEUS was more sensitive in depicting endoleaks. Due to the dynamic character of the examination the differentiation between Type II and type III endoleaks was more precise compared with CTA. The imaging technique shows even small blood flow and can be depicted due to the real time imaging endoleak in detail. Especially in patients with contraindication for contrast agents for example because of renal failure or allergy it provides a good alternative and reduced additional costs and exposure to radiation.

MS-CT in evaluation of complex cystic renal masses in comparison to Contrast-Enhanced Ultrasound findings

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To compare the diagnostic performance of MS-CT in evaluation of atypical or complex cystic renal masses in comparison to contrast enhanced ultrasound (CEUS) and in unclear cases to the surgery findings.
32 consecutive patients with 36 atypical or complex cystic renal masses at MS-CT underwent CEUS. CEUS was examined with low MI-technique using 1,6 to 2,4 ml intravenous injection of SonoVue (Bracco, Italy) and using a multifrequency transducer 2-4 MHz (Siemens, Sequoia, Acuson). Eleven masses were resected, the remaining 25 lesions were followed up for periods ranging from 3 months to 2 years. Images and digital cine clips of all lesions were evaluated by blind readers. Basing on MS-CT appearance the lesions were assigned to the Bosniak classification. Similar criteria modified for US imaging were used to score atypical cysts at CEUS. For each lesion, the number of septa, thickness of wall and septa, presence of calcifications, and contrast enhancement were evaluated with both techniques. The scores of resected masses were correlated with pathology reports.
Due to the Bosniak classification in MS-CT lesions were scored as category II (n=15), IIF (n=8), III (n =8), and IV (n =7). All type IV and 6/8 type III and 1/8 type IIF lesions were surgically removed. All category IV and 3/8 category III lesions of the surgical group were malignant the one type IIF lesion was benign. All class II and IIF cysts except one were stable after a follow up periods ranging from 3 months to 2 years. In 7/36 lesions (19%) lesions CT and CEUS scores were differences, while in 29/36 (81%), there were equivalent. CEUS depicted more thin septa than CT, or upgraded wall thickness resulting in Bosniak score upgrade in 3 lesions from category II to IIF. One cystic renal cancer was unclear by MS-CT but due to the additional information of CEUS, surgical findings confirmed a small cystic renal cancer.
CEUS with SonoVue allows an early evaluation of atypical or complex cystic renal masses. It is an additional examination to MS-CT. Due to the dynamic examination additional information about perfusion of the cystic septa or cystic renal cancer can be gained.

Blood rheology abnormalities and vascular cell adhesions mechanisms in sickle cell trait carriers during exercise

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Sickle cell trait (SCT) is usually considered a benign disorder compared with sickle cell anemia (SS hemoglobinopathy). However, several authors reported cases of exercise sudden death in this population. Among mechanisms that could be involved in these fatal complications, those involved in the vaso-occlusive processes of SS hemoglobinopathy could play a role. Therefore, we have developed different studies in order to assess the effects of exercise on biological parameters potentially involved in the vaso-occlusive processes.
In a first set of experiments, we have analyzed the hemorheological response to a short and intense exercise and observed higher blood viscosity in SCT carriers compared to normal subjects. In a second set of studies, we have compared the kinetics of red blood (RBC) rigidity at rest, during a prolonged and intense exercise, and during the immediate and late recovery between a group of SCT carriers and a control group. We found that RBC rigidity increased above resting values in the two groups 24 and 48 hrs after exercise but the increase was greater in SCT carriers. Finally, we have followed the kinetics of several adhesion molecules in response to three repeated maximal ramp exercise tests. Molecules such as the vascular cell adhesion molecule (VCAM-1), which plays a role in the firm adhesion of reticulocytes to endothelial cells, and L-selectin which plays a role in leucocyte rolling on vascular wall, have been tested. Although VCAM-1 was not different between the two groups and remained unchanged with exercise, we found an increase in L-selectin in the two groups and this increase was faster in SCT carriers compared to a controlgroup. L-selectin increased above basal values 24 hrs after the end of exercise in the control group whereas, this increase was observed immediately at the end of exercise in SCT carriers.
In conclusion, we can suggest that the risks for micro vascular complications in SCT carriers in response to exercise could be dependant on changes in blood rheology and vascular adhesion processes.

Influence of alcohol on hemorheological parameters and platelet function

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Alcohol has a dual effect on vascular events. Regular consumption of moderate quantities has a protective effect (so-called French paradox), whereas an acute ingestion of larger quantities increases the risk. We have analysed the influence of acute alcohol exposure in vivo and in vitro on blood flow properties and platelet function.
12 healthy male volunteers drank either 4.36 ml red wine/kg body weight (= 0.5 g ethanol/kg) or water at 06.00 p.m. under fasting conditions. Blood was drawn immediately before, and 1, 2, 4, and 13h (i.e. 07.00 a.m. on the next morning) after alcohol ingestion. In vitro, blood was incubated with 0, 12.5, 25, 50, and 100 mmol/l ethanol for 1h at 37°C. Hemorheological measurements were: whole blood viscosity and blood viscosity at a standardized hematocrit of 45%, plasma viscosity, hematocrit (Hct), erythrocyte count and indices. Platelet function was tested with a platelet function analyser (PFA-100®), which measures the closure time (CT) of a pore by aggregating platelets under high shear flow (5000 s-1).
Alcohol ingestion had a detectable osmotic effect on erythrocytes. The mean cellular volume (MCV) was significantly smaller 1 and 4h after alcohol ingestion compared with water. Whole blood viscosity remained unaffected, at a standardized Hct of 45%, however, blood viscosity at high shear rate (94.5 s-1) was increased 2 h after wine ingestion. In the morning, 13h after wine drinking, platelet aggregatory function was increased, since the CT with epinephrine as an activator was reduced from 139 ± 25s at baseline to 117 ± 16s ($p = 0.003$) and with ADP from 94 ± 13s to 83 ± 17s ($p=0.009$), a circadian variation, which was not seen to that extent after water drinking. Since this effect was not observed with ethanol in vitro, this might indicate an endothelial release of von Willebrand factor under alcohol in vivo, to which the PFA-100® instrument is very sensitive.
Acute exposure to alcohol has only modest effects in vivo on hemorheological parameters (erythrocyte shrinkage) and on platelet aggregatory function (increased aggregability in the morning after wine drinking) and no effect in vitro. Although an increased platelet aggregability may contribute to cardiovascular events, these minor hemorheologic changes are probably negligeable and other factors must be involved especially in the beneficial effects of wine drinking.

The Coronary „Slow-Flow" Phenomenon: Mechanistic Evidence

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Objective: to investigate the mechanism of the coronary slow flow phenomenon (CSFP) in patients presenting with angina and normal epicardial coronary arteries
Design: a parallel study comparing subjects presenting with the CSFP with subjects presenting with normal coronary flow.
Setting: a cardiac catheterization laboratory.
Patients: 15 subjects undergoing diagnostic coronary angiography for low-threshold angina were enrolled. All patients had no epicardial stenosis. Eight subjects presented a delayed progression of the dye in at least one vessel (CSFP group). Corrected TIMI frame count in the CSFP group was 36+/-13 versus 19+/-3 in the control group, P<0.001.
Interventions: All subjects underwent measurement of coronary microvascular resistances at rest and after intracoronary infusion of the vasodilator papaverine.
Main outcome measures: the index of microvascular resistance, as measured by the product of a parameter of blood flow times mean intracoronary pressure.
Results: At rest, parameters of microvascular resistances were significantly higher in the CSFP group (CSFP: 10431 versus 5327, P<0.01). This difference was abolished after induction of hyperemia (CSFP group: 34+/-22; control: 22+/-15, P=ns). Coronary flow reserve was normal in the subjects with the CSFP (3.6+/-1.6).
Conclusions: We provide the first evidence that microvascular resistances are elevated in patients presenting with the coronary slow flow phenomenon. At the same time, we show that coronary flow reserve is normal in these patients.

Stimulation of Monocytes and Macrophages: Possible Influence of Surface Roughness

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The aim of this study was to understand the mechanisms of interaction of monocytes/macrophages and Foreign Body Giant Cell (FBGC) with implant materials, especially the roughness and the solubility of calcium phosphate based coatings. Anderson et al. [1] showed that the presence of FBGC's and moncytes/macrophages influence the strength of the implant integration and that more monocytes/macrophages rested on smooth surfaces compared to rough surfaces. We seeded human bone marrow cells on uncoated ultrasmooth polished TiAl6V4 samples as well as on TiAl6V4 discs of the same diameter with a coating consisting of two different calcium phosphates, monetite (DCPA) and hydroxyapatite (OHAp) with rougher surfaces.
On uncoated ultrasmooth polished TiAl6V4 discs (UUTi, Diameter 16mm, thickness 2mm) and on TiAl6V4 discs of same diameter coated with OHAP or DCPA, Human Bone Marrow Cells (HMBC) were seeded and cultivated under standard conditions for 90 days without any inducing substances like ascorbic acid, Na-β-glycerophosphate or dexamethasone. The roughness of the virgin samples were assessed with atomic force microscopy and light profilometry. After 90 days a fraction of the samples were stained with HE and examined under light microscopy.
Uncoated TiAl6V4 samples had a roughness (Ra) of 0,001µm, the DCPA coated discs a Ra of 4µm and the OHAp coated discs a Ra value of 3µm. The examination of HE stained samples showed a high number of FBGC and monocytes/macrophages on the UUTi samples, on the DCPA coated samples there were less FBGC and monocytes/macrophages and on the OHAp we could not find any FBGC and monocytes/macrophages. Also the matrix we found on the UUTi samples was finer and thinner than on the coated samples. The matrix was vastly spread and not dense on the UUTi samples in contrast to the calcium phosphate coated samples, where the matrix was thicker and stronger.
The ultrasmooth surface of the uncoated TiAl6V4 samples, this material is accepted to be biocompatible, evidently induced the differentiation of cells of the monocytic lineage and the formation of FBGC out of the cell populations present in the human bone marrow.

[1] Anderson, J.M.: Cellular Cascades of Wound Healing In: Davies, J.E. (Ed.): Bone Engineering, Chapter 7, pp 81-93 em2 incorported, 2000, Toronto, CAN

Rheological Properties of Erythrocytes in Patients with High Risk of Cardiovascular Disease

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Rheological properties of erythrocytes from patients with high risk of cardiovascular disease (CVD) were analyzed in relation to individual patient risk factors as well as to the medication. Additionally, comparative statistical analysis was performed considering plasma concentration of the following mediators of vascular endothelium: adhesion molecules (sVCAM-1, E-Selectin), interleukin-6 and prostaglandin 6-keto-F1α a stable metabolite of prostacyclin.
In 53 patients (25 men, 28 women, average age 57.7) with the presence of at least one risk factor of coronary heart disease, deformability, stiffness and aggregability of red blood cells (RBC), the serum concentration of cell adhesion molecules (sVCAM-1, E-Selectin), serum level of interleukin-6 (IL-6), and of 6-keto-prostaglandin F1α; (PGF1α) were measured.
Most of the patients (37) suffered from essential hypertension (HA), 20 of which were under antihypertensive treatment (14 exclusively with angiotensin-converting enzyme inhibitor (ACEI)). Among all patients, 17 had Type 2 diabetes mellitus and 35 persons were diagnosed as hypercholesterolemia. Erythrocyte deformability and aggregability were measured by shear stress laser diffractometry and erythrocyte laser aggregometry, respectively. RBC stiffness, expressed in terms of Young Modulus (YM), was determined using the atomic force microscope. Our data were analyzed by comparing the mean values of a given measured parameter for various groups of patients (ANOVA test). In parallel, correlation between measured parameters and accompanying CVD risk factors (hypertension, diabetes mellitus, low density lipoproteins (LDL), cigarette habit, body mass index (BMI)) or other independent factors (sex, age, hemoglobin level) were assessed using the multiple regression analysis.
As most of the patients were characterized by more than one CVD risk factor, correlation between measured parameters and the specific risk factor had to be unfolded using a complex statistical analysis. Such procedure was performed with the use of multiple regression analysis that took into account multiple and mutually connected impacts of the accompanying risk factors and other parameters characterizing patients, like sex, and age, on parameters measured for a given individual. The use of ACEI and the hemoglobin level were found to be significant, independent factors affecting deformability and aggregability of RBC. In hypertensive patients treated with ACEI, erythrocyte deformability was significantly increased (beta=5.7, $p<0.02$), whereas aggregability was significantly diminished (beta=-3.2, $p<0.05$) as compared to normotensive patients. Higher hemoglobin level was accompanied by decrease of RBC deformability (beta=-4.1, $p<0.0007$) and increase of aggregability (beta=0.8, $p<0.0006$). Statistically significant correlation was also found for RBC stiffness (Young,s modulus) and LDL concentration (beta=0.5, $p<0.05$) as well as between plasma concentration of sVCAM-1 and patient,s age (beta=28.8, $p<0.0001$).
Our data indicate that antihypertensive therapy with ACEI is accompanied by improvement of RBC rheology. It is noteworthy, however, that the measurements of deformability and aggregability of RBC strongly depend on the level of hemoglobin. Therefore this factor should be explicitly taken into consideration when investigating rheological properties of erythrocytes.

Interaction of blood with body foreign surfaces -Haemocompatibility-

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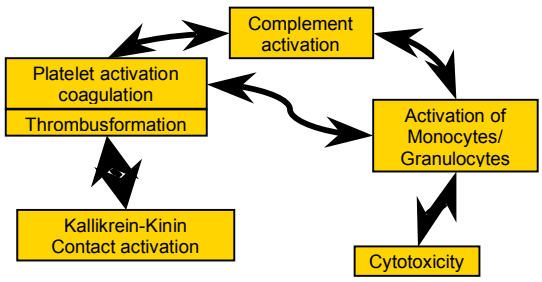
Fields of Application High amounts of blood contacting medical-/pharmaceutical products are used as blood bags and connecting tube systems for the acquisition, storage and supply of blood and blood products, pacemakers and defibrillators, vascular stents, vascular prostheses and heart valves, sensoring devices in blood contact, blood substitutes and contrast enhancing media for imaging technologies. There are some important therapeutic processes with high blood contacting areas which either eliminate unwanted constituents from blood (e.g. haemodialysis, therapeutical aphereses) or which change the composition of blood gases (e.g. extracorporeal blood oxygenation)

How to assess Haemocompatibility Changes in haemocompatibility can be demonstrated by activation or damages of blood constituents and/or blood vessel walls. Some of the blood constituents interact and network in a highly complex manner.



Description of changes in haemocompatibility

Blood components relevant for haemocompatibility	Denomination pathological process	Description parameters
Kallikrein-Kinin-System	Contact activation	Kallikrein-Kinin-activity
Complement System (C1-C9)	Complement activation	Complement components C3a, C5a Terminal Complement Complex (TCC)
Thrombogenic System	Thrombusgeneration (fixed) Embolisation (loose)	
- platelets		Platelet activation
- thrombin-fibrinogen-fibrin system	Coagulation	Thrombin activation/fibrinogen-fibrin-conversion
- plasmin-fibrinolytic system	Fibrinolysis	Fibrin degradation products (FDP)
Erythrocytes	Haemolysis	Liberated haemoglobin
Vascular cells relevant for haemocompatibility		
Leukocytes	Activation	
- monocytes	- e.g. burst reaction	ROS /IL1□ release
- granulocytes	- phagocytosis	
- lymphocytes	- acute reactions	
Endothelial cells	Endothelial damage	NO-/prostacyclin release
	Procoagulant-conditions	Plasminogen-activator-inhibtors

Determinants of haemocompatibility: surface roughness, corrosion-/degradation properties, effectivity of tolerance enhancing measures, wettability, product design, influence on vascular wall.

Influence of radiographic contrast media on the buckling of endothelial cells

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The intraarterial application of x-ray contrast enhancing agents (RCM) can induce perturbations of the blood velocity in subsequent capillaries (observed in patients with coronary heart disease) as well as a decrease in the tissue oxygen partial gas pressure (observed in the pig myocardium). It is unclear whether changes in the endothelial cell morphology contribute to the observed deteriorations of the microcirculation. To elucidate this problem, in the following examination human endothelial cells in vitro where incubated with RCMs in order to learn whether and how endothelial cells change their morphology and thus may reduce the lumen of blood vessels. Four RCMs which are routinely used in clinical practice (Iodixanol 320, Iohexol 350, Iopromid 370, Imeron 350) were added to the culture medium of human umbilical venous endothelial cells (HUVEC) and used for the short time incubation of these cells.

There were major differences in the appearance of the cultured endothelial cells after the incubation of the cells in the modified cell culture media supplemented with 30% v/v of the respective RCM. The addition of human serum pool served as control. Iohexanol ($p=0,6377$) and Iodixanol ($p=0,6309$) did not influence the endothelial thickness after 1,5 minutes incubation. A strong buckling and increase in endothelial thickness appeared after incubation in Iopromid (the cell thickness increased for 95% compared to cells incubated under control conditions; $p=0,0065$). The effects after incubation in Iomeprol were not as strong, however, there was an increase in endothelial thickness of 61,6% compared to cells incubated under control conditions; $p=0,0051$. After five minutes of incubation in the different RCMs there were no longer apparent influences on the endothelial thickness in comparison to the incubation in standard culture media (each p value $> 0,05$).

Riede et al (1) demonstrated in the lungs of septic shock patients (ARDS) that tremendous buckling and blebbing of endothelial cells can occur during the stage like progressing in shock lungs. This is thought to coincidence with an increase in lung capillary obstruction and vascular wall permeability. The buckling of endothelial cells observed in this examination in vitro after the incubation in some of the RCMs, might be the reason why the bolus injection of Iopromide in vivo into the left coronary artery was followed by a 50% decrease of oxygen partial pressure in the tissues supplied by this blood vessel. The bolus injection of Iodixanol did not induce such effects.

1. Riede U., Joachim H., Hassenstein J., Costabel U., Sandritter W., Augustin P., Mittermayer Ch. (1978) The pulmonary air – blood barrier of human shock lungs (a clinical, ultrastructural and morphometric study) Path. Res. Pract. 162, 41 – 72.

Influence of paracetamol, diclofenac and aspirin alone and in combination on platelet aggregation under high shear conditions in vitro

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Since the observation that cyclooxygenase(COX)-2 inhibitors increase the risk for cardiovascular events, all nonsteroidal anti-inflammatory drugs (NSAID) have come under scrutiny for their interference with aspirin and increase in cardiovascular risk. This has prompted the present in vitro study.
Citrated blood from healthy volunteers was incubated with 25 µg/ml paracetamol, 0.5 µg/ml aspirin, 0.04 µg/ml diclofenac, or the same volume of buffer. After 10 minutes a second of the above mentioned solutions was added. Specimens were incubated for 20 minutes at room temperature. Platelet aggregation was then assessed with a platelet function analyser PFA-100® (Dade Behring, Düdingen, Switzerland). In this instrument, blood is aspirated at high shear rates (5000-6000 s-1) via a glass capillary (diameter 200 µm) through a membrane pore (diameter 150 µm) coated with 2 µg type I collagen and 10 µg epinephrine. Platelets adhere to the collagen, become activated by epinephrine and aggregate. They finally plug the pore stopping blood flow, which is measured as closure time (CT), an inverse measure of platelet aggregation.
Paracetamol alone did not affect platelet aggregation compared with control (CT 129 ± 23 s and 120 ± 13 s, respectively), aspirin and diclofenac both increased CT (184 ± 69 s, $p< 0.01$, and 196 ± 54 s, respectively, n=10, $p< 0.001$). Combinations of aspirin and diclofenac, aspirin and paracetamol as well as diclofenac and paracetamol all increased CT further (290 ± 22 s, 281 ± 36 s, 288 ± 25 s, respectively, n=10, $p< 0.001$ compared with either control, aspirin or diclofenac alone). The time sequence of drug application was important: when diclofenac was added 10 minutes before aspirin, CT was less prolonged than when aspirin was added before diclofenac (227 ± 67 s versus 283 ± 29 s, n=20, $p< 0.001$). When paracetamol was added before aspirin, CT was also less increased than when aspirin was added before paracetamol (218 ± 71 s versus 257 ± 51s, n= 20, $p< 0.05$).
We conclude that paracetamol by itself does not affect platelet aggregation, but augments the antiaggregatory effect of aspirin or diclofenac. When diclofenac or paracetamol are added before aspirin, they partially inhibit the antiaggregatory effect of aspirin, suggesting an interference at the site of action (COX). These observations may have clinical implications for aspirin medication.

Influence of Chronical HELP-Apheresis on Microcirculation in Cardiac Allograft Recipients

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Hyperlipidemic heart transplant patients who develop cardiac allograft vasculopathy benefit from HELP-apheresis (**H**eparin-induced **E**xtracorporeal **L**DL **P**recipitation). There is evidence that HELP-apheresis improves microcirculation by normalizing the function of endothelium and also improves hemorheologic defects.
In heart transplant patients with cardiac allograft vasculopathy weekly HELP-apheresis was performed for one year. Mean velocity of red blood cells (vRBC) in nail fold capillaries as well as skeletal muscle oxygen partial pressure were measured before, during, and after apheresis.
8 men 51±9 years, 79±10 kg, 175±4 cm were investigated 8-36 months after transplantation. Three patients suffered from familial hypercholesterolemia, in 5 patients the level of lipids were not sufficient decreased despite diet and medical treatment.
The vRBC before treatment was pathologically lowered (0,133±0,08 mm/s) and increased during the first apheresis significantly (vRBC max 0,424±0,27 mm/s). One hour after apheresis higher velocities were measured (0,17±0,11 mm/s) as compared with baseline. After one year of treatment vRBC at the start of single apheresis was 0,324±0,136 mm/s and increased during apheresis until nearly normal values (0,605±0,30 mm/s). Beyond the velocity increase discontinuity of blood flow in capillaries significantly decreased and the velocity profile smoothened. During the first 3 months of HELP-treatment reactive hyperemia were completely lacking. One year of weekly HELP-therapy significant reactive hyperemia of 82±63 s duration was observed.
The patients presented with 11.6±3.8 mmHg (m. tibialis anterior) a significantly and pathologically reduced intramuscular pO_2 ($p<0.001$). LDL apheresis resulted in a significant increase in pO_2 in the anterior tibial muscle. Thirty minutes after the end of HELP-apheresis, intramuscular partial oxygen pressure had increased by 162 % and showed values at this point of 30.3±9.8 mmHg, which is similar to those found in healthy subjects.
Conclusion: Even the first HELP-apheresis leads to a significant improvement of microcirculation in skin and muscle. Weekly HELP-apheresis over one year led to a normalization of cutaneous microcirculation and restores the regulation of capillary blood flow.

Influence of Nicotinic acid on Cutaneous Microcirculation in Patients with Coronary Heart Disease and Hyperlipidemia

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Background: Nicotinic acid leads in vitro as well as in vivo to a vasodilatation and to an increase of blood flow. This effect seams to be dependend on dose. Under a dose of 600 mg there is no increase in oxygen tension in poststenotic muscle but a too high dose leads in the sequel of a steal-effect to a considerable decrease of microcirculation.
Method: Therefore we examined, whether the intake of 1g nicotinic acid (Complamin spezial retard®, Riemser Arzneimittel AG, Germany) on-top of the existing medication leads to an influence on the cutaneous microcirculation. The investigation was conducted in the framework of result quality control.
We investigated 6 patients with coronary heart disease. Coronary risk factors: hyperlipidemia 6, hypertension 5, diabetes 2, current smoker 3, former smoker 1. Age 73,7±10,2 years, weight 78±6,7 kg, height 171±7,1 cm. Male/female 4:2.
Before and 1 hour after intake of 1 g nicotinic acid a capillary video-microscopy was conducted in the skin of the nailfold of one finger. Recorded were the middle velocity of RBCs in the capillary in rest, the maximum speed during reactive hyperemia, the time till the maximum velocity of RBCs during reactive hyperemia (peak-time) and the duration of hyperemia.
Results: Blood pressure and heart rate did not change. The mean velocity of RBCs in the capillaries and the maximum velocity of RBCs during reactive hyperemia increased smoothly after 1 g nicotinic acid, but did not change substantial. On the contrary a distinct increase in the duration of reactive hyperemia could be observed. The peak-time remained unchanged.
One patient suffered a feeling of heat in the whole body.
Conclusion: Nicotinic acid in a dose of 1 g seams to improve cutaneous microcirculation in patients with coronary heart disease.

The Preconditioning-mimetic Effect of Nitroglycerin

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Objective: Nitroglycerin (GTN) modulates tissue damage induced by ischemia and reperfusion (IR) in a mechanism that is similar to ischemic preconditioning. We set out to study 1) using a human model of endothelial IR injury, whether GTN-induced endothelial preconditioning is mediated by reactive oxygen species (ROS) formation and/or opening of mitochondrial permeability transition pores (mPTP); 2) in vitro, whether GTN-induced ROS production depends on opening of mitochondrial potassium ATP-dependent channels (K-ATP), mPTP opening and/or GTN biotransformation.
Methods and Results: In two double-blind, randomized, parallel studies, a total of 66 volunteers underwent measurement of radial artery endothelium-dependent, flow mediated dilation (FMD) before and after local IR. Transdermal GTN (0.6mh/hour/2 hours, administered 24 hours before IR) significantly reduced the impairment of FMD caused by IR. This protective effect was lost when vitamin C (2 grams i.v. at the time of GTN administration) or cyclosporine (an inhibitor of mPTP, 100 mg 2 hours prior to GTN administration) were co-administered. In vitro, vitamin C prevented GTN-induced mitochondrial ROS production, while inhibitors of 1) K-ATP channels, 2) GTN biotransformation or of 3) mPTP opening did not modify it.
Conclusions: GTN triggers mitochondrial ROS production independently of the opening of mitochondrial channels and/or GTN biotransformation. We show, for the first time in humans, that ROS release, as well as mPTP opening, mediate GTN protection against IR-induced endothelial dysfunction.

Endothelial ischemia and endothelial preconditioning

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The biology of cardiac and peripheral ischemia and reperfusion (IR) injury are extremely complex. In recent years, the endothelium has been shown to play a critical role both in protecting from ischemic damage as well as in determining this damage. Due to its strategic location and its intense synthetic activity, the vascular endothelium is particularly sensitive to IR. At the same time, the endothelium can be preconditioned against IR damage, i.e., it is able to develop a protective phenotype that defends itself and the tissues from IR. We will discuss how the endothelium is the first casualty in the setting of IR, and at the same time how this tissue can be protected by physical and pharmacological stimuli.

The Effect of Prostaglandin E1 on Nailfold Capillary Blood Pressure and Red Blood Cell Velocity in Humans

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This study investigated whether capillary red blood cell velocity and capillary pressure were increased by acute intra-venous infusion of PGE1.
In a double blind randomised placebo controlled study the effect of Alprostadil (PGE1, Prostavasin®, intra-venous, infusion rate: 0.38 µg/h/kg,) on skin nailfold capillary blood pressure (CP) and capillary red blood cell velocity (CBV) was investigated in 16 healthy volunteers (placebo: 5 male, 3 female, age: 27.7, range: 22-29 years; Alprostadil: 5m, 3f, age 27.1, 22-38y), using the electrical impedance servo nulling technique and spatial shift alignment method respectively.
Initial finger tip temperature, systemic blood pressure, heart rate, CP, capillary pulse pressure amplitude (CPPA) and CBV showed no significant differences between the two groups (placebo: 23.6±3.0°C, 123±13 / 83±5 mmHg, 63±11 beats/min, 15.6±3.9 mmHg, 1.5±1.8 mmHg, and 425 µm/s (290-800); Alprostadil: 23.4±2.7°C, 121±9/82±10 mmHg, 65±9 beats/min, 14.4±3.7mmHg, 1.8±1.3mmHg, and 680 (140-1090µm/s)). Twenty minute infusion with either Alprostadil or placebo had no significant effect on any of the parameters measured.
Thus, in healthy volunteers, skin capillary blood pressure, capillary pulse pressure amplitude and capillary red blood cell velocity are unaltered by acute administration of PGE1 at ambient temperatures.

Erythrocyte Sedimentation Rate in 20 s?

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Purpose: evaluation of a new system for determination of erythrocyte sedimentation rate (ESR) in 20 s: Test 1, Alifax, Italy) and comparison with "classic" 1 hr Westergren ESR technique: StaRRsed,Mechatronics, the Netherlands.
Methods: Clinical blood samples were tested on both instruments and in addition, red blood cell aggregation indices (LORCA, Mechatronics), plasma viscosity(Contraves LS-30) and micro-hematocrit were determined. Similarly, blood samples manipulated to end up in a range of hematocrit values and/or plasma dilutions, were tested.
Results: In contrast to previous reports, Test 1 results are affected by the hematocrit. The results correlate closely with the rate of rouleaux formation (Tf), while StaRRsed results show a high correlation with the strength (or tendency) to aggregate (Ythr).
Conclusions: Test 1 does not measure sedimentation, the results have a fair correlation with ESR only in normal blood samples i.e. with minimal sedimentation, having ESR values 0 -15 mm/hr. The term „Length of Sedimentation Reaction in Blood (LSRB), suggested for Test 1 results, is obsolete.

Role of the Microcirculation in diabetic Foot Ulcerations

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Diabetes mellitus is associated with micro- and macroangiopathic complications which cause major morbidity and mortality in diabetic patients.
As microangiopathy is implicated in the pathogenesis of retinal damage, nephropathy and neuropathy, it has been postulated that abnormalities in foot microcirculation could play a significant role in the devolepment of foot ulcers. Various structural and functional abnormalities of the microvasular system have been described in the lower limb of diabetic patients. Although it has been shown that occlusive microvascular disease is not a complication of diabetes, recent work suggests that the microcirculation is impaired in diabetic patients in a different manner: Microvascular dysfunction may be described as increased vascular permeability and impaired autoregulation of blood flow and vascular tone. It has been presumed that hyperglycemia and insulin resistance cause metabolic derangements, working synergistically to bring about microcirculatory changes. Poor glycemic control is associated with hemorheological disturbances.
It has been postulated that these findings contribute to the risk of foot ulceration and impairment of wound healing. However, there is little evidence for this. In clinical practice the sensory polyneuropathy with loss of pain perception and coexisting peripheral arterial occlusive disease are the most important risk factors for amputation and mortality in diabetic patients with foot ulcerations.

Modulation of High Density Lipoprotein Cholesterol Metabolism

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Low high-density lipoprotein (HDL)-cholesterol (C) is an important risk factor for coronary heart disease. In vitro, HDL exerts several potentially anti-atherogenic effects including reverse cholesterol transport (RCT) from peripheral cells to the liver. Hence, raising HDL-C has become an interesting target for anti-atherosclerotic drug therapy. Levels of HDL-C and the composition of HDL subclasses in plasma are regulated by apolipoproteins, lipolytic enzymes, lipid transfer proteins, receptors, and cellular transporters. The interplay of these factors leads to RCT and determines the composition and thereby the anti-atherogenic properties of HDL. In several controlled and prospective intervention studies, patients with low HDL-C and additional risk factors benefited from treatment with fibrates or statins. However, in only some of the fibrate trials were prevention of coronary events in patients with low HDL-C and hypertriglyceridemia related to an increase in HDL-C. This may be because currently available drugs increase HDL-C levels only moderately and because HDL levels per se do not necessarily correlate with the functionality of HDL. Recent findings suggest that the mechanism of HDL modification rather than a sole increase in HDL-C determines the efficacy of anti-atherosclerotic drug therapy. The strongest HDL increasing effect of all commercially available drugs with increments of up to 30% has nicotinic acid, while fibrates and statins only moderately increase HDL-C by 6-18% and 5-10%, respectively. While nicotinic acid and fibrates are thought to increase reverse cholesterol transport, the pleiotropic effects of the statins make a prediction on reverse cholesterol transport impossible. Therefore, effective modification of HDL metabolism remains an attractive target for the development of new regimens of anti-atherogenic drug therapy. Several novel targets to modify RCT have emerged from the recent understanding of HDL synthesis, maturation and catabolism. Two drugs emerged that inhibit CETP and increase HDL-C levels up to twofold but inhibition of CETP blocks reverse cholesterol transport and may not prevent coronary events. Other investigated targets include delivery of recombinant HDL or Apo-AI mimetic peptides, novel PPAR activators and endocannabinoid receptor blockers.

In vitro 3D assay to test angiogenic effects of human CD14+ monocytes seeded on macroporous PLGA/CaP based polymers with a nanostructured surface

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In this study we present data from a in vitro 3D-model for angiogenesis in the context of tissue engineering. The assay allows evaluation of the angiogenic potential of scaffold based implants seeded with stem-/progenitor cells. Therefore primary human endothelial cells of the capillary bed (microvascular endothelial cells) are cultured on an extracellular matrix generated by endothelial cells themselves and covered by a modified agarose gel. Cell seeded scaffold is placed on top of this gel thus microvascular endothelial cells have to migrate actively through the gel towards the scaffold respectively angiogenic stimulus. Evaluation is done by standardized methods of immunhistochemistry.
For assay testing we used a scaffold consisting of a macroporous biodegradable polymer, which has in vivo already proven clear interaction with endothelial cells and endothelial progenitor cells respectively. To determine scaffold shrinkage and degradation scaffold volume was coated on a special way with CaP. To enhance cell adherence furthermore polymer surface was modified by loading with CaP nanoparticles.
For seeding of the scaffold human monocytes were used. Monocytes as fraction of white blood cells are easy to isolate and thus attractive especially for autologous cell therapy. Mononuclear macrophages, the tissue-resident macrophages and the monocyte-derived macrophages, express different functions dependent on microenvironmental signals. Induction of in vitro differentiation of monocytes to macrophages and following stimulation by the alternative way of activation leads to a macrophage fraction, which promotes angiogenesis, tissue remodelling and tissue repair due to the secretion of growth factors, cytokines, chemokines and enzymes.
The presented study was aimed to evaluate the developed angiogenesis assay and to get more information about the angiogenic potential of alternatively stimulated macrophages in the process of scaffold-vascularisation for improved integration of scaffold based implants in the surrounding tissue.

Comparative evaluation of two newly developed devices for capillary viscometry

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Viscometry is an often applied method in clinical chemistry. A variety of studies demonstrate an association of parameters related to blood viscosity with human pathology of varying origin. Whole blood and plasma viscosity are considered to be clinically useful indicators in the diagnostic workup and therapy monitoring of certain diseases.
In this study, we compare the "Waegeviskosimeter" (WV) described in previous publications with a newly developed device, the "Reverse Flow viscometer" (RFV). Both viscometers are capillary flow viscometers. Both overcome the disadvantage of common viscometers of the Ubbelohde and Cannon-Fenske type which require large amounts of plasma and which can be only applied to Newtonian fluids. The accuracy of the measurements of both viscometers, requiring less than 1.0 ml sample volume, is superior to most conventional methods. The major distinction in the functionality of the WV and the RFV is that the WV measures the kinematic viscosity whereas the RFV directly estimates dynamic viscosity without the requirement of additional density measurement.
We found good reproducibility of viscosity with coefficient of variation CV < 1.1% for both viscometers. Quality assurance measures have been carried out. Because no quality assurance scheme according to the guidelines proposed by the German Medical Association exists for plasma or whole blood viscosity, we tested reference material Lyphochek Unassayed Chemistry Control Level 1 and Level 2 (Bio-Rad Laboratories). We determined the viscosities 1.40 mPa s and 1.08 mPa s (37°C) and the between-run precision from daily quality control runs with CV of 1.4% and 1.2% for the WV, and 1.7% and 1.4% for the RFV.
For direct comparison reasons, we determined the viscosity in seventy human plasma and serum samples by both methods. Using the regression analysis described by Passing-Bablok, the RFV and the WV methods are highly correlated and show only little variations (r=0.990, t=0.896). The regression equation is $y_{WV}=1.035*x_{RFV} - 0.056$ with a mean deviation of 0.4 + 3.6%.
We conclude that both new devices for viscosity assessment fulfill all quality requirements as prescribed for clinical chemical laboratories. One advantage RFV is to measure the dynamic viscosity directly.

The Effect of Gender Differences and Social Environment on the Values of Hemorheological Parameters in Oldest Old Residents

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Debrecen is a Central European city where we started a project 2005 under this title: Debrecen Longevity Study (DLS). The aim is to assess the social-psychological, clinical and biological conditions of the residents over 90 years old, since the number of people over 90 is expected to grow over the following decades, according to demographic prognoses.
The total population of Debrecen is 207.308 and the proportion of people over 90 is 0.4% of the population. The investigation has involved 310 subjects (233 women, 77 men) randomly selected from the total oldest-old population. 18 routine (mostly hemorheological) laboratory parameters were compared between men and women and in different residential districts such as in the city, in housing estate, in the suburbs, in the elite zone and in elderly people,s home. Most of the examined parameters fell within the reference range of healthy adult population, although with some exceptions. The cholesterol, triglyceride, the HDL-cholesterol and collagen cross links levels were higher while the hematocrit, hemoglobin level and the red blood cell count were lower in females.
To explain the effect of social environment on different laboratory parameters needs a detailed analysis of the state of health, the lifestyle, living conditions, nutritional habits etc. And comparative studies in various districts of Debrecen. The statistical evaluation of these comparative studies is progressing steadily. Differences in the laboratory values between the genders were studied using the Mann-Whitney test. Associations among the variables were assessed with Spearman,s correlation coefficient.
The study was supported by the Health Science Council of the Ministry of Health and the Debrecen Health Service Public Benefit Company.

Features of rheological properties of blood in the patients with cerebro-vascular pathology.

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A wide specter of researches of analysis of pathogenesis of cerebrovascular pathology (CVP) points important and determinative role of haemorheological abnormalities in development of ischemic CVP. Usually significant shears of rheological properties of blood is being discovered under condition of marked morphological damages of vessels of arterial system of head and heard, but at the same time an influence of attendant metabolic disorders such as hypercholesterolemia, hypertriglyceridemia, hyperinsulinemia and others are exist. The questions of estimate of status rheological properties of blood and optimization of correction of determinative abnormalities in the patients with CVP is very actual in connection with variety of associated and interactional biochemical, coagulological, and biomechanical processes in flow blood and endothelium.

The purpose of the research was to study the status of rheological properties of blood in patients with acute ischemic strokes and chronic CVP with estimate of efficacy the preparations with vasoactive and antiaggregate properties used in neurological clinics. There were 60 patients with acute stroke included that were examined within first 48 hours, then in 5-7 days and 21 day after course of treatment. The second group (n=28): patients with chronicle CVP and discirculator encephalopathy (DE). The third group (n=25): patients with arterial hypertension (AH). The main background disease in these patients was atherosclerosis and AH. 42 patients had metabolic syndrome which showed insulin resistance, abnormalities of lipid metabolism and increased arterial pressure of more then 140/90 mm.

The research of rheological properties of red blood cells (RBC) was being analysed by LoRca (Netherlands). Viscosity of whole blood was analyzed by the rotary viscosimeter AKR-2 (Russia). Aggregability of platelets was analyzed by the aggregometer Biola (Russia). It was estimated an influence the medications with antiaggregate properties on the function of platelets and RBC in vitro. All the analyses were done before and after „cuff test" and were compared to data of control group of healthy persons (n-20).

It was determined that increase of aggregation index (AI) and strength of aggregates, shortening of the times of spontaneous aggregation T1 and T2 were more expressed in the patients with AH compared to healthy subjects. Analysis of data showed that a gradual decrease of AI took place in the patients with stroke from the acute phase to 21 day of disease. At the same time there was increase of strength of aggregates, which could influence negatively to microcirculation. The decrease of maximal index of RBC deformability with shear stress 60 mPA (DImax) in acute phase of disease became much lower by 7 day but increased by 21 day. Studying of influence of short ischemia of upper arm „cuff test‰ on rheological characteristics of RBC in the examined groups of patients determined not only worsening number of researching haemorheological parameters but also let us establish individual effects of medications with vasoactive and antiaggregate properties in the patients with CVP.

The results of longstanding experience of using of testing of individual sensibility of platelets and RBC to medications that was applied in practice of our laboratory researches demonstrates not only need an individual selection of medication but also working out the ways of efficacy of their influence.

Studies on the aggregation behavior of pegylated human red blood cells with the Zeta sedimentation technique

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Introduction Covalent binding of poly(ethylene glycol) [PEG] to red blood cells (RBC) surface have two significant effects. First, it leads to masking of the RBC blood group determinants and in this way reduces the risk of adverse blood transfusion reactions. Second, the PEG layer on the cell surface sterically hinders RBC-RBC and RBC-plasma protein interactions and in this manner prevents RBC aggregation and improves blood flow and oxygen supply. Here we report our preliminary results on the aggregation behavior of pegylated RBC with the Zeta sedimentation technique, which allows modifying the cell-cell interactions pressing them toward each other applying centrifugal forces of various magnitude and duration.

Material and methods Washed human RBC were used throughout this study. Covalent linking of linear mPEG of various molecular mass (2000, 5000, 20000) was achieved at room temperature by incubation of the cells in isotonic phosphate buffered saline (PBS, pH = 7.4) containing the polymers at different concentrations. After two additional washes the RBC were suspended at hematocrit of 0.40 (v/v) in PBS containing free dextran (MW 70 000) at different concentrations as aggregating agent. The Zeta sedimentation was followed up with home made apparatus using the standard procedure – 4 cycles of rotation with 45 second duration, read outs after every cycle. In addition the centrifugation force was varied. The RBC surface modification was also followed up by particle electrophoresis.

Results The electrophoretic mobility (EM) of the RBC decreases with increasing of chain length and concentration of PEG used for surface coating, as found also by other investigators. It may be due to elevation of viscosity in the shear plane or to shifting of the shear plane caused by the presence of the polymer at surface of the RBC.

The aggregation behavior of the pegylated RBC was found to be a complicated function of concentration and PEG chain length on the surface, but also to depend on the concentration of aggregating agent and on the centrifugation force used to enforce the cells toward each other. As a rule at low centrifugation forces the increase in chain length and concentration of PEG linked to RBC surface reduces the aggregation probably via elevation of the steric repulsion, which counteracts the depletion force generated by the free polymer in the surrounding medium. However with increasing the centrifugation forces on the cells in particular situations in the energy balance of RBC-RBC interactions a contribution of polymer cross linking may also play a significant role reversing to some extend the steric hindrance.

Conclusions Our results suggest a very complicated situation in the interaction energy balance between RBC-RBC and in this way in their aggregation behavior when depletion force, steric hindrance and mechanical enforcement are varied at the same time. To predict the aggregation state of the RBC it is afforded to weight very carefully all the energetic contributions in their interactions energy balance, which requires further experimental and theoretical investigations.

Evaluation of quantitative contrast harmonic imaging to detect the malignancy of hepatic tumors: A controlled prospective two centre study

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PURPOSE To establish the extent to which contrast enhancement with SonoVue in combination with quantitative evaluation of contrast medium dynamics facilitates the detection of hepatic tumors
METHOD AND MATERIALS 100 patients with histologically confirmed malignant or benign hepatic tumors, were analysed (maximum size of 5 cm). Contrast-enhanced ultrasonography (bolus injection of 2.4 ml SonoVue) was carried out with the intermittent breath-holding technique using an multifrequency scanner (2.5 – 4 MHz, Logic 9, GE). Native vascularization was analysed with power doppler. The contrast-enhanced dynamic ultrasound investigation was carried out with Contrast Harmonic Imaging (CHI) in true detection mode during the arterial, portovenous and late phase. MI was set at 0.15. Perfusion analysis was performed by postprocessing of the raw data (TIC analysis). The cut-off of the grey value differences between the tumor and normal liver tissue was established using ROC analysis. The 64-line multi-slice CT served as reference method in all cases. MRT findings were used in addition in 19 cases
RESULTS 100 patients with 59 malignant (43 colon, 5 breast, 2 endocrine metastases, 7 HCC and 2 kidney cancers) and 41 benign tumors (15 hemangiomas, 7 focal nodular hyperplasias, 5 complicated cysts, 2 abscesses, 12 local fatty changes) were included. The late venous phase proved to be the most sensitive for classifying the tumor type. 58/59 malignant tumors were classified as correct positive, one patient as false negative. This results in a good sensitivity of 98.3%. Of the 41 benign tumors, 37 were classified as correct negative and four as false negative, which corresponds to a specificity of 90.2%. Altogether, 95.0% of the diagnoses were classifed correctly compared to the histological classification. There was no investigator dependence (p=0.23).
CONCLUSION The results show that it appears possible to predict the malignancy of hepatic tumors with a probability of 93.5% (positive predictive value in the late venous phase, or rule it out with a probability of 97.4%.

On the Effect of Microstructural Changes of Blood on Energy Dissipation in Couette Flow

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Red blood cell (RBC) aggregation affects significantly the flow of blood at low shear rates; not only the behaviour of the fluid deviates from its Newtonian characteristics, but, depending on the shearing history of the flow, the non–Newtonian characteristics may be influenced. It is not clear how the time-dependent microstructural characteristics of the fluid affect its mechanical properties, when they are measured under dynamic flow conditions. The present study aims to improve understanding of the effect of dynamic flow conditions on aggregate formation and consequently on the mechanical properties of the fluid. Results from four different tests are correlated and discussed in relation to the energy dissipation across the gap of a Couette flow; viscosity and viscoelastic measurements on anti-coagulated, whole blood samples from healthy volunteers (adjusted at 0.45 hematocrit) were taken with a double-walled Couette rheometric cell, under quasi-unsteady flow conditions. The aggregation extent index Aa, and the microstructural integrity index AI, which accounts for the inter-aggregate branching characteristics, were assessed, at flow conditions comparable to rheometric ones, with a plate-plate optical shearing system and image analysis. Wall slip and sedimentation effects in the Couette flow have been minimised by using appropriate surface roughening, shearing gap sizes, and flow parameters. Initial results for flows at different shear rate variations with time show that changes in the non–Newtonian characteristics of the fluid can result from an increase of aggregation (Aa) and a decrease in the microstructural integrity (AI) of the fluid. More specifically, at low shear rate variations with time the aggregation index Aa increases, viscosity decreases and the structural integrity index AI decreases.
It is suggested that energy losses in a rheometric Couette flow, in addition to other characteristics of the fluid suspension, depend on both the extent of aggregation and the structural integrity of blood at different stages of the flow. A detailed analysis and discussion of the results will be presented in the conference.

The impairment of platelet function and plasmatic coagulation in faecalperitonitis and endotoxemia are dependent on the volume challenge

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Introduction: Volume administration is one of the mainstays in shock treatment. The administration of large amounts of fluid may interfere with coagulation. We hypothesized, that the severity of derangement of coagulation in sepsis is related to the underlying disease and volume management.
Methods: Pigs were randomly assigned to control (C), infection (endotoxemia (E), faecal peritonitis (P)), and other disease (hypoxia (Hy; n=8), pericardial tamponade (T; n=9)). High (-H) and low (-L) volume (20ml/kg/h [¾ Ringer´s Lactate (RL), ¼ Voluven] vs 10ml/kg/h [RL]) were given in C (CH; n=7) / CL; n=7), E (EH; n=5/ EL; n=5) and P (PH; n=7/ PL; n=6). In Hy and T only low volume was administered. Changes in whole blood coagulation, the plasmatic and platelet component were assessed by computed thrombelastography (TEG). Blood lactate, muscle pO2 and arterial doppler flow were measured simultaneously and correlated to TEG.
Results: A non significant decrease of the coagulation status was observed in the Hy- and T-group. P and E significantly decreased haemostasis (n<.05). High volume deranged the status more than low volume application in C (ns) and infection ($p<0.05$). In the high volume group and in precocious death in the low volume group, the contribution of platelets to clot strength decreased ($p<0.05$). Altogether, clot strength was positively correlated to arterial flow and negatively correlated to lactate level.
Discussion: Peritonitis and endotoxemia derange the coagulation status in pigs. High volume administration additionally aggravates hypocoagulability. A correlation between coagulation, blood flow and metabolic changes may be suspected, but requires further investigations.

The impact of hemorheological oxygen carrying capacity on cardiac morbidity and mortality

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A number of conventional risk factors and some hemorheological parameters have been associated with the mortality of coronary heart disease (CHD) patients. We aimed to compare the predictive power of these parameters and the hematocrit per blood viscosity (Hct/BV) ratio as the hemorheological oxygen carrying capacity of the blood to assess hospitalisation and mortality risk of CHD.
In a retrospective cohort study elective coronary angiography was performed on 109 consecutive CHD patients between 1996 and 1997. In 78 cases (72%) complete follow-up information was obtained in February 2006. During the follow-up time (mean 8.9 years) 10 patients died due to cardiac cause (group C). Two patients died due to non-cardiac cause and 66 were still alive at the end of the follow-up period (group NC, n=68). Among parameters measured at the time of coronary angiography, only mean Hct/BV ratios were significantly different between groups C and NC (87+/-5 and 93+/-9 Pa-1s-1, SD, respectively, p=0.022). Other conventional risk factors (body mass index, serum cholesterol, fibrinogen, hematocrit, plasma and blood viscosity, cardiac index, left ventricular ejection fraction) provided no statistical differences. Kaplan-Meier survival analysis showed only the impact of fibrinogen and Hct/BV ratio on cardiac mortality (p=0.029 and 0.009, respectively). Receiver operating characteristic curves proved only Hct/BV ratio to be able to differentiate between groups (area under curve: 0.716, p=0.028). Hct/BV ratio showed significant negative correlation with the number of hospital admissions due to cardiac reasons in patients who were alive at the end of the follow-up period (r= -0.377, p=0.03).
Conclusion: Hct/BV ratio can be regarded as a potentially reliable marker for the risk of cardiac death in CHD patients. This conclusion needs further examination involving greater patient cohorts.

Plasma viscosity: a forgotten variable?

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Evaluation of plasma viscosity has been under-used in clinical practice. Plasma viscosity is determined by water-content and macromolecular components. Plasma is a highly concentrated protein solution, therefore weak protein-protein interactions can play a role that is not characterized by electrophoresis. The effect of a protein on plasma viscosity depends on its molecular weight and structure. The less spheroid shape, the higher molecular weight, the higher aggregating capacity, and the higher temperature or pH sensitivity a protein has, the higher plasma viscosity results. Plasma is a Newtonian fluid, its viscosity does not depend on flow characteristics, therefore it is simple to measure, especially in capillary viscosimeters. Its normal value is 1.10-1.30 mPas at 37°C and independent of age and gender. The measurement has high stability and accuracy, thus little alterations may be pathologically important. Inflammations, tissue injuries resulting in plasma protein changes can increase its value with high sensitivity, though low specificity. It can increase in parallel with erythrocyte sedimentation rate (ESR), but it is not influenced by hematocrit (e.g., anemia, polycytemia), or time to analysis. Based on these favorable features, in 1942 plasma viscosity was recommended to substitute ESR. In hyperviscosity syndromes plasma viscosity is better in follow-up than ESR. In rheumatoid arthritis, its sensitivity and specificity are better than that of ESR or C-reactive protein. Plasma fibrinogen concentration and plasma viscosity are elevated in unstable angina pectoris and stroke and their higher values are associated with higher rate of adverse clinical events. Elevation of plasma viscosity correlates to the progression of coronary and peripheral artery diseases. In conclusion, plasma viscosity should be measured routinely in medical practice.

Incidence of transfusion requiring bleedings after cardiac surgery operations

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Main reasons for intra- and postoperative blood transfusions after heart surgery are inadequate surgical technique, hemostatic disorders or a combination of both. This combination leads to an increase in postoperative morbidity and mortality, because blood transfusions are an important factor for long term survival after cardiac surgery [1]. The operative strategy as well as the kind and duration of extracorporeal circulation influence the dilution of clotting factors, fibrinolysis and the extent of thrombocyte function disturbances and consequently the risk of postoperative bleeding and requirement for blood transfusion [2].
In our current registry the data of 4.429 patients were reviewed retrospectively looking for the number of intra- and postoperative transfused red blood cells concentrates fresh frozen plasma and thrombocytes.
1.483 of the 4.429 patients received blood transfusions. Overall 11.250 units of red blood cells concentrates were given; this is a mean of 2.6 units per patient. 295 (19.9%) of the 1.195 patients who received transfusions got more than 10 units of red blood cells concentrates. As main reason for this, insufficient surgical hemostatsis has to be assumed. Additional thrombocytes were given in 158 (10.8%) patients, 20 patients got more than 2 units of thrombocytes. 40 units of fresh frozen plasma were given intraoperatively, another 704 units during the postoperative course until discharge.
Summary and conclusion of our results: 1. There is no need to interrupt the therapy with thrombocyte aggregation inhibitors preoperatively (current strategy heart center Dresden). 2. The consumption of blood products increases about 43.8% with the complexity of surgery. 3. With postoperative heparin administration the need for blood transfusion increases. 4. Perioperative mortality was higher in patients with older age and those who required blood transfusions.

DEBRECEN Longevity Study: The Hemorheological and the General Health Status in Oldest Old Residents

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The population of Hungary is aging rapidly due to a longer life expectancy coupled with a dramatic decline in the fertility rate. Among the elderly population the number and proportion of the oldest-old (defined as age 90 years or older) will increase most significantly in the near future. Since there was no similar study on this target group in Hungary, we decided to make a systemic analysis of the social, clinical and biological conditions of them under this title: Debrecen Longevity Study. Debrecen is the second largest city in the country, and the oldest old residents represent 0,4 % of the total population (800 residents). The investigation has involved 310 subjects randomly selected from the total oldest-old population.
The probands were examined in their homes with an interview, based on a questionnaire which contained questions about their nutrition, health status, physical activity, etc. After the interview medical and laboratory investigation were performed in order to evaluate the general health status of the subjects. Among other things we examined 18 routine laboratory parameters. Since age-specific reference values are not available for the oldest-old persons neither in clinical practice, nor in the scientific literature, our results could be compared only to the general, adult reference values. Most of the investigated parameters were in the normal range. The alterations were not extreme, but they were statistically significant. The erythrocyte sedimentation rate and plasmaviscosity were slightly increased. There was a moderate reduction in red blood cells, hematocrit and hemoglobin concentration. The lipid parameters (cholesterol, triglyceride, HDL-cholesterol) have been found in the n ormal range. We compared these results with our earlier laboratory screening test (completed in 2001) of Debrecen residents between 60-74 and 75-89 years respectively. Thus we had the opportunity to compare three age groups. We have experienced significant decreasing trends at cholesterol and triglyceride levels, while there is an increasing trend at HDL-cholesterol level.
During aging the population becomes increasingly selected, namely over 90 years only the „survivors" are alive. As the proportion of "survivors" increases, some parameters stop worsening or even show some improvement. Finally we studied the correlations between the values of hemorheological parameters and prevalence of age-associated diseases (hypertension, diabetes, cardiac infarction, cerebrovascular disorders, neoplastic disease, incontinence, limitation of motion, visual and auditory disturbances) in the oldest-old residents.
The study was supported by the Health Science Council of the Ministry of Health and the Debrecen Health Service Public Benefit Company.

Comparison of rheological properties of erythrocytes in patients with claudication after treadmill and cycloergometer training. Examination with LORCA device

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Objectives: to assess the effect of different methods of training on red blood cell deformability and aggregation.
Design: randomized – controlled trial of exercise training
Setting: patients was recruited from vascular outpatient clinic.
Material and Methods: A total of 45 patients with peripheral arterial occlusive disease (stage II according to Fontaine) were randomized to the treadmill (n= 21) or the cycloergometer group (n=24). Thirthy seven patients completed the study (16 in the treadmill group and 21 in the cycloergometer group) Every patient was exercising 3 times a week for 3 months. Each session consisted of 30 minutes repetitive efforts either on the treadmill or cycloergometer. Exercise was performed till 85% of the pain free walking or cycling time, stopped and restarted again. The training program was supervised by a physioterapist and doctor. Changes in erythrocytes deformability (EI- Elongation Index) and erythrocytes aggregation (T ½ -the time to reach one half of the maximum aggregation) measured by LORCA device before and afterthe intervention.
Results: Before the treatment groups were similar in all observed parameters. A significant improvement of walking ability was observed after the treatment. Cycloergometr training group increased red cell deformability ($p<0,02$), respective changes in treadmill training group was only borderline significant ($p<0,065$). Changes in deformability depended on EI before the treatment ($p<0,0001$), type of exercise ($p<0,004$, treadmill had higher impact), hematocrit($p<0,01$), hemoglobin ($p<0,025$), and less on erythrocytes count ($p<0,06$). Aggregation was decreased in both group after the training and effect was correlated with fibrinogen level ($p<0,0001$), platelet count ($p<0,02$), erythrocytes count ($p<007$) but not with type of exercise.
Conclusion: Model of pain-free exercise training improved rheological properties of erythrocytes in both group. Changes in deformability were higher in the cycloergometer group, however, mode of exercise had no effect on aggregation. Initial EI, type of exercise, hematocrit, hemoglobin, erythrocytes count influenced on deformability while fibrinogen and platelet count influenced on aggregation.

Regulation of para- and transendothelial barrier function by caveolin-1

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The endothelium regulates the exchange of small solutes and macromolecules by a transcellular and a paracellular route. While paracellular permeability requires an activation of the VE-cadherin/catenin complex, a transcellular transport, e. g. of albumin is mediated by caveolae. In the regulation of both permeability routes caveolin-1, the main scaffold protein of caveolae, seems to be critically involved. Here we demonstrate a critical role of caveolin-1 in the down regulation of the paracellular endothelial barrier function after thrombin stimulation by a dynamin II- and eps15-dependent endocytosis of junctional proteins. On the other hand, in caveolin-1 negative endothelioma cells the constitutive endocytosis of albumin was two fold enhanced compared to caveolin-1 wt cells or cav-1 -/- cells re-expressing caveolin-1. These data indicate that caveolin-1 has a dual role on the endothelial barrier function. It is required for the opening of intercellular junctions but it negatively regulates albumin uptake in endothelial cells.

Ophthalmological Diagnostics of Endothelial Dysfunction. Impact of the Dynamic Vessel Analyzer

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The fundus of the eye is an excellent choice for the diagnostics of systemic vessel changes as it allows non-invasive observations. Dynamic vessel analysis (DVA) is a new method which is very promising and has a potential for many applications. It has been further developed from static vessel analysis (SVA) that uses a retinal image to determine the artery/vein-ratio of retinal vessels. However, SVA lacks the possibility of functional analysis and has therefore a limited significance.
The Retinal Vessel Analyzer (RVA) has been further developed and equipped with the technology for DVA in addition to it's standard features for quantitative measurements of vessel calibres (SVA).
The RVA now allows observations on vessel rigidity and on contracting and dilating behaviour of specified vessel segments. Apart from that, important information on the auto-regulation of microcirculation can be obtained from the quantification of vessel diameter changes during a certain time unit.
First results of vessel studies using DVA will be presented. These include the age-dependence of dynamic vascular response. A significant decline of the overall amplitude of responses (vascular dilatation plus constriction) has been found with increasing age. They also include the influence of physical activity on vascular dynamics. Probands that were initially physically inactive show an improvement of retinal vessel responsiveness after training units of 1.5 hours per week.
For the interpretation of the results, the normal ageing process has to be taken into consideration and be distinguished from pathological changes.
The RVA offers a technique that can be standardised and has a high potential for monitoring micro vascular risk. It can also be extremely valuable for tests on vessel-dilating effects of drugs. DVA shows great promise to become an important means for the evaluation and follow up of therapeutic concepts in a number of medical fields.

Anticoagulant properties of a sulphate low molecular weight galacto-mannan derivative from yamopsis tetragonoloba (L.) Taub. seeds

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Polysaccharides with anticoagulant and antithrombotic activities have been found in mammals, marine alga, nontoxic mushroom Auricularia auricula, medicinal plant Porana volubilis and in-vertebrates.
The purpose of this work ˆ research of an anticoagulant activity of a sulfate galactomannans (GM) with different molecular weights (MW) (for deriving low molecular weight GM ˆ LMW GM - with the enzyme hydrolizis help it was used GM from yamopsis tetragonoloba (L.) Taub. seeds); evaluation of the possibility of a complex formation between obtained samples and protamin sulphate. For determination of antithrombin specific activity (anti-IIa activity) of the LMW GM we used a method based on thrombin abilities to split synthetic oligopeptide substrates. As a standard we took 5-th International UFH standard. Heparin specific activity against factor Xa (anti-Xa activity) determined with the help of the 1-st International LMWH standard. In order to determine anticoagulant effects of LMW GM anti-IIa and anti-Xa amidolytic activi-ties were measured. Biospecific electrophoresis in 1% agarose with protamin sulphate was used for LMW GM/sulfate protamin complexes determination. Precipitation zones in the „rocket‰ form were generated. The „rocket‰ squares estimated with the help of PhptoM program. LMW GM wiht MW 12.6; 54,2; 81,9; 132,3; 200,1; 245.6 kDa demonstrated anti-IIa activity from 64 IU/mg to 87 IU/mg, anti-Xa activity from 6,8 IU/mg to 13,0 IU/mg. Any connection between MW and anticoagulant activities it is not revealed. LMW GM derivatives produced a concentration-dependent prolongation APTT and TT tests. All from obtained LMW GM created complexes with sulphate protamin. It was observed moderate positive connection between height/square of the precipitation zones and antithrombin activity. LMW GM drivatives were obtained with the enzyme comlex hydrolisis help have specific anti-thrombin activity more 70 IU/mg, that is necessary for antithrombotic preparations. The height/square of the precipitation zones with sulphate protamin were found to increase with in-creasing antithrombin activity of LMW GM.
This work was supported by the Russian Foundation for Basic Researches, Grants 05-04-08100-1086;fr-1072; 8470; 05-03-32883-a; 06-04-08140-ofr

Effects of the new ampicillin derivative KKP723 on the intestinal microcirculation in experimental endotoxemia

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We evaluated the effects of the KKP723 (KKP), a derivative of ampicillin, on the intestinal microcirculation in experimental endotoxemia using intravital microscopy (IVM) in order to search for side effects of this newly developed ß-lactam antibiotic.
Four groups of animals were studied: control group, endotoxemic group (15 mg/kg i.v. LPS from E. coli), ampicillin (50 mg/kg i.v.) treated endotoxemic group and endotoxemic group treated with KKP (67.4 mg/kg i.v.). Ampicillin treatment resulted in a significant reduced number of firmly adhering leukocytes in intestinal submucosal venules. KKP treatment did not show this effect on leukocyte activation. We found no changes of the functional capillary density (FCD) of the intestinal wall by treatment with ampicillin or its derivative KKP. The increased leukocyte adherence in the KKP treated LPS animals may be explained by a loss of a possible ampicillin-related anti-inflammatory effect by the biotransformation process.
The endotoxemia IVM model is useful to detect side effects of older and newer antibiotics in an impaired microcirculation.

Sodium selenite administration improves intestinal microcirculation without affecting cytokine release in experimental endotoxemia

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We evaluated the effects of sodium selenite (SEL) administration on the intestinal microcirculation and the release of the cytokines TNF-?, IL-1ß, IL-6 and IL-10 in experimental endotoxemia (induced by lipopolysaccharide - LPS). Three groups of animals (n=30) were studied: control group, endotoxemic group (15 mg/kg i.v. LPS from E. coli) and SEL treated LPS group (100 µg/kg SEL i.v.). SEL treatment resulted in a significant reduced number of firmly adhering leukocytes in intestinal submucosal venules and reduced significantly the impairment of the intestinal functional capillary density. Despite of the improvement of microcirculatory parameters, we did not detect any changes in the pattern of cytokine release. In conclusion, administration of the antioxidant sodium selenite attenuates leukocyte adhesion and improves capillary perfusion within the intestinal microcirculation without affecting release of the cytokines TNF-?, IL-1ß, IL-6 and IL-10 in experimental endotoxemia.

Hemorheology in Stable Angina and Acute Coronary Syndrome: a Rheological Basis for Transition?

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The transition from stable angina (SA) to acute coronary syndrome (ACS) primarily results from the rupture of vulnerable plaque due to fluid forces associated with local blood flow. Inasmuch as elevated blood rheological factors lead to increases of these forces, we tested the hypothesis that ACS blood would exhibit higher viscosity and greater RBC aggregation than SA blood.

Blood samples were obtained from 51 angina patients at the LAC-USC Medical Center just prior to diagnostic cardiac angiography. Subsequent patient classification indicated 16 SA and 35 ACS subjects; the latter group was sub-classified as 16 unstable angina (UA) and 19 acute myocardial infarction (AMI). Rheological measurements included whole blood viscosity, plasma viscosity, and RBC aggregation via erythrocyte sedimentation rate (ESR) and by aggregation indices at stasis (M) and low shear (M1) using a Myrenne aggregometer; routine clinical laboratory data were also obtained.

Compared to SA patients, ACS subjects had significantly worse rheological parameters: 1) Higher whole blood viscosity at low shear (17-36% increase, $p<0.001$), and elevated plasma viscosity (1.71±0.12 vs. 1.59±0.07 cP, $p<0.001$); 2) Enhanced RBC aggregation as judged by ESR (58.2±25.3 vs. 35.7±15.5 mm/hr, $p<0.001$), and by RBC aggregation indices at stasis (49% higher M, $p<0.005$) and at low shear (52% higher M1, $p<0.005$); 3) Elevated WBC (8.65±2.44 vs. 7.19±1.87x109/L, $p<0.05$) and hs-CRP (3.89±3.34 vs. 1.17±1.44 mg/L, $p<0.001$). The rank order of rheological abnormalities was AMI>UA>SA. No significant differences were found for age, sex, BMI, and cholesterol profiles.

Our findings of significant hemorheological differences between SA and ACS patients suggest: 1) a progressive worsening of blood rheology promoting the development of ACS; 2) the possibility of an unstable equilibrium between plaque vulnerability and a critical level of rheological factors required for triggering plaque rupture. Prospective studies are required to test this hypothesis: if validated, therapy aimed at regulating blood rheology in SA patients may lead to improved patient care, clinical outcome and better prevention.

Hemorheological Abnormalities in Cardiac Syndrome X as a Cause of Microcirculatory Dysfunction

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Cardiac syndrome X (CSX) continues to be of basic science and clinical interest, yet the mechanism(s) involved have not been fully elucidated. However, it is well known that elevated RBC aggregation and blood viscosity can adversely affect in vivo microcirculatory blood flow: we thus tested the hypothesis that CSX is characterized by such hemorheological abnormalities.

Blood samples were obtained from adult angina patients at the LAC-USC Medical Center just prior to diagnostic cardiac angiography. Rheological measurements were carried out within six hours, with CSX subjects later identified based upon angiographically-normal coronary arteries, typical angina, and a positive stress test. Twenty-one CSX subjects were studied and compared to 21 age and sex matched controls without angina or clinical evidence of coronary arterial disease. Rheological measurements included whole blood viscosity (0.5 to 1500 sec-1 shear rate), yield shear stress, plasma viscosity, RBC aggregation via erythrocyte sedimentation rate (ESR) and by aggregation indices at stasis (M) and low shear (M1) using a Myrenne aggregometer; clinical laboratory data and TIMI frame counts(CTFC) were also obtained.

CSX patients had markedly abnormal blood rheology: 1) Higher RBC aggregation as judged by ESR (3-fold greater, $p<0.001$); 2) Enhanced RBC aggregation index at stasis (49% higher M, $p<0.005$) and at low shear (52% higher M1, $p<0.005$); 3) Elevated whole blood viscosity ($p<0.001$ at lower shear rates), plasma viscosity (1.66 ± 0.11 vs. 1.56 ± 0.10 cP, $p<0.001$), and yield stress (38% higher, $p=0.002$). CSX patients had high WBC counts (7.6 vs. 5.8x109/L, $p<0.05$) and CRP levels (2.85 ± 2.00 vs. 0.51 ± 0.58mg/dl, $p<0.001$), and a mean CTFC of 25.8 ± 5.6.

Our findings suggest that abnormal hemorheological parameters contribute importantly to the etiology of CSX, presumably via adversely affecting blood flow and RBC flux in the coronary microcirculation. Further, they indicate the possibility of therapeutic measures aimed at normalizing blood rheology (e.g., plasmapheresis) and hence microcirculatory flow.

Erectile dysfunction – a local or systemic microvascular disorder?

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Round about one half of the men at the age between 40-70 years suffer from any form of erectile dysfunction (ED). The prevalence of ED is age related and increases threefold from 5% to 15% at the age of forty or seventy, respectively. Moreover, the prevalence of ED is much higher in patients with coronary artery disease (CAD). Two thirds of the men with a coronary three vessel disease and chronic stable angina have ED. Cardiovascular risk factors such as hypertension, hypercholesterolemia, diabetes mellitus or cigarette smoking are known causes of a disturbed homeostatic equilibrium of vascular endothelial cells, referred to as endothelial dysfunction, which plays a pivotal role in the pathophysiology of vasculogenic ED. Among others, endothelial dysfunction is associated with an impaired release and activity of nitric oxide (NO)/cyclic guanosine monophosphate (cGMP). NO is a vasodilatory neurotransmitter, required for the relaxation of arterial smooth muscles by cGMP.
The impairment of the NO-pathway is the critical determinant for the decrease in vasodilatory capacity, especially in the microvasculature, in patients with endothelial dysfunction. The penis is a highly vascularized part of the body and the concentration of endothelial cells in it is relatively many times that amount of other organs. Due to the small diameter of the cavernosal arteries of approximately 100 µm, which belong to the microvascular bed, it can be assumed that ED belongs to the group of microvascular disorders. Firmness and maintenance of the penile erection is a result of the highest increase of blood flow compared to all other vascular regions of the body, which requires the vasodilatory effect of the NO system. This is counterregulated by the cleavage of cGMP by phosphodiesterase type 5 (PDE-5). In early stages of atherosclerosis without clinical symptoms, high concentrations of PDE-5, in particular of the subtype PDE-5 A2, and a reduced release NO can be found in both, the smooth muscle cells of the cavernous body and of coronary arteries. Furthermore, a systemic mismatch of other vasodilating and vasoconstricting mediators like prostacyclin or endothelin develops in the course of atherosclerosis which in the first place affects the regulation of the microcirculation and reveals its early clinical symptoms around the penile vasculature. Consequently, striking evidence for endothelial dysfunction as the common underlying pathophysiological process and, therefore, a close clinical association of ED and CAD has accumulated, in which ED seems to precede CAD.

Can exercise training improve endothelial dysfunction?

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Impaired endothelial function has been well documented in individuals with baseline dysfunction such as the elderly, asymptomatic subjects with cardiovascular disease (CVD) risk factors, and patients suffering from CVD. Emerging evidence supports that physical inactivity may be considered an important and independent risk factor for vascular events, predisposing sedentary healthy individuals to increased CVD morbidity and mortality. Presently, there is growing interest in defining whether physical inactivity in healthy subjects may induce endothelial dysfunction, probably due to an increased susceptibility to oxidative stress, and whether a short-term exercise intervention may improve circulating biomarkers of endothelial health. This study investigated in a cohort of sedentary healthy subjects the effects on plasma nitrate/nitrite (NOx) levels as an index of nitric oxide (NO) bioavailability, extracellular superoxide dismutase (EC-SOD) activity, plasma redox status, lipoprotein-li pid profile, and native low-density lipoprotein (LDL) oxidation, after an acute bout of strenuous exercise, in order to induce a transient oxidative condition, and (about one year later) after 20 weeks of aerobic exercise training programme. The results indicate that both acute strenuous physical stressor and exercise training increase plasma NOx content with respect to pre-exercise values, but no statistically significant differences were detected between plasma NOx levels measured after a single bout of exercise and training program. Notably, a significant decrease of EC-SOD activity and total plasma antioxidant defences as well as an increased oxidation of LDL in vitro were observed after acute strenuous exercise, while each of these parameters resulted improved after moderate regular exercise. Taken together, the results suggest that physical inactivity predisposes to an altered endothelial function via a significant susceptibility to oxidative stress, whereas short-term exerci se intervention may improve endothelial function by enhancing the compensatory mechanisms toward an oxidative insult.

Regulation of myocardial microcirculation

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The undisturbed microcirculation is an absolute prerequisite for the sufficient oxygen supply of tissue as well as the metabolism. The oxygen partial pressure of the myocardium is determined by the convectional O_2-supply and the oxygen need: Both a reduction of O_2-supply and an increase of the oxygen need would lead to less local oxygen partial pressure and thus, depending on the extent, to a decrement of supply or even ischemia.
The heart is almost entirely depending on oxygen supply to keep up its aerobic metabolism. Its oxygen capacity allows only a low oxygen fault. The oxygen storage of the myocardium (hemoglobin and myoglobin) is about 0.4 µ mol/g. If one assumes an ATP turnover of 20 -30 µmol/min/g and an oxygen consumption of 4-5 µmol/min/g, the reserve time of the myocardium is only few seconds at interruption of the circulation before considerable functional consequences appear. Irreversible damages or necroses will take place within 20 minutes at normothermal ischemia. To ensure myocardial circulation, the organism has therefore developed a number of regulatory mechanisms.
The three essential determinants of the O_2 need are heart frequency, contractility and myocardial wall tension. The primary determinant is the heart frequency: Doubling the heart frequency leads to a doubling of the oxygen need. This can be maintained approximately up to a heart frequency of 110 beats/minute under physiological conditions, beyond that frequency the myocardial pO_2 decreases. Contractility is increased by inotropic stimuli (e.g. $Potassium^{++}$, Catecholamine). The net effect of these stimuli on the O_2 need is the result of two contrary mechanisms: the wall tension (which decreases as a consequence of a diminution of the heart size) and myocardial contractility (which increases). Physiologically heart size and with that the wall tension is not reduced fundamentally. With that an increase of contractility leads to a clear rise of the oxygen need.
Convectional supply of oxygen to the myocardial tissue is ensured by a sufficient artery system and an undisturbed flow into the microcirculation. Of equal importance is uptake, binding, and release of oxygen. Any disturbance of oxygen supply, binding or oxygen diffusion or local oxygen consumption leads to an imbalance of tissue oxygen homoeostasis and thus to myocardial dysfunction.
Like in every vessel system the mode of myocardial blood flow is, driving pressure of the arterial inflow and vessel resistance. Regulation of the convectional oxygen supply is made by a variation of the vessel diameters. Already a small change in vessel diameter causes a strong increase of blood flow (Poiseuille law). The diameter of coronary vessels is influenced by a variety of different mechanisms. Neural and neurohumoral effects, myocardial metabolism, endothelial function, autoregulation, myogenic control and extra vascular pressure gradients. Capillary recruitment and rheological factors might as well influence the microcirculation. These regulatory mechanisms are not isolated entities but rather interconnecting and dependent of each other. Additionally each mechanisms targets at a different level and at a different vessel size.

Apheresis in coronary artery disease: Findings in PET-studies²

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Hypercholesterolemia impairs endothelial function and subsequently decreases coronary vasodilatatory capacity. We examined the quantitative effects of one single LDL apheresis on vasodilatatory capacity. Using N-13 ammonia as a tracer for dynamic quantitative positron emission tomography (PET), mean myocardial perfusion measurements were performed before and 20 hours later after LDL apheresis, both under resting conditions and after pharmacological vasodilatation with dipyridamole. LDL apheresis was performed using the H.E.L.P. (heparin induced extracorporal LDL precipitation) procedure.
Patients and Methods: We examined 47 patients (12 women and 35 men), with angiographically proven coronary artery disease. All of them suffered from hyper-cholesterolemia. 35 patients were subject to chronic weekly H.E.L.P. procedure (group A), while H.E.L.P. procedure treatment was started for the first time in 12 patients, who were subsequently enrolled in a chronic apheresis program (group B). H.E.L.P. was combined with cholesterol lowering drugs in all patients.
Results: Both groups underwent positron emission tomography twice (prior to LDL apheresis and 20 hours later). In group A, LDL cholesterol levels decreased from 175±50 mg/dl to 60±21mg/dl immediately after H.E.L.P. (77±25 mg/dl before the second PET). Corresponding values for fibrinogen levels were 287±75 mg/dl to 102±29 mg/dl (155±52 mg/dl), minimal coronary resistance dropped from 0.56±0.20 to 0.44±0.17 mmHg∗100g∗min/ml ($p<0.0001$). Plasma viscosity decreased by 7.8%.
In group B, LDL cholesterol decreased from 187±45 mg/dl to 75 ±27 mg/dl (85±29 mg/dl) and fibrinogen from 348±65 mg/dl to 126±38 mg/dl (168±45 mg/dl). Minimal coronary resistance was reduced from 0.61±0.23 to 0.53±0.19 mmHg∗100g∗min/ml ($p<0.01$). Plasma viscosity was observed to decrease by 7.6%.
Conclusion: The strong LDL drop in patients under chronic H.E.L.P. treatment has a significant impact on coronary vasodilatatory capacity within 20 hours leading to an improved overall cardiac perfusion. Nearly the same effect can be observed in patients after the first H.E.L.P. treatment.

Hematocrit and sympathovagal balance in young athletes during different sporting seasons

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Exact physiological mechanisms of hematocrit regulation in athletes are not known. Exercise training increases parasympathetic tone and/or decrease the sympathetic nervous system activity at rest which may contribute to the reduction in hematocrit associated with regular exercise. The aim was to study relationships between hematological parameters and sympathovagal balance at rest as LF/HF ratio.
Methods. Venous blood was analyzed in 19 young skiers (14-16 y) and 9 young controls (14-16 y). The blood was analyzed (36 h post exercise in athletes) using autoanalyser. Sympathovagal balance was assayed by power spectral analysis of heart rate variability (LF/HF ratio). The sporting seasons were categorized into three periods: autumn (training phase), winter (competition phase), and spring (recovery phase).
Results. Ht increase in winter (44.5±1.7%) compared to autumn (42.7±2.0%, p=0.0009) and spring 43.7±0.9% (p=0.01). MCV was higher in winter (p<0.01) and spring compared to autumn (p<0.01) (82.3±5.7, 85.4±5.5, 86.7±4.9 in autumn, winter and spring respectively). RBC levels were lower in spring compared to winter and autumn (all p<0.05) (RBC: 5.00±0.40, 5.06±0.32, 4.88±0.33 in autumn, winter and spring respectively). The LF/HF ratio in athletes increased in winter and remain higher in spring compared to autumn (all p<0.01) (LF/HF ratio 0.32±0.2, 0.95±0.45. 1.20±0.90 in autumn, winter and spring respectively). LF/HF ratio change (∆LF/HF) correlated to ∆Ht (r=0.535. p=0.018), ∆MCV (r=0.509, p=0.026) but not correlated to ∆RBC (r=0.35, p=0.12).
Conclusion. Our results indicate that in competition phase of season activation of sympathetic nervous system may play a role in increase in hematocrit in athletes via erythropoiesis and/or release erythrocyte with higher MCV from bone marrow.

Tissue perfusion and thermal imaging: recent advances

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Infrared imaging allows the representation of the surface thermal distribution of the human body. The evolution of technological advances in infrared sensor technology and image processing has resulted in new methods of research and use in medical infrared imaging. New detector materials with improved thermal sensitivity are now available and production of high-density focal plane arrays (640×480) have been achieved. Advance read-out circuitry using on-chip signal processing is in common use. These breakthroughs permit relatively low-cost and easy-to-use camera systems with thermal sensitivity less than 50 mK, as well as spatial resolution of 25–50 μm, given the appropriate optics.
The skin temperature distribution of the human body depends on the complex relationships defining the heat exchange processes between skin tissue, inner tissue, local vasculature, and metabolic activity. All of these processes are mediated and regulated by the sympathetic and parasympathetic activity to maintain the thermal homeostasis. Therefore, infrared imaging may provide quantitative and relevant information, given the appropriate modelling, on micro and macro circulatory phenomena and tissue perfusion.
Several studies have been performed so far to assess the contribution that infrared thermal imaging may provide to the clinicians. In this paper, we will review some of the important recent clinical applications of the thermal imaging and modelling of thermal imaging data of our group. In addition, we will describe some innovative applications of thermal imaging to psychometrics, stress measurements and psycho-neurophysiology.

RBC Aggregation In Vivo: Why Bother? Normalize if Treat the Disease?

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Studies of RBC aggregation have been conducted for decades, and have resulted in new information relevant to cellular factors, disease states, and possible mechanisms. However, until fairly recently, less was known about quantitative relations between aggregation and in vivo phenomena.

Newer investigations have begun to explore this field in greater detail, with publications describing changes in areas such as venous vascular resistance, endothelial NO synthase expression, flow resistance, and myocardial hematocrit gradient. The effects of disease-specific therapy on RBC aggregation are also beginning to be examined in order to determine if correcting the pathology also normalizes rheological parameters. Studies to date have included RBC aggregation and aggregability subsequent to improved glycemic control, recovery from severe sepsis, and correction of hyperlipidemia and hyperfibrinogenemia. Additional work should provide the information needed to firmly link cause-effect-therapy.

Indicative Force of Hemorheological Factors in Hyposplenic and Asplenic States after Splenectomy and spleen Autotransplantation. A Research Summary

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Twenty years ago one of the spleen autotransplantation techniques being known as the Furka,s spleen chip method was developed, related to the preservation of splenic function after traumatization of the healthy spleen, in order to prevent postsplenectomy complications. In the first canine experimental series ultrastructural investigations of autotransplanted spleen chips by electronmicroscopy showed intracellular degeneration of red blood cells and reticular cell necrosis. The erythrocytes in this region were of polygonal shape and highly deformed. In the next comparative experimental series there were two lethal events: one in splenectomy group and one in autotransplantation group, and prior to exitus in both animals red blood cell deformability (relative cell transit time) was markedly impaired. In this dead autotransplantated animal the autopsy and histological analyses revealed the atrophy of transplanted spleen chips. These findings focused our interest on postoperative following-up hemorheological factors after splenectomy and spleen autotransplantation for investigating the changes in splenic function. In the further experimental series in mongrel dogs and later in A/J and BALB/c inbred mice (1999-2006) and inbred beagle dogs (2005-date), besides complex immunological, hematological and hemostaseological tests hemorheological investigations (erythrocyte deformability and aggregation, whole blood and plasma viscosity, fibrinogen concentration) were performed.

Compared to the control, erythrocyte filterability impaired significantly after splenectomy, while autotransplantation improved these values, presenting irregular periodicity during the postoperative weeks and months. Among the functional investigations, changes in erythrocyte deformability and phagocyte activity of peripheral blood could signal functional hyposplenia or possible asplenia, supporting and confirming the results of previous light and electron-microscopic morphological investigations.

In the ongoing research in inbred murine and inbred beagle canine models we compare autotransplantation with various amount of splenic mass and different resection techniques (partial and subtotal spleen resection) with comparative examinations to follow-up the splenic functions.

Grant sponsors: OTKA T 022630, T 034211 and T 049331, ETT 6100/1/2000 and 387/2006.

Reversibility of echinocyte formation of erythrocytes after contact with radiographic contrast agents

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Various radiographic contrast agents (RCA) significantly influence the morphology of erythrocytes, especially the formation of echinocytes [1, 2]. Capillary microscopic studies, however, have shown that these changes of erythrocyte morphology are possibly reversible [3].
The aim of this study was to proof if the RCA-induced echinocyte formation can be reversed by a resuspension in autologous plasma.
The RCM induced echinocyte formation (after suspension of erythrocytes in plasma/RCA mixture for 10 minutes at 37°C) was in parts reversible after resuspension in autologuos plasma (resuspension for 5 minutes at 37°C). Especially for Iomeprol and Iopromid - the RCAs which induced the strongest echinocyte formation after 40% addition of RCA to plasma – a reduction from 94.2% to 44.5% echinocytes and for Iopromid from 80,6% to 50.4% occurred. The echinocyte formation was influenced by the type of RCA as well as by the concentration of RCA. The same was true for the reversibility of echinocyte formation due to resuspension in autologous plasma (type of RCA: p=<0,0001; concentration of RCA: p=0,0847). Iodixanol was associated with the fewest echinocytes formation (after suspension in the plasma/RCA-Mixture as well as after the resuspension in autologuos plasma). A complete reversibility back to discocytes was observed in none of the experimental series.
In conclusion, a significant reversibility of RCA-induced echinocyte formation in autologous plasma could be observed.

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Red Cell Microrheological Properties under Chemotherapy Drug Infusion in Patients with Gastric Cancer

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There are some data about hemorheological changes in patients with malignant tumors. However it is not enough information concerning to red cell microrheology under chemotherapy. The aim of this study was to investigate red cell aggregation and their fluidity under one session of chemotherapy with cisplatin and 5-flouracil and their in vitro effect.
Venous blood samples were obtained from gastric cancer patients (men, n=40; age range 42-73 years). Red blood cell aggregation (RBCA) and red blood cell suspension viscosity (RBCSV: in phosphate buffer, Hct=40%) were registered before and after cisplatin (50mg/m2) or 5-Fluorouracil (FU - 500 mg/m2) intravenous chemotherapy infusion. In vitro RBCA and RBCSV were measured after 15 min at 370C RBC incubation with cisplatin (0.01 mg/ml) and 5-FU (0.025 mg/ml). In addition it has been estimated microrheology effect of combinations (in vitro) chemotherapy drug 5-FU with drugs, having positive rheological efficiency (pentoxifylline, vinpocetine, drotaverine: concentrations ˆ 10-6 M). Concentrations of used drugs for in vitro red cell microrheology study were the similar to that it could be in blood of patient after intravenous therapeutic infusion.
RBCA was significantly increased in patients vs. control and RBCSV was changed only slightly. Chemotherapy drugs increased RBCSV by 5-7%. RBCA was reduced a little but remained high enough. In vitro incubation RBCs with chemotherapy drugs gave two variants of RBCA changes: if it was initially relatively low significant increase ($p<0.01$) and vice versa. As for RBCSV it was found a little rise of this microrheological parameter. Rheological active drugs (pentoxifylline, vinpocetine, drotaverine) decreased RBCA by 35-60% ($p<0.01$) and improved RBC fluidity by 12-15% ($p<0.05$). If incubation of red blood cells with 5-FU resulted in RBCA increase that was combination „Pentoxifylline+5-FU‰ or „Vinpocetine+5-FU‰ inhibited proaggregative effect of 5-FU and decreased RBCVS significantly. The similar effect was found when we incubated red cells with EGTA (10^{-3} M) and with tandem „EGTA+5-FU‰. On the contrary, calcium ionofore (A23187, 10^{-6} M) increased RBCA markedly ($p<0.01$).
According to the red cell microrheological changes after drug treatment it is possible to suppose three variations of drug effect on red cell microrheology: 1) Drug doesn,t exert influence significantly upon the red cell aggregation and deformation and their transport capacity doesn,t change: it is neutral effect; 2) Drug decreases red cell fluidity and increases red cell aggregation: it is negative effect; and 3) Drug increases red cell fluidity and decreases red cell aggregation (RBCA): it is positive drug effect. Therefore for effective transport of chemotherapy drugs into tumor microregions it is possible to combine them with hemorheologically positive ones.

Role of the Extracellular Signaling Pathways in the Change of Micro-rheological Red Blood Cell Properties: in-vitro Study

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Background. Drugs and signaling molecules can affect on microrheological properties of blood cells (red cell aggregation and deformation). The signaling pathways for delivery ligands to membrane receptors may be some elements of the para-and endocrine systems. Hormones and prostaglandins can be bound with membrane receptors and activate cellular response, e.g. the change of red blood cell aggregation (RBCA). To check this hypothesis we studied RBCA under cell incubation with some hormones and prostaglandins.

Methods. Venous blood samples were obtained from healthy adults (men, n=28; age range 19-25 years) via withdrawal into sterile vacuum tubes containing heparin (1.5 mg/ml blood). Red blood cells were separated from the blood by centrifugation at 1,400 g for 15 min and washed 3 times with 10 mM phosphate buffered saline (PBS) (pH=7.4). The washed RBCs were then resuspended in PBS at a hematocrit of 40%. In each of the research sessions these RBC suspensions were divided into two aliquots and exposed to: 1) drug at 37°C for 15 min; 2) The remaining aliquot (red cell suspension with PBS) was kept at 37°C for 15 min and served as the control. Concentrations of used drugs for in vitro red cell microrheology study were about 10^{-6} - 10^{-8}M. Following treatment or control periods, the cells were resuspended in autologous plasma at a hematocrit of 0.5% and then used for aggregation measurement. Red blood cell aggregation (RBCA) in native plasma was assessed by direct microscopic methods with computer image analysis.

Results. It has been found that incubation RBCs with epinephrine, norepinephrine, phenilephrine, clonidine (agonist of □2-adrenoceptors) and metaproterenol (nonselective agonist of □-adrenoceptors) in concentration of 10^{-6}M led to an increase of RBCA. The most pronounced aggregative effect had clonidine ($p<0.01$) and the lowest one – -agonist. The combination of clonidine with phosphodiesterase (PDE) activity inhibitor papaverine (10^{-4}M) or with stable analog of cAMP (dB-cAMP; 510□M) removed the proaggregative effect of 2-agonist. The similar reduced RBCA was found when we used the combination of 1-agonist phenylephrine with verapamil (Ca^{2+}-channel blocker; 10^{-5}M). As for insulin it was found that it decreased RBCA markedly ($p<0.01$) and at low concentration (less then 10^{-10}M) in particular. To test paracrine signaling pathway RBCs were incubated with prostaglandin E1, E2 and F2□. PGE1 and PGE2 reduced RBCA significant ($p<0.01$) and PGF2□ had opposite effect.

Conclusion. Thus obtained data showed that red cell aggregation can be changed under activation both para-and endocrine extracellular signaling pathways and it is the most probably ligand-receptor interaction can stimulate adenilate cyclase – cAMP or Ca^{2+}-calmodulin intracellular signaling systems.

This work was supported by RFBR Research Grant N 07-04-01225-a.

Red Cell Aggregation as a Factor Influencing Margination and Adhesion of Leukocytes and Platelets

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In order to carry out their protective functions in inflammation and haemostasis, leukocytes and platelets must adhere to the wall of blood vessels in the presence of flowing blood. An important limiting step in this process is the delivery of the cells to the wall, often referred to as margination. In general, this requires leukocytes and platelets to be excluded from the central flow of the much more numerous red blood cells. This exclusion is believed to be promoted by red cell aggregation. Various studies suggest that promotion of aggregation by added agents such as dextrans or by reduction in fluid shear rate, increase margination of leukocytes and increase efficiency of attachment to the vessel wall. Interestingly, however, fewer studies exist for platelets, and these suggest that margination is actually promoted by increasing shear rate. Our own recent studies in horizontal tubes suggest that sedimentation effects in slow-flowing blood cause greater inequalities between adhesion on upper and lower surfaces for leukocytes than platelets. Thus, red cell aggregates in flowing blood may have quite different effects on delivery of platelets compared to leukocytes, perhaps depending on how these different-sized cells can incorporate into the aggregated stream.

Hemorheological Measurements in Experimental Animals: Applications in Surgical Research

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In basic and applied surgical research dealing with ischemia-reperfusion and organ or tissue transplantation, species-specific differences in laboratory animals need to be considered during evaluation of the results. These differences may influence the ability to obtain correlations with basic, mainly clinical questions. Thus, when considering parameters such as red blood cell deformability, erythrocyte aggregation and blood viscosity, laboratory measurements should be appropriate for the species being studied and standardized in order to allow comparative analyses.

In order to address the above issue, we have carried out comparative hemorheological investigations using A/J inbred mice, CD outbred rats, mongrel and beagle dogs. Using a bulk filterometer (Carat FT-1 filterometer), we investigated the filtration behavior of red blood cell suspensions at 1, 2, 3, 4 and 5% hematocrit (Hct) flowing through 3 or 5-micron pore sized polycarbonate filters. In associated studies using CD outbred rats and inbred beagle dogs, analyses were focused on the relation of viscosity to Hct and the effects of Hct on the Hct/viscosity ratio at different shear rates. Hematological parameters were determined using a Sysmex F-800 microcell counter, whole blood viscosity was measured via a Hevimet-40 capillary viscometer, and RBC aggregation evaluated using a Myrenne MA-1 aggregometer.

Results showed that red blood cell deformability differs among animal species and is related to the cell size/pore size ratio in filtration tests; differences were also noted between mongrel and beagle dogs. In addition, the effects of Hct and cell size/pore size ratio on filtration behavior differed among species. In outbred rats and inbred beagle dogs the correlation between Hct and blood viscosity was nearly linear but with various slopes. The optimum (i.e., highest) points of the bell-shaped curves for Hct/viscosity ratio versus Hct and for erythrocyte aggregation index versus Hct differed between animal species.

In summary, our findings indicate that the relations between hematocrit, blood viscosity and erythrocyte aggregation have diverse optima among animal species, and suggest that these differences may indicate different responses to various pathophysiological challenges.

Grant sponsors: OTKA T032571, T049331, ETT 048/2001, 387/2006, KPO 0147/2006.

The role of macromolecules in red blood cell interactions

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Cell-cell interactions are governed by interplay of various cell-receptor-mediated interactions and non-specific forces, with non-specific forces (e.g., electrostatic repulsion) often responsible for allowing or preventing cells approaching close enough to establish adhesion via lock and key forces. One non-specific force that has only recently been recognized as being important for cell-cell interaction is macromolecular depletion interaction. Polymer depletion occurs at cell surfaces if adsorption energy is low, and if depletion zones of adjacent surfaces overlap; osmotic forces move fluid away from the intercellular gap and cell-cell attractive forces develop.

The present study was designed to examine the impact of depletion forces on cell adhesion. Human red blood cells (RBC) were allowed to settle onto albumin-coated glass surfaces in polymer-free buffer and in solutions of 10 to 500 kDa dextran. Subsequently RBC adhesion and dissociation were investigated via interference reflection microscopy and a parallel plate flow system. Our results indicate decreasing cell-substrate separation and significantly faster cell-substrate binding (increase of the on-rate) in the presence of higher molecular weight dextrans (> 40 kDa). The off rate constants, which were calculated based on the lifetimes of the adherent cells under flow, revealed that the presence of large polymers during cell settling to a decrease of the off rate constant of up to two orders of magnitude.

In conclusion adhesion of RBC is significantly faster and stronger in the presence of these large polymers due to depletion interaction. These results demonstrate the importance of depletion forces for RBC-surface interactions and indicate relevance to a wide variety of cell-cell and cell-surface interactions.

Modulation of erythrocyte deformability by PKC activity

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Protein Band 3 is the protein of erythrocyte membrane in highest quantity. It is phosphorylated/desphosphorylated by phosphotyrosine kinases (PTK)/phosphotyrosine phosphatase (PTP), acetylcholine (ACh) and velnacrine are protein band 3 effectors by intermediate of Acetylcholinesterase (AChE). Besides its role in erythrocyte metabolism, protein band 3 plays a role in the maintenance of erythrocyte stability and shape by is interaction with cytoskeleton proteins that require protein kinase C (PKC) phosphorylation. At this point our question is: how does PKC activity modulate erythrocyte deformability in absence and presence of erythrocyte protein band 3 effectors of it phosphorylated degree? To answer this we used several inhibitors like AMGT, Syk, Calpeptin and Chelerythrine Chloride, respectively inhibitors of PTK (Lyn and p72 syk), PTP and PKC, in presence and absence of band 3 effectors. We also measured in whole blood suspensions glucose, 2,3-BPG, osmolality, pH, ionogram, tHb, O2Hb and p50, and erythrocyte deformability and aggregation.
We observed that erythrocyte deformability was affected by inhibition of PKC, causing a decrease in this, especially when it was conjugated with Syk plus ACh. We propose that PKC activity is essential for maintenance of erythrocyte deformability and the degree of phosphorylation of band 3 plays a role to this effect.
These results may contribute to establish a relation between cytoskeleton proteins, which require activation by PKC activity, and band 3 phosphorylation, to better understand some diseases caused by deficiency in proteins that could be involved in this mechanism like band 3, 4.1, 4.2, spectrin-actin and several others.

Microrheological Blood Properties in Patients with Chronic Cerebrovascular Diseases and Metabolic Syndrome

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To study the state of microrheological blood parameters in patients with chronic cerebrovascular diseases against the metabolic syndrome (MS) background. Materials and methods: 44 patients suffering from chronic cerebrovascular diseases were examined; MS was diagnosed in 22 cases. A LORRCA aggregometer was used to investigate and analyze erythrocyte aggregation behavior (aggregation amplitude, aggregation index and full disaggregation time) along with erythrocyte deformability index. Moreover, using a Biola aggregometer ADP- and adrenaline-induced thrombocyte aggregation indices were analyzed.
Erythrocyte aggregation was found increased in all the patients, however aggregation changes were more pronounced in the presence of MS. The aggregation amplitude reflecting the size of aggregates was definitely higher in MS patients as compared to non-MS patients, being 9.92 and 8.7 arbitrary units, respectively. The erythrocyte aggregation index against the MS background reached 67.1% and without MS it was 60.8% (normal value is 50%). The full disaggregation time that reflects aggregate strength was definitely longer in MS patients than in non-MS patients, being 150 s and 120 s, respectively. Erythrocyte deformability index against the MS background proved to be 0.53, which is lower as compared to 0.56 for non-MS cases. A similar situation was observed in thrombocyte aggregation indices: ADP-TA and Adr-TA indices averaged 51% and 55%, respectively, for non-MS patients and 58% and 62% for MS patients.
Microrheological blood properties deteriorate considerably in the patients with chronic cerebrovascular diseases. In this situation a concurrent MS has significant adverse effect worsening the rheological properties of blood cells.

Magnetocardiograpahy - a new tool for the detection of myocardial ischemia

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Magnetocardiography (MCG) is a new modality which utilizes superconducting quantum interference devices for the detection of the weak magnetic fields (picoTesla range) generated by the heart's electrical currents. The magnetic field map picture, which is created by the measurements of the magnetic field, reflects the electrophysiologic state of the heart. When there is an abnormality in cardiac depolarization or repolarization, such as in ischemia, this is reflected in an abnormality in the magnetic field map.
The value of MCG for the detection of cardiac electrical disturbances associated with myocardial ischemia has been prospectively studied in patients with acute coronary syndromes as well as in patients with stable angina pectoris.
For the prediction of CAD in patients presenting with acute chest pain and without ST-segment elevation an admission MCG test was superior to an admission ECG, echocardiography, and troponin-I. In addition MCG could accurately detect myocardial ischemia in bundle branch block - patients presenting with acute chest pain, a condition, in which ECG is non-diagnostic. The possibility of accurate, rapid, and no risk diagnosis of ischemia could potentially impact healthcare for a large group of subjects by avoiding a delay in diagnosis of ischemic patients while avoiding unnecessary hospital admissions and testing of non-ischemic subjects.
Exercise ECG is an imperfect test for the detection of coronary artery disease. We prospectively investigated the accuracy of high-dose dobutamine stress MCG (DS-MCG) and ECG (DS-ECG) among patients with suspected significant coronary artery stenosis. Patients with an intermediate probability of coronary artery disease before elective x-ray coronary angiography underwent DS-MCG using a multichannel magnetometer ARGOS 50 (AtB, Pescara. Italy). Patients were examined at rest and during a standard dobutamine-atropine scheme until submaximal heart rate was reached. Significant reduction of epicardial current strength/density (at QRSmax) superposed on a virtual heart model during stress indicates stress-induced myocardial ischemia. The epicardial current distribution was reconstructed from the magnetic field map. Among patients referred for their first x-ray coronary angiogram, DS-MCG yielded a significantly higher accuracy for the detection of severe coronary artery stenosis in comparison to DS-ECG.
Widespread clinical acceptance of MCG is highly likely after corroborative evidence is generated and further technical improvements such as enhanced signal to noise ratio are introduced.

Cutaneous Microcirculation Test Predicts Clinical Efficacy of Tadalafil in Patients with Coronary Artery Disease and Erectile Dysfunction

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Background: Atherosclerosis is the major cause of endothelial and erectile dysfunction. We prospectively investigated the effect of the phosphodiesterase-5-inhibitor tadalafil on cutaneous microcirculation and erectile function in patients suffering from coronary artery disease (CAD) and impotence.
Methods: A total of 25 patients with angiographically proven CAD suffering from erectile dysfunction underwent erythrocyte velocity measurement in the nail-fold using video capillary microscopy. Postischemic hyperemia was evaluated after 3 minutes of suprasystolic upper arm compression before and 2 hours after 20 mg of tadalafil. Erectile function before and during 4 weeks of the use on demand of tadalafil was assessed using the International Index for Erectile Function (IIEF).
Results: The patients (mean age: 61±7.7 yrs; mean marriage yrs: 33±14.4) suffered from erectile dysfunction since 4.1±2.6 yrs. The maximal postischemic erythrocyte velocity **v**max increased by 14 % from 1.35±0.65 to 1.54±0.61 mm/s 120 min after 20 mg tadalafil ($p=0.0281$). IIEF score increased markedly after 4 weeks of the use on demand of 20 mg tadalafil from 6.8±4.7 to 24.1±4.7 ($p<0.0001$). There was a significant correlation between the change in the duration of postischemic hyperemia (DpH) after a single dose of 20 mg tadalafil and the change in IIEF with 4 weeks of the use on demand of tadalafil ($r=0.596$; $p=0.0021$). In 5 clinical non-responders to tadalafil, DpH was shortened or unchanged. DpH was increased in all 20 patients, in whom IIEF was significantly increased. In 6 patients mild side effects were reported (3x back pain; 2x flush; 1x headache).
Conclusions: In CAD-patients suffering from erectile dysfunction tadalafil significantly improves cutaneous postischemic hyperemia and IIEF. Comparison of postischemic hyperemia measurement before and 2 hours after 20mg tadalafil predicts the clinical efficacy of tadalafil in CAD-patients with naive erectile dysfunction.

Combination fibrinolysis and early invasive strategy versus facilitated PCI by upstream tirofiban in acute ST-segment elevation myocardial infarction: results of the Alteplase and Tirofiban in Acute Myocardial Infarction (ATAMI) trial

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Direct percutaneous coronary intervention is the generally accepted superior strategy in acute ST-segment myocardial infarction. The ASSENT IV trial demonstrated that facilitated PCI by fibrinolysis had disadvantages in terms of mortality. Furthermore, combination fibrinolysis using half-dose reteplase and glycoprotein IIb/IIIa antagonists such as abciximab and integrilin turned out to have higher rates of open infarct-related coronary arteries without reduction of mortality (ASSENT III, GUSTO V and ADVANCE-MI). The FASTER trial using half-dose tenecteplase and tirofiban was stopped prematurely in the light of ASSENT III and GUSTO V results. After a pilot trial of facilitated PCI with combination fibrinolysis using half-dose alteplase and tirofiban published in 2002 with excellent efficacy and high safety we prospectively randomised 151 patients (96 males, mean age 67.4 ± 8.7 years, range 37 – 83 years) to combination fibrinolysis with 50mg alteplase and tirofiban in modified RESTORE dosage over 24 hours and early invasive strategy within < 48 hours and 162 patients (103 males, mean age 65.6 ± 9.4 years, range 42 – 84 years) to facilitated PCI with upstream administration of tirofiban in modified RESTORE dosage over 24 hours. TIMI II-III or III flow of infarct-related vessel before intervention as the primary endpoint and 30-day mortality, bleeding complication and angiographic proven stent thrombosis as secondary endpoints were assessed.
TIMI II – III or III flow in the infarct-related vessel could be demonstrated in 131 out of 151 patients (87%) in the facilitated PCI group and in 68 patients (42%) in the acute PCI group ($p < 0.0001$). 30-day-mortality was 0.7% (n=1/151) in the facilitated PCI group and 5.5% (n=9/162) in the acute PCI group ($p < 0.025$), although initial hypotension (< 100mmHg systolic pressure) and tachycardia (> 100 bpm) were present in 14% in the facilitated PCI group and in 9% in the acute PCI group. No differences could be assessed in major (1.3% versus 1.2%, respectively) and minor bleeding complications (2% versus 1.9%, respectively). Stent thrombosis occurred in none of the patients with combination fibrinolysis and in 2 patients (1.5%) in the facilitated PCI group.
Using half-dose alteplase and tirofiban and early invasive strategy significantly higher TIMI flow rates of the infarct-related vessel with low 30-day mortality and low bleeding complications could be assessed in comparison to facilitated PCI with upstream tirofiban administration. This strategy needs to be further investigated in larger trials and could optimise acute myocardial infarction management even without 24-hour service of catheter laboratories.

Acute cigarette smoking induces different effects in skin and muscle microcirculation in healthy chronic smokers

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Chronic cigarette smoking is accompanied by pronounced atherosclerotic changes. Smoking can also acutely impair microvascular endothelium-dependent vasodilatation. The aim of this study was to evaluate the acute effects of smoking on cutaneous and muscular baseline and post-ischemia blood flow in habitual smokers.
Twenty healthy smokers (age: 40, 19 - 58 years) (mean, range) were investigated before and after smoking one cigarette. Blood flow was assessed using laser doppler flowmetry (O2C, LEA Medizintechnik, Giessen, Germany) and measurements were performed in 2 mm (skin) and 8 mm depth (muscle) on the hypothenar site of the hand. We measured the baseline and maximal post-ischemic blood flow during reactive hyperemia following a 4.5 min suprasystolic ischemia of the forearm before as well as 30 min. after smoking.
After smoking, the baseline blood flow values decreased in both 2 and 8 mm depth by 49% and 35% respectively ($p<0.005$ for both) when compared to the baseline levels before smoking. The absolute blood flow in 2 mm decreased from 179 ± 22 AU (arbitrary units) (mean ± SEM) to 92 ± 23 AU after smoking, and in 8 mm from 351 ± 26 AU to 228 ± 40 AU. When compared to the values before smoking, the maximal blood flow during reactive hyperemia decreased after smoking in 2 mm by 16% (258 ± 17 AU vs. 217 ± 19 AU, $p<0.05$), but remained unchanged in 8 mm (456± 11 AU vs. 462± 15AU). Smoking caused a reduction in baseline blood flow both at 2 mm and 8 mm depth and also impaired the cutaneous reactive hyperemia (in 2 mm), while it did not significantly change the post ischemic blood flow into the muscle (8 mm). This suggests comparable regulation of baseline blood flow after smoking in the skin and in the muscles, but different reactivity to smoking of the maximal dilatatory capacity of the microcirculation at the two levels.

Comparison of Genetic Polymorphisms in Plasminogen Activator Inhibitor-1 Gene-675 4G/5G in Women with Venous THROMBOEMBOLISM or Early Spontaneous Abortion During Pregnancy

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Genetic polymorphisms in plasminogen activator inhibitor-1 gene-675 4G/5G (PAI-1 4G/5G) are claimed to contribute to an increased risk of venous thromboembolism. Inherited thrombophilia, on the other hand, is associated with the occurrence of spontaneous abortions. The objective of this study was, to explore the significance of genetic polymorphisms of PAI-1 4G/5G with particular emphasis on 4G alleles in pregnant women suffering from venous thromboembolism or early spontaneous abortion, respectively.Therefore PAI-1 4G/5G polymorphisms, detected by PCR and reverse hybridization analysis, were studied in 91 pregnant females, suffering from venous thromboembolism (n=60) or from spontaneous abortion (<20 week, n=31), respectively. Healthy volunteers (n=238) were taken as controls. The frequencies of 4G alleles (4G/4G or 4G/5G genotypes) of PAI-1 were significantly higher in venous thromboembolism (RR: 3.89, p=0,023) and slightly higher, but not significantly, in abortions (RR: 1.74; p=0,200) compared to controls. The incidence of 4G-carriers in females presenting with abortion was 0.52 (-48%) compared to women suffering from venous thromboembolism alone. We conclude from these data, that the occurrence of PAI-1 4G/4G or 4G/5G genotypes, respectively, is clinically significant for the pathogenesis of venous thromboembolism in pregnancy but not for early abortions.

Haemorheological changes in the blood of the syrian hamster (mesocricetus auratus)

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In hibernating animals the metabolic rate is decreased due to a subsidence in body temperature. This leads to a decline of blood flow at about 10% of the basic volume. Since the Blood viscosity is temperature dependent it should increase dramatically. Therefore we investigated the blood viscosity (at native hibernating temperature of 8°C and at 37°C with LS30 Contraves) erythrocyte (RBC) aggregation and deformability (Myrenne, Roetgen Germany; LORCA Mechatronics, Hoorn, Netherlands) A routine hematologic and blood chemistry profile was obtained from each animal. Hamsters were divided into 3 groups: 5 animals were brought into a cold (0 - 10°C) and dark place for hibernation. After 3 months blood was taken by heart puncture. 5 animals were controls. The blood from 5 animals was cooled down to the hibernating body temperature of 8°C and blood viscosity was measured again.
The viscosity measured from the animals kept in a warm environment had the lowest viscosity followed by the hibernating animals. The cooled blood from non hibernating animals showed a significant increase in blood viscosity.
It can be assumed, that due to adaptional procedures the blood of hibernating animals is able to maintain its physiological viscosity.

Connection between genetically determined blood coagulation factors and haemorheology

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Background: The characteristics of phenotypes of blood clotting factors are well known, however, few data have been documented about their effects on haemorheology. The connection among genetic polymorphisms, haemorhological factors and vascular mortality is studied also poorly.
Purpose: Our first aim was to study six genetic polymorphisms of blood clotting factors, which present the role of platelet-plasma protein-endothel system in thrombotic course. Furthermore, we studied the connection of genotypes and haemorheologic factors and both with five years vascular mortality in ischaemic stroke patients.
Patients and methods: The genetic polymorphisms of GP IIb/IIIa Leu33Pro, prothrombin gene G20210A, ACE I/D, fibrinogen gene -455G/A, Leiden mutation and MTHFR C677T alleles or genotypes in blood samples of 433 ischaemic stroke patients by PCR were studied. Haematocrit values, plasma fibrinogen (FIB) concentration, whole blood viscosity (WBV) at 90 sec^{-1} and plasma viscosity (PV) were measured. Vascular mortality of patients were followed for five years and studied by Kaplan-Meier curves.
Results: A higher plasma FIB concentration in non smoker patients, carrying A alleles of FIB gene was present compared to wild types ($p<0.05$). Also an increase of WBV in smoker and non-smoker patients with A alleles was found compared to wild types ($p=0.11$ and $p<0.05$, respectively). The highest quartile of PV showed a connection with Leiden mutation in whole group of patients ($p=0.01$), in subgroup of young patients (<50 years, $p=0.03$) and also in non smoker groups ($p<0.05$) as compared to patients having wild types. No association could be detected between different genetic polymorphisms and vascular mortality, however, it was observed significant mortality increasing in patients having PV above 1.51 mPas ($p=0.03$).
Conclusion: Certain genetic polymorphisms of coagulation system could result unfavourable haemorheological changes, however, none of them increases mortality. The connection between higher mortality and PV focuses the attention for the necessity of PV measuring in stroke patients.

Value of High Resolution Ultrasound and Contrast Enhanced US Pulse Inversion Imaging for the Evaluation of the Vascular Integrity of Free-Flap Grafts.

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The aim of this study was to evaluate the clinical value of color coded Doppler sonography (CCDS) and contrast-enhanced harmonic imaging (CHI) for ultrasound (US) monitoring the integrity of free-flap vascular grafts. Patency of microvascular anastomoses and perfusion as well as microcirculation of the transplanted tissue were analysed.

Fifteen free parascapular flap grafts performed over a period of three years by a single surgeon were examined with CCDS and CHI. The patients (12 male, 3 female) ranged in age from 16 to 60 years (average age 40±12). The follow-up period ranged from two weeks to 2.5 years. CCDS were performed with a multifrequency linear transducer (5-10 MHz, Logiq 9, GE) with 3 D flow detection. For detection and characterization, B scan of the flap tissue was compared to tissue harmonic imaging (THI) and Cross Beam with Speckle Reduction Imaging (SRI). US Pulse Inversion Harmonic Imaging (PIHI) after bolus injection of 2.5 ml Sonovue® was used for contrast enhancement.

Border and tissue structure of the flaps could be detected best in all 15/15 cases using Cross Beam Technology with SRI and THI. Correlations were found for flow parameters of the common femoral artery, popliteal artery and lower leg artery to the anastomotic vessels. 3 D imaging with CCDS facilitated flow detection of elongated and small anastomotic vessels in 4/15 cases. Contrast-enhanced US with PIHI allowed dynamic flow detection of the microcirculation of the transplanted tissue over a depth of up to 3 cm with quantitative perfusion curves of the tissue microcirculation. Reduced US contrast enhancement with modified perfusion curves was seen in 2/15 cases with low anastomic flow in CCDS.

Assessment of microvascular perfusion with contrast-enhanced ultrasound can provide valuable information on free flap viability. Contrast-enhanced US enables dynamic and quantitative flow detection of free flap tissue.

Contrast Harmonic Ultrasound and Indocyanine-Green Fluorescence Video Angiography for Evaluation of Dermal and Subdermal Microcirculation in Free Parascapular Flaps

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Contrast harmonic ultrasound (CHI) with a linear transducer is a new diagnostic approach that allows dynamic and quantitative flow detection of tissue perfusion in microsurgery. The aim of the study was the evaluation of perfusion of the dermal and subdermal layers of microvascular tissue transplants with CHI in comparison to ICG-fluorescence angiography.
In a prospective clinical study Indocyanine-Green Fluorescence Video Angiography and Contrast Enhanced High Resolution Ultrasound (5-10 MHz; linear transducer; Logiq 9; GE) were used for evaluation of the microcirculation in 10 transplanted free parascapular flaps. Two regions were analysed, the centre of the flap and the region of the anastomosis. The perfusion patterns of both methods were compared.
The perfusion indexes measured by ICG-fluorescence angiography correlated very precisely in all patients with the quantitative perfusion curves of contrast-enhanced US with CHI.Two flaps with slow filling and low dye intensity showed low contrast enhancement in CHI with modified perfusion curves with slow increase. In two cases a reduced perfusion and filling were found. There were no statistical differences between the two diagnostic methods ($p>0.01$).
CHI improves US detections of dermal and subdermal microcirculation in comparison to ICG fluorescence angiography. CHI is a new diagnostic method for postoperative monitoring of free flaps.

Resistance to plastic deformation is decreased in RBC of preterm neonates

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The red blood ceil (RBC) membrane forms tethers in response to shear forces acting on tiny membrane points. Tether formation depends on viscous and elastic membrane properties and has been used as indicator of membrane fragility. RBC in newborn infants differ in various properties from RBC in adults: the mechanical fragility of neonatal RBC is increased; the membrane of neonatal RBC shows decreased resistance to elastic deformation; RBC survival time is about 120 days in adults and only 60 days in preterm newborn infants.
A micropipette technique was used to study time dependent tether formation and tether relaxation of individual RBC in adults, term and preterm (26-29 SSW) newborns. Point attached RBC were aspirated at a constant negative pressure into a micropipette with an internal diameter of 7.8 µm. If tether formation occurred and the tether reached a length of approximately 16 µm, the pipette was carefully pulled back. The RBC left the orifice of the micropipette and the tether relaxed and pulled the main body of the RBC back to the attachment point. Aspiration pressures of -5, -3 and -2 cm H2O were applied. Tether formation occurred in all three groups at an aspiration pressure of -5 cm H2O, at an aspiration pressure of -3 cm H2O in RBC of term and preterm newborns and at -2cm H2O only in RBC of preterm neonates. The onset of tether formation at an aspiration pressure of -5 cm H2O was earlier in preterm RBC (3 s) than in term RBC (4 s) and adults (10 s). Tether growth was fastest in preterm RBC followed by term RBC and adult RBC. The relaxation of the tethers was exponential and was not different in the three groups. The time constant for tether relaxation was 0.14 s which is similar to the time constant for recovery of entire RBC from extensional elastic deformation. Repeated tether formation and relaxation of the same RBC led to an earlier begin of tether formation and changed the behaviour of tether growth, although the relaxation time did not change.
We conclude that the resistance to plastic deformation is decreased in RBC of preterm neonates and that tether formation results in irreversible alterations of the RBC membrane.

Effect of Endurance-Training on Peripheral Microcirculation

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Peripheral microcirculation is involved in cardiovascular adaptation which occurs in response to endurance training. Studies performed by means of laser Doppler flowmetry (LDF) showed a higher skin vasodilator response to acute physical exercise in endurance trained subjects, which contributes to a more efficient transport and elimination of heat during effort. More recently, studies based on Fourier spectral analysis of LDF signal, showed an increased spectral amplitude of skin blood oscillations, the so called flowmotion, in endurance trained subjects. In particular, the skin blood flowmotion component with frequency interval of 0.009-0.02 Hz, referred to endothelial activity, and of 0.06-0.2 Hz, referred to spontaneous activity of the vascular smooth muscle cells, showed the highest amplification both under basal conditions and following acute physical exercise in trained subjects. This could represent an important mechanism of peripheral microcirculatory adaptation in response to endurance-training. Effect of endurance training has been also demonstrated on skeletal muscle microcirculation. Studies showed that intense intermittent endurance training induces an increase in endothelial cell proliferation and in capillarization at level of exercised human skeletal muscle. The capillary growth occurs within 4 weeks of training and appears to be transient, as evidenced by a reduction in proliferating endothelial cells and no further capillarization after 7 weeks of training. Proliferative compounds released in response to exercise are likely to be important for training-induced angiogenesis. Consistently with these morphological findings, our preliminary LDF data on skeletal muscle microcirculation showed an increase in spectral amplitude of the all different blood flowmotion components together with a higher blood flow under basal conditions and following ischemia in endurance trained subjects. If further observations confirm these data the LDF study of skeletal muscle microcirculation could be useful in Sport Medicine for evaluating the efficacy of different endurance training programs in inducing microcirculatory skeletal muscle adaptatio

Whole blood viscosity and erythrocyte fluidity are related to endothelium-dependent vasodilatation and coronary risk in the elderly

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It has been shown that an elevated blood viscosity predicts cardiovascular events. One putative mechanism might be an impaired endothelial function. Further, erythrocyte fluidity, representing erythrocyte deformability, is a characteristic rheologic feature of importance for the flow properties of the blood, especially in the smallest vessels. The present study evaluates the relationships between erythrocyte fluidity, whole blood and plasma viscosity, coronary risk and endothelial vasodilatory function.
In a population-based study on 1016 subjects aged 70, endothelium-dependent vasodilatation (EDV) was evaluated by a) the invasive forearm technique with acetylcholine given in the brachial artery, b) the brachial artery ultrasound technique with measurement of flow-mediated dilatation (FMD) and c) pulse wave analysis with β-2-agonist (terbutaline) provocation. Erythrocyte fluidity, and whole blood and plasma viscosity were measured in a random sample of 573 subjects. Whole blood and plasma viscosity were related to Framingham risk score ($r=0.20$, $p<0.0001$). EDV was inversely related to both whole blood and plasma viscosity ($r=-0.16$, $p=0.0004$ and $r=-0.14$, $p=0.0015$, respectively). So was also the pulse wave response ($r=-0.20$, $p<0.0001$ and $r=-0.09$, $p=0.045$, respectively), but not FMD ($r=0.01$-0.02). Erythrocyte fluidity was inversely related to the Framingham risk score ($r=0.12$, $p=0.0009$), EDV ($r=0.12$, $p=0.0064$) and to the pulse wave response ($r=-0.17$, $p=0.0002$), but not to FMD ($r=-0.01$). Multiple regression analysis showed whole blood viscosity and erythrocyte fluidity to be significantly related to EDV and the pulse wave response independently of haematocrit gender, hypertension, smoking, hypercholesterolemia, obesity and diabetes.
Whole blood and plasma viscosity as well as erythrocyte fluidity were related to coronary risk. Acetylcholine-induced vasodilatation in the forearm and terbutaline-induced changes in pulse wave reflection were both inversely related to whole blood viscosity and erythrocyte fluidity independently of traditional risk factors in elderly subjects, indicating a pathophysiological link between impaired haemorheology and coronary risk.

Coated or implanted polyurethane foil, which kind of modification leads to a better biocompatibility?

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Each biomaterial implanted into the body induces a different response in local or systemic processes. Polyurethanes (PUR) became recognized in the 1970s and 1980s as a blood contacting material. The permanent problem was their relative sensitivity to biodegradation.
In cooperation, we developed new modified polyurethane foil surfaces. The foils are created by zircon coating, carbon and nitrogen implanting, respectively. After in vitro tests (done by the cooperation partners) we investigated the implants in different animal models. Here we like to report our results concerning the implantation of PUR-threads in the abdominal aorta of rabbits.
All over we used 32 adult female New Zealand White rabbits weighing 2.5 to 3.0 kg. These animals are housed and treated according to accepted standards for the care of research animals. 4 groups were investigated: uncoated (group 1), carbon- (group 2) or nitrogen-implanted (group 3) and zircon coated (group 4), respectively. Threads of 0.2 x 0.2 mm diameter and a length of 15 mm were implanted oblique to the blood flow. The study duration was 14 days. On 3 time points (implantation, 1 week, explanation) blood and tissue samples were collected for final examinations like: standard clotting tests, histological examinations and scanning electronic microscopy (SEM).
Results of standard blood investigations did not show any significant differences between the groups except a small increase of platelet count one week after implantation in group 2 and 3, respectively. For the activation of inflammatory response and the complement system no differences were found between all groups.
In histological findings a lot of erythrocytes and platelets were identified in group 1, 2 and 3, whereas group 4 showed no platelets and only single erythrocytes. The whole threads of both implanted materials (group 2 and 3) were turned around by fibrous tissue material.
SEM findings confirmed the light microscopy results: Cellular bodies like erythrocytes and platelets are identified by morphology to be prominent in group 2 and 3. Group 1 showed a smaller amount of both cellular types, additionally crystalline structures (of normal saline) were seen. The zircon coated threads showed areas of scaly detaching and missing cellular body accumulations.
All animals survived the surgical procedure and the study period without further investigation or medical support. The decrease of the number of platelets count as sign of formation of thrombosis material let us accept that the material of group 2 and 3 are lesser suitable for biomaterials without permanent systemic anticoagulation. Instead, PUR coated with zircon, without detaching cells, seems to be biocompatible and stable without any anticoagulation.

The development of animal models is an important point in research work

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The objective of research can be the developing of new animal models. In our studies we planed to created models for testing different vascular or heart implants without circulatory support. The surgical aim was the survival of the animals without systemic anticoagulation and other difficulties.
On study begin Sprague Dawley rats with a weight of 250 g were used for thread implantations into the abdominal aorta. Under the implantations a lot of bleeding complications with a rising mortality were observed, caused by stiffness of the implant material. So we changed the animal model from rats to rabbits. For all our thread and patch implantations New Zealand White rabbits with a weight of 2.5 to 3.0 kg were used. After changing the animal size no bleeding or other complications occur during the whole study period. The thread implantation was performed in Seldinger technique with outside fixation. For the patch implantation a shunt, like for off pump surgery performed, were necessary to.
For comparable results with reactivity in the human body a model of a larger animal was important. The decision falls on a domestic swine. The blood system and blood volume is comparable to humans. The processes of blood coagulation are similar or up regulated in swine against humans.
The plan was the implantation of a modified PUR tri-leaflet valve into the descending aorta without any circulatory support and neurological complications after the successful operation. The operation was performed in generally anaesthesia during a left sided thoracotomy (7./8. intercostal space). First after preparation the operations were done with cross clamping and dividing between two vascular clamps. The valve graft was then interposed between the two stumps of the descending aorta with 5/0 Prolene sutures. The main problems are the hemodynamic instability and the postoperative neurological injury, like the paraplegia of the lower extremity. In literature the risk of paraplegia in swine are described with 15 to 20 %, because of the minimal tolerance of the spinal cord for ischemia.
So the operating methods become changed to an intermediated use of a shunt to overcome the instability and complications. After the change no similar or new problems occurred. All swine were doing well after the valve implantation for 4 weeks. The paraplegia rate was drop down to overall 7.7 %, which is comparable to operations of the descending aorta in humans and a very good result for decreasing the paraplegia rate in animal surgery.
The model allowed repeated results for sufficient survival without major complications to examine hemodynamic and coagulation parameters over a longer time.
In conclusion we have 3 different animal models for testing implantation materials, operated without circulatory support.

Endothelial cell junctions: critical structures in vascular biology

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The functional integrity of the entire endothelium depends on the endothelial cell by itself and the organization of the intercellular junctions that differ organ and vascular segment-specific. Intercellular junctions consist of complex interwoven cell-cell adhesion structures displaying components adherents, tight and gap junction. The vascular endothelial (VE) cadherin/catenin complex provides the structural and regulatory backbone of the cell junctions and is critically involved in assembling of its organisation. In inflammation, angiogenesis and wound healing intercellular junctions have to be activated to allow dynamical reorganization of cell junctions as well as cell migration. Tyrosine phosphorylation of the VE-cadherin/catenin complex is assumed to be critical but the effect is controversially discussed. Using a number of different stimuli that leads to junction activation as re-calcification, treatment with VEGF and flow that leads to cell alignment and shape change we show that tyrosine phosphorylation of the VE-cadherin/catenin complex is associated with an transient increase in intercellular cell adhesion. In contrast, application of pro-inflammatory cytokines or supernatants of activated macrophages lacks a transient TER increase and caused a long lasting down regulation of intercellular adhesion and barrier function. Under these conditions tyrosine phosphorylation of the VE-cadherin/catenin complex was never observed. This data indicate that tyrosine phosphorylation of the VE-cadherin/catenin complex is a special form of junction activation which leads to tightening but not to dissociation of the cell junctions.

Future Therapy of Acute Coronary Syndrome : Advanced Role for Thienopyridines or Triple Antiplatelet Therapy ?

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Thienopyridines have become important in supporting the invasive therapeutic approach of acute coronary syndromes in recent years. Clopidogrel is nowadays the preferred agent due to its better safety profile and faster onset than ticlopidine, which belonged to the first generation of thienopyridines. As coronary interventions became more frequently and developed from an elective to an urgent procedure an even faster anti-platelet effect was warranted. In this context GPIIbIIIa inhibitors became important and were the agent of choice to inhibit platelet aggregation to an extent of more than 80% very rapidly. By administering higher doses of clopidogrel directly to patients, which had not been pretreated before but were scheduled for PCI, the protective antiplatelet effect could be achieved earlier. Loading doses of 300 mg and 600 mg clopidogrel are nowadays broadly used and the 600 mg loading dose has shown to provide a sufficient inhibitory effect of platelet aggregation in elective (low risk) patients undergoing PCI (ISAR REACT). In contrast to this ACS patients with an elevated troponin experienced a remarkable additional benefit when they received abciximab on top of 600 mg clopidogrel before PCI (ISAR REACT-2) so that current guidelines recommend triple antiplatelet therapy for ACS patients with an elevated troponin.
The comparison of 300 mg, 600 mg and 900 mg loading doses revealed no further effect of the 900 mg dose in recent clinical trials (ISAR CHOICE, ALBION): A threshold seems to be reached, which could be explained by the metabolization of the pro-drug clopidogrel to the active compound in several steps leaving 85% of the original substance as inactive metabolite aside. This bottleneck and an impaired metabolization process could count responsible for the phenomenon of non-responsiveness to clopidogrel, which can be observed in up to 30% of patients. New agents (prasugrel, cangrelor, AZD6140), which are currently under investigation in ongoing phase III studies give hope that they will provide more efficient and reliable inhibition of platelet aggregation. In how far they will be able to replace clopidogrel and/or GPIIbIIIa inhibitors needs to be further elucidated in randomized clinical trials.

Study of reference control material in measurements of erythrocyte deformability

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Even though the red blood cell deformability (RBC-D) is frequently measured for research and clinical applications, there is a lack of relevant reference materials for both procedure standardization and/or instrumental calibration. The objective of the present study is to investigate whether glutaraldehyde (GA) treated RBCs could be used as such a reference material for RBC-deformability measurements.
Aliquots of stabilized RBCs were prepared by fixation with glutaraldehyde and the variability of RBC deformability stored at 4.0 degrees C during periods of up to 4 weeks was examined with a microfluidic ektacytometer, RheoScan-D in terms of elongation index (EI). The deformability of GA-treated RBCs shows an apparent decrease in GA concentration- and incubation time-dependent manners. For erythrocytes incubated in 0.75 mM and 1mM (0.008%) of GA solutions, the coefficient of variation in EI was not greater than instrumentation precision (2.6%) up to 2 weeks. However, after two weeks, the EI of the GA-treated erythrocytes decreased rapidly. It was confirmed that, as long as it is stored at 4℃, this material is stable up to 2 weeks with acceptable variation and enables daily and/or long-term monitoring of instrument accuracy and precision.

Measurement of RBC aggregation in a microchip using magnetic stirring-assisted cell disaggregation mechanism

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The aggregation characteristics of red blood cells (RBCs) play an important role in the microvascular flow system and increased RBC aggregation has been observed in various pathological diseases, such as thrombosis and myocardial infarction. Available techniques for measuring RBC aggregation require large amount of blood sample as well as washing after each measurement, which prevent from being used in clinical environments.
This paper presents a novel microfluidic RBC aggregometer that can be used at clinical environments. The present technique is based on a disposable microchip with a magnetic stirring mechanism and a laser-light backscattering detection system. The magnetic stirring mechanism was found to be effective for disaggregation of RBCs, which used to require a high-cost and complex system. The present results show good agreement with those of a rotational aggregometer (LORCA). The essential feature of the present technique is the incorporation of a rotating stirrer in a disposable microchip for RBC disaggregation, which enables the present system to be used in a clinical setting with ease and accuracy.

A new shear stress-scanning microfluidic aggregometer

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An optical method based on shear stress-scanning microfluidic rheometry was developed to meaasure red blood cell (RBC) aggregation in blood and RBC suspensions. Measurements of backscattered light intensity and shear stress were made in a microchannel with respect to time. The time record of the backscattered light intensity (syllectogram) consists of an upward convex curve with peak point, which reflects the transition from disaggregation to aggregation processes in RBC-plasma suspensions. The aggregation-time and -shear stress corresponding to the peak point, as newly proposed indexes of aggregation in the present study, were compared with conventional indexes and showed good agreements between them. It is experimentally shown that the new indexes quantitatively represent the aggregation characteristics in RBC-plasma suspensions with varying fibrinogen concentrations. The microfluidic measurement of RBC aggregation characteristics offers a new concept for the study of blood rheology and great potential for point-of-care use.

Endothelial dysfunction of arteries due to thrombophilia and vascular risk factors

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The detection of the DNA-sequence of human coagulation factors, inhibitors and endothelium and lipid metabolism has introduced the possibility of differentiated mutation analysis in patients with arterious thrombosis. Since endothelial dysfunction of arteries and thrombophilia is a multifactorial disease we have to perform also - besides genomic analysis - functional testing of fibrinolytic capacity. We have to identify and categorize patients at an increased risk to develop dysturbances of microcirculation, myocardial infarction, cerebral infarction and venous thromboembolism. Therefore, it is of major interest to define inherited thrombophilic disorders , in which genetic diagnosis and diagnosis of risk factors is of clinical relevance. Genetic analysis seems only useful in patients if combined with exposition factors, endothelial functions' testing and clinical data and risk factors.

Erythrocyte deformability does not seem to be altered in Behçet,s disease

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Behçet,s disease (BD) is a chronic systemic vasculitis characterized by recurrent oral and genital ulcers, uveitis, and skin lesions. It is also associated with an increased risk of developing thrombosis, although the prothrombotic mechanisms are not clearly defined. The role played by rheological alterations in the development of thrombotic events in BD is not well defined, existing little information on whether erythrocyte deformability (ED) may be involved in this issue. Therefore we aimed to evaluate ED by ektacytometric techniques in a large group of patients with BD in a non-active phase of the disease at sampling and in a well-matched control group, in order to establish a possible association between alterations in ED and the presence of thrombotic events.
The patient group comprised 45 patients with BD (22 male, 23 female aged 42 ± 14 years) and the control group comprised 46 healthy volunteers (23 male, 23 female aged 45 ± 13 years). Twelve of the 45 patients with BD had a previous documented history of deep vein thrombosis at least six months before entering the study, and the other 33 did not. Erythrocyte elongation indexes (EI) at the three shear stresses tested (EI12, EI30, EI60) were not statistically different between patients and controls (EI12: $P = 0.453$; EI30: $P = 0.411$; EI60: $P = 0.403$). There were no significant differences in these parameters, either, when patients with and without previous thrombotic events were compared (EI12: $P = 0.272$; EI30: $P = 0.215$; EI60: $P = 0.171$).
Our results suggest that ED is not compromised in BD and does not seem to be involved in the development of thrombotic events in these patients.

Erythrocyte deformability in anaemic patients with reticulocytosis determined by means of ectacytometric techniques.

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It is not clearly established whether reticulocyte deformability is lower than that of the mature erythrocytes, as most of studies published on this matter have evaluated this rheological parameter by means of micropipette techniques, which are unsuitable for routine measurements. Information is scarce as regards the evaluation of reticulocyte deformability by means of ectacytometric techniques, routinely used in clinical laboratories.
We aimed to evaluate erythrocyte deformability (ED), with ectacytometry, in samples of 44 anaemic patients with peripheral reticulocytosis (reticulocytes: 260 ± 150 x103/μL) and in 60 healthy non-anaemic volunteers with a normal reticulocyte count (reticulocytes: 60 ± 20 x103/μL). We also determined other factors that may influence ED, such as erythrocyte indices (MCV, MCH, MCHC), glucose, total cholesterol and triglycerides. ED was evaluated determining the elongation indices (EI) at 12, 30 and 60 Pa, by means of the Rheodyn SSD. At the three shear stresses tested, patients showed statistically lower EI than controls, higher reticulocyte count, lower cholesterol levels and higher MCHC ($P<0.001$, respectively). A statistically significant negative correlation ($P<0.01$) was found between the reticulocyte count and the EI at 12, 30 and 60 Pa ($r = -0.643$, $r = -0.678$ and $r = -0.692$, respectively), and between the EI and the MCHC (correlation coefficients: -0.743, -0.741 y -0.738; $P<0.01$). As the differences in ED could be attributed partly to alterations in erythrocyte indices and plasma lipid levels, a linear regression analysis was performed, showing that EI is independently associated with the reticulocyte count.
Our results suggest that reticulocytes are responsible for the decreased ED observed in anaemic patients with peripheral reticulocytosis, when this hemorheological parameter is evaluated by means of ectacytometric techniques.

Plasmapheresis and his influence to hemorheology

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According to the actual problems of hemorheology, structure blood cells and its motion in blood vessels are general studies of investigation in vitro models of microcirculation.
Mostly fragments of microcircular system is the capillaries. Relationship between rheological characteristics of blood cells and range of thickness of layer solutions studied by method of piezoquartz resonator.
It could be shown, that viscoelastic properties of blood from patients with ischemic stoke is different from its blood after plasmapheresis. Thus, it has been shown, that method quartz resonator makes it possible to estimate rheological properties of blood cells and proteins using only 4 ml of volume samples.

Quantitative Expediency Assessment of the ZETA Sedimentation Ratio and the Plasma Viscosity in Arterial Hypertension Research

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The Westergren method (WM) known as earliest *in vitro* test and yet considered the gold standard of the erythrocyte sedimentation rate (ESR) in whole blood sample (WBS) is in immutable discussion for its limitations on account of uncontrolled shear conditions, dependence on HCT scatter, differences in viscosity and density of WBS. Some authors propose the plasma viscosity (PV) as an alternative to WM but there exist communications taking into account a high false negative rate of PV. The Zeta sedimentation ratio (ZSR) has been recommended from ICSH as independent especially from limitations in HCT (within 0.25 - 0.47) and sensitive to minimal elevations of "acute phase proteins". The aim of the study was to test the diagnostic utility of the three mentioned methods in excerpt of healthy non-pregnant (n = 57), healthy pregnant (n = 57) and pregnant women with hypertension and pre-eclampsia (n = 41) as well, where the last two mentioned groups has been recognized recently as a transient likeness of incipient cardiovascular disease in general. Data of each study population were processed for normal distribution (test of Kolmogorov-Smirnov), descriptive statistics and comparison and correlation tests. For diagnostic verification were calculated sensitivity (SE), specificity (SP), efficiency (EFF), positive predictive value (PPV) and the mean coefficient of variation (CV) of each tested method. Because of established normal distribution of all study populations it was possible to set bounds to normal range (average value ± the double SD). So the cut off for ZSR was in line with 70 % and for PV 1.8 mPa.s. The standard ESR cut off for the population of Bulgarian women up to 50 years is about 25 mm/h. The receiver operating curve (ROC) analyses supply support for the established cut offs of ZSR and ESR but not for PV. The diagnostic characteristics cover SE= 59 %, SP= 88 %, PPV= 63 %, EFF= 80 % and CV=7% for ZSR; SE= 80 %, SP= 69 %, PPV= 44 %, EFF= 71 % and CV= 49 % for ESR; SE= 10 %, SP= 97 %, PPV= 57 %, EFF= 74% and CV= 6 % for PV. We were able also to find reliable proofs that certain metabolic indices (for protein and lipid fractions) are correlates to our elaborated hemorheological tests. ZSR seems to be reliable, satisfactory substitute in clinical situations for its greater SP, PPV, EFF and much smaller CV than ESR. Besides in WBS by excessive protein level, stable HCT - readings and extremely high ESR the WM is recommended as likely more suitable mode. For the present the very low SE of PV could be explained by appearance of irreversible protein-lipid complexes in the settings of WBS.

Rac-dependend adaptation of paraendothelial barrier function under fluid flow

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Endothelial cells constitute a barrier between blood and tissue that is required for a controlled exchange of water and solutes between these compartments. Due to their localisation the cells are continuously subjected to shear stress of the blood flow. This mechanical stimulus activates several signalling pathways, which in turn results in a reorganisation of the endothelial cytoskeleton and alterations in protein expression. Here we investigated the effect of acute and chronic flow on junction regulation. Using an extended experimental setup that allows analyses of endothelial barrier function under flow conditions, we found a flow-induced upregulation of the transendothelial electrical resistance (TER) within minutes. This was accompanied by an increase in actin filaments along the junctions and vascular endothelial (VE)-cadherin clustering, which was identified at nanoscale resolution by stimulated emission depletion (STED) microscopy. In addition, a transient tyrosine phosphorylation of VE-cadherin and catenins occurred within minutes following the onset of flow.
Chronic shear stress application over 24 hours results in a moderate and reversible TER decrease and an increase in cell border length, which was due to cell elongation. Junctional VE-cadherin and actin distribution were maintained under chronic flow and associated with the upregulation of VE-cadherin and □-catenin expression, thus compensating for the increase in cell border length. Importantly, all observed effects were rac1 dependent as verified by the inhibitory effect of dominant negative N17rac1. These results show that adaptation of endothelial cell to shear stress occurs while intercellular junctions remain intact. The data place rac1 in a central multimodal regulatory position that might be important in the development of vascular diseases, such as arteriosclerosis.

Effects of Swimming Exercise IN Cold Water (4º C) on RBC Rheology and Membrane Fatty Acids Composition

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There are few studies concerning rheology of blood cells and studies concerning effects of swimming exercise on red blood cells rheology are especially scarce. In order to better characterize physiological properties of erythrocytes from animals subjected to such conditions studies were expanded and the levels of fatty acids (caprylic, capric, lauric, tridecanoic, myristic, myristoleic, pentadecanoic, palmitic, palmitoleic, heptadecanoic, stearic, elaidic, oleic, linoleic, arachidic, cis 11eicosenoic, linolenic, behenic, erucic, arachidonic) isolated from erythrocyte membranes and their percentage relationships determined. Also whole blood glucose level and percentage relationship of poikilocytotic erythrocytes to normal ones and the number of reticulocytes have been studied. These studies together with erythrocyte rheology could answer if there is any influence of physical exercise carried out at low temperature on the studied parameters. Studies were carried out on rats swimming in water at 4º C (5 minutes long physical exercise connected with thermal shock). The second group consisted of rats swimming in water at 25°C (1 hour – maximal effort), and in the third one there were sedentary rats. In rheological studies the following parameters were taken into consideration: EI, AI, T½, Amp, FSAR. The most prominent differences concerned: in animals from both groups subjected to exercise vs. Sedentary ones there was an increase in: AI (34%), Amp (79%) and a decrease in T½ (52%), FSAR (2,8%). Among hematological parameters there was a decrease in WBC by 17%, and PLT by 60%. There was also an increase in poikilocytosis by 38% in both groups subjected to exercise. The most prominent differences concerned RBC membrane fatty acids (listed above). In both groups exposed to exercise there was an increase in palmitic and linolenic acids content while a decrease in stearic acid content. There were no statistically important changes in the remaining parameters studied. It should be noted that the studies did not affect life of the studied rats and no side effects have been observed.

Coagulation Activation and Hyper viscosity – not Just an Epiphenomenon in Malignancy

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For more than 150 years it is known that cancer patients are at increased risk for the development of thrombotic complications and in the past 3 decades considerable progress has been made in the understanding of the causing mechanisms. Moderate to extensive coagulation activation that is found in most malignant disease is not only a result of inflammatory actions and concomitant infectious disease in these patients but can be induced by tumor-cell mediated actions as well. Physiological healing of injured tissues resembles invasion and progression of malignant tumors in many kinds whereas the latter is a chronic not ending state that permanently initiates and degrades clot.
While extent of coagulation activation is often correlated with advanced stages of the malignancy, its prognostic impact for the outcome of cancer is rather weak. On the other hand, high blood viscosity and a high plasma viscosity in particular was an independent prognostic factor in some cancer types such as gynaecologic and breast cancer patients. Tumor cells themselves are potentially capable to induce systemic coagulation activation through tissue factor expression on tumor cells that binds activated FVII or through tumor procoagulant that directly activates F X. In many cancer types TF expression has now been well analysed and is thought to play a key role in tumor angiogenesis and mechanisms needed for tumor -detachment, - settlement and thus tumor growth. However promotion of the tumor-cell growth via TF seems not to be restricted to its procoagulant features. Although anticoagulation therapy has been shown to improve survival rates in some cancer patients inhibition of the TF pathway that effectively reduced tumor growth and settlement of metastasis *in vitro* is a promising strategy for anti cancer strategies in the future.

Red blood cell aggregation under adrenergic action

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Elevated red blood cell aggregation has been associated with a number of diseases. However, the mechanism and physiological relevance of this phenomenon remains to be clarified. More information is needed about hormonal regulation of the rheological blood properties under various physiological and pathological states. Hormonal status also markedly modifies red cell aggregation. It is well known that epinephrine, norepinephrine and dopamine are the main hormones, which are responsible for the acute and chronicle stress adaptation. Catecholamines provide complex integrated metabolic response. The elevated levels of blood catecholamines are registered under intensive physical exercises, emotional stress. Local increase of catecholamines content takes place under cardiovascular and cerebrovascular disorders. The aim of our study was to investigate the erythrocyte aggregation process under adrenergic action in blood samples of young healthy volunteers (n=27), patients with cardiovascular (n=24), and cerebrovascular diseases (n=16).

It was shown that adrenoreactivity under pathology was markedly decreased as compared to healthy persons adrenoreactivity. In presence of elevated level of catecholamines (epinephrine and norepinephrine) the initial high extent of red blood cell aggregation in both groups of patients (with cardiovascular and cerebrovascular diseases) remained almost unaltered, while the erythrocytes aggregability of healthy volunteers was significantly increased. The detail investigation of this stimulative influence of catecholamines on RBC aggregation process in physiological state showed that the action of both epinephrine and norepinephrine was mediated by the activation of erythrocyte membrane α-adrenoreceptors. The effect of epinephrine was markedly impeded in the presence of α1- and α2-adrenergic blockers (corynanthine and yohimbine), the effect of norepinephrine was inhibited in the presence of α1-adrenergic blocker (corynanthyne). The effect of catecholamines and α1-agonist phenylephrine on α1-adrenergic erythrocyte membrane receptors caused activation of the signal transduction pathway increasing intraerythrocyte Ca^{2+} level and inducing K^{+}-loss (Gardos-effect).

Our studies demonstrated the individual differences in RBC aggregative response to adrenergic action in blood of healthy volunteers from sample to sample. It was shown that the degree of RBC aggregation under catecholamines stimulation depends on the ABO blood group of individual. The more pronounced aggregative effect we registered among blood group O samples (in the absence of AB antigens on RBC membrane). Alpha-agonists caused minimal effect on RBC aggregation in the presence of A blood group antigen on erythrocytes membrane (in the case of A(II) and AB(IY) blood groups). In turn, the effect of β-agonists on erythrocyte aggregation was markedly less in the presence of B antigen (B(III) and AB(IY) blood groups) on the membrane surface.

Bovine blood viscosity as evaluated by a rheometer and a torsional-oscillation viscometer

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Blood viscosity is an important factor to determine the blood flow characteristics. Its value depends, among other factors, on the hematocrit, the ability of the erythrocytes to deform and orient in the flow as well as on the plasma viscosity. Aim of the present study was the viscosity characterization of bovine blood at different hematocrit values using two different techniques: a typical rheometer (AR500, TA Instrument) and a torsional-oscillation viscometer (Viscomate VM10AL, CBC Europe).
The equation $\eta = \mathrm{A} \cdot e^{\mathrm{B} \cdot x} + \mathrm{C}$ was adopted to describe the dependence of viscosity on hematocrit and cell suspension, where: A represents the plasma viscosity; B and C are coefficients, and x represents the relative hematocrit values, expressed as percent. Linear correlation between rheometer measurements (at different shear rates, s^{-1}) and viscometer measurements was found (see Table 1), as estimated by Bonferroni test. The obtained results appear to be promising for the use and feasibility of the new torsional-oscillation viscometer in a hemorheologic laboratory. An analogous evaluation method between the two different viscometric techniques using human blood is ongoing.

Table 1. Linear correlation between rheometer and viscometer measurements

Shear rate AR 500 [s^{-1}]	r^2	p
8 upward curve	0.8417	< 0.0001
10 upward curve	0.9065	< 0.0001
15 upward curve	0.9206	< 0.0001
18 upward curve	0.8173	< 0.0001
20 upward curve	0.7884	< 0.0001
25 upward curve	0.7653	< 0.0001
25 downward curve	0.9083	< 0.0001
20 downward curve	0.8599	0.0002
18 downward curve	0.9045	< 0.0001
15 downward curve	0.9150	< 0.0001
10 downward curve	0.9157	< 0.0001
8 downward curve	0.9009	< 0.0001

Hemorheological parameters as independent risk factors for deep vein thrombosis

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In 1884, Virchow postulated the theory of a triad of abnormalities, i.e., vessel wall disorders, stasis (blood flow alterations) and increased coagulability, to explain the etiology of thrombosis. In the last few years it has become evident that thrombotic risk is a dynamic process resulting from synergism between acquired risk factors (e.g. immobilization, surgery trauma, estrogen intake, pregnancy, malignancy, infection and inflammatory states) and inherited or acquired thrombophilias (e.g. antithrombin, protein C and protein S deficiencies, factor V Leiden, prothrombin G20210A mutation, antiphospholipid antibodies, hyperhomocysteinemia). Although the contribution of thrombophilic defects and its prothrombotic potential are well established for the above mentioned thrombophilic risk factors, it still has not been determined whether hemorheological alterations (blood flow alterations) constitute independent risk factors for deep vein thrombosis (DVT) . The rheological hypothesis for venous thrombosis is supported by the association of many risk factors for DVT with systemic rheological alterations and the association of some rheological alterations with DVT in case-control studies. In this sense increased fibrinogen levels and plasma viscosity have been suggested as independent risk factors for DVT, although little information exist regarding other rheological parameters. Most of these studies lack an appropriate design, have only a small sample size and do not exclude the potential confounding factors. In order to find out the contribution of the rheological alterations themselves on DVT, prospective studies should be carried out although they are difficult to perform and time consuming.

Another way to find out the influence of rheological parameters on DVT may be only considering spontaneous (unprovoked) events in order to avoid the influence of the reactant phase/status of systemic diseases characterized by increased fibrinogen levels and other proteins, which in turn may modify rheological blood behaviour. The presence of concomitant cardiovascular risk factors should not be neglected, as obesity and hyperlipidemia may also modify the rheological profile. In addition, given the prothrombotic potential of some thrombophilic defects, patients carrying these anomalies should also been excluded to find out the real influence of rheological parameters on thrombotic risk.

Given the scarcity of spontaneous DVT, multicentric trials should be performed in order to achieve the desired sample size. However a further problem is that all rheological measurements should be performed in a single centre to avoid further methodological confounders, although given that red blood cell properties deteriorate 2 hours after blood collection, it is a difficult issue. All these considerations help us to understand why the role of rheological parameters as independent risk factors for DVT still remains a question for debate.

The effect of aspirin on hemorheological parameters of patients with diabetic retinopathy

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Introduction: Hemorheological factors play an important role in the pathogenesis of different cardiovascular diseases and retinal disorders. The severe complications of diabetes are well known, and among them diabetic retinopathy is the leading cause of blindness in patients aged 20-65 years. In our study we investigated the effect of aspirin on the hemorheological parameters in patients with different diabetic retinopathies.
Patients and Methods: Hemorheological parameters (hematocrit, plasma fibrinogen, plasma and whole blood viscosity in capillary viscosimeter –Hevimet 40, Hungary; red blood cell aggregation in Myrenne aggregometer) of diabetic patients with non-proliferative (n=14, mean age: 66 years) and proliferative retinopathy (n=8, mean age: 48 years) were measured. The results between the two groups were compared: twelve patients were taking aspirin (group A), while ten patients were not (group B).
Results: Plasma and whole blood viscosity were significantly higher ($p < 0.05$) in patients with diabetic retinopathy who did not take aspirin (group B) than in those who took (group A). No significant difference was observed in hematocrit level and red blood cell aggregation parameters between the two groups. We could not find any significant difference in the measured parameters between patients with non-proliferative and proliferative diabetic retinopathy.
Conclusion: According to our results, all the measured hemorheological parameters were in the pathological range, although aspirin treatment could decrease these factors and thus may help to prevent the progression of severe diabetic retinopathy and blindness.

Hemorheological Disturbances in Cerebrovascular Diseases

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The study aimed to evaluate the possible relationship of the hemorheological disturbances with the clinical characteristics and some risk factors in 92 patients (38 women, 54 men at mean age of 58 years) with cerebrovascular diseases (CVD).Forty eight of the patients were with chronic unilateral cerebral infarctions (UCI) and 44 with transient ischemic attacks (TIAs). The diagnosis of the CVD was based on a history about survived cerebral ischemic event and CT and/or MRI. A questionnaire including 11 main risk RF for CVD was filled. Ultrasound examination with duplex scanning of the carotid and vertebral arteries and TCD monitoring of the basal cerebral arteries was also performed. The hemorheological examination included evaluation of hematocrit (hematological analyzer), fibrinogen in g/l (coagulation method Clauss), blood and plasma viscosity in mPa.s (rotational Couette viscometer Contraves Low Shear 30 at shear rate range of 0.0237 s-1 to 94.5 s-1), plasma viscosity in mPa.s (capillary viscometer). Also some lipid variables (cholesterol, triglicerides and HDL) were measured. The values of the hemorheological and lipid variables were compared to a control group of 42 healthy subjects.
The impairment of the hemorheological parameters was more pronounced in the group with UCI. Their pathological changes predominated in patients with large vessel in comparison to small vessel disease and this was confirmed by their correlations with the neurosonographic parameters. Hematocrit and whole blood viscosity correlated significantly with some carotid atherosclerosis markers. The hematocrit and fibrinogen values were also higher in the anterior circulation ischemic lesions, while plasma viscosity was elevated in localized in the posterior circulation ischemic lesions. Hematocrit and plasma viscosity were found to be increased in patients with cerebral hemispheric hypodense lesions on the CT and plasma viscosity was increased in patients with brain atrophy. Fibrinogen and plasma viscosity were the hemorheological parameters which correlated best with age and the lipid variables.
The diagnostic value of the hemorheological investigations in CVD was discussed.

Hemorheological Disturbances and Cognitive Function in Patients with Cerebrovascular Disease

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To test the hypothesis whether cognitive functions are associated with alterations of some hemorheological variables seventy three patients (29 females, 44 males, mean age 56,32 years) with ischemic cerebrovascular disease (CVD) - 30 with transient ischemic attacks (TIAs) and 43 with chronic cerebral infarctions (CCI) were investigated. The examined hemorheological variables were hematocrit, hemoglobin, fibrinogen and plasma viscosity. The psychological investigation included tests for evaluation of attention, memory, intellectual function, personality qualities and global cognitive capacity.
The hemorheological examination showed increase of fibrinogen and plasma viscosity in both patients` groups in comparison to 80 presumed healthy controls, while hematocrit remained within normal ranges. The psychological testing revealed impairment of the attention distribution and turning over and it was moderate in patients with TIAs and severe in CCI. The memory fixation and reproduction and the cognitive capacity were slightly to moderately disturbed.
It was established that the relationship between the hemorheological variables and the cognitive functions predominated in the patients with CCI, especially in those with bilateral CCI. Fibrinogen and plasma viscosity correlated best with the psychological scales. Their increase was associated with impairment of attention and memory and decrease of cognitive capacity. The correlations of the hemorheological variables with the attention deficit were the most pronounced. Additional data indicate the influence of the systemic and the cerebral hemodynamics on the hemorheological-psychological relationship.

Hemoglobin Levels during 2nd Trimester and at Term in Patients with Moderate and Severe Pre-Eclampsia

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Introduction: During second trimester of pregnancy physiological plasma volumen expansion results in a 10 to 20 % reduction of haemoglobin levels. Large population based trials from the U.K. and Scandinavia found a statistically significant correlation between the rate of adverse outcome events e.g. pre-eclampsia and high haemoglobin levels during pregnancy or at term. In a previous study we found statistically significantly higher mean haemoglobin levels during 2nd trimester in women who developed pre-eclampsia as compared to those who had normal outcome of pregnancy.
Aim of the Study: It was the aim of the present study to compare mean haemoglobin levels during 2nd trimester and those at term in patients with moderate and severe pre-eclampsia.
Among 4,985 konsecutively recorded pregnancies 423 women developed pre-eclampsia (BP ≥140/90 mmHG and proteinuria ≥ 300 mg/24h) of which 24 had severe pre-eclampsia (BP≥180/110; proteinuria ≥ 3g/24h).
Methods: haemoglobin values (hb) between gestational week 14 and 28 were obtained from the maternal records and means were calculated. At term Hb levels were estimated before delivery.
Results: Mean BP and proteinuria was statistically significantly higher in patients with severe pre-eclampsia (m±SD: 181±14/116±8 mmHg vs. 143±14/88±10 mmHg; p<0.001) 7,543±1,820 mg/24h vs. 1.253±650 mg/24h; p<0.001) at term. All Patients with severe pre-eclampsia had caesarean section, had early birth (<37 SSW) and delivered stat. significantly lower birth weight newborns compared to patients with moderate pre-eclampsia. Mean hb levels during 2nd trimester were slightly but not stat. significantly higher in patients who later developed severe compared to those who had moderate pre-eclampsia (12.7±0.9 g/dL vs. 12.5±1.0 g/dL) while at term values were stat. significantly lower 11.7±1.6 g/dl vs. 12.4±1.3gdL; p=0.01) in patients with severe pre-eclampsia.
Conclusion: According to our results there seems to be no association between 2nd trimester haemoglobin levels and severity of disease in patients with pre-eclampsia. However, at term severe pre-eclampsia was coincided with lower hb levels. Although the later most likely is the consequence of markedly intravasal haemolysis due to severity of pre-eclampsia others have found significantly positive correlation between hemoconcentration at term and stage of pre-eclampsia.

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Blood Rheology at Term in Normal Pregnancy and in Patients with Adverse Outcome Events – Results from a Population Based Trial

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Introduction: In the course of pregnancy, activation of the hemostatic system is up-regulated while fibrinolysis is down-regulated leading to a markedly increase in fibrinogen turnover. High levels of split-products of coagulation activation may effectively influence hemorheological variables while physiologic plasma volume expansion counteracts this mechanism. Adverse outcome of pregnancy and pre-eclampsia in particular - however - is associated with even more pronounced coagulation activation and is accompanied by hemoconcentration. Thus blood viscosity in these patients may be considerably increased at the cellular and plasmatic level as well.
Aim of the Study: On the basis of 4,985 consecutively recorded singleton pregnancies in whom plasma viscosity and RBC aggregation as well as haemoglobin-levels and hematocrit were determined routinely directly before delivery ranges of values in women with normal pregnancy outcome and those from patients with adverse outcome events were calculated.
Methods: Plasma viscosity (pv) was determined using KSPV 1 Fresenius and RBC aggregation (stasis E0 and low shear E1) using MA1- Aggregometer; Myrenne.
Results: Seventy-nine point four percent (n=3,959) had normal pregnancy outcome while 1,026 had adverse outcome of pregnancy 8.4% (n=423) had pre-eclampsia, 9.5% (n=473) delivered a low birth weight newborn (< 2,500 g), 9.3% (n=464) had early birth (before week 37), and in 5.0% (n=250) IUGR was diagnosed. In women with normal pregnancy outcome means (±SD) of pv were 1.32±0.08 mPas of E0 were 21.6±5.3 and of E1 were 38.4±7.9 while in women with adverse outcome means for all rheological parmeters were statistitically significantly different e.g. 1.31±0.08 mPas for pv (p=0.006), 22.1±5.5 for E0 (p=0.002) and 39.5±8.4 for E1 (p=0.0006). Subgroup analysis revealed stat. sign. lower pv in women who either delivered pre term or a low birth weight child ($p<0.005$) as compared to women who had normal pregnancy outcome while RBC agg (stasis and low shear) was markedly and stat. significantly higher in patients with pre-eclampsia. None of the rheological variables were stat. significantly correlated with maternal BMI, gestational age at term, apgar score and venous pH in the umbilical cord in woman with normal pregnancy outcome while there was a stat. significant but weak correlation between haemoglobin and pv (spearman $r=0.15;p<0.0001$).
Conclusion: To our knowledge this is the largest trial that consecutively assessed blood rheological parameters in pregnant women at term. Distribution of pv and RBC agg was studied in women with normal and complicated pregnancy. We found lower pv and an increased RBC aggregation in patients with adverse outcome of pregnancy compared to normal pregnancy. Fibrinogen concentration which is markedly increased in pre-eclampsia due to enhanced coagulation activation is a weighty co factor of pv but seems not to influence pv under these conditions. In contrast, RBC aggregation is markedly increased in pre-eclampsia which may potentially contribute to the decreased blood flow in the materno-fetal unit

Specifics of Hemodynamics and Endothelium-Dependent Vasodilation in Patients with Inherent Platelet Dysfunction

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Platelet dysfunction (PD) is based on disorders of one or several functions of thrombocytes. PD clinical manifestations include bleedings (epistaxis, hypodermic hemorrhages, metrorrhagia, menorrhagia in women and others). Interaction of platelets with the vascular wall, the von Willebrand factor, creates a system of primary hemostasis. Shear stress significantly influences primary hemostasis condition. Shear stress is a mechanical effect of blood flow on endothelium surface. Hydrodynamic forces influence endothelium condition and functional activity of thrombocytes. The goal of this research was to study some particular hemodynamic parameters and the endothelium-dependent vasodilatation condition in patients with inherent PD forms.
20 patients with PD (7 men and 13 women) were subject to this study. The average age of patients is 21.4 years. In all patients bloodstream parameters (peak systolic (Vps), diastolic (Ved) and volume (Vvol) speed of blood flow), shear stress (τ), sensitivity of brachial artery to shear stress (К), and also endothelium-dependent vasodilatation (Celermajer D.S., 1992) were identified. In patients with PD higher Vps, Vvol and shear stress (by 1.5 times compared to a similar parameter in healthy persons) was detected. No endothelium-dependent vasodilatation and K was identified. The absence of endothelium dysfunction attests that endothelium cells have retained synthesis of nitric oxide (NO). NO inhibits the platelet function. A higher shear stress (τ) leads to disturbance of the processes of interaction between thrombocytes, with the von Willebrand factor and endothelium.
The discovered changes in some particular hemodynamic bloodstream characteristics enhance the existing qualitative defect of platelets and have hemorrhagic directivity.

Influence of nicotinic acid on Lp(a)

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The cholesterol lowering effect of nicotinic acid has been known since the investigation of Altschul et al [1]. The main effects include the inhibition of fatty acid mobilisation from the peripheral tissues, resulting in the reduction of triglyceride synthesis and hepatic VLDL secretion [2]. Additionally, nicotinic acid can prevent the transformation of VLDL to LDL [3]. With maximum therapy, the LDL concentration can be lowered by up to 30% [2]. The newest studies demonstrate now that nicotinic acid can also decrease Lp(a) concentrations. [4,5].

This effect is especially interesting as the increased Lp(a) concentrations have as yet hardly been amenable to any medical therapy. Even the modern, cholesterol concentrations drastically reducing statins, do not influence the Lp(a) concentrations.

During our investigation, a total of 50 patients with an Lp(a) concentration of more than 60mg/dl were treated with xanthinol nicotinate (Complamin retard). The therapy was initiated on a once daily basis, after 7 days the dosage was increased to twice, and after 14 days to three times daily.

From the total of 50 patients included, 31 patients completed the study as intended. Among the study completers, neither the triglyceride nor the HDL concentrations changed significantly ($p=0.1121$ bzw. $p=0.1196$). The total cholesterol levels decreased from 228.1±40.3mg/dl initially, to 194.3±38.8mg/dl after 28 days, and to 193.3±42,1mg/dl after 84 days of xanthinol nicotinate therapy ($p<0.0001$). The LDL cholesterol concentrations were 152.7±40.3mg/dl initially, 109.6±29.7mg/dl after 28 days, and 115.6±34.5mg/dl after 84 days of xanthinol nicotinate therapy ($p<0.0001$). The Lp(a) levels were 92.6±37.7mg/dl initially, 86.2±34.6mg/dl after 28 days, and 89.3±33.7 mg/dl after 84 days of xanthinol nicotinate therapy ($p=0.033$).

It must be taken into consideration that in 14 patients a statin was administered concurrently.

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Resting Perfusion in Viable Myocardium distal to Chronic Coronary Stenosis is Reduced: Characterization by Magnetic Resonance Imaging and Radiolabeled Microspheres in the Rat

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Chronic coronary artery stenosis results in patchy necrosis in the dependent myocardium and impairs global left ventricular (LV) function in rats in vivo. The aim of the present study was to compare blood flow in poststenotic myocardium using magnetic resonance [MR] and microspheres [MS], and to assess whether or not resting blood flow in the viable poststenotic tissue remains normal.
MR Imaging (Bruker Spectrometer) and radiolabeled MS measurements were performed on consecutive days in 11 anesthetized rats with two weeks stenosis of the left coronary artery. Post mortem, the extent of fibrotic tissue was quantified (histology).
Perfusion (ml/min/g) in the remote myocardium was 4.05±0.50 and 3.85±0.36 using MR and MS, respectively. Poststenotic perfusion was significantly reduced to 2.21±0.30 and 2.11±0.31 using MR and MS, respectively, and the correlation of MR and MS was not different from the line of identity (MR=0.98*, MS+0.11, r=0.77, $p<0.001$). Assuming perfusion in scar tissue to be 30±2 % of perfusion of remote myocardium – as measured in 5 additional rats – and in remote myocardium to be 116±14 % of normal myocardium – as assessed in 5 sham rats, – the calculated perfusion in partially fibrotic tissue samples (35.7±5.2% of analyzed area) was 2.50±0.20, whereas measured MS perfusion was only 1.99±0.15 ($p<0.05$).
These results indicate that MR can adequately assess perfusion in poststenotic myocardium of rats, and that resting perfusion in viable poststenotic myocardium is moderately reduced. Alterations in global LV function are therefore secondary to both, patchy fibrosis and reduced resting perfusion.

Central venous oxygen saturation does not correlate with the venous oxygen saturation in the operation field during visceral surgery

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Purpose: Measurement of the central venous oxygenation saturation has become a surrogate parameter for fluid administration, blood transfusions and treatment with catecholamines in goal directed therapy of acute septic patients. Because of totally different conditions in surgical patients these strategies can not be transferred easily for the postoperative management in visceral surgery.
Methods: Our study population consisted of 17 patients (10 female/7 male) undergoing elective visceral surgery: 6 gastrectomies, 5 major liver resections, 2 pancreatic head resections and 4 lower anterior rectum resections. Surgery was performed due to primary or secondary malignancy. The mean patients age was 65.9 (44-84) years. Blood samples were taken from indwelling central venous lines as well as from the draining veins of the field of surgery. Blood gas analyses to determine the oxygen saturation were immediately performed at a maximum of 4 time points during surgery. All patients were operated in standardized general anesthesia including epidural analgesia and in a balanced volume status.
Results: The average values of the oxygen saturations in central venous blood and venous blood from the draining veins of the operation fields showed a correlation of 71-88 %. But overall, these values are widely spread due to high intra- and interindividual differences: 83-100 % in gastrectomies, 21-97 % in major liver resections, 100 % in pancreatic head resections and 26-75 % in lower anterior rectum resections.
Conclusion: Our results show a lack of correlation between the central venous oxygenation and the oxygen saturation in the draining veins in the field of surgery during visceral surgery. Therefore measurement of the central venous oxygenation is not a good surrogate parameter for determining the perioperative treatment in visceral surgery even under standardized conditions.

Portal venous stasis during major liver resection does not affect platelet aggregability

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Purpose: Platelet aggregability may be affected by circulatory stasis. To reduce intraoperative blood loss in major liver resections a surgical bleeding control is often performed by selective vascular clamping or complete inflow obstruction of the liver by a Pringle manoeuvre leading to a portal venous stasis.
Methods: A study population of 17 patients (31 to 74 years old, 9 female and 8 male) with hepatic tumours underwent elective major liver resection. Selective vascular clamping was performed in 11 patients. A Pringle manoeuvre with a duration of up to fifty minutes was used in 6 patients. Blood samples for platelet aggregability were taken from peripheral veins as well as via direct punctation of the portal vein. Platelets were aggregated with ADP, collagen and ristocetin (according to Born).
Results: Mean maximal amplitudes of platelet aggregation were comparable for selective vascular clamping (SVC) and Pringle manoeuvre. With the use of SVC ADP-induced aggregation decreased from 68.4% to 68.3%, collagen-induced aggregation decreased from 67.8% to 62.8% and ristocetin-induced aggregation increased from 81.5% to 81.8%. Pringle manoeuvre lead to a decrease in ADP-induced aggregation from 73.3% to 68.2%, collagen-induced aggregation decreased from 67.8% to 62.8% and ristocetin-induced aggregation decreased from 73.3% to 67.7%. Komolgoroff-Smirnoff statistic assay did not detect a significant difference between the pre- and postoperative platelet aggregability in both groups.
Conclusion: Induced platelet aggregability is not affected by the method of surgical bleeding control used in major liver resection. Platelet aggregability seems to be resistant even to portal stasis of up to 50 minutes during Pringle manoeuvre.

Influence of preanalytic conditions on soluble CD40L determination

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Markers reflecting an inflammatory response are increasingly offered to improve the risk stratification of patients with acute coronary syndrome (ACS). So current evidence suggests that soluble CD40 ligand (sCD40L), an immune-modulatory substance largely expressed by CD4+-T-cells and activated platelets, is elevated in patients with ACS. But only a few data are available to evaluate the influence of preanalytic conditions on sCD40L values. Therefore we determined sCD40L levels under different sampling techniques and storage conditions.
Blood samples of five healthy blood donors were collected in tubes without additives and in EDTA- or citrate-filled tubes at various storage conditions (25° C, 37° C, 1 h and 2 h). Additionally, the number of thrombocytes was modified by serum dilution, and sCD40L was measured after platelet activation in platelet-rich-plasma (PRP) and in whole blood. sCD40L levels were determined by an commercially available ELISA-Kit (R&D Systems).
Immediately after blood sample assessment, sCD40L levels in serum samples were elevated (1258 ± 820 pg/ml) compared to EDTA (64 ± 32 pg/ml) and citrate (60 ± 8.5 pg/ml) values showing no significant differences between each other. Additionally, sCD40L levels were dependent on storage duration (e.g. citrate values after 1 and 2 h were found to be 174 ± 131 and 271 ± 149 pg/ml). After platelet activation, sCD40L levels were significantly increased to 8278 ± 2453 pg/ml and were significantly correlated to platelet count ($r = 0.96$).
Soluble CD40L levels were clearly influenced by preanalytic conditions and were dependent on storage duration, sample technique, platelet activation and platelet count. These influences should be considered by the determination and evaluation of sCD40L concentrations.

Hemorheology in different laboratory rat strains

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Four laboratory wildtype and 2 genetic mutant (Dahl/SS/JrHsd, ZDF/Crl-Leprfa) rat strains were tested for variability in whole blood viscosity (LS30, Contraves, Switzerland; shear rates: 0.7, 2.4, 94*s-1), plasma viscosity (OCR-D, Paar, Austria), RBC aggregation (Myrenne, Germany; M0, M1), and RBC deformability (Lorca, Mechantronics, Netherlands; SS ½).
Wistar (W) and Long Evans (LE) rats showed decreased WBV, the latter due to low hematocrit. At standardized hematocrit (40%), WBV was comparable in Fischer (F), Hairless (H), and LE, but decreased in W. Plasma viscosity and RBC aggregation indices did not differ between these strains. SS½ was between narrow ranges (1.79 – 1.90) in F, H, and LE, but increased in W (2.54). Blood chemistry and hematological values were within normal ranges. In Dahl/SS and ZDF rats, WBV was increased due to an increase in RBC aggregability; plasma viscosity was increased as well. SS½ in ZDF was in the range of W rats (2.56), whereas in Dahl/SS, SS½ was only lightly higher than in F, H, and LE (2.00). Blood chemistry showed increases of plasma lipids and glucose in diseased rats. Whole blood viscosity and RBC deformability may differ between healthy rat strains. RBC were observed to be less deformable in Wistar than in other rats. Hematocrit is lower in Long-Evans rats than in other strains which led to the differences in WBV. In case of metabolic imbalance, parameters may vary distinctively from those of healthy rats (WBV 0.7*s-1: ZDF: 56.04±19.7; Dahl/SS: 50.76±21.5 mPa*s. M1: ZDF: 14±3, Dahl/SS: 12±9).

Albumin permeability and composition of the adherens junction core complex in the rat lung as affected by ischemia/reperfusion and preconditioning with NO

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Previous work in our lab has shown that short-term inhalation of NO before ischemia/reperfusion (I/R) ameliorates the detrimental I/R-induced consequences on lung function. In order to further study the underlying mechanisms, an in situ rat model of normothermic pulmonary ischemia was established.
After left lateral thoracotomy, left lung ischemia was maintained for 60 min, followed by 30 min reperfusion (ischemia group). In the NO-group, inhalation of NO (10 min, 15 ppm) preceded I/R. Animals in the control group underwent sham surgery without NO inhalation or ischemia. Lung injury was assessed in terms of oxygenation (arterial pO2) and lung albumin permeability (Evans blue extravasation). The composition of the adherens junction core complex was analyzed in detergent (0.2 % NP40) lung protein extracts by immunoprecipitation using anti-E-cadherin and anti-VE-cadherin antibodies and quantitative immunoblotting.
After 30 min reperfusion, the animals in the I/R group developed severe pulmonary I/R injury, including reduction of the arterial pO2 to 26.9 % of controls and a 2.2-fold increase of Evans blue accumulation in left lung tissue. These effects were completely prevented (Evans blue accumulation) or ameliorated (pO2 76.9 % of controls) by short term NO inhalation. A significant increase of cadherin-bound αcatenin was determined in the NO-group compared to the I/R-group, whereas the ratios of p120/cadherin and □-catenin/cadherin were not affected by any treatment. The binding of □-catenin to E-cadherin was increased over baseline in the NO-group and in the control group without ischemia. The nuclear fraction of □-catenin and the abundance of all these adherens junction components did not vary among groups.
Since the composition of the adherens junction complex directly affects the interaction with the cytoskeleton and the cell contact in epithelia, the remodeling of the complex by pretreatment with NO may have played a role in preventing the I/R-induced increase in permeability.

Role of red blood cell aggregation in determining endothelial function

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The role of shear forces in determining endothelial function is well known. The shear forces affecting on the endothelial cells are in turn determined by the velocity and local viscosity of the fluid flowing adjacent to the vessel wall. Aggregation may influence the cross-sectional distribution of red blood cell (RBC) in tube flow and affect the local composition and viscosity of blood near vessel wall. It has been previously demonstrated that chronically enhanced RBC aggregation in rats leads to down-regulation of endothelial nitric oxide synthase (eNOS) expression and function in skeletal muscle small arteries and increased arterial blood pressure. Down-regulation of eNOS expression could also been demonstrated in endothelial cells cultured inside of glass capillaries and perfused either by normal and hyper aggregating blood samples. These experimental data suggests that the alterations in shear stress affecting on the vessel wall due to the modification of RBC aggregation can be monitored by eNOS activity and expression.

Gender-Related Alterations in Erythrocyte Mechanical Activities under Desfluran or Sevofluran Anesthesia

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Alterations in erythrocyte mechanical activities under the influence of anesthesia have been observed and discussed among the responsible factors for the deterioration of tissue and organ perfusion related to anesthetic procedures.
21 female and 17 male Swiss Albino rats were used. Female (f) and male (m) rats were divided into 3 gorups; control (f: n=7); m: n=5)), sevoflurane treated group (f: n=7);m: n=5)), desflurane treated group (f: n=7); m: n=7)). %2 of sevoflurane or %6 desflurane were applied to the rats with inhalation in a adjustable cage for one hour. The deformability indexes of the erythrocytes were measured by a laser diffractometer (Myrenne Rheodyne SSD).
Sevoflurane anesthesia has improved the deformability of erythrocytes in male rats whereas there were not any significant changes in female rats. However desflurane has improved the deformability of erythrocytes in both gender significantly.
Volatil anesthetic agents sevofluran and desflurane has improved the mechanical properties of the erythrocytes in male rats compared to their controls. However, these changes were not significant with sevoflurane in females. The results in male rats may be due to the effects of testosterone on the flexibility of the erythrocytes leading them to tolerate to the environmental changes. These results reveal that the inhalation anesthetics like sevoflurane and desflurane are appropriate anesthetics which can improve the deformability of erythrocytes during surgery.

Hemorheological effect of desmopressin as one of possible mechanism its haemostatic actions

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Desmopressin has haemostatic effects by increasing the plasma levels of coagulation factor VIII and von Willebrand factor. The precise effects of desmopressin are not known. Taking into consideration haemorheology and the haemostatic parameters interrelation the case is of interest of viscosity of blood viscosity and the condition of the basic factors which determine it at influence of this preparation. For the purpose of studying haemorheological parameters dynamics there was an experimental research. During this investigation the male rats (n = 9) were made injections of 0, 02 mkg of desmopressin twice a day. It was noticed that desmopressin injections raise blood viscosity (12 %, $p<0.001$), plasma viscosity (10 %, $p< 0,001$). The RBC aggregation extent has considerably increased (automatic aggregometer of erythrocytes MA 1, Myrenne) up to 40 %, $p<0,001$. The considerable rise glycosamineglycane (GAG) maintenance in serum was revealed ($p<0,001$). Ability of these high-molecular components of conjunctive tissue to give rise reversible aggregation of red blood cells was showed in researches in vitro and in vivo. It,s supposed that aggregate action of GAG is one of their universal biological functions which become apparent not only in the erythrocytes relations but in other cages and different tissue elements. It,s known that erythrocyte aggregates influence intensively on microvessels, activating a vascular component of haemostasis. Besides this, the concentration of cellular elements in aggregate and deceleration of circulation of the blood at increase of blood viscosity is a necessary condition of a blood clot formation.

Effects of desmopressin on thrombocytic membrane glycoproteins and platelet aggregation in healthy volunteers on clopidogrel

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The use of clopidogrel is standard in interventional cardiology. Haemorrhage occurs in some patients demanding a non-transfusional therapy. Desmopressin showed its efficacy as an antidote of acetylsalicylic acid. In this trial the effects of desmopressin on platelet glycoproteins and the platelet,s ability to aggregate under the influence of clopidogrel are studied.
The trial is conducted as an open, prospective, single-centre, randomised pilot study with n=17 healthy volunteers in a parallel-group design. 1 h after an oral loading dose of 375 mg clopidogrel the effects of a single-dose of 300µg of desmopressin nasal spray (n=9) on platelet aggregation, activity of platelets on the density of membrane-bound receptors are measured.
Ristocetin cofactor and platelet reactivity rise significantly after the administration of Octostim® nasal spray with 31.9% and 5.3%, respectively (p=0.03; p=0.04). The ADP-induced platelet aggregation rises after the administration of desmopressin nasal spray by approximately 20% (p=0.056). The fraction of CD62- and CD63-positive platelets does not change after clopidogrel nor after desmopressin (p=0.4; p=0.7). The density of GPIIb/IIIa receptors per platelet does not change after desmopressin (p=0.9). The density of GPIb/IX receptors per platelet rises after desmopressin without reaching the level of significance (p=0.08). In the desmopressin group alone the receptor density rises by 5.5% (p=0.07).
The administration of desmopressin improves the primary haemostasis when given in addition to a clopidogrel therapy. Patients undergoing a heart catheter procedure with clopidogrel might benefit from the use of desmopressin when having a bleeding episode.