Joint Meeting

Society of Microcirculation and Vascular Biology (GfMvB)
and
Dutch Society for Endothelial Biology (DEBS)
Society for Microcirculation and Vascular Biology (MiVaB)

The Young Investigator Forum

PROGRAMME

Mannheim, September 27-29, 2012
Medical Faculty Mannheim
University Heidelberg

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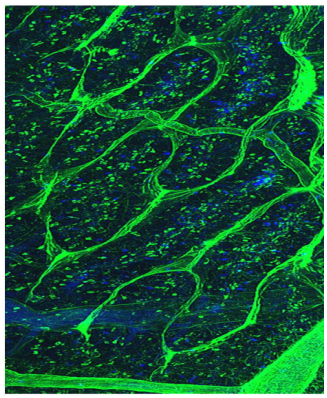

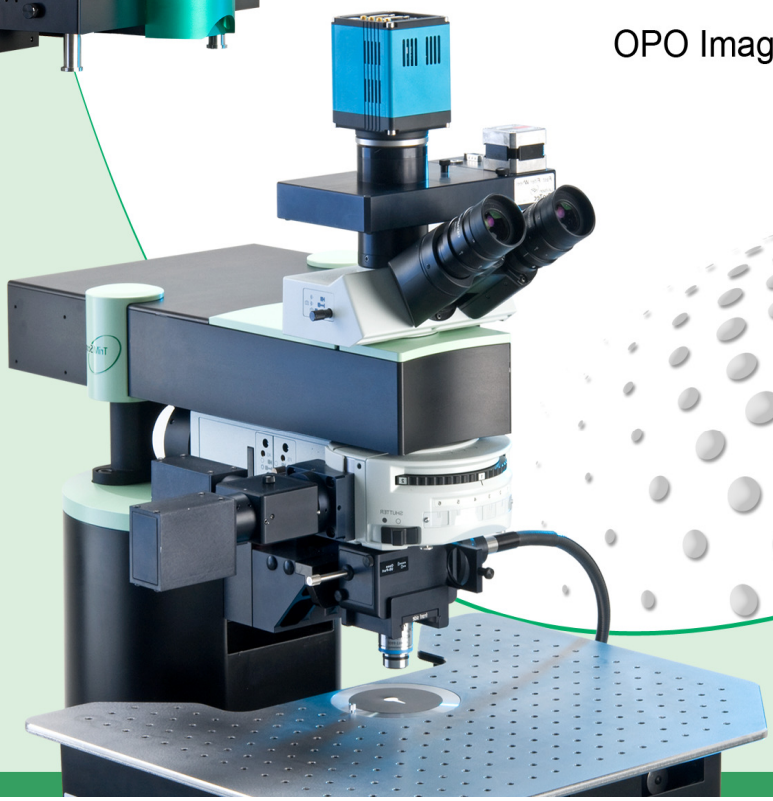

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Table of Content

Participants of the meeting will have access to the internet via WLAN

username: wlan
password: vid45

Organisation and Imprint

Venue (see also map on p. 79)
Heidelberg University
Medical Faculty Mannheim
Alte Brauerei
Röntgenstrasse 7
68167 Mannheim

Organizing Societies
Society for Microcirculation and Vascular Biology (GfMvB)
Dutch Society for Endothelial Biology (DEBS)
Dutch Society for Microcirculation and Vascular Biology (MiVaB)

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Prof. Dr. Rudolf Schubert
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Centre for Biomedicine and Medical Technology Mannheim (CBTM)
Medical Faculty Mannheim, Heidelberg University
Ludolf-Krehl-Str. 13-17, 68167 Mannheim, Germany

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Meeting Organisation
Prof. Dr. Rudolf Schubert
Medical Faculty Mannheim, Heidelberg University
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Welcome Note

Dear Colleagues,

It is a pleasure for me to welcome you at the Joint Meeting of the Society of Microcirculation and Vascular Biology (GfMvB), the Dutch Society for Endothelial Biology (DEBS) and the Dutch Society for Microcirculation and Vascular Biology (MiVaB) at the Medical Faculty Mannheim of Heidelberg University, September 27-29, 2012.

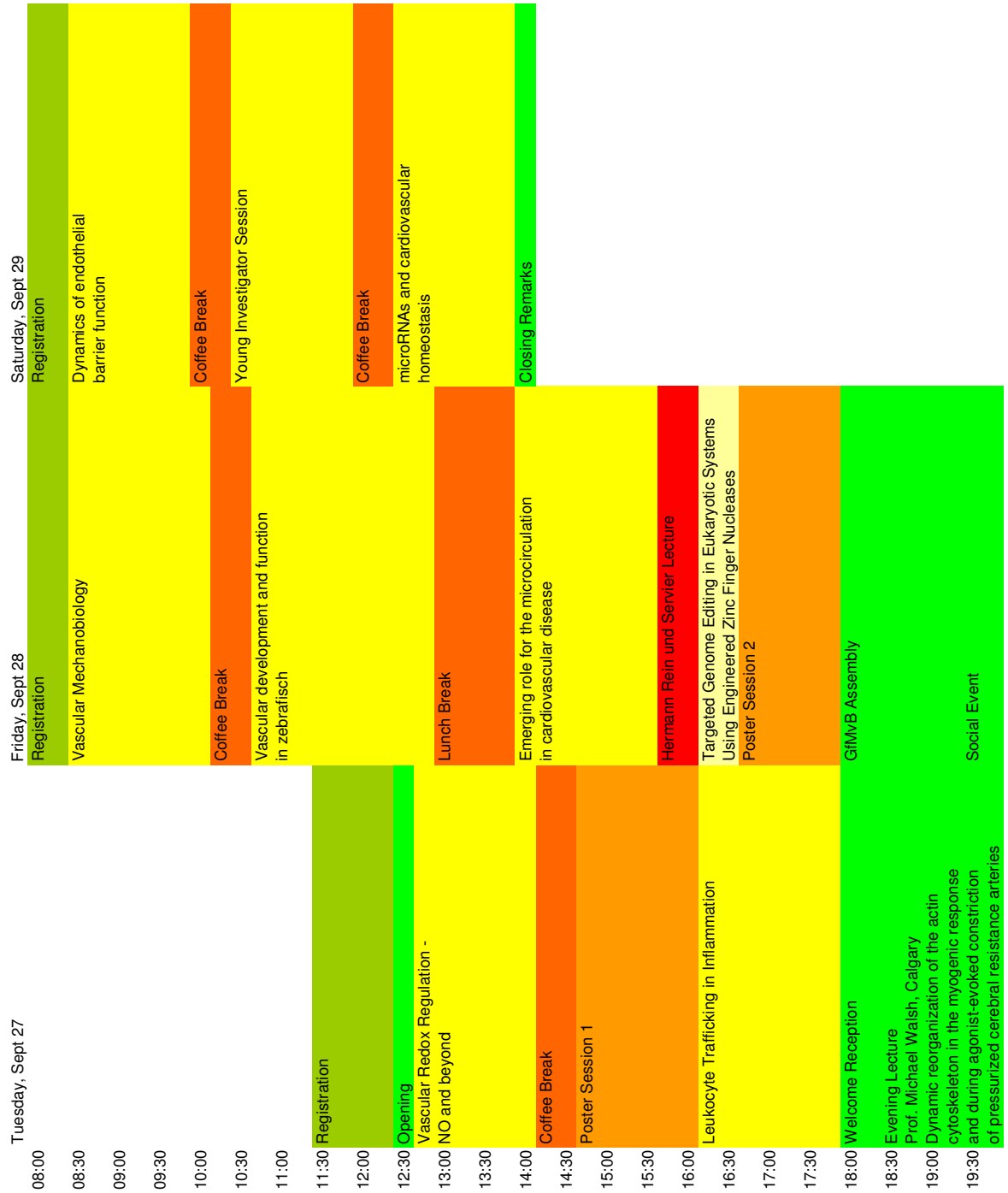
Due to the invaluable support by colleagues from the Netherlands (Boy Houben), the greater Rhein-Main Area (Ralf Brandes, Ingrid Fleming, Markus Hecker, Jens Kroll) and the executive board of the GfMvB (Hans-J. Schnittler, Barbara Walzog) as well as the many contributions from the abstract submitters an interesting programme is offered. In particular, the programme contains presentations by invited speakers, renowned specialists in their fields, and a large number of talks by young investigators, including the Young Investigator Award session, clearly underscoring the motto of this meeting, The Young Investigator Forum. Thereby, the meeting will be a place to follow the intriguing progress in many fields of microcirculation and vascular biology, to stimulate the exchange of experiences and ideas, and, last but not least, to take pleasure in science.

Enjoy your stay in Mannheim and the scientific interaction at the meeting!

Best regards

Prof. Dr. Rudolf Schubert
Meeting Organiser

Programme Overview



Scientific Programme – Thursday, September 27, 2012

12:30 – 12:45
Opening

12:45 - 14:15
Session „Vascular Redox-Regulation: NO and Beyond“
Organizer: Ralf Brandes (Frankfurt, Germany)
Chair: Ralf Brandes (Frankfurt, Germany) and **Agnes Görlach** (Munich, Germany)

12:45 – 13:05
Circulating NO Stores: Emerging role of nitrite and nitrate in cardiovascular function
T. Rassaf (Düsseldorf, Germany)

13:05 – 13:25
Vascular ROS generation: The case of inflammatory leukocytes
Ph. Wenzel (Mainz, Germany)

13:25 – 13:40
Folic acid partially restores hypoxia-induced remodelling in pulmonary vascular bed
D. Kračun (Munich, Germany)

13:40 – 13:55
The existence of an endothelial brake on agonist-induced contraction may restrict the blood pressure level during early postnatal development
D. Gaynullina (Mannheim, Germany)

13:55 – 14:15
Interplay of NO and ROS in exercise: Implications for vascular aging
Ch. Werner (Homburg, Germany)

14:15 – 14:45
Coffee Break

14:45 – 16:15
Poster Session 1

16:15 – 18:00
Session “Leukocyte trafficking in inflammation”
Presented by the SFB 914 “Trafficking of Immune Cells in Inflammation, Development and Disease”
Organizer: Barbara Walzog (Munich, Germany)
Chair: Barbara Walzog (Munich, Germany) and **Markus Sperandio** (Munich, Germany)

16:15 – 16:40
Intracellular signaling pathways in leukocytes leading to integrin activation and leukocyte recruitment
A. Zarbock (Münster, Germany)

16:40 – 17:05
Role of S100A8/A9 in leukocyte recruitment in vivo
M. Prünster (München, Germany)

17:05 – 17:30
Coronin 1A is critically required for b2 integrin (CD11/CD18)-mediated neutrophil recruitment during the acute inflammatory response
R. Pick (Munich, Germany)

17:30 – 17:40
Impact of the EphrinB/EphB system on pro-inflammatory monocyte-endothelial cell interaction
H. Liu (Heidelberg, Germany)

17:40 – 17:50
Von Willebrand Factor interacts with Extracellular DNA thereby facilitating Leukocyte adhesion
S. Grässle (Mannheim, Germany)

17:50 – 18:00
Eliminating or blocking 12/15-Lipoxygenase reduces neutrophil recruitment in mouse models of acute lung injury (ALI)
J. Rossaint (Münster, Germany)

18:00 – 18:30
Welcome Reception

18:30
Evening Lecture "Dynamic reorganization of the actin cytoskeleton in the myogenic response and during agonist-evoked constriction of pressurized cerebral resistance arteries"
M. Walsh (Calgary, Canada)

Scientific Programme – Friday, September 28, 2012

8:30 – 10:15
Session "Vascular Mechanobiology"
Organizer: Markus Hecker (Heidelberg, Germany)
Chair: Markus Hecker (Heidelberg, Germany) and **Cor de Wit** (Lübeck, Germany)

8:30 – 8:50
The role of stretch in vascular remodeling
E. van Bavel (Amsterdam, Netherlands)

8:50 – 9:10
Zyxin, a novel mechanotransducer in vascular cells
M. Cattaruzza (Heidelberg, Germany)

9:10 – 9:30
Shear stress induced mechanosensitive signaling pathways in atherosclerosis
R. Krams (London, England)

9:30 – 9:50
Shear stress-induced MAP kinase phosphatase-1 activation in endothelial cells – pathophysiological implications
P. Evans (Sheffield, England)

9:50 – 10:02
The contribution of GPCRs to myogenic response in murine mesenteric arteries
S. Blodow (Munich, Germany)

10:03 – 10:15
Visualization of endothelial actin cytoskeleton in vivo
E. Montanez Miralles (Munich, Germany)

10:15 – 10:45
Coffee Break

10:45 – 13:00
Session "Vascular development and function in zebrafisch"
Presented by the SFB/TR23 "Vascular Differentiation and Remodeling"
Organizer: Jens Kroll (Mannheim, Germany)
Chair: Jens Kroll (Mannheim, Germany) and **Carmen Ruiz de Almodovar** (Heidelberg, Germany)

10:45 – 11:15
Development of cardiovascular form and function in zebrafish
D. Stainier (Bad Nauheim, Germany)

11:15 – 11:35
Action at the junction: Dynamic cell-cell interactions shape the vascular network in the zebrafish embryo
H.-G. Belting (Basel, Schweiz)

11:35 – 11:55
Guidance events and control of organogenesis
F. le Noble (Berlin, Germany)

11:55 – 12:15
The role of Notch signaling during sprouting and pruning of the zebrafish brain vasculature
A. Siekmann (Münster, Germany)

12:15 – 12:35
miR-10-mediated modulation of angiogenesis
D. Hassel (Heidelberg, Germany)

12:35 – 12:47
Protein kinase D1 (PKD1) regulates physiological and tumor angiogenesis in zebrafish
S. Cramer-Stoll (Mannheim, Germany)

12:48 – 13:00
Depletion of Nucleoside Diphosphate Kinase B Inhibits Angiogenesis
N.M. Wolf (Heidelberg, Germany)

13:00 – 14:00
Lunch Break

14:00 – 15:45
Session "Emerging role for the microcirculation in cardiovascular disease"
Joint Session with the Dutch Societies for Endothelial Biology (DEBS) and for Microcirculation and Vascular Biology (MiVaB)
Organizer: Boy Houben (Maastricht, Netherlands)
Chair: Boy Houben (Maastricht, Netherlands)

14:00 – 14:25
The TNFalpha/sphingosine-1-phosphate/CFTR signalling axis as a central regulator of microvascular function in diseases with a systemic inflammatory component
S.-S. Bolz (Toronto, Canada)

14:25 – 14:35
Uridine Adenosine Tetraphosphate (Up4A)-Induced Coronary Vasodilation Is Blunted in Swine with Aortic Banding: a Role for Prostanoids?
Z. Zhou (Rotterdam, Netherlands)

14:35 – 14:45
Microvascular Dysfunction Increases the Risk of Type 2 Diabetes Mellitus. A meta-analysis
D. Muris (Maastricht, Netherlands)

14:45 – 14:55
AAV2.9-mediated Thymosin ß4 Overexpression Induced Therapeutic Neovascularization in a Pig Model of Chronic Myocardial Ischemia
W. Husada (München, Germany)

14:55 – 15:05
Increased vascular permeability induced by Angiopoietin-2 overexpression is antagonized by AAV-mediated PDGF transfection
V. Pfetsch (Munich, Germany)

15:05 – 15:15
Alterations in the nitric oxide/ soluble guanylyl cyclise pathway enhance the risk of myocardial infarction
C. de Wit (Lübeck, Germany)

15:15 – 15:25
Insulin does not redistribute microvascular blood volume from skeletal muscle to subcutaneous adipose tissue in obese women
R. Meijer (Amsterdam, Netherlands)

15:25 – 15:35
Early impairment of skeletal muscle endothelial glycocalyx barrier properties in diet-induced obesity in mice
B. Eskens (Maastricht, Netherlands)

15:35 – 15:45
Protective effects of the tyrosine kinase inhibitor imatinib in sepsis
G.P. van Nieuw Amerongen (Amsterdam, Netherlands)

15:45 – 16:15
Hermann Rein und Servier Lecture "The role of microbiota-induced coagulation factor signalling in intestinal vascular remodelling"
Ch. Reinhardt (Mainz, Germany)

16:15 – 16:45
Sponsor Lecture "Targeted Genome Editing in Eukaryotic Systems Using Engineered Zinc Finger Nucleases"
H. Lehrmann (Sigma-Aldrich, Germany)

16:45 – 18:00
Poster Session 2

18:00
GfMvB General Assembly

19:30
Social Event
Restaurant MARUBA
Feudenheimer Str. 2 (see Map on p. 79)

Scientific Programme – Saturday, September 29, 2012

8:30 - 10:00
Session "Dynamics of endothelial barrier function"
Organizer: H.-J. Schnittler (Münster, Germany)
Chair: H.-J. Schnittler (Münster, Germany)

8:30 - 9:00
JAMs and beyond in the regulation of vascular tight junction
B.A. Imhof (Geneva, Switzerland)

9:00 - 9:20
Cadherin mechanosensing in the control of endothelial junction remodeling
J. de Rooij (Utrecht, Netherlands)

9:20 - 9:40
Dynamic remodeling of endothelial cell junctions
A. Abu Taha (Münster, Germany)

9:40 - 9:50
Unique cell type-specific junctional complexes in vascular endothelium of human and rat liver sinusoids
K. Evdokimov (Mannheim, Germany)

9:50 - 10:00
Investigation of the causal contribution of selective blood-brain barrier glucose transport processes to brain edema formation and functional deterioration after experimental focal brain ischemia and traumatic brain injury
C. Förster (Würzburg, Germany)

10:00 - 10:30
Coffee Break

10:30 -12:00
Young Investigator Session

10:30 – 10:45
Regulation of Endothelial Nitric Oxide Synthase as a Compensatory Mechanism After Inhibition of NADPH Oxidase4
C. Brunssen (Dresden, Germany)

10:45 – 11:00
The polarity protein Scrib regulates directed endothelial cell migration by modulating integrin α5 turnover
U.R. Michaelis (Frankfurt, Germany)

11:00 – 11:15
Role of FOXQ1 in the transmigration of monocytes in inflammatory conditions
I. Ovsiy (Mannheim, Germany)

11:15 – 11:30
Combined actions of transcription factors promote lymphangiogenic specificity and lymphatic vessel growth via microRNAs
M. Tjwa (Frankfurt, Germany)

11:30 – 11:45
Circumferential wall tension elicits the nuclear export and degradation of myocardin in vascular smooth muscle cells
L. Pfisterer (Heidelberg, Germany)

11:45 – 12:00
The presence of connexion37 in endothelial cells depends crucially on CX40 in mice in vivo
K. Schmidt (Lübeck, Germany)

12:00 - 12:30
Coffee Break

12:30 – 14:00
Session "microRNAs and cardiovascular homeostasis"
Organizer: Ingrid Fleming (Frankfurt, Germany)
Chair: Ingrid Fleming (Frankfurt, Germany)

12:30 – 12:55
microRNAs modulating endothelial behaviour: new mechanisms and therapeutic chances
T. Thum (Hannover, Germany)

12:55 – 13:20
Vasculoprotective effects of flow-induced microRNAs
R. Boon (Frankfurt, Germany)

13:20 – 13:35
The AMP-activated protein kinase regulates endothelial cell angiotensin-converting enzyme expression via p53 and microRNA-143/145
K. Kohlstedt (Frankfurt, Germany)

13:35 – 13:50
Cardioprotective Potential of LNA-mediated miR-92a Suppression
S. Zühlke (Munich, Germany)

Scientific Programme – Thursday, September 27, 2012

Session „Vascular Redox-Regulation: NO and Beyond“

0031

Folic acid partially restores hypoxia-induced remodelling in pulmonary vascular bed

*D. Kračun[1], K. Chalupsky[1], I. Kanchev[1], A. Görlach[1]
[1]German Heart Center, Experimental and Molecular Pediatric Cardiology, Munich, Germany

Disruption of the balance between nitric oxide (NO) and superoxide ($O_2^{\cdot -}$) has been linked to pulmonary vascular remodelling and pulmonary hypertension (PH).

PH is also well known to develop in response to chronic hypoxia. We therefore investigated the impact of hypoxia on the NO/$O_2^{\cdot -}$ balance in pulmonary vascular cells.

We found that exposure to 24 h hypoxia lowered NO levels in human pulmonary artery endothelial cells (HPAEC) and murine pulmonary arteries (PA). Concomitantly, the ratio between tetrahydrobiopterin (BH_4), a cofactor for NO synthase (NOS) activity, and dihydrobiopterin (BH_2) was reduced. Interestingly, expression of dihydrofolate reductase (DHFR) which controls BH_4 recycling from BH_2, was decreased under hypoxia while treatment with folic acid (FA) which induces and activates DHFR, restored hypoxic DHFR and NO levels and BH_4/BH_2 ratio.

On the other hand, $O_2^{\cdot -}$ levels were decreased in HPAEC and PA upon exposure to hypoxia, while acute reoxygenation restored $O_2^{\cdot -}$ levels. Interestingly, treatment with the NOS inhibitor L-(G)-nitro-L-arginine methyl ester further decreased hypoxic $O_2^{\cdot -}$ levels in HPAEC , while $O_2^{\cdot -}$ levels in response to reoxygenation were increased. FA partially restored hypoxic $O_2^{\cdot -}$ levels suggesting that uncoupled NOS may contribute to hypoxic $O_2^{\cdot -}$ levels.

Subsequently, blood NO levels and BH_4/BH_2 ratio as well as $O_2^{\cdot -}$ levels were decreased in mice with pulmonary vascular remodeling and right ventricular hypertrophy upon exposure to chronic hypoxia. Treatment with FA restored NO availability and DHFR levels, and was able to partially reduce pulmonary vascular remodeling and RVH in mice exposed to chronic hypoxia. These findings suggest that NOS is uncoupled under hypoxic conditions thereby shifting the NO/$O_2^{\cdot -}$ balance. Since FA not only ameliorated the NO/$O_2^{\cdot -}$ balance but also diminished pulmonary vascular remodeling under hypoxic conditions rrestoration of DHFR function by FA treatment may be a novel therapeutic option for PH under hypoxic and possibly also non-hypoxic conditions.

Scientific Programme – Thursday, September 27, 2012

Session „Vascular Redox-Regulation: NO and Beyond“

0052

The existence of an endothelial brake on agonist-induced contraction may restrict the blood pressure level during early postnatal development

*D. Gaynullina[1], L. Lubomirov[2], S. Sofronova[2,3], V. Kalenchuk[3], T. Gloe[1], G. Pfitzer[2], O. Tarasova[3], R. Schubert[1]
[1]Heidelberg University, Mannheim, Germany
[2]University of Cologne, Cologne, Germany
[3]Moscow State University, Moscow, Russian Federation

Introduction: Maturation of mammals is associated with a rise in arterial blood pressure accompanied by an increase of vascular resistance. However, the mechanisms of this adaptive process are not fully understood. The endothelium may produce vasodilating factors, thereby decreasing vascular resistance and blood pressure. In addition, the endothelium has been shown to elicit an anticontractile effect during vasoconstrictor action. Whether this effect is altered during maturation is unknown. Thus, the hypothesis was tested that the impact of the anticontractile effect of the endothelium on agonist-induced contractions changes during early postnatal development. Methods: We studied isometric contractions to methoxamine (MX, α_1-adrenoceptor agonist) in saphenous arteries of young (1-2-week-old) and adult (2-3-months) rats. qPCR and Western Blot were performed to evaluate the levels of mRNA expression and protein phosphorylation, respectively. Results: MX-induced contractions of arteries form young rats with intact endothelium were significantly weaker compared to adults. Endothelial denudation increased the sensitivity to MX in young, but not in adult vessels. The endothelial influence on MX-induced contractions in arteries of young rats was completely abolished by the eNOS inhibitor L-NNA as well as by the guanylate cyclase inhibitor ODQ. The sensitivity of endothelium-denuded vessels to exogenous NO-donors did not differ between the two age groups, indicating an endothelial origin of the effect. The expression level of eNOS mRNA was prominently higher in arteries from young compared to adult rats. L-NNA alone induced tonic contractions of endothelium-intact arteries from young but not from adult animals that were associated with corresponding changes in phosphorylation of the myosin regulatory light chains, the regulatory subunit of smooth muscle myosin light chain phosphatase, and vasodilator-stimulated phosphoprotein, the latter two being considered to be good markers of NO/sGC/PKG pathway activity. Conclusion: Our results show that agonist-induced contractions in arteries of young rats are limited by an endothelial brake mediated by an active NO-pathway. The active NO-pathway is due to a constitutive eNOS activity that disappears with age.

Scientific Programme – Thursday, September 27, 2012

Poster Session 1

0021

In Vivo Measurement of Obesity Induced Endothelial Dysfunction in Mice Using Optical Coherence Tomography

*H. Langbein[1], A. Leuner[1], C. Brunssen[1], P. Cimalla[2], W. Goettsch[1], C. Eickholt[1], E. Koch[2], H. Morawietz[1]
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[2]Dresden University of Technology, Clinical Sensoring and Monitoring, Dresden, Germany

Consumption of high amounts of saturated fat is a risk factor of patients with cardiovascular diseases. An important step in the pathogenesis of arteriosclerosis is the development of endothelial dysfunction. Induction of endothelial dysfunction by high-fat diet (HFD) is difficult in mouse models and controversial in the literature. In this study, we show a reproducible development of endothelial dysfunction using a very HFD.

C57BL/6J mice were fed a diet containing 60% kcal from fat for 20 weeks. Body weight and the amount of epididymal, retroperitoneal, mesenteric and perivascular fat were increased after HFD. Diet-induced obesity elevated blood glucose, triglyceride, total cholesterol, and LDL plasma levels. This study is the first showing an analysis of endothelial function by self-developed optical coherence tomography devices using flow-mediated vasodilation in the murine saphenous artery in vivo. In addition, we performed ex vivo analysis of endothelial function in aorta thoracalis from these animals using a Mulvany myograph. Both methods showed an impaired endothelium-dependent relaxation. As control, endothelial dysfunction was shown by the same approach in atherosclerotic Ldlr-/- mice. Application of COX-1/2 inhibitor diclofenac inhibited this effect. Next, we studied the role of H_2O_2 in the thoracic aorta. Catalase increased EC_{50} values in endothelial function only of mice on standard chow. Moreover, a reduced H_2O_2 release was measured in thoracic aorta after HFD compared to mice on standard chow. This might contribute to the reduced endothelium-dependent vasodilation after HFD.

In conclusion, we were able to induce endothelial dysfunction in a reproducible manner in murine models using a very HFD. This study showing endothelial dysfunction by self-developed optical coherence tomography devices using flow-mediated vasodilation in mice models in vivo. A reduced release of H_2O_2 might contribute to the impaired endothelium-dependent vasodilation after HFD.

Scientific Programme – Thursday, September 27, 2012

Poster Session 1

0039

Leptin-induced neuronal NOS (nNOS) expression:
A compensatory mechanism for lack of endothelial NO

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[2]University , Physiology, Greifswald, Germany
[3]University, Cardiovascular Medicine, Tohoku, Japan

In the absence of metabolic syndrome or diabetes II, obesity is only a weak risk factor for the development of cardiovascular diseases and endothelial dysfunction. We hypothesized that adipokines may contribute to maintain vascular function. Herein we focused on leptin and determined its effects beyond endothelial nitric oxidase synthase (eNOS).

Wildtype (WT) and $eNOS^{-/-}$-mice were infused with or without leptin. Isolated vessel recordings in presence of diclofenac revealed that leptin treatment improved acetylcholine (ACh)-induced relaxation of aortic segments from $eNOS^{-/-}$-mice. Blocking total NOS activity by L-nitro-arginine abolished the improved relaxation in response to ACh suggesting a NOS mediated effect of leptin-treatment. TRIM, a selective i/nNOS-inhibitor blocked the augmented endothelial relaxation in rings from $eNOS^{-/-}$-mice by leptin. Accordingly in vivo leptin failed to enhance ACh-induced relaxation in segments from e/nNOS double knockout mice. Importantly leptin rescued angiotensin II induced endothelial dysfunction in a disease model where we infused WT-mice with or without leptin.

Quantitative PCR of human and murine isolated vessel segments revealed that nNOS expression was induced by leptin-treatment. In human endothelial cells, acute leptin stimulation increased Jak2, Stat3 and p38 phosphorylation, signals controlling nNOS expression. Pharmacological inhibition of Jak2/Stat3 or p38 pathway prevented leptin-induced nNOS expression in vessels. In contrast only blocking of Jak2/Stat3 but not of p38 prevented leptin-induced improvement of ACh-induced relaxation in vivo.

We conclude that leptin increases the vascular production of NO and thereby improves ACh-induced relaxation by induction of nNOS via the Jak2/Stat3-pathway.

Poster Session 1

0007

C-C motif chemokine CCL3 and canonical neutrophil attractants mediate neutrophil recruitment through common and distinct mechanisms

*D. Puhr-Westerheide[1], Z. Gabriele[1], B. Uhl[1], N. Berberich[2], S. Zahler[2], M.P. Wymann[3], B. Luckow[4], F. Krombach[1], C.A. Reichel[1]
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[2]Department of Pharmacy, Ludwig-Maximilians-Universität, München, Germany
[3]Department of Biomedicine, Institute of Biochemistry and Genetics, University of Basel, Basel, Switzerland
[4]Klinikum der Universität München, medizinische Poliklinik Campus Innenstadt, München, Germany

Introduction

C-C motif chemokines (e.g. CCL3) have initially been thought to exclusively mediate the migration of mononuclear leukocytes. Interestingly, however, there is growing evidence that these chemotactic proteins are also involved in the recruitment of neutrophils. The underlying mechanisms remain largely unclear.

Material & Methods

Usingin vivotransillumination microscopy on the cremaster muscle of male Balb/c mice, intravascular rolling, firm adherence and transmigration of leukocytes were analyzed 3 h after intra-scrotal injection of CCL3 or of canonical neutrophil attractants including the C-X-C motif chemokine CXCL1 and the lipid mediator platelet-activating factor (PAF). Phenotyping of transmigrated leukocytes was performed by immunostaining of paraffin-embedded tissue sections.

Results

Stimulation with CCL3, CXCL1, or PAF induced a significant increase in numbers of intravascularly adherent and transmigrated neutrophils (>80% Ly-6-G$^+$ leukocytes) as compared to PBS-treated controls. Inhibition of G-protein receptor coupling or of PI3Kg-, but not of PI3Kd-dependent signaling almost completely abolished CCL3-elicited neutrophil responses, but only partially reduced neutrophil recruitment upon CXCL1 or PAF. Antibody blockade of b_2-integrins (Mac-1, LFA-1) or of a_4-integrins as well as of their (putative) counter receptors ICAM-1 and VCAM-1 significantly diminished intravascular adherence and transmigration of neutrophils elicited by CCL3, CXCL1, or PAF. Blockade of ICAM-2 or PECAM-1 selectively reduced transmigration of neutrophils in response to CCL3, but not upon stimulation with CXCL1 or PAF.

Conclusion

These data demonstrate that the C-C motif chemokine CCL3 and canonical neutrophil attractants exhibit both common and distinct mechanisms for the regulation of intravascular adherence and transmigration of neutrophils.

Scientific Programme – Thursday, September 27, 2012

Poster Session 1

0010

Inflammatory tissue damage in ischemia/reperfusion injury induces unmasking of pro-inflammatory CRP epitopes thus aggravating the inflammatory response

*J.R. Thiele[1], E. v. Dobschuetz[2], K. Peter[3], H. Bannasch[1], G.B. Stark[1], S.U. Eisenhardt[1]
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[2]University of Freiburg Medical Center, General and Visceral Surgery, Freiburg, Germany
[3]Baker Heart Research Institute, University of Melbourne, Melbourne, Australia

Introduction:C-reactive protein (CRP) is a pentameric plasma protein consisting of 5 identical subunits and proposed to be not only a marker, but also a mediator of inflammatory disease. We recently showed that ischemia/reperfusion injury (IRI) after free flap surgery induces a molecular switch leading to dissociation of pentameric CRP (pCRP) to monomeric CRP (mCRP), however the pathophysiological relevance remains elusive. Here we investigated the effect of CRP-dissociation in an in vivo model of IRI by intravital microscopy.

Methods:25 µg/ml pCRP was fluorescently labeled and intravenously injected for real-time in vivo tracking in the microcirculation of the cremaster muscle of the rat after IRI. Binding of pCRP and co-localization with activated damaged membranes was determined by in vivo Annexin V fluorescence stain and dissociation to mCRP was detected by immunofluorescence and native Western blot analysis using conformation specific antibodies. Leukocyte activation under CRP dissociation was analyzed by quantification of leukocyte adhesion/rolling and by reactive oxygen species (ROS) detection via electron spin resonance spectroscopy (ESR). Treatment with pre-dissociated mCRP (25 µg/ml) and pCRP stabilized with 1,6-bisPC-Hexane (PCH) in a decameric conformation served as control.

Results:IRI of the rat cremaster muscle provoked binding of pCRP to the activated vascular endothelium and to activated leukocytes, resulting in dissociation of pCRP to mCRP in vivo. The newly formed mCRP induced a significant increase in leukocyte rolling/adhesion as well as in ROS formation ($p<0.05$) and was deposited in the inflamed perivascular tissue. These findings were mimicked by the pre-dissociated mCRP control. Stabilization of pCRP and therefore inhibiting dissociation to mCRP by treatment with PCH abrogated these effects.

Conclusion:These results suggest for the first time an in vivo conformational change in the molecular structure of pCRP induced by inflammatory activated membranes. mCRP formation is a causal event in the pathological cascade of inflammatory tissue damage in IRI and enhances the pro-inflammatory properties of CRP. Stabilization of pCRP inhibits membrane binding and dissociation, thereby preventing mCRP induced inflammatory events and might be a feasible therapeutic strategy.

Scientific Programme – Thursday, September 27, 2012

Poster Session 1

0016

Tissue plasminogen activator and plasminogen-activator inhibitor-1 promote tissue integration of polyethylene biomaterial

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[2]Department of Otorhinolaryngology, Head and Neck Surgery; Ludwig-Maximilians-München, Munich, Germany, München, Germany
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Introduction

Rapid vascularization and a moderate inflammatory response are thought to be the prerequisites for engraftment of porous polyethylene (PPE) implants. The serine protease plasmin is known to be the key enzyme in the fibrinolytic system and has recently been implicated in tissue integration of biomaterials. Tissue plasminogen activator (tPA) is the principal plasminogen activating protease in fibrinolysis, whose activity is tightly controlled by plasminogen-activator inhibitor-1 (PAI-1). So far, the functional relevance of tPA and PAI-1 for the integration of biomaterials *in vivo* is unclear.

Methods & Materials

PPE implants were inserted into dorsal skinfold chambers in female C57Bl/6, $tPA^{-/-}$, and $PAI\text{-}1^{-/-}$ mice. Vascularization and leukocyte-endothelial cell interactions were analyzed on days 7, 10, and 14 after implantation using *in vivo* fluorescence microscopy. Integration was analyzed on day 14 by measurement of the dynamic strength needed for mechanical removal of the implant out of the host tissue.

Results

No significant differences were observed in numbers of intravascularly rolling and firmly adherent leukocytes as well as in the vessel density surrounding the implant among all experimental groups. In contrast, leukocyte responses as well as the increasing vessel density within the implant were significantly reduced in tPA- or PAI-1-deficient mice as compared to wild-type mice on day 7 and 10 after implantation. In addition, the dynamic strength needed for desintegration of the implant was significantly reduced in tPA- or PAI-1-deficient mice.

Conclusion

Our data indicate that both endogenous tPA and PAI-1 are critically involved in the functional engraftment of PPE biomaterial through effects on leukocyte recruitment and implant vascularization.

Poster Session 1

0038

Inflammatory stimuli generate distinct surface patterns on endothelial cells

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During inflammation, the extravasation of leukocytes into tissues is controlled by endothelial cells. Inflammatory cytokines activate the endothelium to promote leukocyte attachment. During the firm adhesion phase, which is a prerequisite for the actual transmigration step, both the leukocytes and endothelial cells form protrusions at the plasma membrane to intensify their interaction: leukocytes form invadopodia to palpate the endothelial cell for suitable entry sites; endothelial cells engulf an attached leukocyte with filopodia. Recently we observed, that a sole activation of endothelium is sufficient for maximal leukocyte transmigration rates (in vitro). Accordingly, we here searched for distinct morphological changes at the endothelial membrane, which could facilitate the capturing of leukocytes. Ultra-topography of endothelial cell surface was recorded using atomic force microscopy (AFM) under physiological buffer conditions. The cell model were human umbilical vein endothelial cells (HUVEC) stimulated with TNFa or interleukin1b, respectively - but without any leukocyte contact. Image analysis was performed using the nAnostic™ method of quantifying *local deviational volumes* (LDV) by computer vision. The number and the LDV of surface objects at least doubled under the influence of cytokines. Inversely, nanostructural patterns indicate the proinflammatory status of endothelial cells.

Scientific Programme – Thursday, September 27, 2012

Poster Session 1

0002

Diltiazem prevents aortic aneurysm formation via inhibition of IL-6-induced AP-1 activity in macrophages

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Abdominal aortic aneurysm is an important manifestation of arteriosclerosis with increasing incidence in the western world. Progressive inflammatory processes involving infiltration and differentiation of monocytes into the vessel wall, proliferation and migration of smooth muscle cells and eventually the degradation of the internal elastic lamina are causal for aneurysm formation. Since calcium channel blockers exert multiple beneficial effects in the vascular system we investigated the effect of the benzothiazepine-type calcium channel blocker diltiazem (DIL) on aneurysm formation.

Angiotensin II infusion (ATII, 4 weeks, 1.44 mg/kg bw/d) induced massive aneurysm formation in ApoE-deficient mice that was blocked by co-treatment with DIL (100 mg/kg bw/day). Additional treatment with phenylephrine (PHE, 18 mg/kg bw/d) to counteract the blood pressure-lowering effect of DIL had no influence on its beneficial effect. Moreover DIL prevented the ATII-induced mRNA expression of pro-inflammatory cytokines in the aortic arch after 6 days of ATII treatment due to a reduction in the amount of locally infiltrating macrophages which was accompanied by attenuated systemic CCL12 serum level.

Ex vivo, DIL (10 µM) did not reduce the ATII-induced (100 nM) expression of promigratory chemokine CCL2 and proinflammatory cytokine IL-6 of vascular segments in organ culture or vascular smooth muscle cells. Moreover, DIL (30 µM) did not affect the recruitement of Ly6C+-monocyte into subcutaneously implanted matrigel plugs filled with CCL2 (600 ng/ml) in ApoE-deficient mice. In contrast, in peritoneal macrophages and RAW264.7 cells, DIL prevented the IL-6-induced (10 ng/ml) mRNA expression of IL-1β and CCL12. As underlying mechanism, DIL abrogated the IL-6 induced activation of AP-1 promoter without affecting NF-kB activity as well as phosphorylation and nuclear translocation of MAPK1 and Stat3 or intracellular calcium.

These observations indicate that DIL prevents aneurysm formation by a mechanism independent of its blood pressure and intracellular calcium lowering effects

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Poster Session 1

0012

Flotillin1 regulates the Toll-like Receptor 3 and 4 trafficking

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Toll-like receptors (TLRs) are responsible for the detection of invading microbes. The transport of TLRs after ligand stimulation to endosomes is essential for their signalling function. The proteins flotillin1 and 2 which form heterodimers are markers of lipid raft fraction but it is unknown whether they contribute to raft formation or endosome internalization. We studied whether flotillin1 is required for the agonist-induced trafficking of TLR3 and TLR4 in human umbilical vein endothelial cells (HUVECs). Knockdown of flotillin1 by shRNA lead to a decreased internalization of TLR3 upon polyIC-rhodamine stimulation. Similarly, flotillin1 shRNA attenuated the TLR3- and TLR4- induction of the adhesion molecules VCAM and ICAM-1 as well as the adhesion of peripheral blood mononuclear cells to HUVECs in response to agonist-stimulation. Importantly, overexpression of flotillin1 in cells exposed to flotillin1 shRNA restored TLR-dependent VCAM and ICAM expression excluding non-specific effects of the shRNA. Flotillin1 silencing also disrupted the TLR-TRIF-dependent signaling pathway as demonstrated by a loss of the phosphorylation of the TLR-downstream target interferon regulatory factor-3 (IRF3). Surprisingly, knockdown of caveolin1, also a lipid raft protein, promotes flotillin1 degradation and reflects the flotillin1 depleted phenotype in HUVECs. Amazingly, knockdown of flotillin2 or combined knockdown of both, flotillin1 and 2 was without effect on adhesion molecule expression. This may suggest that not the lack of flotillin1 but an aberrant action of flotillin2 in the absence of its binding partner flotillin1 mediates the anti-inflammatory effect observed. Conclusion: Down-regulation of flotillin1 elicits an inhibitor effect on TLR signaling which is mediated by flotillin2.

Scientific Programme – Thursday, September 27, 2012

Poster Session 1

0032

RAGE cooperates with ICAM-1 to mediate anti-inflammatory functions of Protein C

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Background and Aims: The receptor for advanced glycation endproducts, RAGE, perpetuates inflammation and mediates leukocyte recruitment through binding the β_2-integrin Mac-1 and activating NFkB. We now asked whether RAGE is involved in mediation of anti-inflammatory properties of protein C (PC) in different mouse models of inflammation.

Methods and Results: As reported previously, RAGE and ICAM-1 collaborate in a stimulus dependent manner to mediate leukocyte recruitment in cremaster muscle models of inflammation (TNFa and trauma model). Notably, RAGE plays a major role for leukocyte recruitment after longer inflammatory stimulation, i.e. with LPS during LPS-induced acute lung injury (10 µg intratracheally, 6h) or LPS-induced endotoxemia (40mg/kg i.p.).

In WT mice PC treatment (Protein C concentrate; Ceprotin ®, 100 U/kg iv) reduced leukocyte recruitment in all used inflammation models. However, in RAGE deficient mice PC induced inhibition of leukocyte recruitment was absent or attenuated, suggesting that RAGE is involved in mediation of anti-inflammatory effects of PC. In ICAM-$1^{-/-}$ mice the efficacy of PC to block leukocyte infiltration was dependent on the model and stimulus used. While PC was effective during TNFa- and LPS- induced inflammation, it failed to inhibit leukocyte recruitment during trauma-induced inflammation of cremaster muscles in ICAM-$1^{-/-}$ mice. Thus, PC interferes with both, RAGE and ICAM, in a stimulus dependent manner in order to mediate inhibition of leukocyte recruitment. The results were supported by survival studies during LPS-induced endotoxemia with and without PC treatment in these mice.

Conclusion: We conclude that the anti-inflammatory potential of PC is mediated by RAGE in concert with ICAM-1.

Poster Session 1

0035

The mAbp1 'interactome' is critical for neutrophil trafficking during the acute inflammatory response

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The mammalian actin-binding protein 1 (mAbp1) has been identified as a novel component of the β_2 integrin-mediated signaling cascade. Here we studied the role of hematopoietic progenitor kinase 1 (HPK1), a known mAbp1 interacting protein, for the β_2 integrin dependent recruitment of polymorphonuclear neutrophils (PMN) to sites of lesion during acute inflammation.

Co-immunoprecipitation was performed with GFP-nanotraps using neutrophil-like differentiated HL-60 cells. Cell surface expression of β_2 integrins was investigated by flow cytometry using specific antibodies. Murine PMN were isolated from the bone marrow of HPK1$^{-/-}$ and wild-type mice. Analysis of PMN adhesion, adhesion strengthening and spreading was conducted using IBIDI µ-slides. Intravital microscopy of inflamed cremaster muscle venules was used to study leukocyte adhesion *in vivo*.

We could confirm the interaction of HPK1 and mAbp1 in myeloid cells and showed that HPK1$^{-/-}$ PMN have normal cell surface expression of β_2 integrins. Under flow conditions, induction of β_2 integrin-mediated PMN adhesion and spreading was severely compromised in the absence of HPK1. Moreover, enhanced detachment of HPK1$^{-/-}$ PMN under physiological shear stress conditions was observed. Intravital microscopy of inflamed cremaster muscle venules demonstrated that leukocyte adhesion was significantly reduced in HPK1$^{-/-}$ mice compared to wild-type control animals.

These findings revealed that HPK1 plays an important role in PMN trafficking during inflammation by regulating induction of PMN adhesion, adhesion strengthening and spreading under physiological flow conditions (supported by SFB 914).

Scientific Programme – Thursday, September 27, 2012

Poster Session 1

0036

Protein C interacts with LFA-1 in mediating anti-inflammatory functions

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Background and Aims: The ß2-integins LFA-1 and Mac-1 mediate firm adhesion of leukocytes to the endothelium by interacting with their endothelial ligands. Since protein C is able to block leukocyte adhesion during inflammation in-vivo, we asked whether protein C (PC) influences ß2-integrin-mediated leukocyte adhesion in different mouse models of inflammation.

Methods and results: While PC treatment (Protein C concentrate; Ceprotin ®, 100 U/kg iv) reduced leukocyte recruitment in cremaster muscle inflammation models in WT- and Mac-1-deficient mice, it failed to inhibit leukocyte recruitment in LFA-$1^{-/-}$ mice. However, the inhibitory effect of PC was retained in WT mice treated with LFA-1-blocking antibodies, suggesting that PC interacts with LFA-1. These results were confirmed in other murine models, as long-term stimulation with LPS during LPS-induced acute lung injury (10 µg intratracheally, 6h), LPS induced endotoxemia (40 mg/kg i.p.) and *in vitro* flow-chamber-studies using LFA-1 deficient mice or leukocytes, respectively.

In addition, we found that Mac-1, although of relevance, is differently involved in the PC induced effects in the investigated inflammation models.

Conclusion: We conclude that the anti-inflammatory properties of PC are mediated by LFA-1.

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Poster Session 1

0022

Deficiency of liver sinusoidal scavenger receptors stabilin-1 and -2 in mice causes glomerulofibrotic nephropathy via impaired hepatic clearance of noxious blood factors

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Tissue homeostasis and remodeling are processes that involve high turnover of biological macromolecules. Many of the waste molecules that are by-products or degradation intermediates of biological macromolecule turnover enter the circulation and are subsequently cleared by liver sinusoidal endothelial cells (LSEC). Besides the mannose receptor, stabilin-1 and stabilin-2 are the major scavenger receptors expressed by LSEC. To more clearly elucidate the functions of stabilin-1 and -2, we have generated mice lacking stabilin-1, stabilin-2, or both stabilin-1 and -2 (Stab1-/-Stab2-/- mice). Mice lacking either stabilin-1 or stabilin-2 were phenotypically normal; however, Stab1-/-Stab2-/- mice exhibited premature mortality and developed severe glomerular fibrosis, while the liver showed only mild perisinusoidal fibrosis without dysfunction. Upon kidney transplantation into WT mice, progression of glomerular fibrosis was halted, indicating the presence of profibrotic factors in the circulation of Stab1-/-Stab2-/- mice. While plasma levels of known profibrotic cytokines were unaltered, clearance of the TGF-β family member growth differentiation factor 15 (GDF-15) was markedly impaired in Stab1-/-Stab2-/- mice but not in either Stab1-/- or Stab2-/- mice, indicating that it is a common ligand of both stabilin-1 and stabilin-2. These data lead us to conclude that stabilin-1 and -2 together guarantee proper hepatic clearance of potentially noxious agents in the blood and maintain tissue homeostasis not only in the liver but also distant organs.

Poster Session 1

0051

ERp46 is involved in regulation of TNF-α induced angiogenesis

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Protein disulfide isomerase (PDI) is an oxidoreductase of endoplasmic reticulum essential for protein folding. A role for PDI in endothelial cell function, such as integrin activation has been established. Erp46 is a PDI family member preferentially expressed in endothelial cells but little is known about its physiological functions. We hypothesized that Erp46 is involved in endothelial receptor-dependent signaling and down-stream events such as angiogenesis. Human umbilical vein endothelial cells (HUVECs) were transfected with different siRNAs against ERp46 or PDI and stimulated with TNF-α (10ng/ml). ERp46 and PDI silencing decreased TNF-α-induced ERK1/2 phosphorylation whereas JNK and p38 MAP kinase action were PDI independent. Additionally ERp46 or PDI silencing did not affect TNFα-stimulated NFkB signaling: TNFα stimulated IκBα degradation and TNF-α induced VCAM and ICAM induction were unaffcted by ERp46-siRNA. In order to investigate the mechanisms involved in ERp46/PDI regulation of ERK1/2 phosphorylation, HUVECs were stimulated with TNF-α in the presence of inhibitors of plasma membrane PDI, scrambled RNase (100ug/ml) or anti PDI neutralizing antibody (1:100). PDI inhibition did not decrease ERK1/2 phosphorylation. Furthermore, addition of exogenous PDI (500nM) did not restore ERK1/2 signaling after PDI or ERp46 knockdown, suggesting that plasma membrane- PDI or ERp46 are not involved in TNF-α-dependent ERK1/2 signaling and thus that alterations of the intracellular Ras/Raf/MEK/ERK pathway are most probable. The transcription factor AP-1 is dependent on ERK activation. To determine the relevance of our findings, we therefore sought for AP-1 dependent genes and studied MMP-9 induction and angiogenic responses in endothelial cells. Indeed, ERp46 silencing decreased TNF-α induced MMP-9 mRNA expression. Moreover, ERp46 siRNA markedly decreased the formation of endothelial cell sprouts in the spheroid assay, used as a model of angiogenesis. Conclusion: ERp46 regulates TNF-α induced angiogenic effects in an ERK1/2 dependent manner.

Scientific Programme – Thursday, September 27, 2012

Session “Leukocyte trafficking in inflammation”

0024

Intracellular signaling pathways in leukocytes leading to integrin activation and leukocyte recruitment

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Leukocyte recruitment into inflamed tissue proceeds in a cascade-like fashion. During rolling on the inflamed endothelium, leukocytes collect several activating signals through selectin-ligands, GPCRs, and outside-in signaling of integrins. Elimination or blockade of one or more molecules involved in this cascade might lead to a reduction or abolishment of leukocyte recruitment into the inflamed tissue. Reduced leukocyte recruitment into the inflamed tissue can be beneficial in the context of abacterial injuries including ischemia-reperfusion injury and acid-induced lung injury. Modulating leukocyte recruitment by the treatment with Natalizumab, a monoclonal antibody against $\alpha_4\beta_1$, is very efficacious in patients with Crohn disease and multiple sclerosis. However, defects in leukocyte recruitment as seen in patients with leukocyte adhesion deficiency (LAD) -leukocytes of these patients express either no or non-functional integrins- lead to a reduced host response. These patients have a reduced life expectancy due to recurring bacterial infections.

Recent papers convincingly demonstrated the important role of selectins in leukocyte activation and recruitment. Selectin engagement induces downstream signaling leading to tyrosine phosphorylation, cytoskeletal rearrangement, transcriptional activation, activation of β_2-integrins, and increased intracellular calcium levels. However, the precise signaling pathway leading to β_2-integrin activation following selectin engagement is poorly understood. Understanding neutrophil activation, signaling, and recruitment can help identify therapeutic targets to selectively inhibit specific functions of neutrophils without affecting others.

Scientific Programme – Thursday, September 27, 2012

Session “Leukocyte trafficking in inflammation”

0047

Role of S100A8/A9 in leukocyte recruitment in vivo

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Question: S100A8 (MRP8) and S100A9 (MRP14) belong to a family of cytosolic calcium-binding proteins expressed in myeloid cells. S100A8 and S100A9 are secreted as dimers by neutrophils and activated monocytes at high levels at sites of inflammation. In this study we investigated the role of S100A8/A9 in leukocyte recruitment in vivo. Methods: We studied leukocyte rolling and adhesion in TNF-α-stimulated cremaster muscle venules of S100a9-deficient and WT mice using intravital microscopy. In addition, ex vivo flow chamber assays were used to observe rolling and adhesion of leukocytes of S100a9-deficient mice. Results: In cremaster muscle venules we observed a decrease in overall leukocyte adhesion and extravasation 2h after intrascrotal injection of TNF-α in S100a9-deficient mice compared to WT mice. Leukocyte rolling of S100a9-deficient leukocytes was slightly increased in comparison to WT leukocytes. In the ex vivo flow chamber assay, the number of adherent S100a9-deficient leukocytes was strongly reduced and the number of rolling leukocytes slightly increased as compared to their WT counterparts. Both, the in vivo and ex vivo findings suggest a defect of S100a9 deficient leukocytes in the transition from leukocyte rolling to arrest. Addition of soluble S100A8/A9 was able to almost completely reconstitute the decreased number of adherent leukocytes in S100a9-deficient cells to WT levels, pointing out a central role of soluble S100A8/A9 in the transition from leukocyte rolling to leukocyte arrest. Conclusion: We identified soluble S100A8/A9 as important pro-inflammatory modulator during leukocyte recruitment in vivo supporting leukocyte arrest on inflamed postcapillary venules and extravasation into inflamed tissue. Additional studies are currently under way to further uncover the molecular mechanisms by which soluble S100A8/A9 regulates leukocyte recruitment in vivo.

Scientific Programme – Thursday, September 27, 2012

Session “Leukocyte trafficking in inflammation”

0034

Coronin 1A is critically required for β_2 integrin (CD11/CD18)-mediated neutrophil recruitment during the acute inflammatory response

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Introduction: Adhesion molecules of the β_2 integrin family are critically involved in the recruitment of polymorphonuclear neutrophils (PMN) to sites of lesion during acute inflammation. The evolutionary conserved protein coronin1A (coro1A), which is predominantly expressed in leukocytes, has been described as an important regulator of lymphocyte trafficking. This study was performed to investigate the impact of coro1A on β_2 integrin-dependent PMN recruitment.

Material & Methods: Murine PMN were isolated from the bone marrow of wild type (WT) and coro1A$^{-/-}$ mice. PMN adhesion, spreading and migration were studied under static and under flow conditions by time-lapse video microscopy. Chemotactic migration was analyzed in Zigmond chambers. Leukocyte rolling and adhesion *in vivo* were monitored in inflamed cremaster muscle venules by intravital microscopy.

Results: The genetic absence of coro1A had no effect on β_2 integrin-mediated PMN adhesion on intracellular adhesion molecule-1 or fibrinogen under static conditions but severely compromised spreading and chemotactic migration. Under physiological shear stress conditions, loss of coro1A resulted in impairment of PMN spreading and mechanotactic crawling and led to enhanced detachment of adherent PMN. In inflamed cremaster muscle venules, no difference in leukocyte rolling between coro1A$^{-/-}$ and WT mice was observed suggesting that leukocyte rolling was independent of coro1A. However, the genetic absence of coro1A completely abrogated induction of firm leukocyte adhesion in mice indicating that coro1A was critically required for β_2 integrin-dependent leukocyte recruitment during acute inflammation.

Conclusion: Our data provide evidence for a fundamental role of coro1A for β_2 integrin-mediated adhesion, spreading and migration of PMN under flow conditions. Thus, we identified a novel molecular player in PMN trafficking during the acute inflammatory response (supported by SFB 914).

Scientific Programme – Thursday, September 27, 2012

Session “Leukocyte trafficking in inflammation”

0013

Impact of the EphrinB/EphB system on pro-inflammatory monocyte-endothelial cell interaction

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Introduction: The binding of the membrane-associated ephrinB ligands to corresponding EphB receptors is pivotal for the orchestrated development of the circulatory system. Despite the importance of this receptor/ligand system for the arteriovenous differentiation of endothelial cells, only little is known about its function in adult blood vessels or the immune system.

Methods: Human umbilical vein endothelial cells (HUVECs) were exposed to clustered soluble EphB2, EphB2-overexpressing myeloma cells or monocytes upon siRNA-mediated knockdown of ephrinB1 or ephrinB2. Based on these experimental setups, we investigated the proinflammatory differentiation of HUVECs and the migration of monocytes through an endothelial cell monolayer.

Results: EphrinB2 as well as ephrinB1 are localized on the luminal surface of endothelial cells and become up-regulated during inflammation, enabling their interaction with circulating leukocytes. Whereas forward signaling downstream of the EphB receptors promotes the migratory and pro-inflammatory activity of monocytes, reverse signaling induces granulocyte-macrophage colony-stimulating factor(GM-CSF) release and a c-Jun N-terminal kinase (JNK)-dependent increase in expression of adhesion molecules such as VCAM-1 or E-selectin in the endothelial cells. Therefore, monocyte attachment to EphB2-stimulated endothelial cells is enhanced. Moreover, ephrinB1 and ephrinB2, which both are partially localized in the intercellular junctions of the endothelial cells, are crucial for the transmigration of monocytes through the endothelial cell monolayer, and their expression is affected by pro-inflammatory stimuli such as tumor necrosis factor-alpha (TNF-α). Thus, ephrinB1/2 expression on endothelial cells promotes the adhesion and transmigration of EphB2-expressing monocytes. In order to analyse these ephrinB/EphB-dependent interactions between monocytes and endothelial cells in more detail, we generated EphB2-overexpressing mouse myeloma cells, which in fact are capable of up-regulating adhesion molecule expression in and inducing the release of von Willebrand factor from the cultured endothelial cells.

Conclusion: Our data reveal that reverse signaling processes promote the pro-inflammatory differentiation of endothelial cells whereas forward signaling facilitates monocyte diapedesis and subsequent differentiation into macrophages in the vessel wall.

Scientific Programme – Thursday, September 27, 2012

Session “Leukocyte trafficking in inflammation”

0023

Von Willebrand Factor interacts with Extracellular DNA thereby facilitating Leukocyte adhesion

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Introduction

Inflammatory conditions have distinct effects on the cells in our vascular system. They provoke the release of Von Willebrand Factor (VWF) from endothelial cells (EC), which then forms ultra-large structures being uncoiled under shear flow. Furthermore, inflammation leads to the formation of large extracellular DNA structures by stimulated leukocytes, which undergo a novel cell death program called NETosis. In this context, we are interested in the question if both molecules VWF and DNA are able to interact to promote the adhesion of leukocytes to the endothelium.

Material & Methods

For unidirectional flow experiments we apply microfluidic devices to mimic conditions of the vasculature. Both functionalized surfaces and intact cell-layers of human umbilical vein endothelial cells (HUVEC) are perfused with isolated DNA or leukocytes from whole blood at different shear rates ranging from 200 s^{-1} to 1000 s^{-1}. DNA-VWF interaction is monitored using fluorescence microscopy or ELISA-based assays.

Results

Isolated DNA as well as DNA released by stimulated leukocytes is able to bind to VWF under shear conditions on functionalized surfaces. Additionally, DNA interacts with VWF-fibers directly released from HUVEC. Non-stretched VWF prevents DNA binding but can be restored upon Ristocetin application. Moreover, DNA-VWF interaction can be blocked using unfractionated Heparin and DNA-VWF complexes attenuate platelet-binding to VWF by 78% (+/-6,8%).

Conclusions

As binding of DNA to VWF is dependent on shear flow or on Ristocetin, it is likely that the VWF A1-domain is mainly involved in the interaction due to electrostatic binding to polyanionic molecules. Other hints for this hypothesis are the possibility to block the binding with Heparin and the impaired platelet-interaction upon DNA perfusion. We hypothesize that VWF mediated DNA binding promotes leukocyte adhesion to the endothelium and thereby facilitates leukocyte trafficking.

Scientific Programme – Thursday, September 27, 2012

Session “Leukocyte trafficking in inflammation”

0029

Eliminating or blocking 12/15-Lipoxygenase reduces neutrophil recruitment in mouse models of acute lung injury (ALI)

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Introduction: Acute lung injury (ALI) is a disease with a high morbidity and mortality. 12/15-lipoxygenase (12/15-LO) produces 12-hydroxy-eicosatetraenoic acid (12-HETE) and 15-HETE from arachidonic acid. 12/15-LO has been shown to regulate vascular permeability during ALI. In this study we investigated the role of 12/15-LO in neutrophil recruitment in LPS-induced pulmonary inflammation and a clinically relevant model of acid-induced ALI. **Materials and methods:** Pulmonary inflammation by LPS inhalation or acute lung injury by intratracheal acid instillation was induced in 12/15-LO-deficient mice or WT mice after blockade of 12/15-LO with an inhibitor. Neutrophil recruitment into the intravascular, interstitial, and intraalveolar compartment was analyzed by flow cytometry. Edema formation was quantified by measuring wet/dry ratio. Lungs were stained for histological analysis. The role of hematopoietic and non-hematopoietic 12/15-LO was investigated with bone-marrow chimeric mice. Chemokines were measured in plasma and lungs and surface expression of CXCR2 was analyzed by flow cytometry. *In vitro* chemotaxis of neutrophils was investigated in a transwell system. **Results**: Neutrophil recruitment, lung wet/dry ratio, and histological signs of ALI after LPS inhalation or acid instillation were decreased in 12/15-LO-deficient mice and inhibitor-treated WT mice. Hematopoietic 12/15-LO was responsible for neutrophil recruitment into the interstitial and alveolar compartment, whereas neutrophil recruitment into the intravascular compartment was also regulated by non-hematopoietic 12/15-LO. 12/15-LO-deficient mice and inhibitor-treated WT mice had elevated levels of CXCL1 and CXCL2 in plasma and the lung, which caused the down-regulation of CXCR2 on neutrophils resulting in decreased neutrophil recruitment. This could be reproduced by *in vitro* chemotaxis assays. **Conclusion:** This study shows that 12/15-LO regulates neutrophil recruitment by modulating chemokine hemostasis.

Scientific Programme – Friday, September 28, 2012

Session “Vascular Mechanobiology”

0001

The contribution of GPCRs to myogenic response in murine mesenteric arteries

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The intrinsic property of resistance arteries to constrict in response to an elevation of intraluminal pressure is known as arterial myogenic tone. To date, the underlying signaling cascade has been well elucidated, whereas the molecular identity of the mechanosensory elements remains indeterminate. Recent studies at the cellular level suggest a subset of G-protein coupled receptors (GPCRs) as possible mechanosensors. By analyzing mRNA-expression levels of specific GPCRs in resistance arteries versus their preceding conduit arteries, an array of $G_{q/11}$-coupled receptors, such as angiotensin II AT_{1B}, vasopressin V_{1A}, endothelin ET_A and ET_B and α_{1A} adrenoceptor, was found to be significantly enriched in resistance vessels. Pharmacological inhibition of the highly expressed GPCRs in isolated murine mesenteric arteries decreased myogenic response ex vivo. Interestingly, blocking of AT_{1A} and AT_{1B} receptors by candesartan and losartan caused the most prominent suppression of myogenic tone. To exclude the contribution of locally produced angiotensin II to myogenic vasoconstriction, vessels of angiotensinogen$^{-/-}$ mice were analyzed. They exhibited an unaltered vascular response, but additional blocking of AT_1 receptors by losartan reduced myogenic tone significantly, indicating agonist-independency of myogenic vasoconstriction. Moreover, investigating the myogenic response of $AT_{1A}^{-/-}$ mice with and without additional blocking of AT_{1B} receptors by candesartan suggested that especially AT_{1B} receptors play a dominant role for mechanosensitivity in mice. This was affirmed by investigating the myogenic response of $AT_{1B}^{-/-}$ mice, which exhibited a significantly reduced myogenic tone. Our results indicate that AT_1, and especially AT_{1B} receptors, serve as pressure sensors and are centrally involved in the development of myogenic tone in mesenteric arteries.

Scientific Programme – Friday, September 28, 2012

Session “Vascular Mechanobiology”

0048

Visualization of endothelial actin cytoskeleton *in vivo*

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Angiogenesis requires coordinated changes in cell shape of endothelial cells (ECs), orchestrated by the actin cytoskeleton. The mechanisms that regulate this rearrangement *in vivo* are poorly understood - largely because of the difficulty to visualize filamentous actin (F-actin) structures with sufficient resolution. Here, we use transgenic mice expressing Lifeact-EGFP and the early postnatal mouse retinal angiogenic model to visualize actin in ECs. We show that in the retina, Lifeact-EGFP expression is largely restricted to ECs allowing detailed visualization of F-actin structures in ECs. Lifeact-EGFP labels actin associated with cell-cell junctions, apical and basal membranes and highlights actin-based structures such as filopodia and stress fiber-like cytoplasmic bundles. Our results indicate that the Lifeact-EGFP transgenic mouse in combination with the postnatal retinal angiogenic model constitutes an excellent system for vascular cell biology research. Our approach is ideally suited to address structural and mechanistic details of angiogenic processes, such as endothelial tip cell migration and fusion, EC polarization or lumen formation.

Scientific Programme – Friday, September 28, 2012

Session “Vascular development and function in zebrafish”

0004

Protein kinase D1 (PKD1) regulates physiological and tumor angiogenesis in zebrafish

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Protein kinase D isoenzymes (PKDs) are serine threonine kinases that belong to the CAMK superfamily. The PKD subfamily consists of PKD1, -2 and -3. PKD1 is the so far best characterized isoform of this family. PKD1 is expressed in endothelial cells and mediates biological responses downstream of the VEGFR. In addition, PKD1 is important for crucial events in angiogenesis, including endothelial cell migration, proliferation and tubulogenesis. PKDs also play a critical role in tumor development and progression, including tumor angiogenesis. However, its role in physiological angiogenesisin vivois as yet poorly characterized. This study was aimed at dissecting the contribution of PKD1 to physiological angiogenesis, lymphangiogenesis and tumor angiogenesis in zebrafish. We could show that PKD1 is widely expressed during zebrafish development. Morpholino-based silencing of PKD1 expression reduced moderately formation of the intersomitic vessels and the dorsal longitudinal anastomotic vessel intg(fli1:EGFP)zebrafish embryos. In addition, silencing of PKD1 resulted in reduced formation of the parachordal lymphangioblast that serves as a precursor for the developing thoracic duct which also was not properly formed. Strikingly, tumor angiogenesis was completely abolished in PKD1 morphants using the zebrafish tumor xenograft angiogenesis assay. Taken together, our data in zebrafish demonstrate that PKD1 contributes to the regulation of physiological angiogenesis and lymphangiogenesis during zebrafish development and is essential for tumor angiogenesis.

Scientific Programme – Friday, September 28, 2012

Session “Vascular development and function in zebrafish”

0033

Depletion of Nucleoside Diphosphate Kinase B Inhibits Angiogenesis

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Nucleoside diphosphate kinase B (NDPK B) regulates a variety of cellular processes, for example G protein mediated signal transduction. However, its role in angiogenesis is still unknown. We evaluated in this study the function of NDPK B in angiogenesis models *in vivo* and *in vitro*.

Firstly, in the zebrafish embryo, the morpholino-mediated knockdown of NDPK B led to a severe vascular phenotype. The NDPK B deficient embryos showed disrupted intersegmental vessels and impaired dorsal longitudinal anastomotic vessel formation. The vessel malformations in the NDPK B knockdown embryos could be rescued by co-injection with NDPK B mRNA. Secondly, ischemia-driven angiogenesis in NDPK B knockout mice was analyzed after excision of the left femoral artery in a hind limb angiogenesis model. In the NDPK B knockout mice the functional vascular recovery was significantly reduced at days 3 and 7 in comparison to that in the wild type littermates. Thirdly, in a mouse model of hypoxia-induced retinal angiogenesis, NDPK B expression was strongly increased under hypoxia in retinal arterioles and neovascularizations in the smooth muscle cells and pericytes. The amount of hypoxia-induced pre-retinal neovascularizations was significantly reduced in NDPK B knockout retinas at p17 by about 70% compared to the wild type in this model. Finally, we used an *in vitro* spheroid angiogenesis model with human umbilical vein endothelial cell (HUVEC). SiRNA-mediated knockdown of NDPK B led to a significant attenuation of sprouting angiogenesis induced by vascular endothelial growth factor (VEGF).

Taken together, our results indicate that deficiency of NDPK B interferes with the VEGF-induced angiogenesis. Suppression of NDPK B upregulation might be beneficial for some diseases with pathological angiogenesis.

Scientific Programme – Friday, September 28, 2012

Session “Emerging role for the microcirculation in cardiovascular disease”

0042

Uridine Adenosine Tetraphosphate (Up4A)-Induced Coronary Vasodilation Is Blunted in Swine with Aortic Banding: a Role for Prostanoids?

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Up4A is a potent endothelium-derived contracting factor that contains purine and pyrimidine moieties and activates purinergic P2X and P2Y receptors. Up4A has recently been found to also have vasodilator effects. Here, we evaluated the vasoactive effect of Up4A in isolated coronary small arteries from sham (S) swine and swine with severe aortic banding (AoB) (2-month systolic pressure gradient of 60 mmHg), and studied the purinergic receptor subtypes and pathways involved. Plasma concentrations of Up4A were much higher in AoB (5.9±3.0 nmol/L) compared to S (0.07±0.03 nmol/L). Surprisingly, Up4A (10^{-9}-10^{-5} M) failed to induce coronary contraction, but produced dose-dependent relaxation of vessels from S and AoB preconstricted with U46619. Sensitivity to Up4A was lower in AoB than S. In both S and AoB, P1 blockade with non-selective antagonist 8PT attenuated the Up4A-induced vasodilation. In contrast, $P2X_1$ blockade with MRS2159, or $P2Y_1$ blockade with MRS2179 attenuated the vasodilator effect of Up4A in S but not in AoB. Furthermore, the effect of Up4A was reduced by nitric oxide synthase inhibition with LNAME in both S and AoB. Subsequent cycloxygenase inhibition with indomethacin further attenuated Up4A-induced coronary vasodilation in S, while it enhanced vasodilation in AoB.

In conclusion, Up4A is a dilator rather than a constrictor in coronary microvessels, which in S occurs via P1, $P2X_1$ and $P2Y_1$ mediated activation of NOS and COX. In AoB, P1 but not $P2X_1$ and $P2Y_1$ receptors are involved in activation of NOS. Elevated plasma levels of Up4A in AoB cause production of vasoconstrictor prostanoids thereby limiting the vasodilator response to Up4A. These findings indicate that in AoB involvement of different purinergic receptors results in activation of other intracellular pathways.

Scientific Programme – Friday, September 28, 2012

Session “Emerging role for the microcirculation in cardiovascular disease”

0040

Microvascular Dysfunction Increases the Risk of Type 2 Diabetes Mellitus. A meta-analysis.

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Introduction: Type 2 diabetes mellitus (DM2) is characterized by microvascular dysfunction. Recent data support the hypothesis that impairment of microvascular function may cause insulin resistance and thus contributes to the development of DM2. The aim of this meta-analysis is to investigate whether microvascular dysfunction contributes to the development of impaired glucose tolerance and DM2.

Materials and methods: We searched MEDLINE and EMBASE for studies published up to Oct. 2011. Prospective studies were included if they focused on microvascular measurements (retinal diameters, skin microvascular endothelium-dependent and -independent reactivity, capillary density, peripheral vascular reactivity, microalbuminuria, or plasma biomarkers of microvascular endothelial dysfunction) in a population-based sample of individuals without DM2 at baseline. We conducted a meta-analysis by use of RevMan5 to determine the associations of microvascular function with glucose tolerance status, using the generic inverse variance method. The pooled relative risk (RR) and 95%CI of the fully adjusted models were estimated by use of the random effects model.

Results: Thirteen studies met our pre-specified inclusion criteria. The pooled RR for incident DM2 was 1.49 [1.36; 1.64] per 1SD higher plasma sE-selectin levels; 1.21 [1.11; 1.31] per 1SD higher plasma sICAM-1 levels; 1.48 [1.03; 2.12] per 1SD lower response to acetylcholine-mediated peripheral vascular reactivity; 1.18 [1.08; 1.29] per 1SD lower retinal arteriole-to-venule diameterratio; and 1.43 [1.33; 1.54] per 1 logarithmically transformed unit higher albumin-to-creatinine ratio. Combining all estimates of microvascular dysfunction resulted in a pooled RR of 1.25 [1.15; 1.36] per 1SD greater microvascular.

Conclusion: These data indicate that various estimates of microvascular dysfunction are associated with a higher risk of developing DM2. This suggests a role for microvascular dysfunction in the (early) pathogenesis of DM2.

Scientific Programme – Friday, September 28, 2012

Session “Emerging role for the microcirculation in cardiovascular disease”

0050

AAV2.9-mediated Thymosin ß4 Overexpression Induced Therapeutic Neovascularization in a Pig Model of Chronic Myocardial Ischemia

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Thymosin ß4 (Tß4), a small 4.9 kDa peptide, has been characterized as a major G-actin-sequestering factor regulating the cytoskeletal reorganisation, thus, cell motility, migration and differentiation. In this study, we investigated the role of long-term overexpression of Tß4 using recombinant adeno-associated viral vector 2.9 (rAAV2.9) in a pig model of normo- and hypercholesterinemic diet in response to chronic myocardial ischemia. **Methods:** Chronic ischemia was induced via reduction stent graft in the circumflex artery, leading to a total occlusion on day 28. Selective pressure-regulated retroinfusion of saline (C) or rAAV2.9 Tß4 (5x10E12 viral particles) was performed on day 28 and compared with the ubiquitous permanent overexpression of Tß4 transgenic pigs. On day 28 and 56, global myocardial function (LVEDP) and fluorescent microsphere immunoassay (FMI) were performed. Subendocardial segment shortening (SES) and post mortem angiography were obtained on day 56. Tissue samples were harvested for histological analysis. Hypercholesterinemia was provoked by high-cholesterol-containing diet. **Results:** Data are presented as mean±SD, $p<0.05$. Tß4 overexpression via rAAV2.9 significantly enhanced both, capillaries (294±31(Tß4) vs. 150±21(C) capillaries/field) and collaterals (9±1(Tß4) vs. 3±1(C)). FMI showed a significant increase of blood flow on day 56 (91±2(Tß4) vs. 78±3(C)% non-ischemic area), which was accompanied by significant improvement of the global (LVEDP 12±2(Tß4) vs. 19±2(C) mmHg) and regional myocardial function (SES at 150 beats/min 73±5(Tß4) vs. 10±6(C)% of non-ischemic area). Similar results were obtained in transgenic pigs (LVEDP 13±1 mmHg). In the pig model of hypercholesterinemia, serum triglyceride level rose from 22±3 to 72±4 mg/dl following high-cholesterol diet. However, the improvement of the neovascularization and heart function could also be achieved, albeit at a lower level. **Conclusion:** These results suggest that post-ischemic rAAV2.9 Tß4 administration enables capillary growth in vivo. This process provides enhanced collateralization and perfusion, thereby resulting in improving myocardial function. A comparable effect is seen in the dyslipidemic hearts, suggesting a therapeutic potential for otherwise no-option patients with ischemic heart disease.

Scientific Programme – Friday, September 28, 2012

Session “Emerging role for the microcirculation in cardiovascular disease”

0053

Increased vascular permeability induced by Angiopoietin-2 overexpression is antagonized by AAV-mediated PDGF transfection

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Ang-2, a co-factor in postnatal angiogenesis interacting at the endothelial Tie-2 receptor, is a potential target fortherapeutic angiogenesis. In earlystage of neovascularisation Ang-2 leads to detachment of pericytes increasingvessel permeability (Hammes 2004), as does an introduction of a PDGF-B retention motiv, which reduces PDGF-function (Nyström 2006). Here we analysed the difference in endothelial cell and cardiomyocyte restricted chronic overexpression of Ang-2 on vascular permeability, pericytes coverage, myocardial morphology and function and the effect of an AAV mediated PDGF-B transfenciton in this scenario.

Methods: Two lines of Ang-2 overexpressing mice were established (expression driven by either Tie-2 or a-MHC promoter) and analyzed. These overexpressing mice were compared to control mice (Ang-2 on vs. Control, MHC Ang-2 on vs. Control). At 12 weeks of age, the Ang-2 on mice were transduced, using a long-acting adeno-associated virus encoding for PDGF-B (rAAV-PDGF-B, 5x1012 PFU). For analysis, mice underwent echocardiography at age 12, 18, and 24 weeks. Furthermore vascular leakage of 4kD-TRITC-dextran following i.v. injection was assessed using a MPLS microscope. At 24 weeks of age, organs were harvested and underwent histological analysis.

Summary and Conclusion: Prolonged endothelial Ang-2 overexpression induces progressive LV-dilation. This effect can not be seen in mice overexpressing Ang-2 in a cardiomyocyte specific manner. The progressive dilation and hypertrophy is antagonized by increased PDGF-B levels. Furthermore, endothelial overexpression of Ang-2 leads to a increase of vascular permeability which again is abolished by PDGF-B. Our results indicate that Ang-2 induced microcirculatory pericyte loss causes systemic cardiovascular alterations (vessel leakiness, cardiac hypertrophy), unless increased PDGF-B levels enhance pericytes recruitment. The cardiovascular impact of prolonged endothelial Ang-2 presence might explain the necessity of its tight expression control, allowing only temporary increases in the earlyphase of angiogenic reactions

Scientific Programme – Friday, September 28, 2012

Session "Emerging role for the microcirculation in cardiovascular disease"

0056

ALTERATIONS IN THE NITRIC OXIDE / SOLUBLE GUANYLYL CYCLASE PATHWAY ENHANCE THE RISK OF MYOCARDIAL INFARCTION

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Introduction

Myocardial infarction (MI) results from sudden atherothrombotic occlusion of a coronary artery. A genetic predisposition for MI was revealed in large cohorts but sometimes MI clusters in families. Herein, genetic analysis through whole exome sequencing may shed light on the pathophysiology of MI and uncover new targets.

Methods

Whole exome sequencing was performed in 3 family members. Identified targets and signalling pathways were analysed in cellular expressing systems, animal knockout models, and platelets from probands.

Results

In a large MI family, heterozygous mutations in two functionally related genes, *GUCY1A3*, encoding the α_1-subunit of the soluble guanylyl cyclase (α_1-sGC), and *CCT7*, encoding a protein of the chaperonin containing TCP-1 complex (CCT), segregate with the disease. All family members carrying the *GUCY1A3* mutation (p.Leu163Phefs*24) and the *CCT7* mutation (p.Ser525Leu) were affected. HEK cells transfected with sGC expressed the protein and exhibited NO-induced cGMP formation, whereas transfecting the *GUCY1A3* mutation was ineffective. In cultured VSM, downregulation of CCT7 by siRNA decreased sGC expression verifying the importance of CCT7 for sGC expression. In platelets of probands heterozygous for both mutations α_1-sGC levels and NO-induced cGMP formation was strongly reduced compared to single mutation or non-carriers. Light-dye induced thrombus formation in the microcirculation revealed the functional relevance of α_1-sGC. Time to arteriolar occlusion was shorter in α_1-sGC deficient mice (α_1-sGCKO) compared to wildtype littermates. Inhibition of endogenous NO formation accelerated thrombus formation only in wildtype and abrogated differences between genotypes indicating an absent inhibitory effect of NO in α_1-sGCKO.

Conclusion

We conclude that dysfunctional NO/sGC signalling may lead to premature MI. We suggest that cGMP formation through α_1-sGC is decisive in continuous inhibition of thrombus formation and thereby prevention of MI.

Scientific Programme – Friday, September 28, 2012

Session “Emerging role for the microcirculation in cardiovascular disease”

0058

Insulin does not redistribute microvascular blood volume from skeletal muscle to subcutaneous adipose tissue in obese women.

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Question:

Hyperinsulinemia increases microvascular blood volume (MBV) in skeletal muscle in lean subjects. This microvascular recruitment promotes the access of insulin to the target tissues. In obese subjects insulin-induced microvascular recruitment is blunted. It is unknown whether the blood is alternatively diverted to adipose tissue in obese.

Methods:

Seven lean, and seven obese females (18-55 years) underwent a hyperinsulinemic eyglycemic clamp. Contrast Enhanced Ultrasound measurements (CEU) were performed in real-time using a Siemens Sequoia 512 ultrasound machine and Sonovue microbubbles. CEU was performed before, and during hyperinsulinemia. MBV was quantified both in skeletal muscle as in subcutaneous adipose tissue.

Results:

MBV of skeletal muscle was similar in lean and obese volunteers. Skeletal muscle MBV increased (18 ± 6%) in lean, but did not increase in obese (-10 ± 19%) during hyperinsulinemia (p=0.018). In subcutaneous adipose tissue, MBV was slightly lower in lean (1.39 ± 0.28) at baseline than in obese (1,88 ± 0.41) (p = 0.43). In both lean (1.33 ± 0.19, p=0.61) and obese volunteers (2.01 ± 0.53, p=0.86), MBV did not increse during hyperinsulinemia, compared to baseline.

Conclusions:

The present study confirms that the microcirculation of skeletal muscle is recruited by insulin in lean, healthy volunteers, and that this effect is blunted in obese. Insulin does, however, not redistribute microvascular blood volume to the subcutaneous adipose tissue in obese. In both lean and obese women the microvasculature of subcutaneous adipose tissue is not recruited by insulin.

Scientific Programme – Friday, September 28, 2012

Session “Emerging role for the microcirculation in cardiovascular disease”

0062

Early impairment of skeletal muscle endothelial glycocalyx barrier properties in diet-induced obesity in mice

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Introduction

Damage to the glycocalyx has been suggested to be an early event in the progression of microvascular dysfunction during exposure to cardiovascular risk factors. In the current study glycocalyx barrier properties in skeletal muscle microcirculation of mice were determined at a relative early and later stage of diet-induced obesity (DIO) using a high-fat diet (HFD).

Material & Methods

The microcirculation of the hindlimb muscle of anesthetized mice, fed chow or HFD for 6 or 18 weeks, was visualized with a Sidestream Dark-field (SDF) camera. In the recorded movies, the variation in RBC column width was used to measure glycocalyx barrier properties. Hereafter, an intraperitoneal glucose tolerance test was performed by bolus administration of 1g/kg glucose and measuring blood glucose levels for 120 minutes. AUC of glucose (AUCg) was calculated as a measurement for glucose tolerance.

Results

In the mice that received chow for 6 weeks the outward movement of RBCs relative to the median RBC column width was 0.81 ± 0.03 µm, and this was not different in the mice that received chow for 18 weeks (0.87 ± 0.03 µm). The outward movement significantly increased to 0.97 ± 0.04 µm in the mice that were fed a HFD for 6 weeks and to 1.02 ± 0.07 µm after 18 weeks of HFD, indicating that glycocalyx barrier properties were impaired. Glucose intolerance appeared not fully manifested after 6 week of HFD (AUCg was 421 ± 51 mmol/L*120min versus 284 ± 38 mmol/L*120min in the 6 week chow mice; p=0.08), yet after 18 weeks of HFD there was a significant increase in AUCg (581 ± 61 mmol/L*120min). AUCg did not change in 18 week chow mice (357 ± 30 mmol/L*120min).

Conclusion

In DIO, glycocalyx barrier properties are impaired before overt glucose intolerance has developed. These observations open novel directions for diagnosis and treatment of microvascular dysfunction at an early stage of DIO.

Scientific Programme – Friday, September 28, 2012

Session “Emerging role for the microcirculation in cardiovascular disease”

0045

Protective effects of the tyrosine kinase inhibitor imatinib in sepsis

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Sepsis carries extremely high morbidity and mortality, which mainly result from massive loss of endothelial barrier function. Since we previously demonstrated that endothelial barrier dysfunction can be reversed by imatinib, we evaluated the effects of imatinib on vascular leakage during sepsis.

Sepsis was induced in mice by cecal ligation and puncture (CLP). Vascular leakage of Evans Blue was compared between mice treated with imatinib 50mg/kg or vector. Imatinib attenuated vascular leakage in the kidneys and reduced the number of mice that developed vascular leakage in the lungs. Comparable results were obtained in a model of VEGF-induced vascular leakage in the skin (Miles assay) in which imatinib attenuated vascular leakage with 50%. Reduced hydrostatic pressure differences or microvascular perfusion were excluded as causes of attenuated vascular leakage.

Transfection of human endothelial cells (ECs) with siRNAs against the imatinib-sensitive kinases revealed that imatinib exerts its protective effect via inhibition of Abl-Related Gene (Arg). Knock-down of Arg had a similar effect as imatinib, whereas imatinib did not have an additive effect in Arg-depleted cells. Interference with Arg function (pharmacological or genetic) increased cell-matrix interaction, resulting in improved adhesion of ECs to the extracellular matrix. Arg-depleted cells showed peripheral redistribution of β1 integrin, showing a ring of β1 integrin at the cell periphery, colocalizing with cortical actin.

Elucidating pathways via which Arg may affect β1 integrin, we found that endothelial stimulation with barrier-disruptive agents (thrombin, histamine and VEGF) results in phosphorylation of CrkL protein at Tyr207. Arg inhibition with imatinib or siRNA revealed that CrkL phosphorylation was Arg dependent. Unphosphorylated CrkL is required for activity of Rap1, which in turn activates integrins. Indeed, inhibiting CrkL phosphorylation with imatinib enhanced Rap1 activity.

These data show that imatinib attenuates vascular leakage during sepsis. The protective effect of imatinib results from inhibition of Arg in ECs, which enhances cell-matrix interaction of ECs via CrkL/Rap1/ β1 integrin signaling.

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Poster Session 2

0008

The polarity protein Scribble is a negative regulator of endothelial shear stress signalling

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The highly conserved epithelial protein Scribble (Scrib) is part of a multiprotein complex, which is responsible for the development and maintenance of epithelial cell polarity. Also vascular endothelial cells express Scrib and show a certain polarization, but the function of the protein in the vascular system is elusive. In their physiological environment, endothelial cells are exposed to shear stress induced by blood flow, which is required to maintain their phenotype. Given that Scrib controls the polarized phenotype of epithelial cells, we hypothesize that also in endothelial cells Scrib contributes to mechanotransduction and phenotype maintenance in the vasculature.

In HUVEC cultured under static conditions, Scrib was mainly present in the plasma membrane. Unidirectional laminar flow induced an alignment of the cells and resulted in a translocation of Scrib to the leading part of the cell diametric to the flow direction. Upon shear stress exposure, HUVEC treated with Scrib siRNA showed an increased cell length and size as well as an enhanced flow-induced alignment as compared to scrambled siRNA transfected cells exposed to laminar shear stress. Suppression of Scrib expression under laminar flow conditions changed the F-actin pattern from circular to stress fibre morphology. Analyzing the role of Scrib in shear stress-induced signalling and gene expression, we found that Scrib knockdown leads to a six fold increase in the mRNA of the transcription factor KLF2, a main driver of shear stress-induced gene expression. As KLF2 is known to potently inhibit inflammatory cytokine mediated induction of the adhesion molecule VCAM, we determined the effect of Scrib in this setting. Indeed, Scrib knockdown attenuated the TNFα-induced VCAM protein expression, suggestive of a vasoprotective effect of Scrib downregulation. To demonstrate the relevance of this effect, cell adhesion was studied. Scrib knockdown in TNFα stimulated HUVEC indeed let to a marked reduction in peripheral blood mononuclear cell adhesion.

Taken together, these observations identify Scrib as a negative regulator of endothelial shear stress signalling. Inhibition of Scrib could be a strategy to enhance the beneficial effects of laminar flow on endothelial cell function.

Poster Session 2

0019

Stretch-induced activation of the transient receptor potential channel C3 in endothelial cells

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Question

Chronic exposure of blood vessels to supra-physiological levels of wall tension leads to remodeling of the vessel wall. Although the clinical consequences of long term remodeling are quite well known, very little is known about the events at the onset of this process. Our recent work shows the involvement of transient receptor potential channel C3 (TRPC3) in stretch-induced activation of the mechano-sensitive transcription factor zyxin. We here analyze the mechanism of stretch-induced TRPC3 activation and its role in zyxin-mediated vascular gene expression.

Methods

Primary human umbilical vein endothelial cells (EC) were cultured on glass cover slips or on stretchable Flexercell membranes. Real time ratiometric Fura-2 fluorescence measurements were made with ECs. Analytical methods such as real time RT-PCR, Western blotting and immunofluorescence analysis were used.

Results

Inhibition of TRPC3 by its specific inhibitor Pyr3 (10 mM) or siRNA directed against TRPC3 efficiently blunted stretch-induced zyxin translocation and zyxin-induced gene expression in EC. Calcium signals in response to stretch also were Pyr3-sensitive. Hypothesizing that TRPC3 channels in ECs may be activated by diacylglycerol (DAG), we exposed ECs to the DAG analogue OAG (100 mM) to activate TRPC3 and, thus, mimic the effects of stretch. Indeed, Pyr-3-sensitive nuclear translocation of zyxin and zyxin-induced gene expression similar to the response to stretch was observed with OAG. Fura-2 imaging revealed that in ECs, OAG induces a strong calcium signal caused by an influx of extracellular calcium. This signal was blunted by parallel exposure of the cells to Pyr3 and by siRNA mediated TRPC3 knock down.

Conclusion

Our data suggest that in endothelial cells TRPC3 is activated by stretch via a release of DAG. The concomitant increase of cytosolic calcium seems to trigger zyxin translocation to the nucleus where the protein regulates a majority of stretch-induced genes

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Poster Session 2

0027

The phosphatase activity of SHP-2 enhances hypoxia induced vessel sprouting and HIF-1α expression

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Background: We previously showed that the tyrosine phosphatase SHP-2 regulates growth factor dependent angiogenesis. Since it exerts functions both by its phosphatase activity and as adapter protein, we investigated which functions of SHP-2 are involved in hypoxia induced endothelial proliferation and vessel growth.

Methods: Overexpression of SHP-2 wildtype (SHP-2 WT), catalytically inactive (SHP-2 CS), constitutively active (SHP-2 E76A) or SHP-2 with lost adapter function (SHP-2 Y2F) in human microvascular endothelial cells (HMEC) and isolated mouse aorta were achieved by lentiviral transduction. Cells were exposed to hypoxia for 4h or 24h. Proliferation was assessed by MTT reduction. HIF-1α was detected by western blot and angiogenesis assessed by the aortic ring assay in matrigel.

Results: Expression of catalytically inactive SHP-2 reduced proliferation during normoxia ($p<0.05$, $n=4$), hypoxia ($p<0.05$, $n=6$) and after hypoxia-reoxygenation (H/R) ($p<0.01$, $n=6$) compared to SHP-2 WT. Likewise aortic vessel sprouting upon H/R ($p<0.05$, $n=4$) and hypoxia inducible factor 1α (HIF-1α) expression ($p<0.05$, $n=4$) were also impaired. In contrast, expression of constitutively active SHP-2 resulted in a 1.7-fold increase in proliferation during normoxia ($p<0.01$, $n=6$) and promoted aortic vessel growth upon H/R ($p<0.01$, $n=5$) as well as enhanced HIF-1α expression ($p<0.05$, $n=4$) compared to SHP-2 WT. However, SHP-2 without adapter function only enhanced proliferation under normoxia ($p<0.001$, $n=24$) but had no significant impact on proliferation and HIF-1α expression during hypoxia or H/R.

Conclusion: SHP-2 is important for proliferation and vessel sprouting upon hypoxia and normoxia. The phosphatase activity contributes to HIF1α upregulation and vessel sprouting in hypoxic conditions, whereas the adapter function appears not to be functionally important for hypoxic processes. Thus, the SHP-2 catalytic activity may be a promising target for angiogenesis induction in ischemic conditions.

Poster Session 2

0041

Induction of apoptosis in endothelial cells by changes of matrix stiffness.

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Introduction: Endothelial cells (EC) are important components of the cellular vascular system and function as sensor for multiple signals. However, the influences of the physical and elastic properties of the vascular wall on those signals are studied insufficiently. Recently we could show that upon chronic exposure to elevated shear stress EC release and/or activate an elastase-like protease. During vascular remodelling processes, proteases are recruited and seem to be critically involved in generation and maintenance of signalling cascades of adaptive vascular remodelling. Furthermore, enhanced elastase activity has been attributed to the initiation of aneurysms. Therefore we follow the hypothesis, that due to those proteolytic activities the matrix stiffness is altered and by that influences cellular signalling responses. Methods and results: EC were seeded on matrix coated polyacrylamide gels exhibiting increasing stiffness (estimated Young's modulus ranged from 7 to 100 kN/m²). Parallel to increasing stiffness, EC showed changes in morphology from a flat and spread phenotype to a more rounded and contracted, verifying that EC are constantly pulling on their adhesion sites. Soft and flexible matrix gels gradually induced apoptosis (tunel assay). A combination of various matrix stiffness and exposure to elastase showed that notably on a firm underground elastase treatment induced an additional increase in apoptosis. To investigate acute signalling effects induced by matrix flexibility, EC were grown on plastic dishes coated with fibronectin and subsequently exposed to elastase (0.3 to 0.5U/ml). As soon as 2-5min after elastase treatment EC appeared to be retracted and showed a substantial reorganization of focal adhesion sites. Conclusion: These results indicate that proteolytic alteration of the matrix induces distinct signalling sites by changes of the focal arrangement of matrix receptors leading to apoptosis (anoikis). Thus fragmentation of matrix proteins show modulator properties for adhesion dependent signalling and might serve not only as a model system to study development and progression of vascular aneurysms but also of arteriosclerosis.

Poster Session 2

0063

A nanomicroscopy technique to study the physical properties of the endothelial surface layer

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Introduction: The endothelial surface layer (ESL) has a pivotal role in maintaining the functions of microvessels. However, due to the lack of an appropriate investigation tool, little is known about the exact physical properties (thickness, density) of the ESL under physiological conditions. These properties could be studied via tracking the motion (Brownian or induced) of fluorescent tracer nanoparticles (FNPs) through the ESL. The aim of this work was to establish a user friendly microscopic technique for single FNP tracking with nanometer resolution. **Material and Methods:** An inverted microscope was equipped with a 100W mercury arc lamp, a piezo objective positioner, a 100x oil immersion objective (NA = 1.3) and a CCD camera (1392´1040). Microbeads (Molecular Probes) with 200 ± 40nm diameter, absorption maximum at 540nm and emission peak at 560nm were used as FNPs. Data analysis was carried out with custom-made software. Brownian motion of FNPs and near-wall flow profile were reconstructed beside high (dextran) or low (PBS) viscosity media. **Results:** Two different Fourier based cross-correlation algorithms were used for 3D tracking of FNPs: the defocus position of a FNP was determined **(A)** by comparing a whole out-of-focus image of the FNP to a specially prepared z-stack image of a point-spread function (PSF); **(B)** the outermost ring radius (ORR) of the FNP's diffraction pattern was correlated with Gauss function and the linear relation between ORR and defocus distance was used. Pattern comparison **(A)** yielded *ca.* 40nm axial resolution, whereas ORR approach **(B)** resulted 22nm resolution on defocus range of 3000-9000nm. MSD value of 3D Brownian motion was in correlation with the media viscosity. The precision of near wall flow profile reconstruction was increased by more viscous media. **Conclusion:** Both defocus imaging nanomicroscopy approaches could be used for 3D FNP tracking in microvessels and as a powerful tool for ESL investigation.

Poster Session 2

005

Regulation of pathophysiological blood vessel development in zebrafish by glucose and glucose metabolite methylglyoxal

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The zebrafish is a well established model organism to study vascular development. Diabetes mellitus is associated with a state of chronic hyperglycaemia and diabetic patients are known to develop various long-term micro- and macrovascular damages, which are mostly caused by the formation of reactive glucose metabolites, called reactive dicarbonyls. One important reactive dicarbonyl is methylglyoxal (MGO), which is excessively formed as a result of increased glucose flux through glycolysis and is known to accumulate under diabetic conditions. MGO modifies proteins, lipids and nucleic acids by forming advanced glycation end products (AGEs) and thereby leading to structural damages and dysfunctional molecules.

In this study we have analyzed effects of high glucose and MGO concentrations on blood vessel formation in zebrafish and identified an abnormal vascular development in the trunk. Likewise, gene expression silencing of glyoxalase 1, a key enzyme in the main degradation pathway of MGO, induced a similar hyperbranching phenotype in zebrafish. Aminoguanidine, which is a MGO scavenger, reduces hyperbranching induced by glucose treatment or glyoxalase 1 silencing. The enhanced angiogenic activity under high glucose and MGO is mediated by vascular endothelial growth factor (VEGF) receptor signalling via its downstream target phosphoinositide-3 kinase (PI3K), as use of the VEGF receptor inhibitor Vatalanib (PTK787) and the PI3K inhibitor LY294002 impaired hyperbranching of the trunk blood vessels.

Together, the data support an important impact of metabolic pathways on vascular development in zebrafish.

Poster Session 2

0014

Monitoring of vascular remodeling after myocardial infarction using flt-1/eGFP transgenic mice

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Question:The vascular endothelial growth factor receptor (VEGFR-1, flt-1) plays an important role in vascular development and different pathologies; in particular it is upregulated and mediates vascularization in the heart during ischemia. To examine these mechanisms a transgenic mouse model with the live reporter gene eGFP driven by the flt-1 promoter was generated.

Methods: At first, transgenic murine embryonic stem (ES) cells were generated using an flt-1 promoter-driven eGFP expression vector. These transgenic ES cells were then applied for diploid aggregation to generate transgenic mice. The mice were subjected to cardiac injury and vascular remodeling was investigated.

Results: The flt-1/eGFP murine ES cells formed extensive GFP+ vascular networks during differentiation; immunostaining with endothelial markers have proven co-localization with the endothelium. In transgenic mouse embryos large and small vessels showed eGFP expression. In contrast, at the adult stage large arteries were eGFP- whereas small vessels were found to be strongly eGFP+ in various organs, e.g. the heart, the lung, the brain and the kidney. In the heart eGFP expression could be detected in small vessels but not in large coronary arteries. When flt-1/eGFP mice were investigated after cryoinfarction or LAD ligation we observed extensive remodeling of vascular structures as well as an increase of the endothelial cell area in the peri-infarct zone at 6 days post-injury.

Conclusion: The flt-1/eGFP ES cell and mouse model provide useful systems for the monitoring of small vessel formation and remodeling in vitro and in vivo. These models should prove helpful to investigate vascular development and re-vascularization in different organs post-injury especially in the heart.

Poster Session 2

0026

SYNJ2BP is a novel modulator of DLL4-Notch signaling during angiogenesis

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Question:

The formation of novel blood vessels is initiated by vascular endothelial growth factor (VEGF) and counterbalanced by Delta-Notch signaling. Notch signals promote the stalk cell phenotype and arterial cell fate during sprouting angiogenesis. We aimed at identifying novel genetic modifiers of Notch signaling in endothelial cells.

Results:

Yeast two-hybrid screening approaches revealed several proteins interacting with the intracellular domains of Notch ligands DLL1, DLL4 or JAG1. SYNJ2BP (Synaptojanin-2 binding protein/ARIP2) was identified as a novel DLL1 and DLL4 interacting protein. Binding of SYNJ2BP to the PDZ motif of DLL4 strongly extended the half-life of DLL4 protein in primary arterial endothelial cells. The elevated DLL4 protein levels were accompanied by enhanced Notch signaling and expression of the Notch target genes HEY1 and ephrin-B2. SYNJ2BP-silenced endothelial cells showed diminished Notch activity, increased migration speed, proliferation rates; and formed a much more complex vascular pattern by sprouting angiogenesis. This could be reversed by re-expression of active NOTCH1. While silencing of SYNJ2BP increased vascular sprout formation and branching, SYNJ2BP overexpression inhibited angiogenesis in a Notch-dependent manner. In line with the function of DLL4, SYNJ2BP diminished the expression of tip cell marker genes and enhanced the stalk cell phenotype. In vivo, the implantation of SYNJ2BP-silenced human endothelial cells into immune-compromised mice led to the formation of a functional blood vessel network with significantly increased vascular density indicating that SYNJ2BP is an anti-angiogenic protein. Consistently, pathological angiogenesis in a mouse model of oxygen-induced retinopathy of prematurity was associated with decreased DLL4, HEY1 and SYNJ2BP expression levels in retinal endothelial cells.

Conclusions:

In summary, we conclude that SYNJ2BP is a novel inhibitor of angiogenesis executing its functions predominately by promoting DLL4 protein stability and DLL4-Notch signaling.

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Poster Session 2

0054

PDGF-B transfection reduces septic hypercirculation, improves cardiac function and survival

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PDGF-B is a key regulator of pericyte recruitment and survival. A introduction of a PDGF-B retention motiv, which reduces PDGF-function (Nyström 2006) results in increased vascular permeability and loss of pericytes. In previous experiments we were able to show, that an LPS induced sepsis leads to loss of pericytes and increased vascular permeability. Here we analyzed the efficiency of a PDGF-B transfection prior to sepsisinduction in reduceing pericyte loss and capillary leakage.

Methods: To induce sepsis, lipopolysaccharides (LPS) [20mg/kg] were injected intraperitoneally into C75BL/6 mice. 14 days before sepsis induction, mice were pre-treated with PDGF-B or LacZ as control using an adeno-associated virus. Measuring IL-6 serum levels proved the induction of sepsis. Additionally a sepsis-severity score was assessed after 6 and 12 hours, followed by non-invasive RR measurements after 12 hours (n=9 per group) or observation of further survival (n=22 per group). Tissue samples (heart and peripheral muscle) were harvested and analyzed for capillary density (CD31+) and pericyte coverage (NG2+). Vascular leakage was assessed by determining the loss of intracellular fluorescence (Δ fl) in ear vessels after injection of 4dK-TRITC-dextran into the tail vain using intravital 2Photon-Microscopy.

Results and conclusion: IL-6 serum levels were significantly increased 12 hours after LPS injection in both groups. Pre-treatment with PDGF-B was capable of reducing pericyte loss in septic mice and in reducing microvascular leakage, as shown in enhanced intravascular fluorescence over time. Non-invasive blood pressure measurements illustrated almost normal mean arterial pressure after PDGF-B treatment, which was significantly higher compared to LacZ-controls. This resistance to LPS-induced hemodynamic changes was reflected by a better clinical situation due to Ang-2 inhibition and significantly improved survival rates (PDGF-B 45% after 36 h vs. LacZ 0%).

Summary and Conclusion: PDGF-B treatment decreases vascular leakage and attenuates the hypercirculatory state in acute inflammation. Moreover, PDGF-B preserves cardiac function and reduces the mortality during LPS induced sepsis. Overall, our findings emphasize the relevance of pericyte recruitment to the microvasculature during sepsis.

Poster Session 2

0055

Thrombus formation in murine fetuses - developmental aspects of platelet function

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Introduction

Thrombus formation is a well investigated process in adult mice and men. Yet, knowledge about in-vivo clot formation during fetal life is still unclear, although in vitro investigations of human fetal platelets display reduced activity which provides some evidence that platelet function might be developmentally regulated. Using a newly developed intravital microscopy model in the mouse fetus, we set out to investigate thrombus formation in yolk-sac vessels of murine fetuses.

Material and Methods

Pregnant C57/Bl6 mice were anesthetized, the abdominal cavity opened, the uterus horn exteriorized and opened, and one living fetus mobilized for intravital microscopy of yolk sac microvessels. Microinjection of phototoxic FITC-dextran into fetal vessels enabled us to induce thrombus formation at the site of observation. Frequency and time course of thrombus formation was examined in fetuses of different gestational ages (E13.5 to E17.5).

Results

Following phototoxicity-induced vessel damage, occurrence of thrombus formation and final vessel occlusion in yolk sac vessels of murine fetuses was significantly reduced in fetuses with the lowest gestational age (E13.5) compared to older fetuses ($p<0,05$). In addition, reflow phenomena after initial occlusion appeared more often in E13.5 mice. Furthermore, time until onset of thrombus formation as well as final vessel occlusion was prolonged in E13.5 fetuses. These results suggest that thrombus formation is ontogenetically regulated and only develops late during fetal life. Because fetal platelet counts were found to be low during fetal life and only increased towards birth, low platelets might contribute to poor thrombus formation early during fetal life.

Conclusion

Our experiments provide the first in-vivo evidence for a developmental regulation of thrombus formation in murine fetuses with reduced clotting performance and stability of thrombi early during fetal ontogeny.

Poster Session 2

0057

Degradation products of hyaluronan produced in the context of tumors are novel regulators of lymphangiogenesis

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Question. Given the importance of tumor-induced lymphangiogenesis, it is important to know how this process is regulated. Enhanced break down of the extracellular matrix component hyaluronic acid can occur in tumors, leading to accumulation of small oligosaccharides (sHA) that can promote angiogenesis. Here we investigated whether sHA can also induce lymphangiogenesis.
Methods. In this study, the lymphangiogenic response to sHA was examined using (i) proliferation, tubule formation and migration of primary lymphatic endothelial cells (LECs); (ii) ex vivo thoracic duct ring assays; (iii) experimental animals.
Results. Low concentrations of sHA increased the proliferation rate of primary LECs and acted additively with VEGF-C and FGF-2. In ex-vivo and in vivo lymphangiogenesis assays, sHA promotes outgrowth of lymphatic capillaries. The sHA-induced lymphangiogenesis is dependent on LYVE-1, a hyaluronan receptor that is widely used as a marker of lymphatic endothelial cells. In contrast, high sHA concentrations inhibited lymphangiogenesis. This anti-proliferative effect is mediated by TGF β, an inhibitor of lymphangiogenesis. To determine the pathophysiological sHA concentrations in the context of tumors we established a novel method that allows analysis of sHA levels in interstitial fluid derived from tumor samples, and found that interstitial fluid from some tumors contains sHA concentrations in the pro-lymphangiogenic range. When sHA-producing 4T1 breast tumor cells were introduced into LYVE-1 knockout animals, metastasis to lymph nodes was significantly reduced compared to that in wild-type mice. We also found that manipulation of the expression of Hyal1 is not sufficient to influence sHA levels in tumor interstitial fluid but nevertheless regulates metastasis formation.
Conclusions. These results suggest that tumor-produced sHA may contribute to lymphangiogenesis and metastasis.

Poster Session 2

0061

Gap Junction blockage in chorioallantoic membrane networks

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Introduction. Studies using theoretical models have revealed the necessity of information transfer along blood vessels to achieve efficient blood flow distribution in microvascular networks. Absence of information transfer, which is most likely mediated via gap junctions, might lead to maldistribution. Here, we investigated the effects of gap junction blockage in chick chorioallantoic membrane (CAM) networks by using intravital microscopy.

Material & Methods. We analysed the effects of the gap junction inhibitors carbenoxolone (175 µM, CBX), palmitoleic acid (100 µM, PA) and GAP27 (1000 µM), a gap junction blocking peptide, on CAM vessel diameter. Additionally, we examined to what extent these effects are caused by change of vessel structure and vascular tone using a dilative cocktail consisting of acetylcholine (10 µM), adenosine (100 µM), papaverine (200 µM) and sodium nitroprusside (10 µM).

Results. All three gap junction inhibitors led to a decrease in both arterial and venous diameter. After 3h of incubation relative diameter of arteries (a.) and veins (v.) was: 0,49 (a.) and 0,36 (v.) for CBX, 0,70 (a.) and 0,59 (v.) for PA and 0,77 (a.) and 0,57 (v.) for GAP27. CBX also caused hemostasis mainly in arterial but also in venous vessels. Vessel reaction upon dilatation diminished with increasing time of CBX incubation indicating possible changes in vessel structure.

Conclusions. Our findings provide evidence for the importance of gap junction based communication for developing microvascular networks. We could show that gap junction blockers cause a decrease in vessel diameter probably by an initial increase in vascular tone and subsequent structural changes leading to maldistribution of blood flow in CAM vasculature.

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Poster Session 2

0025

Luminal released von Willebrand factor supports *S. aureus* adhesion to intact endothelial cell layer under shear stress

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Introduction: *S. aureus* is able to bind to undamaged cardiac endothelium causing infective endocarditis, but the molecular mechanisms are not well defined. During inflammation activation of the endothelium leads to a von Willebrand factor (VWF) release followed by a shear-dependent VWF fiber formation. Typically, these VWF fibers bind platelets at sites of vascular injury. But, VWF fibers can also be formed on an intact endothelium promoting inflammatory processes. In this study we analyzed ULVWF as potential interaction partner for *S. aureus* adhesion to intact endothelium with regard to infective endocarditis.

Material & Methods: During *in vitro* experiments HUVECs were perfused with fluorescence-labelled *S. aureus* at high flow conditions. Bacterial adhesion to VWF was quantified after immunofluorescence staining. *In vivo* experiments were performed with the dorsal skinfold chamber of VWF knock-out and WT mice by using intravital fluorescence microscopy.

Results: Our *in vitro* experiments demonstrated that VWF almost exclusively triggers *S. aureus* adhesion to intact endothelium under high shear flow. This interaction was dependent on the A1 domain of endothelial VWF and can be inhibited by ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) and heparin. While protein A can be exclude as binding factor for *S. aureus* - VWF adhesion at high shear rates, low binding capacity of several mutants lacking adhesive surface molecules indicated a cooperation of binding factors for bacterial adhesion to VWF. *In vivo* experiments revealed a strongly reduced bacterial adhesion in VWF knock-out mice which documented the high physiological relevance of VWF in *S. aureus* binding to an intact endothelium.

Conclusion

Conclusion: The *in vitro* as well as *in vivo* results indicate the potential role of VWF in the pathogenesis of infective endocarditis. Since bacterial binding is influence by heparin and ADAMTS-13 novel therapeutic approaches are envisioned.

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Poster Session 2

0028

Site directed vascular gene delivery by ultrasonic destruction of magnetic microbubbles

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Background: Site specific vascular gene delivery is a highly desirable therapeutic tool but efficient delivery upon systemic administration is still inefficient. Here, we tested whether a combination of standard ultrasonic microbubble technology and local concentration of magnetic microbubbles by magnetic fields enhanced site specific gene transfer after systemic microbubble application *in vivo*.

Methods: Vascular gene expression was optically assessed in the dorsal skin fold chamber of C57BL/6 mice by intravital microscopy 48-72h after intra-arterial application of magnetic microbubbles (MMB) carrying pDNA encoding dsRed (2.4 µg pDNA/g body weight) and magnetic field (MF) (B=1023mT, 5min) and ultrasound (US) (1MHz, 2W/cm^2, DC50%, 30sec) exposure. Reporter gene expression in tissues was detected by quantitative real-time PCR.

Results: Microbubbles readily associated with positively charged MNP and pDNA as well as lentivirus (n=3) thereby enhancing their capacity for gene delivery to endothelial cells in vitro. In vivo, intra-arterial applied MMB circulated in the vascular system for up to 30min. Local MF and US exposure of the skin fold tissue after injection of pDNA coated MMB enhanced local dsRed expression over sixtyfold as compared to the conventional microbubble technique (p

Conclusion: These results demonstrate that microbubbles can be successfully coated with MNP and pDNA or lentivirus. In vivo by a combination of MF application and US, they can be targeted to a specific site after systemic administration, thereby enhancing local vascular gene delivery significantly. This technology may improve targeted gene delivery in the vascular system for both research and therapeutic purposes.

Poster Session 2

0030

Dasatinib causes endothelial leakage in a SRC tyrosine kinase dependent manner

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Introduction:

The multiple kinase inhibitor Dasatinib, used in clinical applications in Imatinib-resistant chronic myeloid leukemia, frequently induces endothelial dysfunction and subsequently pleural effusion (10-35% incidence). To investigate the effect of Dasatinib on the lung vessels in clinically relevant concentrations we examined human pulmonary artery and microvascular endothelial cells exposed to the drug *in vitro*.

Materials and Methods:

Human pulmonary artery endothelial cells (hPAECs) and human pulmonary microvascular endothelial cells (hPMVEC) were obtained from Lonza and treated for up to 24 hours with 1, 10 or 100nM Dasatinib, 1µM PP2 or 1µM PP3. Continuous transendothelial electric resistance measurements were carried out with a CellZScope (Nanoanalytics). The monolayer permeability was assessed by fluorescently labelled dextran. The changes in the cell junctions were visualized by staining for ZO-1 and VE-cadherin. The cell viability was assessed by MTT assay and the amount of apoptotic cells was determined by PI and Annexin V staining by fluorescent activated cell sorting.

Results:

We observed a dose dependent decrease in the electrical resistance of the endothelial monolayer upon 30 hours of clinically relevant concentrations of Dasatinib treatment. The monolayer permeability had an average of 2-fold increase in hPAEC and a 4-fold increase in hPMVEC upon Dasatinib treatment (100nM). The immunofluorescent stainings showed disruption of the monolayer upon 24h of Dasatinib treatment (100nM) with discontinuous staining for VE-cadherin and ZO-1. We observed the same effect on the cells treated with PP2 (Src-kinase blocker), whereas PP3 (inactive analogue of the Src-kinase inhibitor) had no effect. Neither cytotoxic effect, nor increase in the number of apoptotic cells was detected upon 24 hours of Dasatinib treatment.

Conclusion:

Dasatinib, used for the treatment of chronic myeloid leukemia, via inhibition of Src tyrosine kinase induces endothelial dysfunction at cellular level, which might lead to lung dysfunction at organ level, causing pleural effusion.

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Poster Session 2

0037

Sphingosine-1-phosphate receptor 3 and activation of Rho mediate sphingosine-1-phosphate induced angiogenesis in HUVECs

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Introduction:

Sphingosine-1-phosphate (S1P) elicits numerous biological responses including cell survival, growth, migration and proliferation of endothelial cells (EC). In vascular EC these responses are mediated by three different types of G-protein coupled receptors ($S1P_1R$, $S1P_2R$, $S1P_3R$). Whereas the G_i-coupled S1P1R is involved in maintaining the endothelial barrier function, the subtype(s) regulating angiogenesis are ill defined.

Methods:

We therefore studied the S1P-induced *in vitro* spheroid sprouting of human umbilical vein EC (HUVEC) and analyzed the concomitant activation of the RhoGTPases Rho and Rac1 by effector pull down assays.

Results:

Physiological relevant concentrations of S1P (100 nM) induce a moderate activation of both, Rac1 and Rho. The activation of Rac1 was sensitive to NSC23766 (NSC), an inhibitor of Rac1 specific guanine nucleotide exchange factors. To investigate the involvement of the $S1P_1R$ pathway in *in vitro* angiogenesis we studied the influence of SEW2871, pertussis toxin (PTX) and NSC. SEW2871 concentration dependently inhibited angiogenesis whereas PTX and NSC had no influence on S1P-induced sprouting. Inhibition of the $S1P_2R$ by its specific antagonist JTE 013 increased basal but had no effect on the S1P-stimulated sprouting. In contrast, the $S1P_1R$ and $S1P_3R$ antagonist VPC23019 suppressed basal and S1P-induced sprouting angiogenesis. Suppression of the activity of the $G_{q/11}$ did not inhibit whereas attenuation of $G_{12/13}$ signaling blunted S1P-induced Rho activation.

Conclusion:

We conclude that angiogenic sprouting of EC is mainly stimulated via the $S1P_3R$ and likely involves the activation of Rho via $G_{12/13}$ dependent pathways at physiological relevant S1P concentrations.

Poster Session 2

0059

Red wine polyphenols improve cardiometabolic risk in obese subjects by alleviating inflammation and microvascular dysfunction

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Introduction:

Epidemiological studies have shown that consumption of alcoholic beverages, red wine in particular, is associated with less cardiovascular mortality. In addition, there are reported beneficial effects of red wine on components of the metabolic syndrome, arguably the most menacing cardiometabolic condition facing us due to the unfolding obesity epidemic.Experimental studies with mixed or separate Red Wine Polyphenols (RWPs) (i.e. without alcohol) have shown beneficial effects on cardiometabolic parameters associated with obesity. Most research has focused on resveratrol, a specific polyphenol components which is quite specific to red wine and has, at least in animal studies, beneficial effects on insulin sensitivity, blood pressure, and endothelial function. Moreover, RWPs have shown to improve endothelial NO-mediated relaxation using the same PI3-kinase/Akt pathway as does insulin. However, data in humans are remarkably scarce.

Primary objective: To study effects of RWPs on insulin sensitivity and microvascular function

Secondary objectives: To study effects of RWPs on blood pressure, markers of low-grade inflammation.

Study design: double-blind placebo-controlled randomised clinical trial

Subjects: Obese (BMI >30; n=60) men or women, aged 18-70 years. To exclude metabolically healthy obese subjects, only subjects with a HOMA-IR index of ≥3 will be included

Intervention: mixed RWP 600mg/day (Provinols™, SEPPIC-France; corresponds to polyphenol content of 6 glasses of red wine) or matching placebo for a total duration of 8 weeks.

Measurements:

At baseline and after 8 weeks of intervention, insulin sensitivity and skeletal muscle perfusion (measured by contrast-enhanced ultrasonography), skin microvascular function (capillary microscopy and laser-doppler fluxmetry with iontophoresis) will be assessed. We will perform a Transcutaneous quadriceps muscle biopsy to study relevant molecules in the insulin signaling cascade. We will measure markers of low-grade inflammation and adipokines. And finally, a 24-hr ambulant blood pressure measurement will be performed.

Expected results: RWPs improve microvascular function, insulin sensitivity and systemic low-grade inflammation. We anticipate that the effects of RWPs are mediated by improving the insulin signalling.

Scientific Programme – Friday, September 28, 2012

Poster Session 2

0060

Vascular smooth muscle BK channels limit agonist-induced contractions by preventingvasomotion

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The high-conductance calcium-activated potassium channel (BK channel) is ubiquitously expressed in vascular smooth muscle. It is activated by membrane depolarisation and increase of the intracellular calcium concentration, both associated with vessel contraction. However, if and how the BK channel is involved in the signalling pathways that lead to vessel contraction, is unknown. Therefore, we tested the hypothesis that the BK channel contributes to agonist-induced vasoconstriction.

Experiments were performed on intact rat tail arteries using isometric myography and FURA-2 calcium fluorimetry.

Methoxamine (MX), an α-receptor agonist, contracted the vessels in a concentration-dependent manner. The MX-induced contraction was associated with an increase of the intracellular calcium concentration $[Ca]_i$. In the presence of iberiotoxin (IBTX), the specific BK channel inhibitor, MX-induced responses were altered in a complex fashion: At low and intermediate MX concentrations, vasomotion occurred and average contraction amplitude as well as $[Ca]_i$ were increased. At high MX concentrations, vasomotion disappeared and contraction amplitude as well as $[Ca]_i$ were not changed. This effect was endothelium-independent.

The effect of IBTX on vessel contractility during MX application was abolished by 3 µM ryanodine, an inhibitor of ryanodine-receptor mediated calcium release and 10 µM cyclopiazonic acid, an inhibitor of sarcoplasmic calcium-pump mediated calcium uptake, but not by 10 µM 2-APB, an inhibitor of IP_3-receptor mediated calcium release. Further, the effect of IBTX was abolished by 1 µM nimodipine, an inhibitor of L-type voltage-gated calcium channel mediated calcium influx and 3 µM SKF-96465, an inhibitor of receptor-operated calcium channel mediated calcium influx, but not by 10 µM Ruthenium Red, 20µM 9-phenanthrol and 3µM Pyr3, inhibitors of TRPV4, TRPM4 and TRPC3 channel mediated calcium influx.

In conclusion, this study shows that BK channels contribute to agonist-induced vasoconstriction by providing an anticontractile effect. This effect requires voltage-operated (L-type calcium channel) and receptor-operated (unknown channel) calcium influx as well as ryanodine-receptor but not IP_3-receptor mediated calcium release.

Scientific Programme – Saturday, September 29, 2012

Session “Dynamics of endothelial barrier function”

0049

Dynamic remodeling of endothelial cell junctions

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•Question

The vascular endothelial (VE)-cadherin/catenin complex comprises the backbone of endothelial cell junctions and is linked to the actin filament cytoskeleton. Cell junction dynamics, particularly the interaction between the VE-cadherin/catenin complex and the junction associated actin filaments, is critical in endothelial cell biology but remains poorly understood.

• Material & Methods

Apart from classical cell biology methods, live-cell imaging was performed by fast spinning disk microscopy on human endothelial cell cultures that express either or both VE-cadherin-mCherry and EGFP-p20, a subunit of the actin-related protein-2/3 (ARP2/3) complex. Furthermore, quantification of the observed dynamical changes was performed.

• Results

A cell density dependent VE-cadherin dynamics was observed that critically depends on ARP2/3-driven actin dynamics and is related to the formation of junction associated intermittent lammellipodia. Using specific ARP2/3 inhibitors and dominant negative mutants of both VE-cadherin and the CA domain of nuclear promoting factors,we show a close interaction between the VE-cadherin and actin polymerization during adherens junction remodelling.

• Conclusions

Our results describe and highlight the critical role of dynamical changes within cell junctions, mediated by VE-cadherin and actin dynamics in close interaction with the ARP2/3 complex, allowing both remodeling and maintenance of junction integrity.

Scientific Programme – Saturday, September 29, 2012

Session “Dynamics of endothelial barrier function”

0006

Unique cell type-specific junctional complexes in vascular endothelium of human and rat liver sinusoids

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Liver sinusoidal endothelium is strategically positioned to control access of fluids, macromolecules and cells to the liver parenchyma and to serve clearance functions upstream of the hepatocytes. While clearance of macromolecular debris from the blood is performed by liver sinusoidal endothelial cells (LSECs) using a delicate endocytic receptor system, vascular permeability and cell trafficking are controlled by transcellular pores, i.e. the fenestrae, and by intercellular junctional complexes. In contrast to blood vascular and lymphatic endothelial cells in other organs, the junctional complexes of LSECs have not yet been consistently characterized in molecular terms. In a comprehensive analysis, we have shown that LSECs express the typical proteins found in endothelial adherens junctions, i.e. VE-cadherin as well as α-, β-, p120-catenin and plakoglobin. Claudin-5 and occludin, transmembrane proteins typical of endothelial tight junction, were not expressed by rat LSECs while heterogenous immunreactivity for claudin-5 was detected in human LSECs. In contrast, junctional molecules preferentially associating with tight junctions such as JAM-A, B and C and zonula occludens proteins ZO-1 and ZO-2 were readily detected in LSECs. Remarkably, among the JAMs JAM-C was considerably over-expressed in LSECs as compared to lung microvascular endothelial cells. In conclusion, our data prove the existence of organ-specific intercellular junctions between endothelial cells of the liver sinusoids characterized by co-occurrence of endothelial adherens junctions proteins, and of ZO-1 and -2, and JAMs. The comprehensive molecular characterization of the specialized intercellular junctions between LSECs corroborates previous ultrastructural findings and provides a framework for further functional investigations of the transendothelial barrier of liver sinusoids in numerous pathological conditions ranging from hepatic inflammation to formation of liver metastasis.

Scientific Programme – Saturday, September 29, 2012

Session “Dynamics of endothelial barrier function”

0017

“Investigation of the causal contribution of selective blood-brain barrier glucose transport processes to brain edema formation and functional deterioration after experimental focal brain ischemia and traumatic brain injury”

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Traumatic brain injury (TBI) is the leading cause of death in children and young adults globally. Malignant cerebral edema plays a major role in the pathophysiology which evolves after severe TBI. Added to this is the significant morbidity and mortality from cerebral edema associated with acute stroke, hypoxic ischemic coma, neurological cancers and brain infection. Therapeutic strategies to prevent cerebral edema are limited and if brain swelling persists beyond 24 h, the risks of permanent brain damage or mortality are greatly exacerbated.

During traumatic brain injury (TBI) and stroke, both, oxygen and glucose deprivation (OGD) may be encountered. Due to a higher demand for glucose in the brain in these cases, enhanced levels of glucose transporters are expressed, facilitated glucose transporter-1 (GLUT1) and sodium-dependent glucose transporter-1 (SGLT1). Besides their function as energy supply, glucose may influence brain water homeostasis by a direct hygroscopic effect; a possiblecause for early brain edema formation. The present study investigated the expression of sodium-dependent glucose transporters (SGLT) and the effect of their deficiency on brain edema formation after experimental TBI.

Scientific Programme – Saturday, September 29, 2012

Young Investigator Session

0020

Regulation of Endothelial Nitric Oxide Synthase as a Compensatory Mechanism After Inhibition of NADPH Oxidase4

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NADPH oxidase isoform NOX4 mainly produces H_2O_2. NOX4 is the major endothelial NOX isoform and constitutively active. Regulation of NOX4 and formation of reactive oxygen species is involved in NO release. The transcription factor NRF2 is a key mediator of cellular adaptation to redox stress. Therefore, regulation of NOX4 on transcriptional level by NRF2 might be directly linked to NO release and endothelial function. Besides endothelial nitric oxide synthase (eNOS), a role of neuronal nitric oxide synthase (nNOS) in endothelial NO and H_2O_2 release has been proposed.

In this study, endothelial cells (HUVEC) were exposed to high laminar shear stress (24 h, 30 dyn/cm²). Application of shear stress stimulated NO formation and induced elongation of the cells in the direction of flow. Overexpression of Nox4 strongly increased H_2O_2 release. Furthermore, application of shear stress caused downregulation of NOX4, upregulation of eNOS and antioxidative responses via NRF2 and its target genes NQO-1 and HO-1. H_2O_2 can affect NO release by eNOS. Here we could show, that downregulation of NOX4 leads to upregulation of eNOS mRNA expression and increased cytosolic protein expression under flow conditions. Determination of NO release confirmed these results. This supports a compensatory mechanism maintaining a stable NO release after NOX4 inhibition in response to flow. Attenuation of NRF2 by shNRF2 inhibited shear stress-dependent induction of NRF2 and its target genes. In addition, shNRF2 enhanced the shear stress-dependent downregulation of NOX4. Finally, we detected a stable mRNA expression of nNOS in endothelial cells which was not affected by flow. Interestingly, downregulation of NOX4 resulted in strong induction of nNOS mRNA expression.

In conclusion, our data suggest a novel mechanism how H_2O_2 released by NOX4 might affect endothelial and neuronal nitric oxide synthase. This can play a role in maintaining a stable NO release in response to flow in endothelial cells.

Scientific Programme – Saturday, September 29, 2012

Young Investigator Session

0046

The polarity protein Scrib regulates directed endothelial cell migration by modulating integrin α5 turnover

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Cell polarity is a fundamental characteristic of many cell types and essential for their function. Several protein complexes mediate cell polarisation one of these being the Scrib/Dlg/Lgl-polarity module. We hypothesized that the polarity protein Scrib also controls directed processes in endothelial cells and is therefore involved in the angiogenic process.

Scrib siRNA blocked directed migration in chemotaxis and transwell assays, whereas random migration in the scratched wound assay was not affected. This effect was accompanied by an increased number and a disturbed orientation of cellular lamellipodia shown by Rac-1 staining. Co-Immunoprecipitation and protein identification by LTQ-Orbitrap-Mass spectrometry identified an interaction of Scrib with integrin α5. Duolink analysis, a new method detecting single-molecule protein interaction events in situ and TIRF microscopy confirmed an interaction of Scrib and integrin α5 which colocalize in the basal membrane. Western blot and FACS analysis showed that Scrib siRNA reduced protein amount and surface expression of integrin α5 as well as RGD-binding capacity. This is the consequence of reduced integrin α5 recycling and protein stability after Scrib siRNA treatment as determined by a cycloheximide time course. By re-inducing the wild type protein of Scrib using a siRNA-resistant expression plasmid we could also re-induce integrin α5 protein expression, but overexpression of a Scrib mutant missing the LRR domain failed to re-induce integrin α5 expression. Consistent with this finding re-induction of wild type Scrib rescued directed cell migration in the transwell assay whereas the LRR domain-missing mutant did not. Showing that the regulation of integrin α5 signalling by Scrib is involved in the Scrib-mediated effect on directed cell migration, directed cell migration was only inhibited on fibronectin, the ligand for integrin α5 but not collagen, the ligand for other α integrins. In flkGFP zebrafish mutants, Scrib morphants showed a delayed sprouting of the intersegmental vessels and malformations of vessels in the brain.

In conclusion, Scrib represents a new endothelial protein mediating directed migration by modulating integrin α5 turnover and is therefore required for sprouting angiogenesis in vitro and in vivo.

Scientific Programme – Saturday, September 29, 2012

Young Investigator Session

0003

Role of FOXQ1 in the transmigration of monocytes in inflammatory conditions

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Introduction: Monocyte polarization in the circulation is a result of the interplay between circulating and tissue-derived molecules. However, the molecular mechanism of the specific activation of monocytes in blood stream is still poorly understood. Recent studies showed that the levels of cytokine IL-4 are increased in the circulation during Th2-associated inflammation. Using microarray assay we found that monocytes respond to IL-4 by strong overexpression of FOXQ1 and observed its upregulation in monocytes of patients with acute atopic dermatitis. However function of FOXQ1 in monocytes was unknown. In the presented study we investigated the expression regulation of FOXQ1 in human monocyte-derived macrophages and analyzed effect of FOXQ1 on monocyte transmigration.

Methods and Results: Analysis of FOXQ1 expression in human monocyte-derived macrophages by qRT-PCR confirmed that cytokine IL-4 induces FOXQ1. We showed that TGF-β1 in combination with dexamethasone amplifies the effect of IL-4. In order to identify FOXQ1-induced genes and analyse function of FOXQ1 murine macrophage-like RAW264.7 cells were stably transfected with mFOXQ1 or empty vector. A microarray analysis revealed that FOXQ1 target genes can be involved in the monocytes motility. In fact, the macrophage-like cells showed increased migration activity during FOXQ1 overexpression. The stimulatory effect of FOXQ1 on monocyte migration correlated with its ability to suppress expression of receptor PLXNC1 known to inhibit migration of monocytes and dendritic cells. In parallel with FOXQ1, PLXNC1 was downregulated in human monocytes upon IL-4 stimulation and in monocytes of patients with acute atopic dermatitis.

Conclusions: Our data indicate that FOXQ1 is upregulated by IL-4 and TGF-β1 and stimulates monocyte migration in response to inflammatory stimuli by suppression of PLXNC1. We hypothesise that FOXQ1 supports increased monocytes extravasation through the activated endothelium during chronic inflammation.

Scientific Programme – Saturday, September 29, 2012

Young Investigator Session

0009

Combined actions of transcription factors promote lymphangiogenic specificity and lymphatic vessel growth via microRNAs

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Particular genes can have both lymphangiogenic and angiogenic properties, but with differential involvement during the *morphogenesis* and/or *maintenance* of lymphatic versus blood vessels in development and disease. However, this differential effector function remains often observational and molecularly unexplained. Here, we demonstrate that genetic knockdown of the transcription factors (TFs) Klf2, Erg or Gata2 impairs the formation of the thoracic duct in zebrafish. In fact, the three TFs act in the same genetic pathway to ensure *lymphatic vessel morphogenesis* in zebrafish. Interestingly, these TFs converge on the promotor, and synergistically regulate the endothelial expression of the endothelial-enriched miR-126, previously shown to regulate *blood vessel maintenance*. Using ChIP and miR-126 promotor reporter constructs, we compared lymphatic endothelial cells (LECs) and blood endothelial cells (BECs), and found differences in TF promotor binding profiles, response to HDAC inhibitors, epigenetic histone marking, and mechanosensitive promotor activation, which might explain LEC-specific effects of miR-126. Mechanosensitive miR-126 expression in LECs is mediated by FAK/beta1 integrin. Forced endothelial expression of miR-126 rescues the lymphangiogenic defects following combined knock-down of Klf2/Erg/Gata2. Consistent herewith, we unveil a conserved cell-autonomous role for miR-126 in *lymphatic vessel morphogenesis* in zebrafish, mice and human LECs. Furthermore, miR-126 targets Spred-1 and CRK in LECs, thereby biasing VEGFR-3/VEGF-C activity via prioritizing Y1230/1231-associated ERK signalling over Y1063/1068-associated JNK maintenance signalling, without affecting VEGFR-3 expression or phosphorylation. In disease, specific inhibition of miR-126 reduced tumor lymphangiogenesis and lymphatic metastasis in an orthotopic mouse model of pancreatic cancer. Thus, we propose a model wherein combined actions of non-LEC specific transcription factors together with a subendothelial-specific epigenetic switch determines the lymphangiogenic effects of a microRNA involved in *lymphatic vessel morphogenesis* versus blood vessel maintenance. With the right mechanosensitive stimuli, this transcriptional regulatory node increases miR-126 expression in LECs, and controls lymphatic vessel growth via changing VEGFR-3/VEGF-C signalling from maintenance (JNK) to activity (ERK).

Scientific Programme – Saturday, September 29, 2012

Young Investigator Session

0015

Circumferential wall tension elicits the nuclear export and degradation of myocardin in vascular smooth muscle cells

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Myocardin is thought to control the expression of genes stabilizing the contractile phenotype of vascular smooth muscle cells (SMC) by binding to the DNA recognition motif (CArG box) of serum response factor. Although originally established as a crucial determinant for development of the embryonic vasculature, its impact on the phenotype of adult SMCs still is poorly understood.

In this context, we observed a significant decrease in myocardin abundance in medial SMCs of mouse arteries upon in vivo exposure to increased wall stress (e.g. DOCA-salt induced hypertension, arteriogenesis), and this was accompanied both by downregulation of myocardin target gene such as calponin and an increase proliferation. Likewise, in SMCs of isolated mouse arteries myocardin abundance strongly decreased in response to a supraphysiological increase in transmural pressure hence wall stress. Moreover, in human arterial cultured SMCs exposed to cyclic stretch for 24 hours - thus mimicking one critical component of wall stress- myocardin abundance was significantly reduced both on the mRNA- and protein- level. Interestingly, this in vitro approach revealed a stretch-induced and HDAC-protein mediated transport of myocardin from the nucleus to the cytoplasm where it was subsequently degraded by the proteasome. This stretch-induced export of myocardin from the nucleus was stimulated by the activation of ERK1/2 through MEK1/2 and resulted in a serine phosphorylation of myocardin in the nucleus.

Collectively, these findings suggest that increased wall stress triggers the export of myocardin from the nucleus and its proteasomal degradation in the cytoplasm of arterial SMCs through an as yet unknown mechanism. This process may determine the switch from the contractile towards the synthetic phenotype and thus control the onset of arterial remodelling processes associated with an increase in wall stress.

Young Investigator Session

0018

THE PRESENCE OF CONNEXIN37 IN ENDOTHELIAL CELLS DEPENDS CRUCIALLY ON CX40 IN MICE IN VIVO

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Introduction

Gap junctions are formed by connexins (Cx) and provide conduits for signalling along the vascular wall. Of the different Cx subtypes expressed in vascular cells, Cx40 is crucial to support the conduction of endothelium-dependent dilations. However, in addition to Cx40, Cx37 is expressed in endothelial cells (EC). In contrast to Cx40, lack of Cx37 does not alter signal conduction. Therefore we studied dependencies of Cx37 expression and function on Cx40 using Cx40-deficient mice.

Methods

Cx expression was detected by immunostaining and qRT-PCR, blood pressure was assessed using a telemetric system, and coupling of EC was determined by measuring the capacitance in isolated EC cell clusters using patch-clamp.

Results

In arterioles, Cx37 expression was not detected by immunostaining in Cx40-deficient mice whereas in the aorta Cx37 was still detected, however, at reduced levels. This downregulation of Cx37 was independent of the hypertension in global Cx40-deficient mice because it was likewise observed in normotensive animals deficient for Cx40 specifically in EC but not in hypertensive animals being deficient for Cx40 in renin-producing cells. Moreover, mice carrying a non-conducting Cx40 exhibited preserved Cx37 expression despite hypertension. Functional assessment of coupling in EC clusters harvested from the carotid artery revealed strong coupling in Cx40 expressing EC, but near complete decoupling in EC clusters from global Cx40-deficient mice. The levels of mRNA for Cx37 studied by qRT-PCR were not reduced in Cx40-deficient mice in the microcirculation or the aorta.

Conclusion

Cx40 seems to be essential for the implementation of Cx37 into endothelial cell gap junctions. This Cx40 function acts beyond the transcription level either during assembly or consolidation of Cx37 in gap junctions. The crucial role of Cx40 may lead to complete absence of EC coupling in animals lacking this connexin providing a model for the lack of this signalling pathway.

Scientific Programme – Saturday, September 29, 2012

Session “microRNAs and cardiovascular homeostasis”

0011

The AMP-activated protein kinase regulates endothelial cell angiotensin-converting enzyme expression via p53 and microRNA-143/145

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Introduction: High ACE-levels are associated with cardiovascular disease development, but little is known about its expressional regulation. ACE is a target of the miR-143/145-cluster in murine vascular smooth muscle cells and can also be affected by AMPK activation in monocytes. Since shear stress activates AMPK in endothelial cells, we determined whether flow alters ACE expression and the involvement of AMPK and miR-143/145.

Methods & Results: Shear stress (cone plate viscometer) decreased ACE expression in cultured human endothelial cells, an effect prevented by downregulating (siRNA) the AMPKα2 subunit, but not AMPKα1. Moreover, AMPKα2-/- (not AMPKα1-/-) mice expressed significantly higher ACE levels in different vascular beds than wild-type littermates and were more sensitive to the hypotensive effect of the ACE-inhibitor Ramipril. AMPKα2-/- mice also demonstrated an impaired bradykinin-induced hindlimb-vasodilatation. The suppressive effect of the miR-143/145-cluster on human endothelial ACE was confirmed by overexpression of pre-miR-143/145 and AMPKα2 downregulation decreased miR-143/145 levels. Since shear stress increased mature and pre-miR-143/145 levels, but not pri-miR-143/145 levels, we focused on the post-transcriptional regulation of miR-143/145 by AMPK via p53. Indeed, p53 phosphorylation (Ser15) increased in response to shear stress, and was sensitive to treatment with AMPKα2 siRNA. p53 siRNA on the other hand increased ACE expression and prevented its shear stress-induced downregulation.

Conclusions: AMPKα2 suppresses endothelial ACE expression via p53 activation and upregulation of miR-143/145. Since AMPK and p53 dysregulation is associated with several diseases (e.g. diabetes, cancer), their effect on miR-143/145 and ACE might underlie disease-associated cardiovascular disorders.

Scientific Programme – Saturday, September 29, 2012

Session “microRNAs and cardiovascular homeostasis”

0044

Cardioprotective Potential of LNA-mediated miR-92a Suppression

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Introduction: In addition to ischemic cell damage, cardiac cells are affected by reperfusion injury after arterial reopening due to rapid change in pH value, release of ROS, microcirculatory disturbances, increased inflammation und apoptosis. A pleiotropic approach may be delivered by miRNA (miR) modulation. miRs are small non-coding RNAs that control posttranscriptionally gene expression via mRNA degradation or translational repression. In order to determine the use of miR-92a inhibition to treat ischemic diseases, we performed a pre-clinical study in pigs.

Methods: Pigs underwent percutaneus ischemia (60min)/reperfusion (72h). Locked nucleic acid-modified anti-sense miR-92a (LNA-92a; 5 mg/kg heart weight) was applied at the end of LAD occlusion either regionally (antegrade (AntimiR-A) or retrograde (AntimiR-R)) or systemically (AntimiR-S) and compared to control LNA (AntimiR-C). Additionally, one group received a 5-fold higher LNA-92a concentration by antegrade delivery (AntimiR-H). Infarct size, left ventricular enddiastolic pressure (LVEDP), regional myocardial function, inflammation and apoptosis were analyzed.

Results: LNA-92a application significantly reduced miR-92a expression in the infarct zone in all three groups studied (LNA-92a A: 27±6%; R: 28±5%; S: 43±10% compared to PBS 100±15%). Regional LNA-92a administration reduced the infarct size (% AAR) from 56±4% (PBS) and 53+2% (C) to 37±4% in the antegrade and 37±5% in the retrograde LNA-92a groups. LVEDP increase was reduced in regionally LNA-92a treated pigs (A: 9.3±0.2 mmHg, R: 9.5±0.2 mmHg, High dose: 8.6±0.5 mmHg compared to PBS: 13±0.8 mmHg), i.v. injection (S) was not sufficient. Analysis of the regional myocardial function revealed similar results. MPO activity assays showed significant reduction in the LNA-92a-treated pigs (A: 51±9%, R: 48±11% inhibition). Apoptosis assessment revealed a significant reduction of cell death in the ischemic areacompared to controls. Effect of LNA treatment on apoptosis rate was also observed in cell culture performing hypoxia assays..

Conclusion: Local administration of LNA-92a leads to decreased miR-92a expression in the heart and increased expression of the miR-92a target Integrin α5. Additionally, it yields to reduced infarct size, improved global and regional myocardial function, reduced inflammation and cell death via endothelial cell protection.

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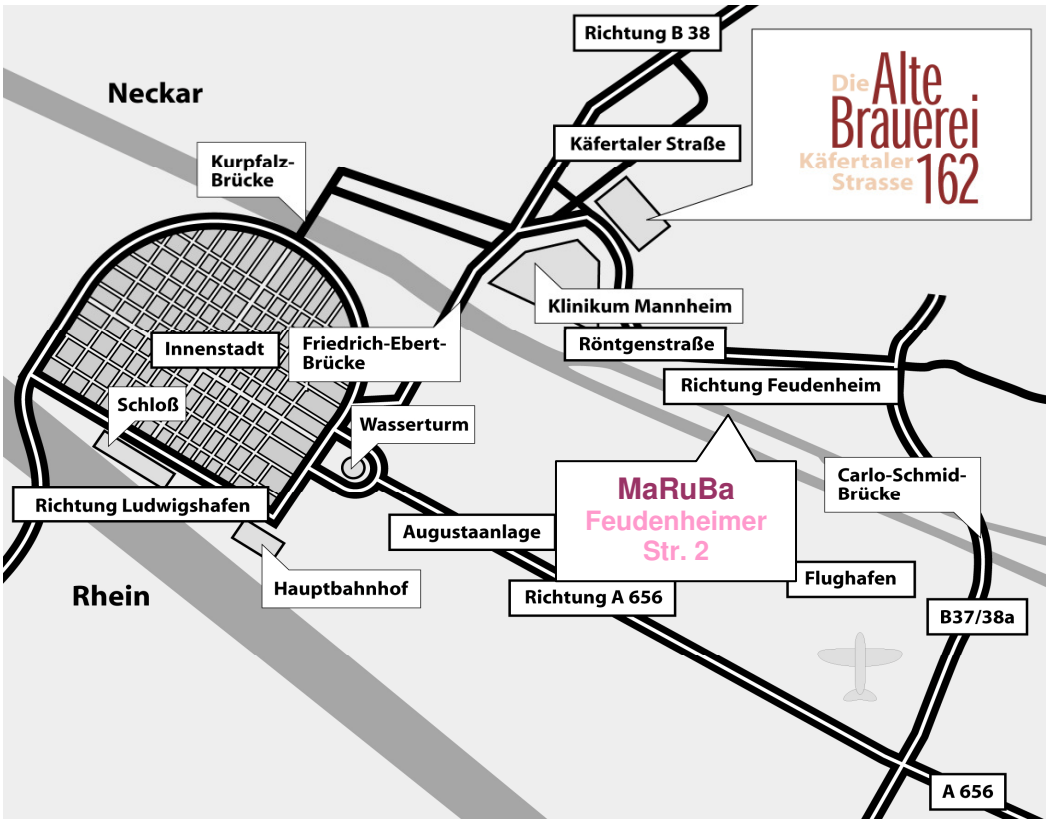

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The core function of the MyoVIEW II is to acquire diameter measurements from pressurized vessels. Flow measurements using the FlowMeter 161FM are now integrated into this single software package and allow the user to perform constant flow experiments and parameters such as vascular resistance and shear stress.

Scan the QR bar code with your smartphone to find out more about Danish Myo Technology A/S

WIRE MYOGRAPH SYSTEMS

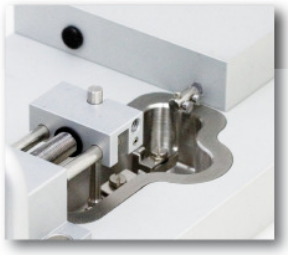
Wire Myograph Systems are used to measure force in small rings of muscle tissue with diameters as small as 40 microns. This instrument range covers systems from a single channel system with an optical window for combining electrophysiology and imaging, to 4-channel high-throughput systems for pharmacological testing of componds.

AUTOMATIC BUFFER FILLER SYSTEM - 625FS

An add-on filler system that provides an automatic, reliable and consistent way to replace buffer in the chambers of your 4-channel Myograph System.

MUSCLE STRIP MYOGRAPH SYSTEM

The 820MS represents a state-of-the-art 4-channel myograph system for muscle strips of up to 25 mm in length. Originally developed to give the skeletal muscle physiologist a sophisticated, high through-put muscle myograph, the rectangular design of the chamber gives this system the flexibility to mount larger, longer muscle strips of a variety of organs.



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Procoralan®

Ivabradin

Reduziert Gesamtmortalität*

Idealer Kombinationspartner zum Betablocker*

Procoralan® Fachinformation. Procoralan® 5 mg Filmtabletten. Procoralan® 7,5 mg Filmtabletten. Wirkstoff: Ivabradin. **Zusammensetzung:** *Procoralan® 5 mg:* 1 Filmtablette enthält 5 mg Ivabradin (entsprechend 5,390 mg Ivabradin als Hydrochlorid). *Procoralan® 7,5 mg:* 1 Filmtablette enthält 7,5 mg Ivabradin (entsprechend 8,085 mg Ivabradin als Hydrochlorid). Sonstige Bestandteile: Lactose-Monohydrat, Magnesiumstearat (E 470 B), Maisstärke, Maltodextrin, hochdisperses Siliciumdioxid (E 551), Hypromellose (E 464), Titandioxid (E 171), Macrogol 6000, Glycerol (E 422), Eisen(III)-hydroxid-oxid x H_2O (E 172), Eisen(III)-oxid (E 172). **Anwendungsgebiete:** Behandlung der chronischen Herzinsuffizienz symptomatische Behandlung der chronischen stabilen Angina pectoris bei Erwachsenen mit Koronarerkrankung und normalem Sinusrhythmus. Ivabradin ist indiziert bei Erwachsenen mit einer Unverträglichkeit für Betablocker oder bei denen Betablocker kontraindiziert sind; oder in Kombination mit Betablockern bei Patienten, die mit einer optimalen Betablockierung unzureichend eingestellt sind und deren Herzfrequenz > 60 Schläge pro Minute (bpm) ist. Behandlung der chronischen Herzinsuffizienz: Ivabradin ist indiziert bei chronischer Herzinsuffizienz der NYHA Klasse II bis IV mit systolischer Dysfunktion, bei Patienten im Sinusrhythmus mit einer Herzfrequenz ≥ 75 Schläge pro Minute (bpm), in Kombination mit Standardtherapie einschließlich Betablocker oder wenn Betablocker kontraindiziert sind oder eine Unverträglichkeit vorliegt. **Gegenanzeigen:** Überempfindlichkeit gegen den Wirkstoff oder einen der sonstigen Bestandteile, Herzfrequenz im Ruhezustand unter 60 Schlägen pro Minute vor der Behandlung, kardiogener Schock, akuter Myokardinfarkt, schwere Hypotonie (< 90/50 mmHg), schwere Leberinsuffizienz, Sick-Sinus-Syndrom, SA-Block, instabile oder akute Herzinsuffizienz, Herzschrittmacher-Abhängigkeit (Herzfrequenz wird ausschließlich durch den Schrittmacher erzeugt), instabile Angina pectoris, AV-Block 3. Grades, Anwendung von starken Cytochrom P450 3A4-Hemmern wie Antimykotika vom Azol-Typ (Ketoconazol, Itraconazol), Makrolid-Antibiotika (Clarithromycin, Erythromycin per os, Josamycin, Telithromycin), HIV Proteasehemmern (Nelfinavir, Ritonavir) und Nefazodon, Schwangerschaft, Stillzeit. **Nebenwirkungen:** *Erkrankungen der Blut- und der Lymphgefäße:* gelegentlich: Eosinophilie; *Stoffwechsel- und Ernährungsstörungen:* gelegentlich: Hyperurikämie; *Erkrankungen des Nervensystems:* häufig: Kopfschmerz, im Allg. während des ersten Behandlungsmonats, Schwindel, möglicherweise im Zusammenhang mit Bradykardie; gelegentlich: Synkope, möglicherweise im Zusammenhang mit Bradykardie; *Augenerkrankungen:* sehr häufig: Sehstörungen visuelle Symptome (Phosphene); häufig: verschwommenes Sehen; *Erkrankungen des Ohres und des Labyrinths:* gelegentlich: Vertigo; *Herzerkrankungen:* häufig: Bradykardie (bei 3,3% der Patienten in den ersten 2–3 Monaten, bei 0,5% der Patienten schwere Bradykardie ≤ 40 Herzschläge pro Minute); AV-Block 1. Grades (Verlängerung des PQ-Intervalls im EKG); ventrikuläre Extrasystolen; gelegentlich: Palpitationen, supraventrikuläre Extrasystolen; sehr selten: Vorhofflimmern, AV-Block 2. Grades, AV-Block 3. Grades, Sick-Sinus-Syndrom; *Gefäßerkrankungen:* häufig: unkontrollierter Blutdruck; gelegentlich: Hypotonie, möglicherweise im Zusammenhang mit Bradykardie; *Erkrankungen der Atemwege, des Brustraums und des Mediastinums:* gelegentlich: Dyspnoe; *Erkrankungen der Gastrointestinaltrakts:* gelegentlich: Übelkeit, Verstopfung, Diarrhoe; *Erkrankungen der Haut und der Unterhautzellgewebes:* gelegentlich: Angioödem, Ausschlag; selten: Erythem, Pruritus, Urtikaria; *Skelettmuskulatur-, Bindegewebs- und Knochenerkrankungen:* gelegentlich: Muskelkrämpfe; *Allgemeine Erkrankungen und Beschwerden am Verabreichungsort:* gelegentlich: Asthenie, möglicherweise im Zusammenhang mit Bradykardie, Erschöpfung, möglicherweise im Zusammenhang mit Bradykardie; selten: Unwohlsein, möglicherweise im Zusammenhang mit Bradykardie; *Untersuchungen:* gelegentlich: erhöhte Kreatininwerte im Blut. **Warnhinweise:** Enthält Lactose-Monohydrat. Patienten mit den seltenen hereditären Krankheiten Galactose-Intoleranz, Lapp-Lactase-Mangel oder Glucose-Galactose-Malabsorption sollten dieses Arzneimittel nicht einnehmen. Weitere Hinweise siehe Fachinformation. Verschreibungspflichtig. Pharmazeutischer Unternehmer: Les Laboratoires Servier; 50, rue Carnot, 92284 Suresnes cedex, Frankreich. Örtl. Vertreter: Servier Deutschland GmbH, Elisenhofervorstadt 53, D-80687 München, Telefon: +49 (0)89/57095 01. Stand: Februar 2012

* Swedberg et al., Lancet (2010); 376: 875–885 – Fachinformation, Stand Februar 2012

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5 mg | 7,5 mg 1-0-1