Book of Abstracts

Thursday, October 23th

SESSION 1: PGE_2 in PATHOPHYSIOLOGY
SESSION 2: PROSTAGLANDINS and CARDIOVASCULAR SYSTEM
SESSION 3: NOVEL LIPID MEDIATORS and ADVANCES in LIPID MEDIATOR ANALYTICS

Friday, October 24th

YOUNG INVESTIGATOR SESSION
SESSION 4: LIPOXYGENASES
SESSION 5: ADIPOSE TISSUE and BIOACTIVE LIPIDS

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The 5th European Workshop on Lipid Mediators has been organized under the auspices of

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And

Istanbul University, Faculty of Pharmacy, Department of Pharmacology

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Program

Thursday, October 23th, 2014

8:00-9:00 *Registration, Coffee*

9:05-9:15 Opening address by organizers

Opening lecture

9:15-10:00 Prostaglandin E_2 - EP3 signaling triggers acute inflammation by mast cell activation
Yukihiko Sugimoto (Kumamoto University, Japan)

SESSION 1. PGE_2 IN PATHOPHYSIOLOGY *Chairs: Per-Johan Jakobsson (Karolinska Institutet, Stockholm, Sweden) and Jane Mitchell (Imperial College, London, UK)*

10:00-10:30 Prostaglandin E_2 And T-Cell Mediated Inflammation
Chengcan Yao (The University of Edinburgh, The Queen's Medical Research Institute, MRC Centre for Inflammation Research, UK)

10:30-11:00 Neuroinflammation: role of PGE_2 in neuron-glial interactions
Helen Wise (Chinese University of Hong Kong, China)

11:00-11:30 *Coffee Break - Poster session and Exhibition visit*

11:30-12:00 PGE_2 in airway diseases
Maria G Belvisi (Imperial College London, UK)

*12:00-12:20 Involvement of microsomal prostaglandin E synthase-1-derived prostaglandin E_2 in chemical-induced carcinogenesis
Shuntaro Hara (Showa University, Tokyo, Japan)

*12:20-12:40 Role of prostaglandin EP4 receptor in adipose tissue
Tomoaki Inazumi (Kumamoto University, CREST, JST, Japan)

12:40-14:10 *Lunch*

SESSION 2. PROSTAGLANDINS AND CARDIOVASCULAR SYSTEM Chairs: Xavier Norel (INSERM U1148, Paris, France) and Jane Mitchell (Imperial College, London, UK)

14:10-14:40 Platelets and vascular cells, prostacyclin and thromboxane receptors
Therese Kinsella (University College, Dublin, Ireland)

14:40 -15:10 The role of platelets in cardiovascular disease and cancer
Paola Patrignani (University Chieti, Italy)

15:10 -15:40 Aspirin and lipid mediators in the circulatory system
Karsten Schrör (Heinrich-Heine University, Düsseldorf, Germany)

*15:40-16:00 1-Methylnicotinamide, a major metabolite of nicotinamide exerts anti-atherosclerotic action in ApoE/LDLR-/- mice; a possible involvement of COX-2/PGI2 pathway
Stefan Chlopicki (Jagiellonian University, Krakow, Poland)

*16:00-16:20 Genetic and lipidomic approach to uncover cardioprotective mechanisms by omega-3 polyunsaturated fatty acids
Makoto Arita (RIKEN Center for IMS, Yokohama, Kanagawa, Japan)

16:20-16:50 *Coffee Break : Poster session and Exhibition visit*

SESSION 3. NOVEL LIPID MEDIATORS AND ADVANCES IN LIPID MEDIATOR ANALYTICS *Chairs: Gerard Bannenberg (Global Organization for EPA and DHA, Madrid, Spain) and Öner Süzer (*Cerrahpasa Faculty of Medicine, Istanbul, Turkey)

16:50-17:20 Lipid signaling and lipidomics in innate immunity and inflammation
Jesús Balsinde (CSIC, Valladolid, Spain)

17:20 -17:50 High-resolution lipidomics
Rob Vreeken (Discovery & Exploratory BA, Discovery Sciences, Janssen Pharmaceutica NV, Beerse, Belgium & Division of Analytical BioSciences/LACDR, Leiden University, Leiden, the Netherlands)

*17:50-18:10 Oxidized phospholipids from lipoxygenase significantly enhance tissue factor dependent thrombin generation in vitro
David Slatter (Cardiff university School of Medicine, UK)

*18:10-18:30 The novel insight on the role of sphingosine-1-phosphate in neurodegeneration/ neuroprotection
Joanna B Strosznajder (Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland)

Closing Remarks for Day 1

Reception at the Foyer of the Heritage Building of the Faculty of Pharmacy

Friday, October 24th, 2014

8:25-8:30 Morning address by organizers

Plenary lecture

8:30-9:15 Roles of leukotrienes and other lipid mediators in asthma and allied airway disease
Sven-Erik Dahlén (Karolinska Institutet, Stockholm, Sweden)

YOUNG INVESTIGATOR SESSION Chairs: Gerard Bannenberg (GOED) and Gökçe Topal (Faculty of Pharmacy, Istanbul University, Turkey)

9:15-11:15 : (10 +2 min Q&A)

* YS1 Human vascular wall remodelling by MMP is inversely regulated by pge_2 and h_2s in aorta aneurysm and saphenous vein varicosity. **Ingrid Gomez**, University of Sheffield , UK – Inserm U1148, Paris, France.

* YS2 Resolvin D1 (RvD1) promotes the resolution of inflammation in obese insulin-sensitive tissues. **Bibiana Rius**, Hospital Clínic-Idibaps-Esther Koplowitz Center, Barcelona, Spain.

* YS3 Novel insights into the regulation of protein kinase B (Akt) by functional lipidomics. **Andreas Koeberle**, Friedrich-Schiller-University, Jena, Germany.

* YS4 Lipoxin A_4 Reduces Intimal Hyperplasia Following Carotid Ligation Through ALX/FPR2 Signaling In Vascular Smooth Muscle Cells. **Marcelo H Petri,** Karolinska University Hospital, Stockholm, Sweden.

* YS5 EP4 and IP receptor down regulation in patients with group 3 pulmonary hypertension is associated with reduced bronchial dilation. **Chabha Benyahia**, Inserm U1148, LVTS, Paris, France.

* YS6 Dietary effects on circulating oxylipins and endocannabinoids. **Sandra Gouveia - Figueira** University of California, Davis, CA, USA.

* YS7 A cell-based model for studying the 5-lipoxygenase-activating protein in leukotriene biosynthesis. **Ulrike Garscha**, Friedrich-Schiller-University Jena, Germany.

* YS8 Mono Mac 6 lipopolysaccharide-stimulated cytokine secretion: a new tool to quantify and delineate the immunomodulatory effects of prostaglandin E_2. **James N Fullerton**, University College London, London, UK.

* YS9 Identification of novel EPA-derived anti-inflammatory metabolites using a targeted lipidomics approach. **Yosuke Isobe**, Laboratory for Metabolomics, Riken Center for Integrative Medical Sciences, Yokohama-City, Japan.

* YS10 N-stearoylethanolamine restores plasma cholesterol content in the lipoprotein fractions of insulin resistant rats. **Oleksandra Onopchenko**, Palladin Institute of Biochemistry of Nasu, Kyiv, Ukraine.

11:15-11:45 *Coffee Break: Poster session and Exhibition visit*

SESSION 4. LIPOXYGENASES Chairs: Dieter Steinhilber (Universität Frankfurt, Germany) and Zeliha Yazıcı (Cerrahpasa Faculty of Medicine, Istanbul, Turkey)

11:45-12:15 5-Lipoxygenase and cardiovascular disease
Magnus Bäck (Karolinska Institute, Stockholm, Sweden)

12:15 -12:45 15-Lipoxygenase; structure and function
Hartmut Kühn (Charité, Berlin, Germany)

*12:45-13:05 15-Lipoxygenase: a novel drug target for treatment of inflammatory lung diseases
Hans-Erik Claesson (Karolinska University Hospital Solna and Karolinska Institutet, Stockholm, Sweden)

*13:05-13:25 Leukotrienes: key mediators of early vascular remodelling in obstructive sleep apnea syndrome. A translational approach
Françoise Stanke-Labesque (Université Grenoble Alpes; INSERM U1042, CHU, France)

13:25-15:25 *Lunch (followed by coffee at poster session)*

SESSION 5. ADIPOSE TISSUE AND BIOACTIVE LIPIDS *Chairs: Joan Clària (Hospital Clínic/IDIBAPS) and Sönmez Uydeş Doğan (Faculty of Pharmacy, Istanbul University, Turkey)*

15:25-15:55 Regulation of vascular tone by adipocytes
Johan van de Voorde (Ghent University, Belgium)

15:55-16:25 Nutritional modification of lipotoxic endoplasmic reticulum stress and inflammation by a bioactive lipokine prevents atherosclerosis
Ebru Erbay (Bilkent University, Ankara, Turkey)

16:25 -16:55 Role of omega-3 fatty acids in obesity, metabolic syndrome and cardiovascular diseases
María Jesús Moreno Aliaga (University of Navarra, Pamplona, Spain)

*16:55-17:15 Potential role of lipid mediators in the induction of fatty acids cycling an energy expenditute in white adipocytes
Jan Kopecky (Institute of Physiology Academy of Sciences of the Czech Republic, Prague, Czech Republic)

*17:15-17:35 One week oral treatment of rats with soluble epoxide hydrolase inhibitor tppu–resulting drug levels and modulation of oxylipin pattern
Nils Helge Schebb (University of Veterinary Medicine Hannover, Germany)

*17:35-17:55 Impact of colonic mucosal lipoxin A_4 synthesis capacity on healing in rats with dextran sodium sulphate-induced colitis
Mehmet Melli (Ankara University, School of Medicine, Turkey)

17:55 Adjourn : Workshop closing address by organizers

(Coffee and beverages for last stayers)

** indicates presentation selected from submitted abstracts*

PROSTAGLANDIN E_2–EP3 SIGNALING TRIGGERS ACUTE INFLAMMATION BY MAST CELL ACTIVATION

Authors: YUKIHIKO SUGIMOTO[1,2], Soken Tsuchiya[1,2], and Shuh Narumiya[3]
Lab adress : 1) Kumamoto University, Kumamoto Japan; 2) CREST, JST, Saitama Japan; 3) Kyoto University, Kyoto Japan

Pro-inflammatory actions of prostaglandins (PGs) in the acute inflammation have been widely accepted. Particularly, PGE_2 has been reported to work as one of the pro-inflammatory mediators during the pathological processes in various peripheral tissues. The actions of PGE_2 are mediated via four subtypes of PGE receptors, EP1, EP2, EP3, and EP4. The EP1 receptor is coupled to intracellular Ca^{2+} mobilization, EP2 and EP4 are coupled to stimulation of adenylate cyclase, and EP3 is coupled mainly to inhibition of adenylate cyclase. EP2 and EP4 receptors also elicit the activation of phosphoinositide 3-kinase via the β-arrestin pathway. By using pharmacological and genetic approaches, it has been identified which EP subtype are involved in each of PGE_2 actions. For instance, EP3 mediates PGE_2-induced fever generation, and EP1 mediates PGE_2-induced thermal sensitization. However, it remains unknown which EP subtypes mediates PGE_2-induced inflammatory response, such as enhancement of vasopermeability, edema formation and leukocyte infiltration. In order to clarify these points, we employed arachidonic acid-induced and PGE_2-elicited inflammation models and examined the effect of EP gene disruption on this model. We finally found that PGE_2-EP3 signaling triggers acute inflammatory responses by mast cell activation in the skin. We would like to discuss on potential roles of EP3-mediated mast cell activation in the other inflammatory diseases.

Invited Speakers

PROSTAGLANDIN E_2 AND T-CELL MEDIATED INFLAMMATION

Author: CHENGCAN YAO
Lab address: MRC Centre for Inflammation Research, The University of Edinburgh, The Queen's Medical Research Institute, Edinburgh, United Kingdom

Prostaglandins (PG) are produced from arachidonic acid in response to various stimuli by the sequential actions of cyclooxygenases and the respective synthases. PGs play essential roles in maintaining body homeostasis and are abundant at inflammation sites. PGE_2 is the best known and most well-studied PG. It exerts its biological actions through binding to four subtypes of G protein–coupled receptors termed EP1, EP2, EP3 and EP4, among which EP2 and EP4 signaling is coupled to a rise in intracellular cyclic AMP (cAMP) concentration. Genome-wide association studies have recently revealed that the PTGER4 (encoding human EP4) gene is associated with many human inflammatory diseases such as multiple sclerosis and Crohn's disease, suggesting a shared mechanism of PGE_2-EP4 signaling in immune inflammatory diseases. For more than three decades, PGE_2 has been known as a mediator of acute inflammation as well as an immunomodulatory substance that suppresses T cell activation and IFN-gamma production by raising intracellular cAMP concentration. On contrary to this conventional thought, we have recently clarified that PGE_2 through EP2 and EP4 collaborates with interleukin (IL)-12 and IL-23 and facilitates type 1 helper T (TH1) cell differentiation and TH17 cell expansion, respectively. Moreover, PGE_2-EP4 signaling in dendritic cells is also required for production of IL-23, a cytokine that is required for TH17 cell expansion. We have also mechanistically defined that PGE_2 promotes TH1 cell differentiation by inducing IL-12 receptor beta2 chain (IL-12Rb2) and IFN-gamma receptor alpha chain (IFN-gammaR1) and therefore amplifying IL-12 and IFN-gamma signalling via activating cAMP-PKA-CREB/CRTC2 pathway. Meanwhile, cAMP-mediated suppression of T cell receptor signalling and T cell activation/proliferation was rescued by simultaneous activation of PI3-kinase by PGE_2-EP2/EP4 signalling and CD28 co-stimulation. Importantly, in vivo treatment with a selective EP4 antagonist or EP4 deficiency (either systemically or conditionally in T cells) was effective at inhibiting a variety of inflammatory diseases, e.g., multiple sclerosis, dermatitis, colitis, arthritis etc in animal models. These findings suggest a novel therapeutic strategy for autoimmune and chronic inflammation by targeting PGE_2 receptors.

Invited Speakers

NEUROINFLAMMATION: ROLE OF PGE_2 IN NEURON-GLIAL INTERACTIONS

Author: HELEN WISE
Lab adress : School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

The classical theory of neuron-glial interactions underlying pain conditions involves mediator release from glial cells in the dorsal horn of the spinal cord, leading to sensitization of neurons and the enhanced and/or persistent experience of pain. Often overlooked are the neuron-glial interactions which occur in the dorsal root ganglia (DRG) and which are undoubtedly influenced by damage to primary sensory neurons in the peripheral nervous system. Primary cultures of isolated DRG cells are the principle pharmacological research tool in studies of the cellular basis of pain, and provide an amenable system with which to study the neuron-glia interactions underlying neuroinflammation. The neuronal soma of primary sensory neurons are normally ensheathed by satellite glial cells (SGCs), thus isolating individual neurons. We have previously shown that when DRG cells are dissociated for *in vitro* studies, prostanoid receptors (EP4 and IP) which were considered exclusive to neurons *in vivo* are now expressed by SGCs as well. Thus, hypothesis testing using biochemical studies of mixed cell cultures may provide misleading conclusions of events occurring *in vivo*. This issue has become even more apparent from our studies of Toll-like receptor-4 (TLR4) signaling in DRG cells. TLR4 is expressed almost exclusively by DRG neurons *in vivo* and its activation by PAMPs such as lipopolysaccharide, or DAMPs such as factors released during tissue damage, leads to COX-2-dependent PGE_2 production. By studying isolated glial cells, we have shown that DRG neurons suppress the expression of cell surface TLR4 by glial cells, perhaps in a similar manner to their suppression of glial cell prostanoid receptor expression. Together these observations raise the following questions: (1) why do neurons down-regulate glial cell expression of receptors for mediators of inflammation? and (2) what is the particular role for PGE_2 in the crosstalk between neurons and glial cells?

Acknowledgement: This work is supported by a grant from the Research Grants Council of the Hong Kong SAR (GRF476710) and a CUHK Direct Grant (MD12312).

Invited Speakers

PGE_2 IN AIRWAYS DISEASE

Author: MARIA G. BELVISI
Institute address: Head, Respiratory Pharmacology Group, Pharmacology and Toxicology Section, NHLI Imperial College London

Prostaglandin E_2 (PGE_2) is an endogenous lipid eicosanoid synthesised by cyclooxygenase mediated metabolism of free arachidonic acid. It is produced in a variety of cells including airway smooth muscle, epithelial cells alveolar macrophages and pulmonary endothelial cells. It exerts its biological effects primarily through the activation of cell surface, G-protein coupled receptors (GPCRs), termed EP_{1-4}, encoded for by the genes *Ptger1-4*. Increased levels of PGE_2 have been reported in the lavage fluid. Selective ligands are now available for the various EP receptors which now enable dissection of the beneficial properties of PGE_2 from the deleterious. Previous work from our laboratory and others has demonstrated that the bronchodilator effects of PGE_2 occur through the activation of the EP4 receptor whereas the irritant effects of PGE_2 such as airway sensory nerve activation and cough appears to be via the activation of the EP_3 receptor. However, although some reports exist, the pro and/or anti-inflammatory effects of PGE_2 have not yet been fully elucidated. This ability to identify the beneficial effects of PGE_2 through activation of specific EP receptor subtypes will allow the development of therapeutics for the treatment of airway disease.

Invited Speakers

PROSTACYCLIN & THROMBOXANE RECEPTORS: TRANSCRIPTIONAL REGULATION & NOVEL SIGNALLING PATHS/GIPS.

Author: B. THERESE KINSELLA
Lab adress: Conway Institute for Biomolecular & Biomedical Research, University College Dublin, Ireland.

The prostanoids thromboxane (TX) A_2 and prostacyclin (or prostaglandin (PG)I_2) play key, opposing roles in the regulation of haemostasis and blood vessel tone and modulate a range of other (patho)physiologic responses, such as within the renal and pulmonary systems. More recently, (TX)A_2 and its receptor, the TXA_2 receptor or, in short, the TP have been increasingly implicated in several prevalent cancers, including prostate and breast cancers.
Both TXA_2 and prostacyclin signal through cognate G-protein coupled receptors (GPCRs). While prostacyclin largely signals through the I Prostanoid receptor or the IP, in short, in humans and primates TXA_2 actually signals through two distinct T Prostanoid receptor (TP) isoforms, TPα and TPβ, which differ exclusively in their carboxyl-terminal (C)-tail domains. Whilst the biologic significance of two TP isoforms is not fully understood, they greatly add to the complexity of TXA_2 responses in humans/primates and there is substantial evidence that they have distinct (patho) physiologic roles. In this presentation, I will outline some of our work on (i) the transcriptional regulation of the IP (PTGIR) and TP (TBXA2R) genes and (ii) secondly on our identification of novel signalling pathways regulated by the IP and by TPα/TPβ.

Invited Speakers

THE ROLE OF PLATELETS IN CARDIOVASCULAR DISEASE AND CANCER

Author: PAOLA PATRIGNANI
Lab adress: Department of Neuroscience, Imaging and Clinical Sciences (Section of Cardiovascular and Pharmacological Sciences) and Center of Excellence on Aging (CeSI), "G. d'Annunzio" University, Chieti, Italy. E-mail: ppatrignani@unich.it

Platelets are chief effector cells in hemostasis but they are also inflammatory cells. In fact, they play a central role in the crosstalk with stromal and immune cells leading to their activation which is crucial in the progression of malignant, inflammatory and metabolic diseases. Thus pharmacological inhibition of platelet responses may indirectly dampen the accomplishment of pathophysiological mechanisms, such as inflammation and oxidative stress, which are common to different diseases, such as atherothrombosis and cancer. This hypothesis is sustained by the findings of clinical trials showing that chronic administration of the antiplatelet agent low-dose aspirin is beneficial in the prevention of vascular occlusion and cancer. The finding of aspirin beneficial effect on the risk of colorectal cancer (CRC) and CRC-related death detected at long-term follow-up may suggest that the drug prevents the early development of an adenomatous lesion. Platelets play a role also in tumor dissemination and metastasis. We have recently found that platelet/tumor cell interactions are associated with the release of platelet-derived mediators, such as prostanoids and PDGF, and enhance the metastatic potential of cancer cells through the aberrant expression of cyclooxygenase (COX)-2 in tumor cells, and the acquisition of a mesenchymal/migratory phenotype. We have also identified the determinants of platelet-tumor cell crosstalk consisting in the interaction between the platelet collagen receptor GPVI and galectin-3, which is expressed in tumor cells and contains a collagen-like domain. These may represent novel targets for pharmacological interventions to affect metastasis formation. Thus, blockers of collagen binding sites, such as revacept, and galectin-3 may be innovative strategies in colon cancer chemotherapy that should be tested in experimental animals, followed by randomized clinical trials in colon cancer patients.

Invited Speakers

ASPIRIN AND LIPID MEDIATORS IN THE CARDIOVASCULAR SYSTEM

Author: KARSTEN SCHRÖR
Lab adress: Institut für Pharmakologie und Klinische Pharmakologie, Universitätsklinikum Düsseldorf, Düsseldorf

Aspirin contains two pharmacologically active groups with different pharmacokinetics and pharmacodynamics within one and the same molecule: reactive acetyl and salicylate which acts similar to though weaker than aspirin in many nucleated cells and additionally as a protonophore at concentrations of 1 mM and more. At oral doses of 100-300 mg of standard aspirin, maximum plasma levels are in the range of 10-20 µM (aspirin) and about 6-8-fold higher levels for salicylate. Thus, the main molecules of interest in the cardiovascular system are those which become acetylated by aspirin.
Best studied acetylation targets of aspirin are COX-1 and (upregulated) COX-2. These are inhibited by aspirin in low-to-medium µmolar concentrations, indicating that prostaglandins and thromboxanes are a major class of lipid mediators, affected by aspirin. The actions of prostaglandins and thromboxane within the cardiovascular system, specifically on platelets and vascular cells will probably be covered in detail by the presentations of Drs. Kinsella and Patrignani. Another class of lipid mediators made by the acetylated COX-2 in cooperation with white cell 5-lipoxygenase are lipoxins, here “aspirin-triggered lipoxin” (ATL). This product has anti-inflammatory properties and might also contribute to improved oxygen defense of endothelial cells by enhanced NO production via the acetylated eNOS. In cooperation with Dr. Rauch (Greifswald) we are working on sphingosines, another class of lipid mediators with widespread biological activities. Sphingosine-1-phosphate (S1P), the mediator of interest, is stored for large extent in the platelets and released from there upon stimulation in an thromboxane-dependent manner. Platelet-derived S1P stimulates monocyte and endothelial cell migration, upregulates thrombin receptors and COX-2 in vascular cells and has a number of other effects inside and outside the cardiovascular system: Aspirin at antiplatelet doses in vivo, markedly inhibits the release of S1P from human platelets, including thrombin-induced platelet stimulation in acute MI. A number of other lipids, including anandamide, are also targets of COX-2, and also of aspirin. However, there role in the cardiovascular system is less clear.

Invited Speakers

LIPID SIGNALING AND LIPIDOMICS IN INNATE IMMUNITY AND INFLAMMATION

Author: JESUS BALSINDE
Lab adress : Institute of Molecular Biology and Genetics, Spanish National Research Council (CSIC), University of Valladolid School of Medicine. Valladolid, Spain.

Lipid droplets (LD) are cytosolic inclusions present in most eukaryotic cells that contain a core rich in neutral lipids such as triacylglycerol (TAG) and cholesteryl esters (CE), and are surrounded by a phospholipid monolayer decorated with a variety of proteins. We have examined the pathways for LD biosynthesis in human monocytes exposed to free arachidonic acid (AA), and studied the signaling cascade and intracellular events leading to LD formation in human monocytes. Mass spectrometry analyses of neutral lipids were conducted to delineate the composition of LD in monocytes exposed to AA. Exposure of human peripheral blood monocytes to AA results in the rapid induction LD formation by these cells. This effect appears specific for AA in that it is not mimicked by other fatty acids, whether saturated or unsaturated. LD are formed by two different routes, namely (i) the direct entry of AA into triacylglycerol and (ii) activation of intracellular signaling leading to increased triacylglycerol and cholesteryl ester formation utilizing fatty acids coming from the de novo biosynthetic route. LD formation can be completely inhibited by selective inhibition of the group IVA cytosolic phospholipase A_2□ (cPLA_2□), pointing out this enzyme as a key regulator of AA-induced signaling. LD formation in AA-treated monocytes can also be blocked by the combined inhibition of the mitogen-activated protein kinase family members p38 and JNK, which correlates with inhibition of cPLA_2 activation by phosphorylation. These results suggest that concomitant activation of both p38 and JNK by AA cooperate to activate cPLA_2□, which is in turn required for LD formation possibly by facilitating biogenesis of this organelle, not by regulating neutral lipid synthesis.

Invited Speakers

HIGH RESOLUTION PROFILING OF LIPIDS AND LIPID MEDIATORS IN (CLINICAL) PHENOTYPES

Author: ROB J. VREEKEN
Lab adress : Discovery & Exploratory BA, Discovery Sciences, Janssen Pharmaceutica NV, Beerse, Belgium&Division of Analytical BioSciences/LACDR, Leiden University, Leiden, the Netherlands

The influence and involvement of lipids and lipid mediators in a multitude of clinical phenotypes and disease area's is becoming more and more apparent in recent years. They are involved in processes, like e.g. acute inflammation, athrosclerosis, cellular communication, angiogenesis and (viral) infectious diseases. Due to the fact that these compounds can contain e.g. multiple fatty acid tails, with or without several double bonds as well as several oxidation stages, the complexity of analyzing the complete lipidome still forms a challenge to the scientific community.
Next the actual involvement of all these different isomers and isobars in the biochemical processes is far from being understood and will require lots of attention in the years to come.
Therefore, in depth analysis of the individual lipids and lipid mediators using novel and improved techniques as well as in depth profiling in specific (clinical) phenotypes will be discussed in this paper.

Invited Speakers

THE GOOD, THE BAD AND THE UGLY: DIVERSE ROLES OF LEUKOTRIENES AND OTHER LIPID MEDIATORS IN ASTHMA AND ALLIED AIRWAY DISEASES

Author: SVEN-ERIK DAHLÉN
Lab adress: The Institute of Environmental Medicine and the Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden

The presentation will review recent findings in asthmatics and human tissues to illustrate how different arachidonic acid-derived lipid mediators (eicosanoids) have distinct roles in the pathobiology of asthma.The Good: Whereas prostaglandin (PG) E_2 mediates inflammation and pain in most tissues, it has a bronchoprotective and anti-inflammatory action in the lung. Aggressive attacks of asthma are thus precipitated in subjects with the syndrome of aspirin/NSAID-intolerant asthma when the protective effect of PGE_2 on EP_2 receptors on airway mast cells is lost by inhibition of its local biosynthesis catalysed by COX-1 in airways. In contrast, inhibition of COX-2 is tolerated by asthmatics.
The Bad: Prostaglandin D_2 is the major eicosanoid released by activated mast cells. It produces bronchoconstriction by activation of TP receptors in the airways and may contribute to inflammation by stimulation of chemotactic responses via DP_2 (CRTH2) receptors. Already at baseline, subjects with asthma have elevated levels of PGD_2 as indicated by increased urinary excretion of its two main urinary metabolites tetranorPGDM and 2,3-dinor-11-β-$PGF_{2\alpha}$. When an asthma attack is provoked by allergen exposure or exercise, there is a further increase in the urinary excretion of PGD_2 metabolites.
The Ugly: The cysteinyl-leukotrienes (CysLTs: LTC_4, LTD_4 and LTE_4) are the most potent bronchoconstrictors within the eicosanoids system, and interventions with their formation or action is the mode of action of established anti-leukotriene asthma medications. Data will be presented to show that the constriction in humans is caused solely by activation of $CysLT_1$ receptors. In a recent study of biomarkers in asthma, it was furthermore found that increased urinary excretion of LTE_4 was the strongest predictor of severe asthma and depressed lung function. Moreover, urinary LTE_4 levels correlated with high total IgE, exhaled NO and eosinophils in sputum or blood, suggesting that urinary LTE_4 is a sensitive biomarker of Th2-driven airway inflammation.
It is concluded that combined antagonism of PGD_2 and CysLTs has potential as new asthma therapy and that inhaled EP_2 agonists may represent a new strategy for inhibition of asthmatic airway inflammation.

5-LIPOXYGENASE AND CARDIOVASCULAR DISEASE

Author: MAGNUS BÄCK

Lab adress: Department of cardiology and Center for Molecular Medicine, Karolinska Institutet and University Hospital, Stockholm, Sweden

Despite major advances in the prevention and treatment of cardiovascular diseases, ischemic and valvular heart diseases still represent major health problems. Acute myocardial infarction remains a leading cause of morbidity and mortality worldwide, and is caused by an underlying atherosclerosis in the coronary arteries, which is characterized by inflammatory infiltration and activity. Calcification and obstruction of the aortic valve, referred to as aortic stenosis is the third most common cardiovascular pathology after ischemic heart disease and hypertension, and may also be largely driven by local chronic inflammation.

The 5-lipoxygenase enzyme is involved in the formation of several lipid mediators derived from arachidonic acid and omega-3 fatty acids. These lipid mediators, which may transduce both inflammation and its resolution, have been implicated in several cardiovascular diseases. For example, 5-lipoxygenase-derived leukotrienes are formed locally in atherosclerotic lesions and in stenotic heart valves. Leukotrienes activate specific leukotriene receptors (BLT and CysLT receptors) that may transduce pro-atherogenic signaling in the vessel wall, immune activation, and calcification of heart valves. Furthermore, dual lipoxygenation of arachidonic acid yields anti-inflammatory lipoxin A4, which may transduce the resolution of inflammation through its receptor termed ALX/FPR2. However, ALX/FPR2 may also transduce proinflammatory signaling when activated by prokaryotic peptides as well as by a number of endogenous ligands, such as amyloidogenic and antibacterial peptides. Together, 5-lipoxygenase-derived lipid mediators and their receptors induce a variety of responses in terms of pro- and anti-inflammatory signaling, as well as mediating the resolution of inflammation, with important implications for cardiovascular disease.

15-LIPOXYGENASES. STRUCTURE, FUNCTION AND EVOLUTIONARY ASPECTS

Authors: HARTMUT KÜHN, Susan Adel, Dagmar Heydeck
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Lipoxygenases (LOX) are lipid-peroxidizing enzymes, which have been implicated in the pathogeneses of inflammatory, hyperproliferative, metabolic and neurodegenerative diseases. LOX are not restricted to mammals but they also occur in plants and in a number of pro- and eukaryotic organisms but their biological roles in protozoa and metazoan are only poorly understood. LOX-like sequences have been described in two (Bacteria, Eucarya) of the three domains of terrestric life but might be absent in Achaea. Unfortunately, because of limited functional data it remains unclear whether these sequences encode for functional enzymes. The human genome involves six functional lipoxygenase genes and among them ALOX5 (pro-inflammatory) and ALOX15 (anti-inflammatory) are considered antipode during the pathogenesis of inflammation. This review is aimed at summarizing our current knowledge on structure (crystal structure, solution structure, motional flexibility) and function (involvement in cell differentiation, inflammation, and atherogenesis) of human ALOX15 and of its orthologs in other mammalian species. Moreover we would like to address evolutionary aspects of ALOX15 research, which includes the alteration in reaction specificity of this enzyme during late primate evolution and its expression in the extinct human subspecies *H. neadertalensis* and *H. denisovan*.

Invited Speakers

REGULATION OF VASCULAR TONE BY ADIPOCYTES

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Adipocytes are no longer considered just as cells related to storage of energy and thermoregulation. Now we know that they release a huge number of paracrine and endocrine biologically active molecules, the so called adipo(cyto)kines. There is growing evidence that these adipo(cyto)kines may link obesity to cardiovascular diseases. The excessive adipocyte hypertrophy in obesity induces hypoxia in adipose tissue. This leads to adiposopathy, the process that converts “healthy” adipose tissue to “sick” adipose tissue. This is accompanied by a change in profile of adipo(cyto)kines released, with less production of the “healthy” adipo(cyto)kines such as adiponectin and omentin and more release of the “unhealthy” adipo(cyto)kines, ultimately leading to the development of cardiovascular diseases. Also perivascular adipose tissue (PVAT) that surrounds almost all blood vessels in the organism secretes adipo(cyto)kines that, because of its proximity, can easily influence vascular smooth muscle cells. The role of PVAT on vascular function can be both protective and deleterious. Normal healthy PVAT, as present in lean subjects, helps to keep the blood vessels dilated as its presence diminishes the effect of vasocontractile agents. Obesity is associated with an increased mass in PVAT. Excessive adipocyte hypertrophy may result in “adiposopathy” in which PVAT attracts macrophages and becomes a more inflammatory phenotype. This leads to a change in profile of the released adipo(cyto)kines, resulting in a decreased vasorelaxing effect of PVAT, which may be linked to obesity-induced hypertension. It also results in smooth muscle cell migration and proliferation and the development of atherosclerotic lesions. The increased knowledge of the functions of adipo(cyto)kines brings up new targets that can be useful to develop novel therapeutic and preventive strategies for obesity related cardiovascular diseases.

Invited Speakers

NUTRITIONAL MODIFICATION OF LIPOTOXIC ENDOPLASMIC RETICULUM STRESS AND INFLAMMATION BY A BIOACTIVE LIPOKINE PREVENTS ATHEROSCLEROSIS

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The prevalence of obesity has increased epidemically, with little hope for effective treatments on the horizon. Rising along with obesity is an aggregation of co-morbidities including insulin resistance, diabetes, hepatosteatosis, dyslipidemia, hypertension and atherosclerosis, collectively known as the metabolic syndrome. Signaling pathways that lie at the interface of metabolism, inflammation and stress are altered by excessive exposure to lipids and other nutrients and profoundly influence these chronic diseases. Despite intense research into the crosstalk between metabolic, inflammatory and stress pathways, there remains a gap in our knowledge regarding the nutritional modification of such crosstalk. What are the nutritional cues that can beneficially modify this crosstalk? Bioactive lipokines offer tools for nutritional modification of the crosstalk between metabolism, inflammation and stress pathways that are major contributors to the formation of atherosclerosis.
Recent studies showed that lipokines and other bioactive lipid species have profound effects on metabolism. For example, palmitoleate (PAO), a lipokine, can be generated from the adipose tissue through *de novo* lipogenesis and exert endocrine effects in distant tissues such as the liver and skeletal muscle. PAO can suppress inflammation in the adipose tissue and improve insulin signaling in skeletal muscle and liver. The impact of PAO on atherosclerotic lesions is also highly relevant to cardiovascular diseases; the links between nutrients, stress pathways and inflammation are central to atherogenesis and its complications, but very little is known about nutritional modification of this crosstalk. Our studies show that PAO can inhibit lipotoxic endoplasmic reticulum stress, a common pathophysiological mechanism that drives obesity, insulin resistance, hepatosteatosis, diabetes and atherosclerosis, and prevent atherogenesis in mouse models. Our findings have important implications for unraveling the pathogenesis of metabolic and inflammatory diseases and can facilitate generation of novel therapeutic approaches utilizing bioactive lipokines in these diseases.

(Supported by the Marie Curie Reintegration Grant/FP7)

Invited Speakers

FATTY ACIDS, ADIPOSE TISSUE AND THE METABOLIC SYNDROME (ROLE OF OMEGA-3 FATTY ACIDS IN OBESITY, METABOLIC SYNDROME AND CARDIOVASCULAR DISEASES)

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Obesity leads to several chronic morbidities including type 2 diabetes, dyslipidaemia, atherosclerosis and hypertension, which are major components of the metabolic syndrome. Low-grade inflammation has been identified as a key factor in the development of metabolic syndrome features affecting obese subjects. White adipose tissue (WAT) metabolism and WAT-derived factors (fatty acids and adipokines) play an important role in the development of these metabolic disturbances. In obesity, the expanding WAT makes a substantial contribution to the development of obesity-linked inflammation via increased secretion of pro-inflammatory cytokines, chemokines and adipokines and the reduction of anti-inflammatory adipokines. The state of chronic low-grade inflammation is powerfully amplified through the infiltration of macrophages into WAT. This dysregulated situation initiated primarily within WAT can affect the function of other metabolic organs, including liver, muscle and pancreas. The n-3 long-chain polyunsaturated fatty acids (n-3 PUFAs) eicosapentaenoic (EPA) and docosahexaenoic (DHA) have been reported to improve some obesity-associated metabolic syndrome characteristics by acting on key metabolic organs including WAT. In adipocytes, n-3 PUFAs have been shown to regulate adipogenesis, lipogenesis, lipolysis and fatty acid oxidation. In rodents, n-3 PUFAs also modulate mitochondrial biogenesis, AMPK activation, glucose transporters expression and insulin signaling pathway. In addition, n-3 PUFAs are able to prevent and/or ameliorate inflammation associated to obesity and metabolic syndrome by inhibiting the formation of n-6 PUFAs-derived pro-inflammatory eicosanoids, and by contributing to the formation of endogenous anti-inflammatory and pro-resolving lipid mediators such as resolvins, protectins and maresins. Interestingly, both n-3 PUFAs and their pro-resolving lipid mediators are capable of regulating the secretion of bioactive adipokines, such as leptin, adiponectin and chemerin involved in the control of body weight, nutrient metabolism, insulin sensitivity and immune function. It is essential that these observations be further explored and to determine whether these fatty acids can effectively prevent and reverse the progression of metabolic syndrome features in obese humans.

ORAL SESSIONS
(selected oral from submitted abstracts)

Thursday, October 23th, 2014

Selected Oral 1

INVOLVEMENT OF MICROSOMAL PROSTAGLANDIN E SYNTHASE-1 - DERIVED PROSTAGLANDIN E2 IN CHEMICAL-INDUCED CARCINOGENESIS

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Microsomal prostaglandin (PG) E synthase-1 (mPGES-1) is a terminal enzyme that acts downstream of cyclooxygenase (COX)-2 in the PGE2-biosynthetic pathway. Here, we examined the role of mPGES-1 and mPGES-1-derived PGE2 in chemical-induced carcinogenesis using a mouse model. Genetic deletion of mPGES-1 significantly reduced both the total number and size of colorectal polyps at 18-20 weeks after azoxymethane (AOM) administration with reduced nuclear translocation of beta-catenin and altered expression profiles of chemokines/cytokines. PGE2 level in tumor tissues derived from mPGES-1-deficent mice was lower than that from wild-type (WT) mice, but the levels of PGs other than PGE2, such as prostacyclin (PGI2) and PGD2, were increased by mPGES-1-deletion. At an early stage (6 weeks), mPGES-1 deficiency significantly reduced the number of aberrant crypt foci (ACF), while its transgenic overexpression increased the number. Contrary to the mPGES-1 deletion, PGI2 synthase (PGIS) deletion increased the number of ACF at 6 weeks and that of large-sized colorectal polyps at 20 weeks after AOM administration. These results indicate that ablation of mPGES-1 not only suppresses carcinogenic PGE2 production but also reciprocally up-regulates anti-carcinogenic PGI2 production, and thereby suppresses chemical-induced carcinogenesis. Furthermore, we found the involvement of EP1, one of PGE2 receptor subtypes, in chemical-induced carcinogenesis using a bladder cancer model. The incidence of bladder tumors in the N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN)-treated mice was significantly decreased by EP1 gene deletion. Malignant potential of bladder tumors in BBN-treated wild-type mice was greater than those in EP1-deficient mice. These results suggested that COX-2-mPGES-1-EP1 axis might play an important role in chemical-induced carcinogenesis.

Selected Oral 2

ROLE OF PROSTAGLANDIN EP4 RECEPTOR IN ADIPOSE TISSUE

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Adipose tissue is important not only for energy storage but also as an endocrine organ that regulates energy homeostasis and insulin sensitivity by secreting adipokines such as adiponectin and leptin. Excess lipid accumulation in adipose tissue results in an imbalance in the secretion of adipokines, leading to diabetes and other metabolic disorders. Hence, understanding of molecular mechanisms underlying physiological regulation of adipogenesis is an important issue both in biological and clinical aspects. Prostaglandins (PGs) are the arachidonate metabolites synthesized by the action of cycloxygenase (COX) as a rate-limiting enzyme. It has been shown that several PGs regulate adipocyte defferentiation or lipolysis in cell culture system. Indeed, we previously identified that PGE2-EP4 signaling suppresses adipocyte differentiation from 3T3-L1 preadipocytes. However it has not been examined the physiological roles of EP4 receptor in adipocyte differentiation or maturation. To elucidate the roles of endgenous PG on adipocyte differentiation, we first employed an adipocyte differentiation system from mouse embryonic fibroblasts (MEF). In wild-type (WT) MEF cells, inhibition of endogenous PG synthesis by indomethacin augmented the differentiation, whereas exogenous PGE2 reversed the effect of indomethacin. In EP4-deficient (EP4KO) cells, basal differentiation was up-regulated to the levels in indomethacin-treated WT cells, and indomethacin did not further enhance differentiation. Differentiation stimuli induced COX-2 gene and protein expression, as well as PGE2 production, in WT MEF cells. These results suggest that PGE2-EP4 signaling suppresses adipocyte differentiation in an autocrine manner. In this presentation, we would like to show the phenotypes regarding adipose tissue development and insulin response of EP4KO mice and discuss on the physiological role of EP4 signaling in the maintenance of adipose homeostasis.

Selected Oral 3

1- METHYLNICOTINAMIDE, A MAJOR METABOLITE OF NICOTINAMIDE EXERTS ANTI- ATHEROSCLEROTIC ACTION IN ApoE/LDLR-/- MICE; A POSSIBLE INVOLVEMENT OF COX-2/PGI2 PATHWAY

Authors: Lukasz Mateuszuk, Agnieszka Jasztal, Edyta Maślak, Barbara Sitek, Agnieszka Serwadczak, Agnieszka Zakrzewska, Agnieszka Kij, Maria Walczak, Renata Kostogrys,STEFAN CHLOPICKI
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Introduction: We demonstrated previously an anti-thrombotic and anti-inflammatory activity of 1-methylnicotinamide, a major nicotinic acid (NicA) and nicotinamide (NA) metabolite, acting via COX-2/PGI2 pathway. The aim of this study was to analyze anti-atherosclerotic and vasoprotective action of MNA in mouse model of atherosclerosis (C57Bl/6J ApoE/LDLr-/-) in comparison with NicA. Materials and methods: 4 month- old ApoE/LDLr-/- mice with advanced atherosclerosis were treated with MNA or NicA (100 mg/kg) for 8 weeks. Plaque area and macrophage content were examined inside brachiocephalic artery (BCA) using ORO/OSMB histochemical stainings and immunohistochemical CD68/MAC3 staining, respectively. Microscopic images were analyzed semi- automatically and automatically by Columbus software. Systemic inflammation was measured on the basis of selected acute phase proteins (haptoglobin, A2M, SAP), while lipid profile by field flow fractionation method (FFF). 6-keto-PGF1alpha and nitrite/nitrate production by aortic rings was measured by ELISA and automated HPLC-based system (ENO-20), respectively. Ex vivo release of TXB2 and TNFalpha from full blood as an index of platelets and monocyte function was also measured by ELISA. Urine and plasma concentrations of MNA and its metabolites were measured by LC/MS/MS. Results: In ApoE/LDLr-/- MNA- and NicA- treated mice plaque area and macrophage- specific area in BCA as well as systemic inflammation were reduced. Aortic rings isolated from MNA- and NicA- treated mice released substantially higher amounts of 6-keto-PGF1alpha, and this response was suppressed by COX-2 inhibitors. Higher concentration of NOx produced by aortic rings and NOHb in blood was also detected in MNA- but not in NicA- treated mice. In turn in both in MNA- and NicA- treated groups TXB2 and TNFalpha release in ex vivo assay was blunted. Concentration of nicotinamide, MNA and its metabolites were higher in MNA- and NicA-treated groups. Conclusions: MNA displays anti-atherosclerotic action in mouse models of advanced atherosclerosis, due to its vasoprotective, anti-inflammatory and antiplatelet action that may be linked to its prostacyclin- releasing activity dependent on COX-2. We also suggest that MNA may be partially responsible for the anti-atherosclerotic activity of NicA.

Selected Oral 4

GENETIC AND LIPIDOMIC APPROACH TO UNCOVER CARDIOPROTECTIVE MECHANISMS BY OMEGA-3 POLYUNSATURATED FATTY ACIDS

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Omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is widely held to be cardioprotective with epidemiological evidence of people who consumed omega-3 PUFA-rich diet having a low incidence of cardiovascular death. Increased cardiac afterload is responsible for the pathogenesis and progression of heart failure accompanied by maladaptive cardiac remodeling, namely cardiomyocyte hypertrophy, inflammatory cell infiltration and fibrosis. Cardioprotective effect of omega-3 PUFAs in the setting of chronic heart failure can be explained by the anti-inflammatory and/or anti-remodeling activities, although the direct evidence and mechanisms underlying such effects have been unclear. In this study, we showed that fat-1 transgenic mice expressing C. elegans omega-3 fatty acid desaturase, which is capable of producing omega-3 PUFAs endogenously, exhibited resistance to pressure overload-induced cardiac remodeling as well as reduced cardiac function. Bone marrow (BM) transplantation experiments revealed that fat-1 transgenic BM cells, but not fat-1 transgenic cardiac cells, contributed to the anti-remodeling effect. Lipidomic analysis revealed selective enrichment of EPA and EPA-metabolite 18-HEPE in fat-1 transgenic macrophages, and that the 18-HEPE-rich milieu in the fat-1 transgenic heart was generated by BM-derived macrophages. 18-HEPE inhibited proinflammatory activation of cardiac fibroblasts in culture, and in vivo administration of 18-HEPE reproduced the fat-1 mice phenotype including resistance to pressure overload-induced maladaptive cardiac remodeling. The use of 18-HEPE may lead to a novel therapeutic application for heart failure based on anti-inflammatory and anti-fibrotic lipid mediators.

Selected Oral 5

OXIDIZED PHOSPHOLIPIDS FROM LIPOXYGENASE SIGNIFICANTLY ENHANCE TISSUE FACTOR DEPENDENT THROMBIN GENERATION IN VITRO

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Agonist-activated platelets generate oxidized phospholipids (PL) via 12-lipoxygenase (LOX). These comprise 12-hydroxyeicosatetraenoic acids attached to either phosphatidylethanolamine (12-HETE-PE) or phosphatidylcholine (12-HETE-PC). Monocytes generate 15-HETE-containing phospholipids via 15-LOX. To characterise HETE-PL effects on coagulation, liposomes (5% PS, 65% PC, 30% PE containing 0-10% HETE-PE or HETE-PC, 4 microMolar) were made incorporating recombinant tissue factor (rTF) and used to trigger coagulation, thrombin generation (TG) being measured with a ThrombinoscopeTM.

In platelet poor plasma (PPP), maximal TG increased from 2 ± 0.5 nM.min-1 (control) to 18.3 ± 0.6/13.5 ± 2.2 nM.min-1 using 12-HETE-PE/PC liposomes, and to 20.0 ± 1.2/31.7 ± 4.4 nM.min-1 using 15-HETE-PE/PC liposomes (n=3). HETE-PL also enhanced TG in FVIII deficient plasma.

Using recombinant coagulation proteins (rTF, VII, IX, VIII, X, V, II) at physiological concentrations in the absence of inhibitors, TG was faster, with control values of 23.8 ± 4.8 nM min-1, rising to 44.1 ± 4.0/41.2 ± 0.5 nM.min-1 (12-HETE-PE/PC) and again to 48.9 ± 3.7/57.1 ± 2.1 nM.min-1 (15-HETE-PE/PC). Adding physiological concentrations of tissue factor pathway inhibitor approximately halved the maximum TG but the action of HETE-PL was unaffected.

At physiological concentrations of activated Va and Xa, TG From the prothrombinase complex rose from 4.6 nM.min-1 (PE) to 14.0/14.9 nM.min-1 (12/15-HETE PE). At 10-fold higher lipid concentrations, HETE-PL stimulation was less apparent, the control of 55.6 nM.min-1 rising only to 64.6/69.7 nM.min-1 (12/15-HETE PE).

Clinical data showed that following cardiopulmonary bypass (CPB), platelets generated reduced HETE-PL and externalised less PS, PE, and HETE-PE compared to pre-bypass samples. Likewise, liposome-induced TG in the post-bypass plasma was significantly reduced unless the liposomes contained 12-HETE-PE or 12-HETE-PC. Liposomes made with15-HETE-PE were also significantly smaller as measured using a Nanosight instrument.

In summary, HETE-PLs effectively stimulate TG, with stronger effects at lower lipid:clotting factor ratios. This may be clinically important for maintaining haemostasis in stressed situations. The procoagulant actions of HETE-PLs represent a novel function for the platelet 12-LOX pathway.

Selected Oral 6

THE NOVEL INSIGHT ON THE ROLE OF SPHINGOSINE-1-PHOSPHATE IN NEURODEGENERATION/ NEUROPROTECTION

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Sphingosine -1-phosphate (S1P) is a very potent lipid messenger involved in cell to cell communication and in intracellular signal transduction. S1P is synthesized by sphingosine kinases (SK1and SK2) and can act in an autocrine or paracrine fashion. It can bind to specific G protein operated receptors(S1P1-S1P5). S1P is involved in angiogenesis, cell viability, migration, proliferation, inflammation and carcinogenesis.
In our study we have evaluated the SKs expression and activity and the role of S1P in oxidative stress evoked by: glucose deprivation/glucose reload (GD/GR), amyloid beta (AB) peptides toxicity and 1-Methyl-4-Phenylpyridinium (MPP+) –cell model of Parkinson Disease. Our data indicated that GD/GR stress up regulates SKs expression and activity, however, endogenous S1P pool is not able to protect the neuronal HT22 cell against death. Exogenous S1P (1uM) through S1P1 and S1P3 receptors mediated PI3/Akt kinase pathway and enhanced cell survival. S1P was responsible for the up-regulation of gene expression of anti apoptotic proteins Bcl2 and Bclx and for suppression of pro apoptotic proteins. We have shown that in AD experimental model performed on PC12 cells transfected with human gene for amyloid precursor protein (APP), wild type (APP wt) or bearing a double Swedish mutation (APPsw) amyloid beta peptides significantly decreased SK1/2 expression and activity. SKs inhibition and lower S1P synthesis is implicated in oxidative stress and up regulation of apoptotic proteins and cell death. Exogenous S1P (1uM) protected APPwt and control PC12 cells against death by receptor independent mechanism. However, in APPsw S1P decreased cells viability. In SH-SY5Y cell's model of PD evoked by MPP+ agonist of S1P receptor(s), S1P itself and its analog P-FTY720 exerted neuroprotective effects. Summarizing, our data indicate that S1P and its analog P-FTY720 depending on the type of stress exert cytoprotective effects in receptor independent or receptor dependent manner. S1P and agonists of its receptors ought to be considered in therapy of some neurodegenerative diseases.

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YOUNG SESSION

Friday, October 24th, 2014

Young Session – Friday, October 24th, 2014

Oral YS1

HUMAN VASCULAR WALL REMODELLING BY MMP IS INVERSELY REGULATED BY PGE2 AND H2S IN AORTA ANEURYSM AND SAPHENOUS VEIN VARICOSITY

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BACKGROUND: Hydrogen sulphide (H2S) is a mediator with demonstrated benefit properties on the cardiovascular system. In rodents, H2S is known to regulate the metabolism of prostaglandin (PG)E2. In this study, we investigated the relationship between H2S and PGE2 and their role on metalloproteinase activity in human healthy (aorta (HA) or saphenous veins (SV)) versus pathological vasculature (aortic aneurysm abdominal (AAA) and varicose saphenous veins (VSV)). AAA and VSV are characterized by respective thinning and thickening of vascular wall.
METHODS AND RESULTS:
HA specimens were collected from post-mortem human aorta. AAA samples were obtained from patients undergoing surgery. Human SV and VSV were collected from patients undergoing heart bypass surgery or vein stripping operations, respectively. The varicose veins were obtained from patients at the clinical stage C2 of the disease.
The enzyme responsible for H2S synthesis (Cystathionine-gamma;-lyase (CSE)) expression was significantly higher in varicose veins than in SV. The endogenous H2S levels generated by CSE were also consistently increased in varicosity when compared to SV. On the contrary, the endogenous H2S levels were lower in AAA than in healthy aorta. The beta-cyano-L-alanine (BCA), a CSE inhibitor, completely inhibited the H2S release in all vessels stimulated with L-cysteine (substrate of CSE). Measurements by ELISA showed lower contents of PGE2 in varicose veins than in healthy veins. In contrast, a higher level of PGE2 was found in AAA than in healthy aorta. Exogenous H2S caused a significant decrease in PGE2 concentration in SV and VSV. In contrast, H2S was ineffective on TXA2 and PGJ2 levels. Furthermore, the content of active MMP-1, which was reduced in varicose veins versus SV, was also down-regulated by H2S. TIMP1 was enhanced, while TIMP2 remained constant. This regulation of MMP/TIMP ratio is responsible for thickness of vascular wall in VSV and thinness in AAA (Yokoyama et al., 2012).
CONCLUSION: Our data show that depending of the endogenous levels of H2S, PGE2 synthesis is decreased in VSV and increased in AAA. This regulation can lead to a subsequent regulation of active MMP/TIMP ratio. Those results support the idea that H2S regulation of PGE2 synthesis is involved in pathological vessel wall remodelling.

Yokoyama U, Ishiwata R, Jin MH, Kato Y, Suzuki O, Jin H, et al. (2012). Inhibition of EP4 signaling attenuates aortic aneurysm formation. PloS one 7(5): e36724.

Oral YS2

RESOLVIN D1 (RvD1) PROMOTES THE RESOLUTION OF INFLAMMATION IN OBESE INSULIN-SENSITIVE TISSUES

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Obesity is a highly prevalent health problem that has acquired in recent years the characteristics of a global pandemic disease. Obesity is associated with metabolic comorbidities, including insulin resistance and non-alcoholic steatohepatitis (NASH). There is strong evidence that the development of obesity-related problems is driven by the presence of a chronic low-grade inflammation in obese individuals. In particular, a persistent un-resolved inflammation in adipose tissue triggers the release into the circulation of a number of inflammatory signals (i.e. adipokines). In addition, NASH, in which the accumulation of fat in the liver is accompanied by un-resolved inflammation, is one of the most prevalent complications of obesity. Therefore, inflammation in insulin-sensitive tissues, specifically in adipose tissue and liver is a hallmark of obesity. Here, we investigated the ability of resolvin D1 (RvD1), a potent pro-resolving molecule, to promote the timely resolution of inflammation in obese insulin-sensitive tissues. Dietary normalization induced weight loss and a reduction of adipose tissue and liver weight, decreased serum leptin and resistin levels, and reduced hepatic steatosis and insulin resistance (i.e. JNK phosphorylation) in obese mice. On top of these changes, mice receiving RvD1 during the dietary intervention exhibited higher adiponectin levels, lower serum insulin and glucose concentrations and a significant reduction of hepatic macrophage infiltrate accompanied by a switch from M1 to M2-like anti-inflammatory phenotype. Moreover administration of RvD1 was associated with a specific liver microRNA signature related to cytokines, monocyte/macrophages and communication between innate and immune responses (i.e. miR-219-5p, miR-199a-5p, miR-149-5p and miR-122). Experiments carried out in precision-cut liver slices (PCLS), which allow the study of intact liver cells, revealed that RvD1 was able to down-regulate hypoxia-induced expression of COX-2, IL-1#beta#, IL-6 and CCR7, effects that were not observed when macrophages were depleted by clodronate treatment. Interestingly, obese transgenic fat-1 mice enriched in omega-3 precursors of pro-resolving mediators showed attenuated sings of hepatic cell stress and injury compared to those in control animals. Conclusion: RvD1 acts as a facilitator of the resolution process in obese insulin-sensitive tissues. Strategies aimed to endogenously increase the formation of this pro-resolving mediator or its use as a therapeutic compound may have potential benefits in metabolic diseases.

Young Session – Friday, October 24th, 2014

Oral YS3

NOVEL INSIGHTS INTO THE REGULATION OF PROTEIN KINASE B (Akt) BY FUNCTIONAL LIPIDOMICS

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Dysregulation of membrane lipids has been associated with disease (e.g., cancer), though the underlying mechanisms are poorly defined. Functional lipidomics combines comprehensive lipid profiling with mechanistic and cell-biological studies to unravel the signalling mechanism of bioactive lipid mediators. We successfully applied this approach to study the role of lipids in cancer-relevant processes as here exemplified for the cell cycle-dependent regulation of protein kinase B (Akt) - a major kinase promoting cell proliferation and survival. Since Akt is recruited to membranes for activation, we speculated that its activity might be regulated by an oscillating membrane lipid component. By monitoring the lipid profile of synchronized mouse fibroblasts during the cell cycle by UPLC-MS/MS, we found an inverse correlation between the proportion of arachidonic acid-containing phosphatidylcholine (20:4-PC) and Akt activity (1). Increasing the ratio of 20:4-PC inhibited Akt membrane binding, Akt (S473) phosphorylation, Akt downstream signalling, S-phase transition and cell proliferation. Thus, our study implies for the first time a potential role of 20:4-PC for regulating Akt activity during the cell cycle. The novel mechanism by which 20:4-PC inhibits Akt activation is surprising due to the direct interference and specificity. Akt activity is usually regulated through the level of phosphatidylinositol-3,4,5-trisphosphate (PIP3) - which anchors Akt to membranes – and not through the affinity of Akt for binding PIP3, and biological effects of 20:4-PC are ascribed in most studies to the release of arachidonic acid and biosynthesis of eicosanoids instead of to the phospholipid itself.

1. Koeberle, A. et al. (2013) PNAS 110: 2546.

Oral YS4

LIPOXIN A4 REDUCES INTIMAL HYPERPLASIA FOLLOWING CAROTID LIGATION THROUGH ALX/FPR2 SIGNALING IN VASCULAR SMOOTH MUSCLE CELLS

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Background: Lipoxin (LX) A4 is produced during vascular injury, and may activate vascular smooth muscle cells (SMC) in the vascular wall by means of its receptor FPR2/ALX. The goal of this study was to explore the role of LX signaling through ALX/FPR2 in vascular injury in vivo, and on SMCs in vitro.
Methods and results. Mice lacking the ALX/FPR2 homologue (Fpr2-/-) and wild type (Fpr2+/+) mice were used for carotid ligation and isolation of aortic SMCs. In addition, mice were treated with either the LXA4 analogue 15-epi-LXA4 (10 microg/kg) or PBS by means of osmotic pump implantation. In Fpr2+/+ mice, 15-epi-LXA4 reduced the neointima formation following carotid ligation; intima/media ratio 1.2± 0.1, compared with 2.7± 0.3 in the PBS-treated group ($P<0.05$). In contrast, 15-epi-LXA4 did not alter the response to carotid ligation in Fpr2-/- mice. In vitro, SMC expressed Fpr2 protein as revealed by immunofluorescence. Vascular SMC derived from FPR2-/- mice exhibited 2.2±0.3 fold higher proliferation compared with Fpr2+/+ SMC ($P<0.05$). SMC migration was assessed by scratch assay, which revealed that Fpr2-/- SMC closed 75±9 % of initial wound over a period of 24 h, whereas Fpr2+/+ SMC closed 45±6 % ($P<0.05$). Additionally, 15-epi-LXA4 (100 nM) reduced Fpr2+/+ SMC migration by 26±3 % ($P<0.05$), while Fpr2-/- cells remained unresponsive to this agonist. Finally, gene expression was assessed by real time PCR and revealed that SMC derived from Fpr2-/- mice exhibited significantly lower mRNA levels of collagen 1A, the collagen maturation enzyme lysyl oxidase and the endogenous collagenase inhibitor TIMP-1 compared with Fpr2+/+ cells, whereas the collagenase MMP-13 was increased in Fpr2-/- cells.
Conclusions: LX signaling induced effective inhibition of intimal hyperplasia following vascular injury. The effects of 15-epi-LXA4 in this model was transduced by the receptor ALX/FPR2 expressed on SMC in the vascular wall, and mediated through direct effects on SMC proliferation and migration as well as on extracellular matrix deposition and maturation. Taken together, these findings indicate a potential therapeutic implication of 15-epi-LXA4 and other FPR2/ALX agonists in vascular diseases.

Oral YS5

EP4 AND IP RECEPTOR DOWN REGULATION IN PATIENTS WITH GROUP 3 PULMONARY HYPERTENSION IS ASSOCIATED WITH REDUCED BRONCHIAL DILATION

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Introduction: Pulmonary hypertension (PH)-group 3 is secondary to chronic lung diseases, including chronic obstructive pulmonary disease, lung fibrosis or emphysema (Montani et al. 2013). In these patients, prostacyclin (PGI2) mimetic therapy led to marked improvements in right heart hemodynamics (Zangiabadi et al. 2014). The beneficial clinical effects of PGI2 mimetics are illustrated by decreased dyspnea and improved capacity to perform physical efforts. These effects could be modulated by changes in bronchomotricity. Previously, it has been shown that PGE2/PGI2 mimetics induce potent relaxations of human bronchi from control patients via EP4 or IP receptors (Benyahia et al. 2012; Norel et al. 1999). However, the effects of PGE2/PGI2 mimetics on the human bronchial reactivity in specimens from PH patients are unknown.

The aim of this study was to investigate the effects of several selective prostanoid receptor agonists on the bronchomotricity of preparations from patients with PH-group 3 compared to control patients.

Methods: The muscular tone of bronchial preparations derived from control and PH-group 3 patients was measured with an organ bath system. The different preparations were stimulated by PGE2/PGI2 mimetics and pharmacological studies were performed with different antagonists. The expression of EP4, EP2, IP and DP receptors were analyzed by Western blot and real-time PCR in both types of preparations.

Results: The relaxations induced by different EP4 agonists, PGE2, L-902688, ONO-AE1-329 and the (IP, EP4) agonist iloprost, were significantly decreased in human bronchi from PH patients as compared to controls. However, the relaxations produced by treprostinil, beraprost, MRE-269 (IP agonists) and ONO-AE1-259 (EP2 agonist) were not different between these tissues. The EP4 and IP receptor densities and mRNA expression were significantly lower (50-70%) in human bronchi from PH patients. CAY10441 (IP antagonist, 1μM) blocked completely and partially the relaxation induced by treprostinil and iloprost, respectively.

Conclusions: This study shows that in human bronchial preparations from patients with PH-group 3 the reduction of the relaxation induced by EP4 agonists (PGE2, ONO-AE1-329, L-902688 and iloprost) was associated with decreased EP4 and IP receptor expression. From these results we hypothesize that; treprostinil may be the treatment of choice to treat PH patients with respiratory diseases.

Oral YS6

DIETARY EFFECTS ON CIRCULATING OXYLIPINS AND ENDOCANNABINOIDS

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The complex network of dietary lipids and studying the effect of diets on the lipidome is a bridge to understand the biological responses of nutritional interventions, unique to each individual.
Endocannabinoids (eCB) and oxylipins are bioactive lipid-related signaling compounds that represent a subset of the lipidome.
eCB include N-acylethanolamines and glycerol derivatives such as AEA and 2-AG, while oxylipins include classical eicosanoids derived via the cyclooxygenase and lipoxygenase pathways, as well as non-classical fatty acid epoxides and diols produced by cytochrome P450. Increasing evidence suggest an interplay between the oxylipin and endocannabinoid pathways.
To investigate the diet impact on the lipidome, a single subject who changed diet from vegan to vegetarian was followed over a long period of time. On three non-consecutive days plasma samples were collected at four different time points (fasting state, 0.5h, 1h and 2h after a well-defined meal) to assess the postprandial response (n=12 for each diet). Metabolite profiling and quantification was done by a sensitive and validated SPE-UPLC-ESI-MS/MS method comprising 15 eCB and 38 oxylipins.
A total of twelve eCB were detected with levels ranging from 0.1 to 211 nM. POEA was found in the highest levels followed by 2-LG, 2-AG and SEA. Thirty-five oxylipins were detected with levels ranging from 0.01 to 76 nM.
Two-tailed and unpaired Student's t-test ($p<0.05$) was performed to detect diet-dependent differences at different time points. TXB2 was found to have a significantly decreased baseline level in vegetarian samples, and seven compounds (NAGly, 9-HODE, 13-oxo-ODE, 9,10-EpOME, 12,13-EpOME, 20-HETE and 11,12-DHET) showed significantly lower levels in vegetarian samples at different time points during the postprandial response.
Differences between time points in the postprandial response within the respective diet was assessed by two-way ANOVA ($p<0.05$). Significantly different levels during the postprandial response were shown by six compounds (POEA, 9,10-DiHOME, 12,13-DiHOME, 13-oxo-ODE, 13-HODE, and 9-HODE) in vegan samples and by three compounds (POEA, 13-HODE and 9-HODE) in vegetarian samples.
These changes are currently further studied to determine if we can capture the inflammatory state by measuring lipid metabolites during the post-prandial response.

Oral YS7

A CELL-BASED MODEL FOR STUDYING THE 5-LIPOXYGENASE-ACTIVATING PROTEIN IN LEUKOTRIENE BIOSYNTHESIS

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Translocation of 5-lipoxygenase (5-LO) to the perinuclear region and interaction with the 5-LO-activating protein (FLAP), which facilitates arachidonic acid transfer to 5-LO, is assumed to be a key step in leukotriene biosynthesis.
Here, we present an experimental cell-based model for studying the functional role of FLAP in 5-LO product synthesis, and its suitability for characterization of FLAP antagonists. 5-LO was stably expressed in HEK293 cells with and without FLAP. Upon Ca2+-ionophore (A23187) stimulation, both cell lines metabolized exogenous arachidonic acid (AA) to 5-hydro(pero)xyeicosatetraenoic acid (5-H(p)ETE) and the non-enzymatic hydrolysis products of leukotriene A4 (LTA4).
Unexpectedly, the 5-LO/FLAP expressing HEK cells could not convert endogenously released arachidonic acid (AA). HEK cells liberated AA in response to A23187, and HEK cells stably expressing platelet-type 12-lipoxygenase (p12-LO) converted endogenously released AA to 12-hydro(pero)xyeicosatetraenoic (12-HETE).
Interestingly, co-expression of 5-LO and FLAP increases the product formation in intact cells by enhancing the conversion of 5-HpETE to LTA4, and thus facilitating the second step of 5-LO catalysis. MK886 was able to inhibit the FLAP mediated increase in 5-LO product formation, whereas in cells expressing only 5-LO, MK886 could not impair the 5-LO activity.
A23187 stimulation caused 5-LO accumulation at the nuclear membrane only when FLAP was co-expressed, and MK886 was able to prevent the 5-LO translocation and co-localization with FLAP.
This cell model enables to study the role of FLAP as a 5-LO interacting protein in intact cells, and for characterization of putative FLAP inhibitors.

Oral YS8

Mono Mac 6 LIPOPOLYSACCHARIDE-STIMULATED CYTOKINE SECRETION: A NEW TOOL TO QUANTIFY AND DELINEATE THE IMMUNOMODULATORY EFFECTS OF PROSTAGLANDIN E2

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Prostaglandin E2 (PGE2) exerts multiple negative functional effects on the innate immune system with recent evidence indicating a mechanistic role in cirrhotic liver disease and critically illness-induced immunosuppression. Mass spectrometry, the current gold standard for measuring eicosanoids, is time-consuming and expensive. With increasing interest in PGE2's pathophysiological potential, novel means of determining both its concentration in biological fluids and functional consequences are required.

Mono Mac 6 (MM6) cells, a human cell line exhibiting morphological and cytochemical characteristics of mature blood monocytes, were variably co-incubated with phorbol 12-myristate 13-acetate (PMA, 10ng/ml), macrophage-colony stimulating factor (M-CSF, 20ng/ml) or 1alpha, 25 dihydroxycholecalciferol (calcitriol, 10ng/ml) for 48 or 72hrs. Co-incubation with calcitriol, but not with alternate differentiating reagents, resulted in a time-dependant increase in CD14 expression and corresponding increase in both sensitivity to (left-shift of dose-response curve), and TNFalpha secretion secondary to, lipopolysaccharide exposure (LPS, Salmonella abortus equi S-form, range 1-100ng/ml). TNFalpha secretion was maximal 6 hours post-stimulation and was suppressed in a dose-dependent manner by pre-incubation with PGE2, a significant reduction being observed at concentrations as low as 100pg/ml (IC50 500pg/ml, complete suppression 5ng/ml). Addition of the selective E-prostanoid (EP) 4 agonist CAY10598, but not the EP2 agonist butaprost (1nM-10microM), mimicked this effect, whilst the PGE2-mediated suppression of TNFalpha was reversed by L161,982 (EP4 antagonist) but not by either AH6809 (EP1-3, DP1 antagonist) or PF-04418948 (EP2 antagonist), indicating a predominantly EP4-mediated effect.

Healthy-volunteer plasma led to an independent reduction in TNFalpha secretion in a concentration dependent-manner however the PGE2-TNFalpha dose-response relationship was preserved. This effect persisted after protein denaturation implicating the involvement of alternate lipid mediators. EDTA, a calcium-chelating anti-coagulant, but neither lithium heparin nor sodium citrate also independently reduced cytokine secretion (TNFalpha/IL-6/IL-1beta). When compared to whole blood LPS-stimulated TNFalpha secretion, an assay accepted as clinically relevant and predictive, MM6 displayed greater inter and intra-subject reproducibility in determining the quantity and immunomodulatory effects of PGE2 in plasma.

MM6 LPS-stimulated TNFalpha secretion in conjunction with EP4-receptor antagonists offers a rapid, reliable, reproducible and inexpensive means of quantifying and interpreting the functional relevance of PGE2 at physiological concentrations in biological samples.

Oral YS9

IDENTIFICATION OF NOVEL EPA-DERIVED ANTI-INFLAMMATORY METABOLITES USING A TARGETED LIPIDOMICS APPROACH

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Omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) are well appreciated to have beneficial effects in many inflammatory disorders. During the course of acute inflammation, EPA-derived anti-inflammatory mediators including E-series resolvins (RvEs) are produced. Using the liquid chromatography tandem mass spectrometry (LC-MS/MS)-based lipidomics approach, we identified 17,18-diHEPE (RvE3) as a new bioactive member of the RvEs that carry potent anti-inflammatory actions. In addition to that, we recently uncovered a novel EPA metabolic pathway via omega-3 epoxygenation, and found a novel bioactive metabolite 12-hydroxy-17,18-epoxyeicosatetraenoic acid (12-OH-17,18-EpETE). Intravenous administration of 12-OH-17,18-EpETE dose dependently blocked acute PMN infiltration in zymosan induced peritonitis. Also, 12-OH-17,18-EpETE at low nanomolar concentrations inhibited leukotriene B4-induced neutrophil chemotaxis and polarization in vitro. The complete structures of two natural isomers were assigned as 12S-OH-17R,18S-EpETE and 12S-OH-17S,18R-EpETE, using chemically synthesized stereoisomers. These natural isomers displayed potent anti-inflammatory action, whereas the unnatural stereoisomers were essentially devoid of activity. These results demonstrate that 17,18-EpETE derived from dietary EPA is converted to a potent bioactive metabolite 12-OH-17,18-EpETE, which may generate an endogenous anti-inflammatory metabolic pathway.

Reference:
Isobe Y, et al. Identification and structure determination of a novel anti-inflammatory mediator resolvin E3: 17,18-dihydroxy-eicosapentaenoic acid. J. Biol. Chem. 287, 10525-34 (2012)
Kubota T, et al. Eicosapentaenoic acid is converted via omega-3 epoxygenation to anti-inflammatory metabolite 12-hydroxy-17,18-epoxyeicosatetraenoic acid. FASEB J. 28, 586-93 (2014)

Oral YS10

N-STEAROYLETHANOLAMINE RESTORES PLASMA CHOLESTEROL CONTENT IN THE LIPOPROTEIN FRACTIONS OF INSULIN RESISTANT RATS

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Weight gain and obesity are the main problems of modern society that closely relate to insulin resistance (IR) and diabetes type 2 developments. Enhanced pool of free fatty acids induced by extensive fat intake, promotes the dyslipidemia and glucose intolerance. Progression of which causes the disturbances in plasma lipoprotein profile and as a result cardiovascular complications. Earlier we showed anti-inflammatory and cytoprotective properties of bioactive minor compound N-stearoylethanolamine (NSE) under pathologies connected to lipid imbalance. Accordingly, in our study we explore the effect of NSE on plasma cholesterol content in lipoprotein fractions at a rat model of IR.
Experimental IR was induced in rats by prolonged high fat diet (58% fats) for 6 month, whereas control rats received a regular chow (4% fats). Rats with glucose intolerance and high plasma insulin level were divided in “IR” and “IR+NSE” group, meanwhile control rats – “Control” and “NSE”. At the end of the experiment «NSE», «IR+NSE» rats were given per os water suspension of NSE (50 mg/kg daily) for 2 weeks. The plasma lipoprotein (LDL, HDL, VLDL+IDL) level was analyzed by using commercial kits and performed as (mmol cholesterol /L) in each lipoprotein fraction.
The lipoprotein composition assay of plasma from obese rats with IR showed a reduction in HDL cholesterol (0.06±0.007; $P<0.05$) content compared to control animals (0.08±0.007). The plasma cholesterol level of LDL (0.14±0.001; $P<0.05$) and VLDL+IDL (1.06±0.105; $P<0.05$) was significantly increased in “IR” group compared to controls (0.09±0.014; 0.79±0.004). These findings suggest the development of IR that can drive enhanced LDL and its precursor VLDL secretion by decreasing lipoprotein lipase activity, enhancing FA and TG flux. The administration of NSE considerably increased HDL (0,10±0,007; $P<0.05$) and decreased LDL (0,11±0,004; $P<0.01$), VLDL+IDL (0,79±0,105) cholesterol level over “IR” group. We suggest that NSE realizes its biological effect by affecting the hepatic lipogenesis. It is known that modulation of SEBP1-c through PPARgamma; activation may regulate the lipogenesis under IR dyslipidemia. Moreover, in earlier studies has been reported the possible activation of PPARgamma; by NSE.
Therefore, NSE restores altered cholesterol level in plasma lipoproteins that may play an important role in IR complications improvement.

ORAL SESSIONS
(selected oral from submitted abstracts)

Friday, October 24th, 2014

Selected Oral 7

15-LIPOXYGENASE: A NOVEL DRUG TARGET FOR TREATMENT OF INFLAMMATORY LUNG DISEASES

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Human airway epithelial cells, eosinophils and subsets of mast cells and dendritic cells constitutively express high amounts of 15-LO-1. Several reports have demonstrated a significantly increased expression and activity of 15-LO-1 in the lung in asthmatics in comparison to healthy subjects. In particular eosinophils but also mast cells and airway epithelial cells can produce eoxins which are cysteinyl-containing metabolites formed through the 15-LO-1 pathway. Patients with severe asthma or aspirin-intolerant asthma have an increased 15-LO-1 activity in eosinophils. In particular 15-HETE levels in eosinophils showed significant correlations with lung function and exhaled NO but also eoxin C4 levels in eosinophil incubations, in the presence of aspirin, correlated with lung function. Furthermore, inhibition of 15-LO-1 activity, during maturation of monocytes to dendritic cells, leads to impaired formation of dendrites. Taken together, evidence for a pathophysiological role of 15-LO-1 in certain inflammatory lung diseases will be discussed. In addition, the chemical structure of some novel specific 15-lipoxygenase inhibitors will be shown.

Selected Oral 8

LEUKOTRIENES: KEY MEDIATORS OF EARLY VASCULAR REMODELLING IN OBSTRUCTIVE SLEEP APNEA SYNDROME. A TRANSLATIONAL APPROACH.

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Leukotrienes are biologically active lipid mediators of inflammation involved in atherogenesis. Obstructive sleep apnea (OSA) syndrome, a multifactorial disease characterized by recurrent episodes of nocturnal intermittent hypoxia, is associated with early atherosclerosis. Compelling evidence derived from clinical and experimental studies suggested that leukotrienes could be molecular links between OSA and atherosclerosis (1).
In OSA patients, the production of leukotrienes is increased in relation to OSA severity (2,3) and obesity (3). Moreover, the leukotriene transcriptional pathway is associated with early vascular remodelling (4).
In ApoE-/- mice exposed for 8 weeks to chronic intermittent hypoxia (CIH), the aortic root lesion sizes and the aortic mRNA levels of 5-lipoxygenase, FLAP, CysLT1 and BLT1 are significantly increased compared to mice exposed to normoxia. Moreover mRNA levels of 5-lipoxygenase, FLAP, CysLT1 and BLT1 are strongly correlated to atherosclerotic lesion sizes. Leukotriene pathway modulation by oral DHA-supplementation prevented CIH-induced atherosclerosis acceleration, an effect associated with a decrease of arachidonic acid incorporation in various tissues and organs (5).
These data suggest that the leukotriene pathway could be an interesting target for the prevention of CHI-induced atherosclerosis.

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Selected Oral 9

POTENTIAL ROLE OF LIPID MEDIATORS IN THE INDUCTION OF FATTY ACIDS CYCLING AN ENERGY EXPENDITUTE IN WHITE ADIPOCYTES

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White adipose tissue (WAT) is essential for energy storage, and through its endocrine functions it is also involved in regulation of both glucose and energy homeostasis. Concerning total energy balance, the role of WAT metabolism itself is usually neglected, reflecting a relatively low specific metabolic rate of WAT. However, several pieces of evidence support the notion that energy expenditure in WAT, not mediated by mitochondrial uncoupling, could influence total energy balance. We focused on a possibility to counteract obesity and associated metabolic disorders by modulating metabolism of WAT. Our results in dietary obese mice demonstrated additive anti-obesity effect of a combined intervention using omega-3 fatty acids and calorie restriction, which was associated with increase in (i) mitochondrial biogenesis; (ii) activity of oxidative phosphorylation (OXPHOS) and beta-oxidation; and (iii) activity of a futile substrate cycle based on lipolysis of intracellular triacylglycerols and fatty acids re-esterification (TAG/FA cycle) in white adipocytes. These changes in WAT metabolism were linked with anti-inflammatory effect of the combined intervention and in situ formation lipid mediators (Flachs et al, Diabetologia 2011; Flachs et al, BBA 2013). To assess the role of the interactions between WAT macrophages and adipocytes in the modulation of WAT metabolism, namely the role of various lipid mediators, in vitro system was developed based on the co-culture of RAW 246.7 macrophages and primary mature adipocytes. Results suggest a role of lipid mediators formed in both cell types, namely PGD2 in M2-polarized macrophages and PGE2 in adipocytes. A new model was developed to describe the role the lipid mediators in the WAT remodeling.
Conclusions: High capacity of mitochondrial OXPHOS linked to inducible TAG/FA cycling activity is essential for metabolic flexibility of WAT, may support leanness, and represents a marker of healthy adipocyte. Treatment of obesity and associated metabolic disorders could be improved by modulating metabolism of WAT while targeting formation of specific lipid mediators in the tissue.

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Selected Oral 10

ONE WEEK ORAL TREATMENT OF RATS WITH SOLUBLE EPOXIDE HYDROLASE INHIBITOR TPPU– RESULTING DRUG LEVELS AND MODULATION OF OXYLIPIN PATTERN.

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Epoxygenated fatty acids (EpFA) from arachidonic acid (AA) and other polyunsaturated fatty acids (PUFA) are potent lipid mediators showing anti-inflammatory, analgesic and vasodilatory effects. Increasing EpFA levels is an emerging strategy for the treatment of hypertension, pain and inflammatory diseases. A promising approach leading to an accumulation of endogenously formed EpFA is inhibition of the soluble epoxide hydrolase (sEH) degrading EpFA to dihydroxy fatty acids (DiFA).

In the present study, we characterized pharmacokinetic parameters and effects of the potent sEH inhibitor TPPU (1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea) on EpFA and DiFA levels. Metabolic stability of TPPU was investigated by incubation with primary rat hepatocytes. Intestinal absorption of TPPU was analyzed using confluent cell monolayers of the human intestinal carcinoma cell line Caco-2 grown on semipermeable membranes. Pharmacokinetic and downstream effects of TPPU on oxylipins were investigated following administration of TPPU with the drinking water at different doses to male Sprague Dawley rats. The concentration of TPPU as well as the blood, plasma and tissue levels of EpFA and DiFA from linoleic (18:2), linolenic (18.3), eicosapentaenoic (20:5), docosahexaenoic (22:6) acid and AA (20:4) and additionally the overall oxylipin pattern were investigated by means of LC-MS.

TPPU showed a high metabolic stability and >90% of the compound was recovered following a 24 h incubation with rat hepatocytes. Together with the high intestinal absorption rate (Papp = 5.4 10-5 cm s-1) it is concluded that oral bioavailability of TPPU is high. Consistently significant blood and tissue levels were found following administration of TPPU by drinking water to rats. For example, 5 mg/L in the drinking water led to a blood concentration of ~1 µM, well above the IC50 for sEH inhibition of 29±5 nM. After 7 days treatment, TPPU reached a steady state level in blood and tissues and no signs for an accumulation were found.

On the poster presentation, the TPPU levels are correlated with the EpFA/DiFA ratio as a marker of endogenous sEH activity and discussed with respect to possible downstream biological effects of the treatment.

Selected Oral 11

IMPACT OF COLONIC MUCOSAL LIPOXIN A4 SYNTHESIS CAPACITY ON HEALING IN RATS WITH DEXTRAN SODIUM SULPHATE-INDUCED COLITIS

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Background & Aims: Ulcerative colitis is a chronic inflammatory disease of the large bowel. There are some evidence that lipoxins, which are important mediators for the resolution of inflammation, may play a role in the pathogenesis of ulcerative colitis. We investigated the lipoxinA4 synthesis capacity of the colonic mucosa in different phases of dextran sodium sulphate-induced colitis model in rats. The effect of 5-aminosalicylic acid and misoprostol therapies on lipoxin A4 synthesis capacity and relationship of this capacity with healing were also evaluated.
Methods: Rats were randomly assigned to healthy, dextran sodium sulphate induced-colitis with no therapy or with misoprostol or 5-aminosalicylic acid therapy groups. Misoprostol, 25 µg/kg/day, and 5-aminosalicylic acid, 100 mg/kg/day, was administered via orogastric route. Rats were assessed for disease severity using disease activity index score, colon weight/length ratio and histopathologic scores, at the fifth (acute phase), fifteenth (chronic phase) and nineteenth day (healing phase) with determination of colonic mucosal lipoxinA4 synthesis capacity as well.
Results: 5-aminosalicylic acid reduced colon weight/length ratio and histopathologic score at the acute phase and reduced disease activity score in the healing phase. Misoprostol reduced histopathologic score in the acute phase, reduced disease activity score, histopathologic score and colon weight/length ratio in the healing phase with a marked increase in colonic mucosal lipoxinA4 synthesis capacity. Our findings suggests that this increase is correlated with beneficial effects of misoprostol.
Conclusions: Marked increase in the colonic mucosal lipoxin A4 synthesis capacity achieved by misoprostol administration may be rewarding in ulcerative colitis especially in the healing phase.

Keywords: ulcerative colitis, inflammatory resolution, misoprostol, 5-aminosalicylic acid
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Poster Session

Thursday, October 23th, 2014

Poster 1

LIPID MEDIATOR PROFILING OF LUNG FIBROSIS IN A MOUSE BLEOMYCINE-INDUCED MODEL USING LC-MS/MS

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Introduction: Idiopathic pulmonary fibrosis is a disease with unknown origin. It is characterized by the apparition of collagen fibers into the lung parenchyma, leading to an excessive and irreversible healing of the tissue, associated with a loss of its function. The clinical course of idiopathic pulmonary fibrosis is dramatic since it is estimated that the 5-year survival is between 20 and 40%, a higher mortality rate than many cancer types, including colon cancer, myeloma multiple and bladder cancer. Following the description by Serhan et al. of specialized mediators of inflammation resolution (SPMs) and their potential involvement in the control of fibrosis, their study in animal model of pulmonary fibrosis might help deciphering mechanisms involved in this pathology and thus opening new potential therapeutic directions.

Methods: For this purpose, mice were inoculated by intranasal challenge of bleomycin. After two weeks, mice were euthanized and lungs were collected and prepared for analysis. SPMs and other mediators issued from fatty acids were analyzed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology to quantitatively evaluate their production as described in J Chromatogr B Analyt Technol Biomed Life Sci. 2013; 932:123-33.

Results: During these experiments, we have shown an increase of PGE2, TXB2, PGD2 and 15-HETE, mediators depending on the arachidonic acid pathways. For metabolites linked to EPA, we were also able to demonstrate an increase of PGE3, 15-HEPE and 18-HEPE the precursor of resolvins of type E. Finally, we also observed DHA metabolites and showed an increase of 13-HDoHE and 17-HDoHE but also of RvD2.

Conclusions: Taken together those results suggest that fibrosis context is associated with an inflammatory status that did not seem counterbalanced by production of SPMs. Indeed, even if RvD2 is increased and the enzymes COX, 5- and 15-LOX activated, none of the other SPMs were detected. These results might thus draw the first evidence of a defect during fibrosis to produce a complete network of SPMs.

Poster 2

A NEW SENSITIVE PANEL FOR IN VIVO MONITORING OF MAST CELL ACTIVATION IN BASELINE AND POST CHALLENGE URINE SAMPLES

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Background: Mast cells are activated in asthma and mast cell activating syndromes (MAS) such as systemic mastocytosis and chronic urticaria. Serum tryptase is used clinically for diagnosis of MAS. The urinary excretion of the prostaglandin (PG) D2 metabolite 11beta-PGF2alpha is established as a sensitive marker of mast cell activation in trigger-factor evoked airway obstruction. More recently, another PGD2 metabolite, tetranor-PGDM has been identified as the major metabolite in urine. Moreover, a new selective LC/MS method for measurement of the major urinary histamine metabolite 1-methyl-4-midazoleacetic acid (t-MIAA) has been introduced (Kolmert et al Anal Bioanal Chem. 2014). Apart from PGD2 and cysteinyl leuotrienes (CysLTs) little is known about the release of lipid mediators following challenge. We therefore performed a first study to compare baseline urinary excretion of metabolites of PGD2 and histamine with measurement of serum (S) tryptase. Also urinary excretion following eucapnic voulountary hypopnea (EVH) was studied.
Methods: Urine and blood collected from 9 healthy volunteers, 11 asthmatics (A), 6 subjects with chronic urticaria (CU), and 19 patients with mastocystosis (MC), all being stable at the time of collection. Analysis of 11beta-PGF2alpha; and tetranor-PGDM were made using both a routine enzyme immunoassay (EIA) and UPLC-MS/MS (Balgoma et al Anal Chem 2013), t-MIAA according to Kolmert et al, and serum-tryptase by EIA at the hospital laboratory. Additionally urine samples from asthmatics undergoing repeated EVH were analysed.
Results: The majority of patients with MC exhibited high levels of all urinary mediators as compared to healthy volunteers, between 1.5-30 fold increases. CU patients showed a small, but significant ($p=0.027$) increase in the urinary levels of 11beta-PGF2alpha. Serum-tryptase was only increased in some patients diagnosed with MC and there was no significant alteration of the measured urinary metabolites among the asthmatic patients. Increased release of several metabolites of prostaglandins as well as leukotrienes, thromboxane and isoprostanes was seen after EVH.
Conclusion: The data support that baseline urinary excretion of lipid and histamine metabolites are sensitive non-invasive markers of mast cell activation. The demonstration of release of lipid mediators may reveal new mechanisms of bronchoconstriction in asthmatics.

Poster 3

ADRENIC ACID IS A SUBSTRATE OF SOYBEAN 15-LOX AND BLOCKS LEUKOTRIENE BIOSYNTHESIS OF PRIMARY HUMAN NEUTROPHILS IN THE MICROMOLAR RANGE

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Adrenic acid (FA22:4 n-6)(AdA) has been shown to be rapidly formed after arachidonic acid supplementation in RAW 264.7 macrophage like cells. Non-arachidonic acid dependent incorporation (and release) of AdA into phospholipids has also been shown, suggesting non-redundant biological functions for AdA. While the formation of dihomo-prostaglandins from AdA is very well-known, only some reports describe the possible formation of lipoxygenase derived products of AdA. Along these lines we questioned if AdA is a possible substrate for the enzymes of the lipoxygenase family and if AdA itself or its oxygenated derivatives might possibly exert anti-inflammatory effects. We present the identification of AdA as a substrate of soybean 15-LOX, the LC-MS/MS and NMR based identification of the major product as 17-hydroxydocosatetraenoic acid and the fact that although AdA is not effectively converted by ionophore activated primary human neutrophils, it does block the formation of LTB4 and its pathway marker 5-HETE in the micromolar range without affecting cell viability. Neutrophil viability was determined both by the reduction of a tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)(MTS) into formazan being proportional to the number of viable cells, and by nucleic acid staining using propidium iodide as well as trypan blue. Interestingly, these findings are restricted to neutrophils, as no down regulation of LTB4 or 5-HETE could be observed in M1 macrophages. The inhibitory action of AdA could be related to a blockage of arachidonic acid release from phospholipids in the absence of a change in intracellular calcium flux after ionophore stimulation. A detailed investigation on the mechanisms by which AdA blocks the release of arachidonic acid and the downstream formation of LTB4 in ionophore stimulated neutrophils is ongoing in our laboratory.

Poster 4

IMMUNOSUPPRESSION IN ACUTELY DECOMPENSATED CIRRHOSIS IS MEDIATED BY PROSTAGLANDIN E2

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Liver disease is one of the leading causes of death worldwide. Patients with cirrhosis display an increased predisposition to and mortality from infection due to multimodal defects in the innate immune system; however, the causative mechanism has remained elusive. We present evidence that the cyclooxygenase (COX)-derived eicosanoid prostaglandin E2 (PGE2) drives cirrhosis-associated immunosuppression.

We observed elevated circulating concentrations (more than seven times as high as in healthy volunteers) of PGE2 in patients with acute decompensation of cirrhosis. Plasma from these and patients with end-stage liver disease (ESLD) suppressed macrophage proinflammatory cytokine secretion and bacterial killing in vitro in a PGE2-dependent manner via the prostanoid type E receptor-2 (EP2), effects not seen with plasma from patients with stable cirrhosis (Child-Pugh score grade A). Albumin, which reduces PGE2 bioavailability, was decreased in the serum of patients with acute decompensation or ESLD (<30 mg/dl) and appears to have a role in modulating PGE2-mediated immune dysfunction. In vivo administration of human albumin solution to these patients significantly improved the plasma-induced impairment of macrophage proinflammatory cytokine production in vitro. Two mouse models of liver injury (bile duct ligation and carbon tetrachloride) also exhibited elevated PGE2, reduced circulating albumin concentrations and EP2-mediated immunosuppression. Treatment with COX inhibitors or albumin restored immune competence and survival following infection with group B Streptococcus. Taken together, human albumin solution infusions may be used to reduce circulating PGE2 levels, attenuating immune suppression and reducing the risk of infection in patients with acutely decompensated cirrhosis or ESLD.

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Poster 5

THE PRESENCE OF LIPID MEDIATORS IN HUMAN SYNOVIAL FLUID OF ARTHRITIS PATIENTS

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Chronic inflammation is a prominent feature of several arthritic diseases, such as Osteoarthritis (OA) and Rheumatoid Arthritis (RA). The mechanisms involved in persistence of inflammation are incompletely understood. We hypothesized that the underlying cause of chronic inflammation in OA and RA could be failure of activating pro-resolving mechanisms, involving poly-unsaturated fatty acids and their biologically active metabolites. To investigate this, we have measured more than 50 analytes, including (pro-resolving) lipid mediators, their pathway markers and accompanying PUFA in the synovial fluid from OA and RA patients. To this end, we have set-up a novel analytical platform, characterized by a simplified work-up protocol and high-throughput capabilities, making it particularly suitable for clinical studies.
By using as little as 40 microliter of synovial fluid, we could demonstrate the presence of significant amounts of 5-HETE; 12-HETE; 15-HETE; 10S,17S-diHDHA; PGE2; 17-HDHA, a series of other hydroxylated analytes and a number of PUFA in synovial fluid of OA and RA patients. Analyses of lipid mediator concentrations revealed higher levels of 12-HETE, 17-HDHA and the pro-resolving 10S,17S-diHDHA in OA versus RA patient samples, indicating the activation of pro-resolving pathways. In addition we analyzed the cell infiltrate in the synovial fluid samples and found a strong negative correlation between numbers of infiltrating cells and the concentration of 17-HDHA, suggesting a possible biological role in cell migration blockage.
In conclusion, our analyses indicated that both inflammatory and pro-resolving pathways are activated in OA and RA synovial fluid. The clear correlation between the presence of 17-HDHA and lower cell infiltrate numbers might pinpoint towards important roles of lipid mediators in the context of cell migration and infiltrate numbers in arthritis patients.

Poster 6

THE CONTRIBUTION OF PROSTAGLANDIN SIGNALLING TO THE VIRULENCE OF THEILERIA-TRANSFORMED MACROPHAGES

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Transforming growth factor beta (TGF-beta) is a potent modulator of immune function implicated in the pathogenesis of many human diseases including cancer. The Theileria-transformed bovine macrophage cell line Ode1 displays many characteristics of cancer cells, such as heightened invasive and migratory capacities. However and importantly, the tumour-like phenotype is reversed upon drug-induced parasite death. Moreover, virulent macrophages can be attenuated by multiple in vitro passages and upon attenuation they loose both adhesion and invasiveness1. Comparing virulent to attenuated macrophages led to the identification of TGF-driven transcriptional programme regulating macrophage adhesion and invasiveness1. Altered expression profiles of the TGF-target genes indicated that PKA might also contribute transformed macrophage adhesion and invasiveness and we focussed on PKIG, PTGS2 and PTGER4. The PKA inhibitor PKIG is upregulated upon attenuation-associated loss of TGF-signalling and conversely, addition of db-cAMP to attenuated macrophages stimulated their adhesion to fibronectin. Thus, a decline in cAMP levels occurs upon loss of transformed macrophage virulence, and levels can be restored by addition of exogenous TGF-beta. Signalling via PTGS2 and PTGER4 is known to elevate cAMP levels in many cell types and adding a selective prostaglandin agonist increased cAMP levels and stimulated the adhesion of attenuated macrophages. We found that cAMP-dependent PKA contributes to virulence of transformed macrophages via phosphorylation of Ser133 of the transcription factor CREB. The exchange factor directly activated by cAMP (Epac) is also expressed and incubation of virulent macrophages with either a specific Epac inhibitor, or a prostaglandin antagonist reduced intracellular Ca2+ and decreased the expression level of calcium/calmodulin-dependent protein kinase II (CaMKII). Thus, TGF-beta2 promotes the adhesion and invasiveness of infected macrophages by regulating prostaglandin signalling to raise cAMP levels that activate PKA and Epac/CaMKII to phosphorylate CREB. ChipSeq analyses of CREB immunoprecipitated from virulent and attenuated Ode macrophages should identify the CRE-mediated transcriptional programme underpinning the virulence of Theileria-transformed macrophages.

Poster 7

SCREENING OF SPHINGOLIPIDS IN SLE – BEFORE AND AFTER TREATMENT

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Background and objectives
Systemic lupus erythematosus (SLE) is an autoimmune disease with plethora of symptoms. There is no cure for SLE and the development of drugs is hampered due to the heterogeneity of the disease. Therefore, it is important to understand and differentiate underlying biological pathways to predict prognosis and guide the choice of drugs for the individual patient.
Sphingolipids are bioactive signaling molecules involved in the regulation of cell growth, differentiation and apoptosis. Sphingosine-1-phosphate (S1P) is increased in juvenile-onset SLE, and ceramides and S1P might be involved in mediating vascular disease in SLE mice. In addition S1P agonist (Fingolimod) is investigated for treatment of SLE. Therefore, sphingolipids are important compounds to study in respect to SLE pathogenesis in humans.
Rituximab is a B-cell-targeting therapy and despite failure in two recent controlled trials, there has been a world-wide off-label use of Rituximab in the treatment of severe SLE. The mechanism of Rituximab is not fully understood and one reason for failed clinical trials is the difficulties in measuring endpoints, i.e., lack of biomarkers.
Our objective is to study sphingolipids in patients treated with Rituximab in order to investigate the mechanism of Rituximab and suggest potential biomarkers to follow treatment.
Materials and Methods
EDTA-plasma samples from 20 SLE patients were extracted by liquid-liquid extraction and analyzed by LC-MS/MS. Samples taken before and after treatment with Rituximab (n=20+20) were screened for 34 different sphingolipids. In addition 10 RA patients and 10 healthy controls were analyzed.
Results
Sphingolipids were generally found to be down-regulated in SLE patients after treatment with Rituximab. SLE patients before treatment had increased levels of sphingolipids compared to RA patients and healthy controls. Significant differences comparing before and after treatment were found for dihydroceramide C16:0 ($p=0.04$) and glycosylceramide C16:0 ($p=0.006$) on group level and for additional seven sphingolipids significance was reached comparing paired samples.
Conclusions
Our results show that the sphingolipids are affected in SLE patients when treated with Rituximab and might be involved in SLE pathogenesis and therefore important drug targets to investigate further. Sphingolipids also show potential as therapeutic biomarkers of response to treatment.

Poster 8

ACTIVATED PGD2 RECEPTORS ON MACROPHAGES ENHANCE NEUTROPHIL RECRUITMENT INTO THE LUNGS

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Inflammatory actions of Prostaglandin D2 are often opposing. Its physiological role is highly dependent on the involvement of the individual receptors (CRTH2 and DP) in the context of the investigated disease and cell type that PGD2 is acting on. In allergic diseases CRTH2 activation has been shown to further promote pro-inflammatory activity – to a large extent by activating eosinophils – while DP activation seems to be mainly related to anti-inflammatory effects.
Given the dominant role of PGD2 in pulmonary diseases, we here investigated the physiological effect of CRTH2 and DP activation in experimental acute lung injury (ALI) in mice. Pathology of this disease is mainly characterized by high neutrophilic infiltration, a process that is driven by an initial macrophage-dependent chemokine-mediated recruitment phase.
Amongst those early phase mediators, prostanoids are released in high amounts by various cell types. The action of PGD2 in the early phase of experimental ALI has been investigated in this study. Using pharmacological approaches we can show that PGD2 acting both via the DP and the CRTH2 axis influences the disease progression of ALI in several ways: (i) PGD2 increases neutrophilic influx via both CRTH2 and DP; (ii) the increased neutrophilic influx is accompanied by worsened inflammatory state and decreased lung function; and (iii) macrophage depletion completely prevents the enhanced inflammatory responses following PGD2 administration.
Therefore, PGD2 greatly alters disease activity by its ability to enhance pro-inflammatory actions of macrophages and the consequent interaction of macrophages and neutrophils.

Poster 9

EVALUATION OF RESULTS OF ASSISTED REPRODUCTIVE TECHNOLOGY IN WESTERN ALGERIA

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Introduction: The infertility is a major problem of public health; it concerns 15% of the world population. The Assisted Reproductive Technology (ART) overcomes this problem and gives the possibilities for infertile couples to realize their parental project and the previously unexpected desire of a child. In front of this situation, many ART centers located in all over the country allow these couples to benefit from these techniques.
Aim: Our study is conducted at four (04) ART centers in Oran region in order to assess their activities in this region.
Materiel and Methods : Our investigation consists on a retrospective study conducted between 2009 and 2012 on 1305 patients aged between 18 and 50 years old.
Results and discussion : The results revealed that the average age of the patients was 33.5± 2 years and the average duration of infertility was 7± 2 years. Men are increasingly infertile because the origin of the couple\'s infertility is male in 50% of cases and female in only 17% of cases. The investigation has also allowed us to assess the ART activities carried on four centers and showed that the pregnancy rate is equal to 15% in Artificial Insemination and 28.9% and 32.6% in In Vitro Fetilization and Intra Cytoplasmic Sperm Injection respectively, an average success rate of 31, 33% equivalent to that of Europe and the United States. However, a substantial portion of infertile couples feel skepticism towards these techniques because of the lack of financial resources (45%) and lack of information about these artificial techniques (35%).
Conclusion: The results obtained in our study show that the success rate in AMP in western Algeria are consistent with those obtained in developed countries.

Poster 10

CHARACTERIZATION OF NOVEL ISOFORMS OF HUMAN 5-LIPOXYGENASE

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Human 5-lipoxygenase (5-LO) is the key enzyme in leukotriene (LT) biosynthesis which play an important role in many diseases like asthma bronchiale, atherosclerosis and in many types of cancer. The 5-LO catalyzes two reaction steps, first oxygenation of arachidonic acid to 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HpETE). In a second step, 5-HpETE is converted to the instable epoxide leukotriene A4 (LTA4) that can be further metabolized by the LTA4 hydrolase to LTB4 or by the LTC4 synthase to the cysteine containing leukotrienes LTC4, D4 and E4, causing chemotaxis, vasoconstriction and tumor growth. 5-LO is expressed in many cell types of the human immune system like polymorphonuclear leukocytes, monocytes/macrophages and B-cells.
Recently, we were able to identify novel in-frame mRNA splice variants in B-cells and T-cells named delta 4 and delta p12. Co-transfection of delta 4 or delta p12 with 5-LO wild type (WT) in HEK293 cells shows an influence of the activity in contrast to transfection of WT alone. In crude cell lysates the effect was less pronounced. Moreover, we made the observation that 5-LO is able to form dimers. Thus, we hypothesize that 5-LO and its isoforms can form heterodimers, regulating its activity. By investigating the cellular localization of WT and isoforms, we could determine that the 5-LO isoforms are only present in the nuclear fraction whereas WT 5-LO can be found in both, nuclear and non-nuclear fractions.

Poster 11

THE FUNCTIONAL EFFECT OF VISFATIN IN HUMAN INTERNAL THORACIC ARTERIES

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Abstract: Visfatin, also known as pre-B-cell colony-enhancing factor (PBEF) and nicotinamide phosphoriboslytransferase (Nampt) is a novel adipocyte-derived cytokine (adipokine). The secretion and expression of visfatin were shown in perivascular adipose tissue of aorta and coronary artery in both experimental animal models and human studies. It was also shown that visfatin could directly affect vascular reactivity. Internal thoracic artery (ITA) is one of the most commonly used arterial bypass graft in myocardial revascularization. The present study was designed to investigate the vascular effect of visfatin on human ITA in vitro and the possible underlying mechanisms. We investigated the functional effects of visfatin and the role(s) of cyclooxygenase, nitric oxide (NO) synthase, endothelium-derived hyperpolarizing factor (EDHF) and endothelium in the effects of visfatin on human ITA rings.

Human ITA was obtained from patients undergoing coronary artery bypass grafting. Isometric tensions of phenylephrine-precontracted ITA rings suspended in 20 mL organ baths, in response to cumulative concentrations of visfatin, alone or in combination with the blockers of cyclooxygenase, NO synthase and potassium channels were recorded with an isometric force transducer connected to a computer-based data acquisition system. Acetylcholine (ACh)-induced endothelium-dependent relaxation or sodium nitroprusside (SNP)-induced endothelium-independent relaxation were also recorded alone and after incubation of various visfatin concentrations.

Visfatin (10-12-10-7 M) produced concentration-dependent relaxation responses in human ITA rings that were significantly higher in endothelium-intact than endothelium-denuded preparations. Cyclooxygenase inhibitor indomethacin (10-5 M) did not cause a significant decrease in relaxant responses to visfatin, while of NO synthase inhibitor L-NAME (10-4 M) caused a significant decrease. The visfatin-induced relaxation was not significantly changed by charybdotoxin plus apamin (10-7 M, both) (Blockers of high- and small- conductance Ca2+-activated potassium channels, respectively). Incubation of human ITA rings with visfatin increased ACh-induced endothelium-dependent relaxations but did not cause significant alterations in SNP-induced endothelium-independent relaxations.

We have provided pharmacological evidence about the functional relaxant effect of visfatin in human ITA preparations. The findings of the present study suggested that endothelium through release of NO played a major role in visfatin-induced relaxation responses.

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Poster 12

POST-TRANSCRIPTIONAL CONTROL OF 5-LIPOXYGENASE GENE EXPRESSION

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5-lipoxygenase (5-LO) catalyzes the first two steps in the synthesis of leukotrienes (LTs), which are potent mediators of inflammatory reactions [1]. 5-LO expression is mainly restricted to immune competent cells (monocytes, macrophages, granulocytes, neutrophils, mast cells and dendritic cells) but is also found in many epithelial tumor cells [4]. An increased level of LTs has been shown to be associated with several diseases, such as asthma and inflammatory disorders [2, 3]. Previous studies demonstrated only marginal differences on 5-LO mRNA level in untreated and 1.5% DMSO differentiated HL60 cells, whereas 5-LO protein could only be detected in differentiated HL60 cells [4]. So we want to identify regulatory components that control 5-LO protein synthesis.
Many RNA binding proteins (RBP) and microRNAs (miRNAs) are known to regulate gene expression at post-transcriptional level. They bind to a target mRNA, thereby regulating mRNA stability, decay and translation repression. In case of the 5-LO mRNA, so far two miRNAs were identified to bind to the 3´UTR and repress translation in myeloid cells and T lymphocytes (unpublished data). Since the miRNAs induce only modest changes in target gene expression (unpublished data), the drastically repression of 5-LO translation we observed in undifferentiated HL60 must be controlled by an additional regulatory component [4].
In case of the reticulocyte 15-LO (r15-LOX), the enzyme is only synthesized in mature reticulocytes, although the r15-LOX mRNA is present in premature and mature cells. Recent studies demonstrated that the translation of r15-LOX mRNA is inhibited by binding of the RBPs hnRNP K and hnRNP E1 to the 3´UTR in premature cells [5].
Since 5-LO and 15-LO fulfill similar functions, we speculate that regulation of gene expression could also be analogous. Therefore we want to investigate pull-down experiments to identify RBPs that bind to the 3´UTR of 5-LO mRNA and influence translation initiation.

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Poster 13

LIPIDS GENERATED IN VISCERAL ADIPOSE TISSUE DURING ACUTE PANCREATITIS MODULATE THE POLARIZATION IN MACROPHAGES BY INTERFERING PPAR-GAMMA ACTIVITY

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There is increasing evidence suggesting a role for the white adipose tissue (WAT) in the progression of inflammation during acute pancreatitis. In this study we analyze the effects of lipids generated by epididymal and retroperitoneal adipose tissue on the switch to the M1 phenotype in macrophages.
Pancreatitis was induced in rats by retrograde perfusion of 5% sodium taurocholate, and samples from different WAT were processed for total lipid extraction. Macrophage-differentiated THP-1 cells were used to evaluate lipid uptake and the effect of lipid extracts on M1 polarization. Changes in gene expression and protein levels were analyzed by quantitative RT-PCR and Western Blot, respectively.
No differences were observed in lipid uptake by macrophages between epididymal and retroperitoneal WAT. However, the epididymal region showed a higher inflammatory potential during acute pancreatitis, becoming the focus of our study. In M1-polarized macrophages, the presence of lipids from acute pancreatitis promoted an additional inhibition of M2 markers as MRC-1 and CD36, thus resulting in a higher M1/M2 ratio. Moreover, these lipids also promoted several changes in PPARgamma activation. When macrophages acquired the M1 phenotype, both RNA expression and protein levels of PPARgamma were reduced as expected, but this inhibition became significantly more pronounced when lipids from acute pancreatitis were present. By contrast, lipid extracts from control animals had no effect. Altogether, our results indicate that during acute pancreatitis, some areas of WAT generate lipid mediators that have the capability to interfere on the regulatory role of PPARgamma in macrophages, thus promoting a marked M1 polarization and a more intense proinflammatory response.

Poster 14

THE EFFECT OF CANNABINOIDS ON DINITROFLUOROBENZENE - INDUCED EXPERIMENTAL ASTHMA IN MICE

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Asthma is a chronic inflammatory airway disease associated with various respiratory symptoms and bronchial hyperreactivity. It has been shown that cannabinoids have significant anti-inflammatory effects in experimental asthma models. However the information about their functional effects in asthma and other inflammatory airway diseases are limited. In the present study, we investigated the effects of cannabinoid agonists on tracheal reactivity and lung inflammation in dinitrofluorobenzene (DNFB)-induced experimental non-atopic asthma model in mice.
Female CD1 mice were epicutaneously sensitized to DNFB and then intranasally challenged by dinitrobenzene sulphonic acid (DNS) which is the water soluble hapten of DNFB. Control mice received vehicle epicutaneously and challenged with DNS intranasally. Some of the groups received cannabinoid CB1 agonist (ACEA) (7,5 mg/kg i.p.), CB2 agonist (JWH133) (5 mg/kg i.p.) or dexamethasone (5 mg/kg i.p.) treatment for two consecutive days starting one hour before intranasal challenge. The tracheas of mice were isolated 48 hours after intranasal challenge and divided into two segments as proximal and distal parts. They are mounted in organ baths and contraction responses to carbachol, serotonin and relaxation responses to isoprenalin were elicited. Airway inflammation is evaluated by inflammatory cell count in bronchoalveolar lavage (BAL) fluid.
Carbachol-induced contraction and isoprenaline-induced relaxation responses were similar in control and DNFB groups. Serotonin-induced contractions were enhanced in the proximal parts but not in the distal parts of the tracheas in DNFB group when compared with control. The enhanced serotonin contractions in DNFB group was abolished in the presence of atropin (1 micromolar) or tetrodotoxin (1 micromolar) which suggest that it occurs through the increased acetylcholine release from the nerves of the airways. ACEA treatment augmented the enhanced serotonin contractions, but JWH133 or dexamethasone treatment had no effect. The number of macrophages were significantly increased in the BAL fluid of DNFB group. However ACEA, JWH133 and dexamethasone treatments did not alter this increase. In vitro incubation of tracheas with ACEA also inhibited the enhanced serotonin contractions whereas JWH133 treatment had no effect.
Our results suggest that activation of cannabinoid CB1 receptors may prevent bronchial hyperreactivity in non-atopic asthma.

Poster 15

PHYSIOLOGICAL STUDY OF PENICILLIUM AURANTIOGRISEUM AND ITS USEFULNESS IN FOOD AND PHARMACEUTICALS INDUSTRY

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Penicilluim aurantiogriseum is a species of P. frequently isolated from olives and other foodstuffs. It is recognized very dreaded by mycotoxins which it secretes. However, it is very useful in food and pharmaceutical industry by the substances biologically active it produces.
Therefore, it will be very useful to develop studies to induce the secretion of these substances likely to be secreted by P. aurantiogriseum and block the production of its toxin, especially since our previous work showed that toxin production depends on the conditions of Growing Media.
The aim of this study is to examine the lipolytic activity of P. aurantiogriseum and mastering the production of Aurantiamine.
The samples collected are cultivated in specific media and observed under a microscope. Different keys will be used to identify strains of P. aurantiogriseum. Once identified, a study of toxin production of P. aurantiogriseum isolated from different foodstuffs will be established and an identification of its mycotoxins.

Poster 16

THE ROLE OF THE N-TERMINUS OF β2-MICROGLOBULIN IN LPA- AND SDS-INDUCED AMYLOID FORMATION

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Beta2-microglobulin (beta2m) is the light chain of major histocompatibility complex type I (MHCI). Dissociating from the complex, it circulates in the blood and degraded by the kidneys. In haemodialysis patients, the serum concentration of this protein is increased to 50-fold and becomes the main component of amyloid depositions in dialysis related amyloidosis (DRA). In addition to the elevated beta2m concentration, some unknown factors must contribute to the fibrillation process. Molecules as glycosaminoglycans, collagen or lipids are supposed to be important in the aggregation process in DRA (Myers et al., Biochemistry, 2006).
We have been shown previously, that lysophosphatidic acid (LPA) induces the amyloidogenesis of β2m in vitro and stabilize the amyloid fibrils (Pál-Gábor et al.,2009). LPA is a bioactive lipid with a negatively charged headgroup and a hydrophobic tail of one alkyl chain; it has important role in cell proliferation, signaling processes and motility, in the development of nervous system and in reproductive processes. Furthermore, it can act as mitogen factor in many types of cancer.
LPA has elevated serum concentration in haemodialysis patients (Ookoshi et al., Nephrol. Dial. Transplant. , 2008). Contrary to other physiologically present lipids, LPA destabilizes the structure of native beta2m (Pál-Gábor et al., Biochemistry, 2009). Using molecular docking, we identified specific binding sites at the N-terminus of beta2m that might establish electrostatic interactions with LPA. To verify this hypothesis, we replaced some positively charged sidechains with Ala and Glu by site-directed mutagenesis. We investigated the effect of LPA on the structure of β2m mutants by circular dichroism spectroscopy. The polymerization kinetics was studied by fluorescence spectroscopy and the morphology of amyloid fibrils was analyzed by transmission electron microscopy. Complementary experiments were carried out with SDS, a typical amyloidogenic factor with structural similarities to LPA. It exhibited a stronger destabilizing effect on the monomers and also could induce the seed-dependent formation of amyloid fibrils. Our results might help to understand the molecular mechanisms of the self assembly of beta2m and provides opportunities for the development of diagnostic and therapeutic agents in the future.

Poster 17

EICOSAPENTAENOIC ACID (EPA) REVERSES THE INHIBITORY EFFECT OF TNF-ALPHA ON SUGAR UPTAKE BY ACTIVATION OF AMPK IN CACO-2 CELLS

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Background: Inflammatory Bowel Disease (IBD) is characterized by chronic inflammation of the gastrointestinal mucosa, presenting high levels of tumour necrosis factor-alpha (TNF-alpha) and, as a consequence, malabsorption of nutrients may occur. EPA is an omega-3 polyunsaturated fatty acid, with beneficial effects in obesity and insulin resistance. EPA directly inhibits TNF-alpha-stimulated lipolysis in adipocytes, by the downregulation of pro-inflammatory pathways such as ERK1/2 and NF-κB, as well as the stimulation of AMPK.

We have previously demonstrated in Caco-2 cells, that TNF-alpha impairs sugar uptake by decreasing the Na+-glucose cotransporter SGLT1 expression in the plasma membrane. Therefore, the aim of the study was to investigate in Caco-2 cells whether EPA could block the inhibitory effect of TNF-alpha on sugar transport and identify the intracellular signalling pathways involved.

Methods: Caco-2 cells were grown and pre-incubated for 1 hour with TNF-alpha (10 ng/ml) and EPA (100 microM) before measuring the apical uptake of 0.1 mM alpha-methyl-glucoside (MG) for 15 min. The involvement of AMPK in the EPA effect was analyzed using the AMPK inhibitor Compound C (CC; 20 microM), and the AMPK activator AICAR (1 mM). The implication of ERK1/2 in TNF-alpha effect was determined using the ERK1/2 inhibitor PD98059 (50 microM). The expression of SGLT1 in Brush Border Membrane Vesicles (BBMV) of Caco-2 cells and the activation of AMPK and ERK1/2 were measured by Western blot.

Results: EPA blocked the inhibitory effect of TNF-alpha on MG uptake and SGLT1 expression in the BBMV. The presence of PD98059 reversed the inhibitory effects of TNF-alpha on MG uptake and SGLT1 expression. The AMPK activator AICAR also prevented TNF-alpha inhibition of sugar uptake by maintaining the levels of SGLT1 in the plasma membrane. The inhibition of AMPK by CC abolished the ability of EPA to prevent TNF-alpha effect.

Conclusions: TNF-alpha reduces MG uptake by decreasing SGLT1 expression in the plasma membrane through the activation of ERK1/2 and inhibition of AMPK pathways. EPA prevents the inhibitory effect of TNF-alpha on sugar uptake by activating AMPK.
These data suggest that EPA could be useful in IBD patients that suffer nutrient malabsorption and in obese people presenting intestinal inflammation.

Poster 18

15d-PGJ2 DECREASE NEUTROPHIL HYPERACTIVITY BY ACTIVATING Nrf2 PATHWAY

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Chronic up-regulation of pro-inflammatory mediators due to disease-related redox imbalance activates pro-inflammatory pathways such as NF-κB and cyclooxygenase (COX)-2. There is an essential requirement for effective co-regulation of anti-oxidant pathways such as the nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2) pathway for normal physiological responses to follow. Previously we have shown that neutrophils from patients with the chronic inflammatory disease periodontitis have an intracellular redox disturbance and the nuclear extract showed reduced Nrf2 binding to its consensus sequence (Dias et al PLOS One 2014). We have investigated the hypothesis that a downstream product of COX-2, 15-deoxy-delta (12, 14)-prostaglandin J2 (15d-PGJ2) has protective effects by activating Nrf2 pathway during oxidative stress.

Neutrophils were isolated by density gradient centrifugation (150g/8mins, then 400g/10mins) from healthy subjects (n=3). Cells were incubated in RPMI (+10% foetal calf serum) for 24hours ± 10μM buthionine sulfoximine (BSO). Cells were treated with 10μM 15d-PGJ2 for 1-24hours. Cell viability was measured by the Trypan blue exclusion assay. Extracellular ROS were measured as lucigenin (100μM) chemiluminescence post- stimulation with 1μM fMLP. Cellular glutathione (GSH) levels were measured by the GSH recycling assay. Cellular Nrf2 and COX-2 levels were determined by immunoblotting.

BSO decreased intracellular GSH (27.2±7.1 nmol/mg protein) compared to untreated control (GSH; 35.2±2.8 nmol/mg protein) and increased the fMLP stimulated respiratory burst of neutrophils (56.6% increase). However, pre-incubation with 15d-PGJ2 for 16 hours or 24 hours prevented the increase seen in the fMLP-stimulated respiratory burst that was due to GSH depletion (10% decrease) compared to non-BSO treated control. 15d-PGJ2 alone lowered GSH levels 30 min after the treatment (26.4±3.2 nmol/mg protein) and a subsequent elevation of GSH levels was observed at 16 hours (40.6±6.4 nmol/mg protein; $P<0.05$). BSO decreased total Nrf2 protein level and decreased COX-2 expression in neutrophils. Pre-incubation with 15d-PGJ2 for 16 hours increased total Nrf2 and decreased COX-2 expression. None of the treatments significantly decreased the cell viability.

Conclusion: This study demonstrates that neutrophil hyper-reactivity induced by oxidative stress can be reduced with 15d-PGJ2 treatments in vivo and the role of the Nrf2 signalling pathway merits further investigation.

Poster 19

RELATION OF VASPIN AND VISFATIN LEVELS WITH THE PRESENCE AND THE SEVERITY OF CORONARY ARTERY DISEASE

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Coronary artery disease (CAD) is the leading cause of death worldwide and linked to obesity. It was speculated that fatty tissue originated adipo-cytokines may play role in pathogenesis of atherosclerosis. Vaspin and visfatin are newly described members of these adipocytokines family. The association of these novel adipocytokines, vaspin and visfatin, with atherosclerotic CAD is still obscure. Aim of the work: To investigate the relationship of vaspin and visfatin adipocytokines with the existence as well as the severity of CAD. Patients and Methods: A total of 87 patients who underwent coronary angiography due to symptoms of stable angina were enrolled in the study. They were divided into two groups; CAD group (56 patients) who have at least single vessel disease of ≥ 70 % and/or left main coronary artery ≥ 50% diameter stenosis and normal group (31 patients) who have normal coronary arteries.
The severity of CAD was assessed using coronary angiography by estimation the number of vessels affected and Gensini score. The CAD group was sub-classified according to the severity of CAD by counting number of diseased vessels as 1-, 2- or 3-vessel disease. Clinical parameters were reported and creatinine, glycemic, lipid profile, vaspin and visfatin levels were assayed. Results: Serum levels of vaspin were significantly lower and inversely serum levels of visfatin were significantly higher in CAD group than controls (1.51 ± 0.99 µg/L versus 4.54 ± 0.69 µg/L for the former and 22.86 ± 4.68 µg/L versus 13.43 ± 1.1 µg/L for the later; $p<0.0001$ for each). Decreased vaspin and increased visfatin levels were correlated with CAD severity as expressed by the number of significantly narrowed coronary arteries and Gensini score ($p<0.0001$ for each). There was a negative correlation between vaspin and the Gensini score and positive correlation between visfatin and Gensini score
($r= -0.727$, $p<0.00001$ and $r= 0.798$, $p< 0.00001$, respectively). In conclusion: Patients with established CAD showed reduced vaspin and increased visfatin serum levels. Moreover, low vaspin and high visfatin levels were significantly correlated with CAD severity suggesting a link between atherosclerosis and adiposity. Therefore, vaspin and visfatin adipocytokines may used for prediction of CAD and estimation of its severity.

Poster 20

MECHANISTIC STUDIES ON 5-LIPOXYGENASE INHIBITION BY SULINDAC SULFIDE

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Human 5-lipoxygenase (5-LO) is the key enzyme in the biosynthesis of leukotrienes, which are mediators of proinflammatory and immune modulatory responses. Since 5-LO is involved in the pathogenesis of atherosclerosis, asthma and several types of cancer, the inhibition of 5-LO enzyme activity offers a possible strategy for treatment of these diseases. Therefore developing specific and selective 5-LO inhibitors is of high interest as only one compound so far reached the pharmaceutical market and many developed 5-LO inhibitors failed in clinical trails due to a lack of in vivo efficacy and strong side effect profiles.
We could identify sulindac sulfide (SSi) as a specific 5-LO inhibitor, which interferes directly with the 5-LO enzyme at clinically relevant concentrations. Mechanistic studies could show that SSi lost its 5-LO-inhibitory potency in sonified cell homogenates (IC50 = 100 micromolar), which was partially restored by removal of membrane particles by centrifugation (S100, IC50 = 40 micromolar). To exclude that the changed redox tone in broken cell preparations impairs 5-LO inhibition by SSi – as true for nonredox-type inhibitors – homogenates were supplemented with thiols (DTT or GSH). However, even under reducing conditions 5-LO-inhibitory potency of SSi in homogenates was not restored. Thus, endogenous cellular components or unknown factors seem to impair suppression of 5-LO by SSi in homogenates.
To investigate whether microsomal vesicles containing phospholipids are the cause for the impaired suppression, different exogenous phospholipids were added to partially purified recombinant 5-LO protein in presence of SSi. Interestingly, phosphatidylcholine (PC) but not phosphatidylserine (PS) or –ethanolamine (PE) reversed 5-LO inhibition by SSi in a concentration-dependent manner. Since the well-characterized binding of 5-LO to PC requires three tryptophan residues (W13/75/102) located within the C2-like domain, SSi may interfere with 5-LO activity by binding at this regulatory domain. This hypothesis was strongly supported by the finding, that SSi failed to suppress the activity of a mutant 5-LO enzyme (W13/75/102A) unable to bind phosphatidylcholine.
Thus, SSi is a novel type of direct 5-LO inhibitors apparently targeting the C2-like regulatory domain and may be a scaffold for the design of a novel class of well-tolerated 5-LO inhibitors with relevance for the clinics.

Poster 21

IN VITRO AND EX VIVO SKIN MODELS TO STUDY THE LIPID MEDIATORS INFLAMMATORY RESPONSE TO STRESS AND ITS MODULATION BY ACTIVE INGREDIENTS

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Human skin is considered as the body's largest organ and constitutes the primary barrier from the outside protecting from injury, infections, water loss, solar irradiations, as well as being an important player of the immune system. Inflammation partakes in physiological mechanism mediation as healing and response to injury including stress resulting from exposure to UV radiation (UVR) in sunlight. The cutaneous response is regulated by mediators such as cytokines and bioactive lipids that can initiate rapid reactions with a controlled inflammation, followed by an efficient resolution. UVR results in oxidative stress with an increase in malondialdehyde (MDA) reflecting lipid oxidation. Furthermore, UVR increases the release of polyunsaturated fatty acids (PUFAs) in the skin by up regulating the synthesis and activity of phospholipase A2 (PLA2) through increased oxidative stress-mediated reactions. Proteins expression such as cycloxygenases (COXs) and lipoxygenases (LOXs) are also up regulated by UVB, along with increased levels of bioactive lipids. The aim of the study was to characterize cellular and tissular models regarding the modulation of active ingredients mainly on the bioactive lipids profile after inflammatory stress. After extraction, we measured lipid peroxidation marker MDA by GC/MS method and lipid mediators involved in UV-induced inflammation, PUFAs and eicosanoids (prostaglandins, HETEs and lipoxins) by LC/MS method. Keratinocytes 2D culture, coculture of immune cells (lymphocytes and monocytes) and a developed skin explant model were complementary models to explore UVR effects on cutaneous lipids to better define active ingredients contributing to preventive or curative efficacy of dermo-cosmetic formulations.

Poster 22

SELECTIVE ACTIVATION OF CANNABINOID RECEPTOR 2 PRIMES EOSINOPHILS FOR ENHANCED MIGRATORY RESPONSIVENESS

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Background:
Accumulation of activated eosinophils in tissue is a hallmark of allergic inflammation. The endocannabinoid 2-arachidonoylglycerol (2-AG) has been proposed to elicit eosinophil migration in a CB2 receptor/Gi/o-dependent manner. However, it was claimed recently that besides CB2 activation, 2-AG metabolites and the 15-lipoxygenase pathway are involved in the regulation of eosinophil migration induced by 2-AG. Here we explored the direct contribution of specific CB2 receptor activation to human and murine eosinophil effector function.

Results:
We observed that the selective CB2 receptor agonist JWH-133 induced only a weak migratory response in eosinophils. However, short-term exposure to JWH-133 potently enhanced chemoattractant-induced eosinophil shape change, chemotaxis, CD11b surface expression and adhesion under flow conditions. The receptor specificity of the observed effects was confirmed using the selective CB2 antagonist SR144528. Moreover, selective CB2 stimulation evoked a transient increase in intracellular Ca2+ and mediated the activation of MAPK-kinase 1/2 (MEK 1/2) and Rho-associated protein kinase (ROCK) via a pertussis toxin (PTX)-insensitive G-protein. Finally, we found that JWH-133 does not affect chemoattractant-induced Ca2+ responses, nor modulate degranulation and respiratory burst.

Conclusion:
These data indicate that direct CB2 receptor activation primes eosinophils for an enhanced migratory responsiveness to proinflammatory agents such as eotaxin and prostaglandin (PG) D2. Given its abundant expression in eosinophils, antagonism of the CB2 receptor may be of therapeutic relevance in allergic inflammation and other eosinophilia-associated disorders.

Poster 23

EICOSANOIDS AS MEDIATORS OF INFLAMMATION IN THE IN VITRO MODEL

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Eicosanoids are one of the most crucial inflammation mediators. Their proinflammatory properties were are well known, however, nowadays the studies about anti-inflammatory
effect of fatty acids derivatives are also performed.
The aim of this study was to evaluate pro- or anti-inflammatory impact of polyunsaturated fatty acids n-3 and n-6 as eicosanoids precursors, on the cells. To evaluate the effect human cell line HUVEC was used. Cells were treated with arachidonic (AA) or eicosapentaenoic acid (EPA) and then incubated for 24 hours. In the next step LPS and/or BaP was added to some cells in order to stimulate them. After incubation with fatty acids or LPS no evaluated levels of apoptosis or decreased levels of viability were observed. Significant differences in the amount of membrane fatty acids were observed. Different ability to synthesize isoPs was observed in the experimental cells. The western blot analysis were performed on properly prepared samples in order to determinate the activity of COX-2. The elevated activity of this enzyme was observed in cells treated with AA and activated with LPS. Moreover, compared to control, cells incubated with EPA showed decreased activity of COX-2. The results of the study have revealed the pro-inflammatory properties of AA, while the EPA had the opposite, anti-inflammatory effect. This study provide information about n-3 fatty acids as anti-infammatory substances, which can be used in the process of developing new anti-inflammatory drugs.
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Poster 24

SUPPRESSION OF LEUKOTRIENE BIOSYNTHESIS BY TARGETING 5-LIPOXYGENASE-ACTIVATING PROTEIN (FLAP) WITH THE NOVEL BENZIMIDAZOLE DERIVATIVE BRP-7

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Leukotrienes (LT) are potent lipid mediators in inflammatory responses derived from arachidonic acid (AA). Upon stimulation, the key enzyme in LT biosynthesis 5-lipoxygenase (5-LO) translocates to the nuclear membrane where it acquires its substrate AA from the membrane-bound 5-LO-activating protein (FLAP). As lack or inhibition of FLAP in cellulo or in vivo precludes LT formation, FLAP inhibitors display an interesting pharmacological drug candidates. We recently identified 1-(2-chlorobenzyl)-2-(1-(4-isobutylphenyl)ethyl)-1H-benzimidazole (BRP-7) as a potential FLAP inhibitor using a combined ligand- and structure-based pharmacophore model. Here, we elucidated the pharmacological potential of BRP-7 to interfere with the 5-LO pathway by FLAP inhibition. BRP-7 potently suppressed LT formation in neutrophils and monocytes (IC50 = 0.15 and 0.03 μM, respectively) by abolishing the cellular interaction between 5-LO and FLAP after Ca-ionophore stimulation. Since FLAP is needed to deliver released AA to 5-LO in intact cells, supplementation of exogenous AA makes FLAP dispensable for LT formation and therefore diminishes the inhibitory potency of BRP-7. Consequently, BRP-7 failed to directly inhibit 5-LO activity in cell-free assays or cell homogenates, a typical feature of FLAP inhibitors. To confirm direct interaction of BRP-7 with FLAP as the proposed target, we performed an affinity chromatography method using an insoluble BRP-7 matrix and solubilized FLAP (from neutrophil membranes) as protein source. Interestingly, BRP-7 hardly inhibited other key enzymes along the AA pathway like cytosolic phospholipase A2 (cPLA2), cyclooxygenase-1 (COX-1) and microsomal prostaglandin E synthase-1 (mPGES-1), implying selectivity of BRP-7 for FLAP. Moreover, BRP-7 was effective in human whole blood and impaired inflammation in vivo, in rat pleurisy and mouse peritonitis, along with reduced LT levels. In summary, BRP-7 displays a promising FLAP inhi
bitor with potential for therapeutic use.

Poster 25

THE LINK BETWEEN SALT SENSITIVITY DETERMINED BY POLYMORPHISMS IN GRK4 GENE, AND MYOCARDIAL INFARCTION

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Myocardial infraction occurs when the blood supply to part of the heart muscle is severely reduced or stopped because of blockage of one or more of the coronary arteries. A well-established risk factor for myocardial infraction is hypertension. Essential hypertension is a major public health problem in many countries due to its high prevalence and its association with coronary heart disease, stroke, renal disease, peripheral vascular disease and other disorders. Dopamine D1 receptors in the kidney increase sodium (and water) excretion in response to an increased sodium load. salt sensitivity in essential hypertension in humans has been associated with decreased coupling of dopamine stimulation to sodium excretion. Polymorphisms in the human G protein coupled receptor kinase 4 gene (GRK4) gene appear to account for some of this variation. To examine the link between myocardial infraction and salt sensitive hypertension determined by GRK4 gene polymorphisms (R65L, A142V and A486V) among Jordanian society, we conducted a case control study consisting of 50 hypertensive individuals with myocardial infraction and 50 hypertensive controls matched for age, gender and geographic area. The three GRK4 variants were genotyped by allele specific PCR; results obtained were confirmed by PCR-RFLP , restriction enzymes used for digestion were AatII, HaeIII and Acil. A486V polymorphism was significantly associated with increased risk of myocardial infraction (p=0.027) among patients studied. Several published have shown an influence of these polymorphisms on blood pressure and hypertension. Salt increase the reactivity of platelets, the tiny blood elements that help the blood to clot. Thus, high dietary sodium might lead to cardiovascular events like stroke, heart attack, and kidney disease directly, even in the absence of hypertension. A486V polymorphisms may signal the increased risk to myocardial infraction among hypertensive subjects in Jordan.

Poster 26

Alox5 (5-LIPOXYGENASE) AS A NEW DIRECT TARGET GENE OF p53

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The p53 tumor suppressor plays a critical role in human cancer. More than 50 percent of human tumors contain mutations or deletions of the TP53 gene. p53 can transactivate or repress a number of target genes in response to diverse stress signals. This activation leads to the induction of cellular responses, e.g. transient growth arrest, DNA repair, cellular differentiation, senescence and apoptosis. In the last years, new target genes of p53 have been discovered and attempts have been made to correlate particular patterns of p53 induced target gene expression and the ensuing cellular phenotypes. Although the crucial involvement of p53 in tumorigenesis has been established, its translation to the clinical application is yet to be accomplished.
Through an unbiased genome-wide ChIP-seq analysis, we have found that 5-lipoxygenase (alox5, 5-LO), which is a key enzyme of leukotriene (LT) biosynthesis, catalyzing the first two steps in the conversion of arachidonic acid into different LTs, is a direct target gene of p53 upon genotoxic stress by Actinomycin D (ActD) or Etoposide (Eto).
We identified a complete p53 consensus binding motif, consisting of two half-sites with the sequence RRRCWWGYYY (R=purin, W=A or T, and Y=pyrimidine) within the p53-binding site in intron 7, which is located far downstream (about 64 kbp) of the transcriptional start site of the alox5 gene. We confirmed the strong binding of p53 to the 5-LO target site in targeted ChIP-PCR experiments.
The Luciferase assays of pN10-intronG-p53motif-luc reporter are showing an around 130-fold increase of luciferase activity only in cells co-transfected with the p53 wild-type protein.
Expression analyses by real-time qPCR and immunoblot further revealed that genotoxic stress by ActD or Eto activated the alox5 transcription and induced expression of 5-LO protein in a wild-type (wt-) functional p53-dependent manner.
Futhermore, treatment of U2OS cells induce accumulation of p53 and 5-LO around the perinuclear region.
Taken together, in vivo ChIP and ChIP-seq analysis show a p53-binding site within the distal region of the alox5 promoter and may act as a distal enhancer for transcription.

Poster 27

INVESTIGATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) GENE EXPRESSION IN HYPOTHALAMUS OF OBESE RATS: MODULATION BY OMEGA-3 FATTY ACIDS

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Obesity is a major global health concern which is currently on the rise given the modern sedentary lifestyle, lack of exercise and unhealthy eating habits. It is characterized by an abnormal increase in fat accumulation which leads to inflammatory responses. This study was designed to investigate the effect of obesity and omega-3 fatty acids on brain-derived neurotrophic factor (BDNF) gene expression, using in vivo and in vitro models, in an attempt to unravel the potential mechanisms of polyunsaturated fatty acids use in obesity. Sprague-Dawley rats were divided into 3 groups. Lean controls were fed normal rodent chow for 14 weeks. Obese controls were fed 60% of their diet as saturated fats for 14 weeks. Omega-3 fatty acid-treated rats were fed 60% saturated fat diet for 14 weeks with concomitant oral administration omega-3 fatty acids, mainly DHA and EPA, from week 12 to week 14. The dose used was 400 mg/kg/day. For the in vitro experiment, hypothalamic cells from obese rats were cultured in different concentrations of omega-3 fatty acids to determine the dose-effect relationship of omega-3 fatty acids on BDNF gene expression. Results of the study show that obesity has a negative effect on BDNF gene expression in rat hypothalamus that was reversed by omega-3 fatty acids. Obese rats showed hypercholesterolemia, hypertriglyceridemia, hyperglycemia and hyperleptinemia. Treatment with omega-3 fatty acids induced opposite effects on all parameters to those seen in obese conditions. No significant differences are shown in the levels of serum insulin. The in vitro study results show that the increase in BDNF gene expression caused by omega-3 fatty acid treatment is dose-dependent. In conclusion, obesity results in a down-regulation in BDNF gene expression that was reversed by omega-3 fatty acids treatment, making them an interesting treatment approach for obesity and metabolic diseases.

Poster 28

NEUTROPHILS-DERIVED-MICROVESICLE COULD PLAY A ROLE IN THE EARLY STAGE OF ATHEROSCLEROSIS VIA PGE2

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Background: Atherosclerosis is the most common underlying cause of cardiovascular disease. Many cells types are involved in the initiation and progression of atheromatous plaques and, until recently, neutrophils were not considered to be among them. Despite their rare detection in plaques, neutrophil depletion has been shown to reduce plaque formation and monocyte recruitment to the vessel wall. Neutrophils may influence plaque initiation and progression through the formation of microvesicles, liberating several molecules such as prostaglandin (PG) E2 responsible for metalloproteinase (MMP) activity (Yokoyama et al., 2012; Gomez et al., 2014) and platelet aggregation.
Aim: To investigate the role of prostaglandins in neutrophil-derived-microvesicles production.
Methods and Results: Blood was collected from healthy donors. Neutrophils were isolated and stimulated with fMLP, AcLDL, indomethacin or PGE2 while control samples were kept in PBS. After centrifugation, the neutrophils, supernatant and microvesicles obtained were used for flow cytometry quantification (using Megamix and AccuCount beads) and PGE2 ELISA.
Neutrophils responded to stimulation with fMLP (1 μM) and AcLDL (20 μg/ml) inducing significantly increased production of microvesicles (respectively, 94.5 % ± 21, $p < 0.0001$ and 181.83 % ± 20.2, $p < 0.0001$), whereas the stimulation with indomethacin (1.7 μM) had no significant effect. Interestingly, indomethacin significantly inhibited the production of microvesicles in response to fMLP or AcLDL. However, co-stimulation with PGE2 (1 μM) and indomethacin induced significantly increased production of microvesicles (75 % ±12.2, $p = 0.0416$). ELISA measurement of PGE2 on neutrophil supernatant and microvesicle pellets after lysis, showed a significantly higher content of this prostaglandin after stimulation with fLMP and AcLDL (respectively, 70.37 % ± 15.58, $p = 0.0010$ and 133.42% ± 11.38, $p < 0.0001$ for neutrophils and 196.56 % ± 18.46, $p = 0.0012$ and 373.61 % ± 25.35, $p < 0.001$ for microvesicles).
Conclusion: Prostanoids are involved in microvesicle production. Neutrophil-derived-microvesicle contain PGE2, which could play a role in the early stage of atherosclerosis by activating MMP-9, participate in vascular wall remodelling and also increase platelet aggregation after stimulating the PGE2 receptor EP3 (Tilly et al., 2014).

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Poster 29

QUANTIFICATION OF OXYSTEROLS IN OB/OB AND DB/DB MICE MODELS OF OBESITY AND STUDY OF THE EXPRESSION OF THEIR METABOLIZING ENZYMES.

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Obesity is an ever increasing health problem in our societies and so are its associated disorders: hypertension, type-2 diabetes, systemic low grade inflammation, disturbance of lipid metabolism… Indeed, cholesterol metabolism is greatly affected in obesity leading to hypercholesterolemia and increased liver cholesterol content. Oxysterols are cholesterol-derived oxygenated bioactive lipids, once considered as the metabolic intermediates toward bile salts synthesis. However, they are now considered as full-fledged bioactive lipids. Indeed, beside their role in controlling cholesterol metabolism, they have been shown to modulate inflammatory tone in several models. Alterations in their levels have been found in several pathologies including atherosclerosis or Alzheimer's disease.
Here we wanted to assess the changes in oxysterol levels in the liver of two genetic models of obese mice, the ob/ob and db/db mice by quantifying their levels in the obese and littermate lean mice. We also sought to study the expression of the enzymes responsible for the synthesis and degradation of these oxysterols. A similar approach was used in the hypothalamus, which plays an important role during obesity, of the db/db mice.
With this approach, we were able to compare the oxysterol profiles obtained in the two most common genetic models of obesity and to correlate the expression of the enzymes involved in oxysterol metabolism with the levels of the corresponding lipid.
With this study, we broaden the knowledge concerning oxysterols in obesity settings. We compared two broadly used genetic models of obesity, therefore contributing to their further characterization. We also open the door to future studies regarding the role of each oxysterols we measured in obesity settings and the possible impact of their modulation.

Poster 30

INVESTIGATION OF EFFECTS OF EPOXYGENASES ON NON-ADRENERGIC NON-CHOLINERGIC (NANC) RELAXANT RESPONSES INDUCED BY ELECTRICAL FIELD STIMULATION (EFS) IN RABBIT CORPUS CAVERNOSUM

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Objectives
Nitric oxide (NO) is the main neurotransmitter involved in relaxation of corpus cavernosum smooth muscle. However the continuation of normal erection in eNOS knockout mice suggests the presence of non-NO signaling pathways and the necessity of these pathways for generation and maintaining an erection. Arachidonic acid is metabolized to 20-hydroxyeicosatetraenoic acid (20-HETE) by ω-hydroxylase belonging to the cytochrome P450 (CYP) enzyme system and to various epoxyeicosatrienoic acids by epoxygenase. The aim of this study was to investigate the contributions of non-specific cytochrome P450 epoxygenase inhibitor, miconazole; specific ω-hydroxylase inhibitor, 17-octadecanoic acid (17-ODYA); the metabolites of arachidonic acid, 14,15-epoxyeicosatrienoic acid (14,15-EET), 11,12-epoxyeicosatrienoic acid (11,12-EET) and 20-HETE epoxides on EFS mediated NO-dependent and -independent NANC relaxation responses in isolated rabbit corpus cavernosum.
Method
Isolated rabbit corpus cavernosum tissues which were obtained from male adult albino rabbits weighing 2,5-3 kg were suspended in organ bath chambers containing aerated Krebs solution to record isometric contractions via force displacement transducers. NO-dependent and -independent submaximal relaxation responses were received in the presence of guanethidine (10-6M) and atropine(10-6M) on the contracted tissues with phenylephrine (3x10-5M). Addition to guanethidine and atropine, Nω-nitro-L-arjinin metil ester (L-NAME,10-4M) was added to the organ bath to obtain NO-independent area.
Results
Miconazole (10-9-10-4M) ve 17-ODYA (10-10-10-5M) increased both EFS induced NO-dependent and -independent NANC relaxation responses but there was no statistical significant difference between the groups. 14,15-EET (10-11-10-8M), 11,12-EET (10-12-3x10-8 M) and 20-HETE (10-11-3x10-8M) also enhanced the NANC relaxation responses induced by EFS in both groups. There was no significant difference between 11,12-EET applied NO-dependent and -independent groups whereas in 14,15-EET applied NO-dependent relaxation responses were found to be increased insignificantly when compared to -independent. In 20-HETE applied NO-dependent group, statistical significant difference was observed at lower concentrations(10-11–10-8 M) when compared to -independent group. Cytochrome P450 mono-oxygenase inhibitors and epoxides didn't produce relaxation response on NO-dependent and -independent groups pre-contracted tissues with phenylephrine.
Conclusions
These results suggest that cytochrome P450 mono-oxygenase inhibitors (miconazole and 17-ODYA) and epoxides (14,15-EET, 11,12-EET ve 20-HETE) contribute to EFS mediated NO-dependent and -independent NANC relaxation responses and exert their effects by presynaptic mechanisms.

Poster 31

INTRALEAFLET HEMORRHAGE AS AN ACTIVATOR OF THE LEUKOTRIENE PATHWAY IN AORTIC VALVE STENOSIS.

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Aim: The most common valvular heart disease is the calcification and narrowing of the aortic valve, referred to as aortic stenosis. Leukotrienes have been implicated as key mediators of inflammation, extracellular matrix degradation and osteogenic signaling in the valvular leaflets, leading to development and hemodynamic progression of calcified aortic valve stenosis. The biosynthesis of leukotrienes requires the iron-containing enzyme 5-lipoxygenase. Interestingly, biomechanical factors acting on the aortic valve may induce intraleaflet hemorrhage and an accumulation of intracellular iron, which has been associated with macrophage infiltration and a rapid progression of aortic valve stenosis. The objective of this study is to investigate the mechanisms that link intraleaflet hemorrhage with the progression of aortic valve stenosis and the potential role of the 5-lipoxygenase and leukotriene pathway in this process.

Methods: The presence of iron in human aortic valves derived from 40 patients undergoing aortic valve replacement surgery was analyzed by means of Perls\' Prussian blue staining. In addition, mRNA levels were analyzed by real time PCR in calcified aortic valve tissue exhibiting either negative (N=14) or positive (N=8) Perls\' staining.

Results: In our cohort of patients, the presence of iron in the aortic valves was positively associated with the degree of calcification. In addition, the presence of iron in human aortic valves was associated with 3.7±1.8 –fold higher levels of mRNA encoding the 5-lipoxygenase activating protein (FLAP; P=0.03). In addition, there was a trend towards increased 5-lipoxygenase mRNA levels, being 2.1±1.2 –fold higher in iron-positive compared with iron negative aortic valve tissue (P=0.12).

Conclusions: Our results demonstrate that intraleaflet hemorrhage is associated with valvular calcification and an increase in the components of leukotriene biosynthesis pathway. Taken together, these findings suggest intraleaflet hemorrhage as a potential activator of leukotriene biosynthesis in aortic stenosis.

Poster 32

CYTOSOLIC PHOSPHOLIPASE A2a ENHANCES MOUSE MORTALITY INDUCED BY PSEUDOMONAS AERUGINOSA PULMONARY INFECTION VIA INTERLEUKIN 6

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Pseudomonas aeruginosa pulmonary infection is a leading cause of death in numerous diseases such as cystic fibrosis (CF). The host cytosolic phospholipase A2a (cPLA2a) releases lipid mediators that play an important role in the pathogenesis of diseases, but its role in lung injury induced by P. aeruginosa infection is still obscure. Using an animal model of P. aeruginosa lung infection, we showed that the CHA strain of P. aeruginosawas was more potent than the PAK strain in inducing mouse mortality and lung injury. Both mouse mortality and lung injury were reduced in cPLA2a-/- as compared to cPLA2a+/+ mice. This was accompanyied by decreased levels of IL6 but not other inflammatory cytokines (IL1b, KC and TNFa) in the bronchoalveolar lavage fluids (BALFs) of cPLA2a-/- mice. Given that CFTR-/- mice exhibit increased cPLA2a activation in the lung, the role of cPLA2a was further examined in our lung infection model. Compared to littermates, P. aeruginosa infection caused increased mortality in CFTR-/- mice and IL6 levels in BALFs which were attenuated by pharmacological inhibition of cPLA2a. Compared to IL6-/- mice, an enhanced mortality was observed in P. aeruginosa infected IL6+/+ mice. Since alveolar macrophages (AMs) are the primary cytokine source in the lung, murine AM cell line (MH-S) were used to investigate the signalling pathways involved in this process. Incubation of MH-S cells with P. aeruginosa induced IL6 production, which was mediated by MAPKs ERK/p38 and was abolished by cPLA2a inhibitors. In addition, among cPLA2 downstream signalling pathways, only 15-lipoxygenase (15-LOX) and cyclooxygenase 2 (COX2) were proven to participate in this P. aeruginosa-induced IL6 expression. Based on all these observations, we conclude that cPLA2a enhances P. aeruginosa-induced animal lethality in part via IL6 induction and that MAPKs ERK/p38, 15-LOX and COX2 signalling pathwyas were involved in this process.

Poster 33

APOPTOTIC CANCER CELL MEDIATED REGULATION OF 5-LIPOXYGENASE IN MACROPHAGES

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5-lipoxygenase (5-LO) is a key enzyme in the synthesis of leukotrienes, which are potent pro-inflammatory lipid mediators involved in chronic inflammatory diseases like asthma, atherosclerosis, or tumor-associated inflammation. 5-LO is mainly expressed in immune cells, but also in various types of cancer cells. 5-LO metabolizes arachidonic acid in a two-step catalysis via 5-hydroperoxyeicosateraenoic acid to the unstable leukotriene A4 with the notion that tumor- promoting functions are attributed to its products.
To understand the role of the 5-LO and its products in the tumor microenvironment, we analyzed its function and expression in tumor-associated macrophages (TAMs). TAMs were generated by co-culturing primary human macrophages with human MCF-7 breast carcinoma cells, which caused cell death of cancer cells followed by phagocytosis of cell debris by macrophages. This model mimics an early tumor/immune cell interaction, with concomitant polarization of macrophages to tumor-promoting cells. Interestingly, RNA as well as protein expression of 5-LO in TAMs were reduced after 4 days of co-culture with cancer cells. In line, the formation of 5-LO products was reduced. Downregulation of 5-LO was dependent on tumor cell death and accompanied by the induction of cytokines/growth factors potentially regulating 5-LO expression. Future experiments will address mechanisms of 5-LO regulation in TAMs and analyze functional consequences.

Poster 34

PREVALENCE OF ANEUPLOIDIES IN PRODUCTS OF SPONTANEOUS ABORTION: INTEREST OF FISH AND MLPA IN THE DETECTION OF CHROMOSOMAL ABNORMALITIES

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Spontaneous abortion (SA) is the loss of the conceptus before 22 weeks of gestation, when fetal weight is less than 500 gr. The genetic etiology accounts for more than two thirds of SA, and autosomal aneuploidies alone account for up to 70% fetal loss. The aim of this study was to highlight the most common chromosomal causes of fetal loss. In this study, 220 products of abortion and in utero fetal death were analyzed by using FISH (AneuVysion™) on interphase nuclei from chorionic villus and by using MLPA (SALSA P036, P070 and P245 kits) on DNA extracted from fetal tissues. The gestational age ranged from the 7th to the 38th week of gestation. Of a total of 151 samples analyzed by using FISH, ten chromosomal abnormalities were observed: four trisomies 21 (one of them was mosaic), a trisomy 18, a trisomy 13, three triploidies and one monosomy X (Turner). From the additional 69 samples analyzed by using MLPA, two anomalies were found: two monosomies X (Turner).

FISH and MLPA are simple, rapid and sensitive tools for the detection of chromosomal aneuploidies. Avoiding the cell culture step necessary for karyotyping, they represent very interestingalternative methods to diagnose genomic disorders in products of abortion in which poor sample quality often leads to cell culture failure.

Poster 35

PI3K/Akt PATHWAY AS AN ANTI-INFLAMMATORY DRUG TARGET

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Objectives
The aim of the present study was to characterize the role of PI3K in inflammation and investigate if PI3K inhibitors could be used as a treatment for inflammation in vivo. Another aim was to investigate in vitro how PI3K inhibition affects the levels of expression of different genes involved in innate immunity in activated macrophages.

Methods
Carrageenan-induced paw edema in mice was used as an in vivo model of acute inflammation. We investigated the effects of PI3K inhibitors LY294002 (non-selective) and IC87114 (PI3Kdelta; selective) on carrageenan-induced inflammatory edema. For in vitro studies LPS was used to activate the PI3K/Akt pathway in J774.2 macrophages. The activation of PI3K/Akt pathway was investigated by measuring the phosphorylation of Akt with Western Blotting. Inflammatory gene expression was measured by RT-qPCR, Western Blotting and ELISA.

Results
We found that pharmacological inhibition of PI3K had anti-inflammatory effects in the carrageenan-induced paw inflammation model. We also found that in vitro these inhibitors lowered the expression of inflammatory genes IL6, MCP1, TNF and iNOS which are known to be involved in mediating the carrageenan-induced inflammation. We propose that the anti-inflammatory effects seen by these inhibitors in vivo are caused by the inhibition of inflammatory gene expression found in the in vitro experiments.

Conclusions
PI3K inhibitors appear to have anti-inflammatory properties in vivo suggesting that these inhibitors could be used to treat aberrant inflammatory responses. The proposed mechanism for this is the attenuation of inflammatory gene expression caused by inhibition of PI3K/Akt pathway which was shown in vitro. This in turn suggests that PI3K could indeed be used as a drug target against inflammatory diseases.

Poster 36

LIPIDOMIC IDENTIFICATION OF DIOXOLANE A3, A FREE ACID AND PHOSPHOLIPID-ESTERIFIED EICOSANOID FORMED VIA CYCLOOXYGENASE 1 IN PLATELETS

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Inhibition of COX is used as antiplatelet therapy in secondary prevention of cardiovascular events, and is playing an emerging role in prevention of cancer metastasis. This indicates the relevance of this pathway for clinical medicine, and that identification of novel COX products is an important goal. Herein, we describe a novel COX product proposed to be 8-hydroxy-9,11-dioxolane eicosatrienoic acid (DXA3) and generated by thrombin activated human platelets, identified using a targeted lipidomic strategy. DXA3 is generated in vitro via oxidation of arachidonate by COX-1/COX-2, or by oxidation of 11-hydroperoxyeicosatetraenoic acid (11-HpETE) followed by cyclization at C9 and further oxidation at C8. Derivatisation and analysis of DXA3 and DXA3-d8 using high resolution LC and GC mass spectrometry established the specific fragmentation pattern, supporting the identification of DXA3 as a novel COX-1 product distinct from previously described eicosanoids. Development of a quantitative assay is underway, allowing measurement of free and esterified DXA3 in biological and clinical samples from human and murine studies. In summary, DXA3 represents a novel COX-derived eicosanoid, generated on platelet activation, that could play a role in COX biology in health and disease.

Poster 37

N-ACYLETHANOLAMINE HYDROLYZING ACID AMIDASE INHIBITION RAISES PEA LEVELS IN THE COLON AND COUNTERACTS MURINE COLITIS

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PEA is an anti-inflammatory, analgesic and neuroprotective bioactive lipid which was recently shown to exert PPAR-alpha-dependent beneficial effects on colon inflammation. The actions of PEA are terminated by its hydrolysis by two enzymes, fatty acid amide hydrolase (FAAH) and the more recently described N-acylethanolamine-hydrolyzing acid amidase (NAAA).
We sought to investigate the effects of inhibiting the two enzymes responsible for PEA hydrolysis in colon inflammation in order to propose a potential therapeutic target for inflammatory bowel diseases (IBD).

Two mouse models of IBD, dextran sodium sulfate (DSS)-induced colitis and trinitrobenzene sulfonic acid (TNBS)-induced colitis have been used to assess the effects of NAAA inhibition, FAAH inhibition and PEA on: macroscopic signs of colon inflammation, macrophages/neutrophils infiltration and the expression of pro-inflammatory mediators in the colon, as well as on the colitis-related systemic inflammation in the spleen, liver and brain.

NAAA inhibition increases PEA levels in the colon and reduces colon inflammation and systemic inflammation, similarly to PEA. FAAH inhibition, however, does not increase PEA levels in the colon and does not affect the macroscopic signs of colon inflammation or immune cells infiltration.

This is the first report of an anti-inflammatory effect of a systemically-administered NAAA inhibitor. Because NAAA is the enzyme responsible for the control of PEA levels in the colon, we put forth this bioactive lipid-controlling enzyme as a potential therapeutic target in chronic inflammation in general and IBD in particular.

Poster Session

Friday, October 24th, 2014

Poster 38

DOPING DRUGS AND OXIDATIVE STRESS

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Usage of doping drugs are the most important issue in Olympic Games. Doping drugs are forbidden by several communities and controlled strictly. But there is a growing danger under sportive community because of using these types of drugs by athletes and bodybuilders. Anabolic androgenic steroids (AASs) are a class of doping drugs that clinically used to treat some disorders in pharmacological doses, but these drugs widely abused at suprapharmacologically doses by athletes and bodybuilders. The abuse of AAS may cause side effects in several tissues at higher doses. Some studies showed that usage of AAS may cause mitochondrial respiratory chain disfunction dependent ROS increase, which leads oxidative stres and cell damage. The aim of this review is to explain AAS drugs caused side effects on several tissues and organs at suprapharmacologically doses.

Poster 39

QUANTIFICATION OF SELECTED ARACHIDONIC ACID METABOLITES IN URINE AND PLASMA OF NO-DEFICIENT MICE USING LC-MS/MS TECHNIQUE.

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Background: Arachidonic acid (AA) can be metabolized by various enzymes (e.g. cyclooxygenases, lipoxygenases, cytochrome P450 izoenzymes) as well as by nonenzymatic pathways. Cyclooxygenases are involved in production of prostanoids including prostaglandins (PGs), prostacyclin (PGI2) and thromboxane A2 (TXA2), which play a major role in the cardiovascular homeostasis and their production change in response to NO-deficiency.
Goals: The aim in this work was to develop a bioanalytical method for quantification of selected eicosanoids (e.g. TXB2, 2,3-dinor-TXB2, 11-dehydro-TXB2, 2,3-dinor-6-keto-PGF1alpha, 6-keto-PGF1alpha) in mice urine and plasma using LC-MS/MS method to estimate the changes in their biosynthesis in C57Bl/6J female mice with NO-deficiency and hypertension induced by L-NAME supplementation. Additionally, mice were simultaneously treated with COX-1 and COX-2 inhibitors such as aspirin and DuP-697, respectively. The platelet activation was also assessed by ex vivo dynamic TXB2 generation assay. Moreover, the concentration of NO2 and NO3 was determined to confirm the NO-deficiency after L-NAME treatment.
Methods: Quantification of investigated eicosanoids was performed using Ultrafast Liquid Chromatograph UFLC Nexera (Shimadzu) coupled to mass spectrometer QTRAP 5500 (ABSciex) equipped with TurboV ion source. The linearity range was estimated for all analytes in both urine and plasma specimens. Samples were prepared by liquid-liquid extraction with acidified ethyl acetate. On the other hand, the level of TXB2 after platelets activation was determined by ELISA. The plasma concentration of NO2 and NO3 was measured using NOx Analyzer ENO-20 (Eicom).
Results and Conclusions: L-NAME treatment resulted in hypertension and a decrease in NO2 plasma concentration confirming the NO-deficiency in mice treated with L-NAME. The ratio of PGI2 and TXB2 urinary metabolites measured by LC MS/MS as 2,3-dinor-6-keto-PGF1α and 2,3-dinor-TXB2 was similar between control and L-NAME groups throughout experimental period. However, in ex vivo assay of dynamic TXB2 generation, the concentration of TXB2 was elevated after 2 weeks but not after 4-8 weeks of L-NAME treatment suggesting transient platelets activation in the early phase of NO-deficiency. We suggest that overactivation of COX-2/PGI2 pathway partially compensate the NO-deficiency, thus a model of NO-deficient mice induced by L-NAME may be well suited for in vivo pharmacology of COX-2/PGI2 pathway action.
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Poster 40

CELLULAR ASSAY METHODS FOR DETECTION OF COMPOUNDS ENHANCING THE GENERATION OF LIPOXINS

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Uncontrolled inflammation is a characteristic of chronic diseases such as rheumatoid arthritis, diabetes and atherosclerosis. Recent findings indicate that the resolution of inflammation is an active process controlled through endogenous mediators and mechanisms that switch off acute inflammation by suppression of pro-inflammatory gene expression and cell trafficking and induce inflammatory cell apoptosis and phagocytosis. Lipoxins (LX) are a unique class of arachidonic acid (AA) derived lipid mediators displaying pro-resolving activities during the resolution phase of acute inflammatory reactions. In exchange between distinct cell types such as neutrophils and endothelial cells, neutrophils and platelets or even in single cells, AA undergoes a double oxygenation by the sequential action of two different lipoxygenases (LO) (either 15- / 5-LO or 5- / 12-LO) to form LX. Besides LO triggered LX synthesis, a series of epimeric 15-LX has been found. This group of 'alternative' LX is aspirin triggered. Here, acetylation by aspirin of cyclooxygenase-2 (COX-2) abolishes prostaglandin H2 (PGH2) synthesis while retaining the oxygenase activity of the enzyme, leading to the production of 15(R)-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15(R)-HETE) instead. Epimeric 15-HETE is then further processed by 5-LO to give rise to 15-epi-LXs which share many anti-inflammatory properties with the regular LX.
Diverse cell-based, in-vitro co-culture systems have been described in literature. Some of them we were able to establish. First of all, we spiked freshly isolated 5-LO positive, peripheral blood mononuclear leukocytes (PMNL) with 10 µM 15(S)-HETE, 15(R)-HETE and 17R-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid (17(R)-HDoHE) as a proof of principle. We were able to detect different epi- and native lipoxins as well as 17(R)-Resolvin D1 (17(R)-RvD1) via chiral LC-MS/MS analysis. In addition, we have detected LX biosynthesis so far in a variety of in-vitro systems. Co-culture experiments with PMNL/platelets, apoptotic PMNL/different macrophage phenotypes (M1/M2) and Human Umbilical Vein Endothelial Cells (HUVEC)/PMNL with/without aspirin provided various native and 15-epi-LXs. Incubations of macrophages alone failed to generate detectable LX via LC-MS/MS. Potential lipoxin enhancing drug candidates are being tested in the different cell-based systems.

Poster 41

COMPARISON OF 14-DAYS AND 28-DAYS ZINC HYDROASPARTATE SUPPLEMENTATION ON ANTI-INFLAMMATORY ACTIVITY OF KETOPROFEN

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Zinc is one of the most important trace element for living organisms. Its anti-inflammatory activity is scientifically proven, as well as its role in wound healing; especially in gastric ulcers. It acts in numerous processes such like proper functioning of enzymes, cell proliferation and differentiation. Zinc acts in metabolic processes, regulate activity of immune system and endocrine system. Studies have revealed that supplementation of zinc improves the anti-inflammatory of non-steroidal anti-inflammatory drugs (NSAIDs) and protect gastric mucosa against ulceration at the same time. Even some compounds of zinc have anti-edematous activity especially in the early phase of inflammation response. Numerous studies revealed that zinc supplementation increases anti-inflammatory activity of NSAIDs and decreases risk of ulcerations at the same time. In experiments, the effect of chronic (14 days and 28 days) administration of zinc hydroaspartate on anti-inflammatory activity of ketoprofen and its effects on gastric mucosa were investigated. It was observed that 14-days supplementation of zinc hydroaspartate gives better results when compound is administered per os. All of gain outcomes were statistically significant. Even administration zinc hydroaspartate only inhibited hind paw edema growth. Intraperitoneal supplementation of zinc hydroaspartate did not revealed statistically significant effects in the first hour of experiment. In 28-days studies it was observed that the zinc hydroaspartate administered intraperitoneally gives better results. In this way of administration zinc hydroaspartate inhibited the increase of paw edema even in first hour of experiment. When the compound was administered per os results were not so rewarding. The influence on analgesic activity of ketoprofen wasn't observed. Generally analgesic activity of ketoprofen was weak independently on way of zinc hydroaspartate administration. Statistically significant effect of zinc hydroaspartate administration on anti-inflammatory activity of ketoprofen was shown. To give a clear answer which way of zinc hydroaspartate administration is the best it is necessary to carry out more experiments with different model of inflammatory states. The present study demonstrated for the first time that chronic treatment with zinc salt exhibits anti-inflammatory activity. Besides, anti-ulcerogenic activity and the enhancing properties of zinc to ketoprofen induced anti-inflammatory and analgesic activity were also shown.

Poster 42

DIFFERENTIAL REGULATION OF CHEMERIN BY SEVERAL FATTY ACIDS AND DHA-DERIVED LIPID MEDIATORS IN CULTURED ADIPOCYTES

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Introduction: Chemerin is an adipokine that regulates adipocyte differentiation, immune function and metabolism through activation of chemokine-like receptor 1. Chemerin is elevated in obesity and strongly associated with markers of inflammation and components of the metabolic syndrome. Some studies have revealed that chemerin exacerbates glucose intolerance in obese and diabetic mice. The proinflammatory cytokine TNF-alpha is a stimulator of chemerin production in vivo and in cultured adipocytes.
Objective: The first aim was to carry out a comparative study of the effects of different types of fatty acids on basal and TNF-alpha-stimulated chemerin production in adipocytes. Moreover, the effects of resolvins (RvD1 and RvD2) were also evaluated.
Methods: Two models of cultured adipocytes were used: 3T3-L1 cells and human subcutaneous preadipocytes from overweight/obese subjects (Zen-Bio). Fully differentiated adipocytes were treated with oleic acid (OA), medium chain fatty acids [caprylic acid (CA) and lauric acid (LAU)], n-3 long chain polyunsaturated fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] (200-250 microM) or RvD1 and RvD2 (10 nM) in the absence or presence of TNF-alpha during 24 h. Changes in mRNA expression were assayed by RT-PCR. The amount of chemerin released into media was determined by ELISA.
Results: DHA reduced both basal and TNF-alpha-stimulated chemerin gene expression and protein secretion in 3T3-L1 adipocytes. In contrast, neither OA, CA, LAU nor EPA were able to reverse the stimulation of chemerin production induced by TNF-alpha. Importantly, the inhibitory effect of DHA on chemerin production, in the absence or presence of TNF-alpha, was also observed in subcutaneous human adipocytes from overweight/obese subjects. Interestingly, treatment with RvD1 and RvD2 also caused a significant downregulation of chemerin in the presence of TNF-alpha.
Conclusion: These findings uncover the ability of DHA and their bioactive lipid mediators RvD1 and RvD2 to reduce chemerin production in cultured adipocytes.

Poster 43

FROM MACROPHAGE CELL LINE TO PRIMARY PERITONEAL MACROPHAGES: STUDY OF LYSOPHOSPHATIDYLINOSITOLS LEVELS IN INFLAMMATION MODELS

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Lysoglycerophospholipids are glycerophospholipids in wich one of two acyl chains is absent and one hydroxyl group of the glycerol remains acylated. They are small membrane-derived lipids mainly generated by phospholipase A2. Lysophosphatidylinositols (LPIs) are a subspecies of lysoglycerophospholipids with inositol as the head group. Although LPIs remain less studied than other lysophospholipids (as lysophosphatidic acid or lysophophatidylcholine), increasing evidence support a role for LPIs as bioactive lipids. The demonstration that LPIs in general, and 2-arachidonoyl LPI in particular, are the endogenous ligands of the recently deorphanized GPR55 receptor sparked a renewed interest in the pathophysiological roles of these bioactive lipids. Indeed, the identification of a receptor for LPIs allows for pharmacological modulation of their effects with the use of agonists or antagonists of the receptor.
In this study, we decided to evaluate LPI levels in several cell lines activated with lipopolysaccharides (LPS) (J774 cells, BV2 microglial cells and primary peritoneal macrophages), using an HPLC-MS/MS method allowing for LPIs quantification from biological matrices.
J774 and BV2 cell lines and peritoneal macrophages were activated by incubation with LPS for 8 h. The levels of 16:0, 18:0 & 18:1 LPIs were increased in all the cell lines whereas 20:4 LPI was decreased in J774 and BV2 cells but not in peritoneal macrophages. 18:2 LPI was increased in J774 cells and peritoneal macrophage but was not affected in BV2 cells, in comparison with the control condition.
Morover, using Real-Time qPCR, enzymes implicated in lysopholipids metabolism, such as cPLA2, PA-PLA1 or ABHD6 were studied. The different expression profiles of these enzymes may explain the different variations in individual LPI levels.
The biological activities of LPIs as endogenous bioactive lipids are being unraveled, thus strengthening the need to study the impact of inflammation on LPI levels, and subsequently the effects of LPIs in inflammation. Here we propose a study of LPI levels using an HPLC-MS/MS method allowing for the determination of the relative levels of LPIs in cells.

Poster 44

AL PROTEOME VARIATION BETWEEN GENOTYPES FOR T. AESTIVUM AND T. DURUM SPECIES

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The aleurone layer (AL) is one of inner tissues removed from the grain with the wheat bran. Knowledge of wheat grain structure and of the composition of storage proteins, starch, lipids, vitamins, and micronutrients is essential for producing high quality grain. The AL of three varieties of each of the two main species of wheat, T. aestivum ABD) and T. durum (AB), were manually dissected and analysed using two-dimensional gelbased proteomics. Comparison within and between species revealed a total of 339 AL significant protein spots. Among these spots, 30.8% differed within T. aestivum and 56.5% within T. durum varieties, whereas only 12.7% differed between the two species. Many other proteins (43 %) which differed significantly between species were characteristic of living cells: glyceraldehyde-3-phosphate dehydrogenase, glucose and ribitol dehydrogenase, phosphoglucomutase, enolase, malate dehydrogenase, dehydroascorbate dehydrogenase; the observed qualitative and quantitative differences resulted from genetic diversity between genotypes. The majority of these enzymes play a key role in glycolysis to produce NADH, and ATP, the energy necessary for pyruvate formation. This metabolite is the starting point for the synthesis of amino acids, proteins, lipids for fatty acid biosynthesis, transport and signal transduction proteins.

Poster 45

INCREASED EPA-METABOLISM IN PBMCS OF HYPERLIPIDEMIC MEN

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Hyperlipidemia is an important trigger for the development of hypertension, atherosclerosis and further diseases of the cardiovascular system. In this study we determine using LC-MS an array of eicosanoids and docosanoids as well as calculated sums and ratios from plasma and PBMC samples of male volunteers. In plasma of hyperlipidemia (HL, n=13) vs control group (CTRL, n=35) cholesterol and triglyceride levels were increased in addition to elevated numbers of lymphocytes. In HL plasma the hypertension marker 20HETE (CTRL: 0.60±0.01 vs HL: 0.92±0.07 ng/ml) as well as various ratios related to eicosapentaenoic acid (EPA) metabolism were increased. In PBMCs the sum of PUFAs was strongly reduced (CTRL: 5231±8 vs HL 3691±35 ng/g), while EPA-metabolism via 5-LOX and CYP-pathways was increased and EPA was identified also as the favoured substrate for other LOX- and CYP-mediated metabolism in PBMCs. Especially the ratios of the resolving E2 precursors 5HEPE / EPA (CTRL: 0.01±<0.01 vs HL: 0.03±<0.01) and 18HEPE / EPA (CTRL: 0.06±0.01 vs HL: 0.28±0.06) were significantly increased indicating increased pro-resolving environment in PBMCs of HL group. In summary, a tendency of partly decreased EPA-metabolism in plasma and significant increased EPA-metabolism in PBMCs indicate strong pro-resolving environment in PBMCs from HL patients possibly to prevent inflammation and atherosclerosis development.

Poster 46

REDUCTION OF SPHINGOLIPIDS IN EXOSOMES AFTER RITUXIMAB TREATMENT IN SLE PATIENTS

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Background
Exosomes are small membrane vesicles produced within all types of blood cells. They are secreted into the blood and are important players in intercellular communications. The exosome membrane is highly enriched in cholesterol and ceramides and could therefore be actively involved in the sphingolipid synthesis. Sphingolipids are important mediators in the signaling cascades involved in apoptosis, proliferation and inflammation.

We have recently seen that certain sphingolipids are elevated in patients with systemic lupus erythematosus (SLE) and normalize following treatment with Rituximab. In order to further understand the mechanistic role of Rituximab on sphingolipids, we investigated the concentration of exosomes in patients with SLE, before and after Rituximab treatment.

Materials and Methods
EDTA-plasma from 18 SLE patients were used before and after Rituximab treatment. Exosomes were extracted by ultracentrifugation at 100 000 g in 60 minutes, at 4°C. Later, the exosome-rich suspension was incubated with CD63 positive beads for roughly 24 hours. Prior to the flow cytometric analysis, the exosome suspensions were also co-incubated with fluorescent labeled anti-ceramide antibody (FITC), which binds both to ceramide and sphingomyelin.

Results
Results showed that the concentration of exosomes (CD63+ events, i.e. tetraspanins) were significantly reduced after treatment (564± 63 vs 443±113 events, p=0.012). Further phenotyping reveled that the amount of sphingomyelin and/or ceramide in the exosome population was also significantly reduced (19.3±4.6 vs 17.2±4.3 mean fluorescence intensity, p=0.01)

Conclusions
In conclusion, we demonstrate that Rituximab treatment significantly reduces the total amount of exosomes and also the amount of ceramide and/or sphingomyelin positive exosomes. This reduction could influence the total levels of sphingolipids measured in plasma, as exosome membranes contain ceramide. Taken together, flow cytometric measurement of exosomes could offer a more accessible method for detection of sphingolipids in plasma and in the future, be used as a biomarker of inflammation and systemic autoimmune diseases.

Poster 47

PROFILING OF OXYSTEROLS BY HPLC-MS IN INFLAMMATION: APPLICATION TO LPS-ACTIVATED BV2 CELLS AND LPS-INDUCED INFLAMMATION IN MICE

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Oxysterols constitute a large family of oxidized derivatives of cholesterol. They are intermediates in the catabolism of cholesterol and contribute to its homeostasis. They are also considered as bioactive lipids due to their actions in several pathological conditions, including in inflammation and immunity. Several of their actions are due to their binding to the nuclear liver X receptors (LXRs) although LXR-independent actions are also being unraveled.
In order to further explore the role played by these bioactive lipids during inflammation we developed a HPLC-MS quantification method from biological matrices. The method allows for the quantification by isotope dilution of twelve oxysterols, from cells and murine tissues, using d_7-24(R/S)-OHC and d7-4β-OHC as internal standards for the oxysterols oxidized on the lateral chain and on the sterol backbone, respectively.
We applied this method to the quantification of oxysterols from LPS-activated BV2 cells, a microglia-like murine cell line. The oxysterol levels found following 4h and 8h of activation were compared to those of control cells.
In mice, we similarly compared the levels of oxysterols, in several tissues, after 4h and 8h of LPS administration.
Our results highlight different variations of the levels of these oxysterols (increase or decrease) after 4 or 8 hours in these two models. Because inflammation does not similarly affect the levels of all oxysterols, our results suggest that the roles of individual oxysterols in inflammatory settings could be different. Therefore some of the effects reported with oxysterols in the literature could be due to the sum of the individual effects of oxysterols, each with their own contributions. However, further studies are necessary to elucidate whether the alterations in oxysterol levels we observed are linked with pro- or anti-inflammatory effects, at the individual level as well as the mechanisms involved in their effects on inflammation and immunity.

Poster 48

SEX-SPECIFIC DIFFERENCES IN THE HUMAN LEUKOCYTE LIPIDOME

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Sex disparities are a widespread phenomenon in inflammatory diseases, though their molecular basis is poorly understood. Initiation and onset of inflammation is driven by leukocytes which release and respond to a variety of lipid mediators, including membrane lipids and their derivatives. Gender differences in the membrane lipid composition of leukocytes have surprisingly not been addressed by comprehensive lipidomic studies so far. To define a potential role of membrane lipids in mediating sex differences, we analyzed the cellular lipid profile of human monocytes and polymorphonuclear leukocytes (PMNL) – major cells of innate immunity - by ultraperformance chromatography-coupled ESI tandem mass spectrometry. More than 200 species of phoshpatidylcholines, -ethanolamines, -serines, -inositols, -gycerols, sphingomyelins, triacylglycerols and cholesterol ester have been detected. We found that the lipid composition of monocytes and PMNL was astonishingly conserved between individuals and gender. Neither the total amount nor the proportion of major structural lipids or arachidonoyl-containing phospholipids (as precursors of pro-inflammatory eicosanoids) was significantly changed. Significant differences were particularly evident for minor phosphatidylcholine and -ethanolamine species containing polyunsaturated fatty acids such as linoleic acid (18:2) and docosapentaenoic acid (22:5). Whether they exhibit lipokine-like function or are converted to bioactive metabolites will be subject to further studies.

Poster 49

NaHS INDUCES A VASORELAXATION RESPONSE IN ISOLATED BOVINE RETINAL ARTERIES INDEPENDENTLY FROM NO OR CYCLOOXYGENASE PATHWAY

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Hydrogen sulfide (H2S), with a characteristic odour of rotten egg, was considered as a toxic gas up to the last two decades. However, there are convincing evidences showing that it is produced endogenously and dominated several physiologically important effects. Limited data is available about the production and functional role of H2S in the eye. Indeed, an endogenous production of H2S was observed in various ocular tissues of bovine eye with the highest levels in retina and cornea. The retinal circulation is lack of autonomic innervation and thus regulated by local factors including vasoactive substances derived from retina and retinal arteries. H2S and H2S producing enzymes were shown to be present and active in the eye, specifically in the retina, and suggested to display several favourable effects through different cellular mechanisms. It is reasonable to assume that H2S, which is produced in the retina or the retinal arteries, might also have a role in the regulation of retinal arterial tone as a local factor. Herein, we aimed to investigate the effectiveness and the mechanism of action of H2S in isolated bovine retinal arteries. For this purpose, the probable vasorelaxant and inhibitory effects of H2S on vascular reactivity were tested comparatively in retinal arteries by using the donor, sodium hydrosulfide (NaHS). Thereafter, in relation to the mechanism of action of H2S, the role of nitric oxide (NO) and endothelial vasodilators of cyclooxygenase pathway were evaluated. NaHS (1μM-3mM) displayed notable relaxation responses over the concentration of 300 μM in both PGF2α and K+ precontracted retinal arteries. Comparatively, in the presence of NaHS, the maximum contractile responses to PGF2α and K+ were significantly reduced. Neither the presence of known inhibitors of NO synthase, guanylate cyclase, cyclooxygenase nor the removal of endothelium modified the relaxation responses to NaHS in retinal arteries. Our results suggested that H2S might play a substantial role in the regulation of retinal arterial tone independently of NO or cyclooxygenase pathway.

Poster 50

HIGH FAT DIET-INDUCED OBESITY CAUSES DELETERIOUS ALTERATIONS IN MYOCARDIUM: AN ELECTRON MICROSCOPIC STUDY USING A RAT MODEL

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Obesity, a worldwide phenomenon, is a serious health problem and causes to decrease in lifespan. Obesity associated with an increase in morbidity and mortality leads also to several forms of heart disease. In clinical and experimental studies is reported that obesity causes to cardiac hypertrophy and reduced myocardial contractility. The aim of this study is to examine effects of obesity on ultrastructure of myocardium in a rat model. Twelve 4-week-old male Sprague Dawley rats were randomly divided into two groups of six rats each and fed with standard rat chow (control group) or high-fat diet (HFD group) for three months. Body weights and naso-anal lengths of the animals were measured periodically and "body mass indices" (BMI) were calculated. At the end of the experiment, myocardium samples from the sacrificed rats were prepared for electron microscopic investigation and ultra-thin sections were examined using a Jeol 100SX transmission electron microscope. Mean BMI values were 5.89 ±0.16 kg/m2 in the control group and 6.25 ±0.18 kg/m2 in HFD group. The difference between BMI values of two groups was statistically very important, indicating that obesity was formed in HFD-fed animals. In microscopic examinations, some shrunken nuclei with condensed chromatin were seen in very close proximity to the sarcolemma within the myocardial fibres of HFD group. But, the nuclei within the muscle fibres of control group were centrally located. Transverse striations of the muscle fibers were less apparent in HFD group in comparison with the control group. Sarcoplasmic edema, many lipid droplets, myofibrillar atrophy and swollen mitochondria were remarkable in myocardial fibres of HFD group. Prominent degenerative changes of intercalated discs attaching adjacent muscle cells to each other were observed in obese rats. These alterations were as follows: opening of discal double membrane and disorganisations of myofilaments close to intercalated discs. Recent animal studies have shown that high fat diet may affect cardiac function negatively. Obesity is also a risk factor for congestive heart failure, but its pathogenic mechanisms leading to myocardial alterations remain unclear. Our study has demonstrated that obesity formed by HFD can trigger structural changes and cellular injury in rat cardiac muscle.

Poster 51

INVESTIGATION OF THE CYTOTOXICITY OF A SPECIAL ESSENTIAL OIL COMBINATION THAT HAS A PROMINENT ANTIMICROBIAL ACTIVITY

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Increasing antimicrobial resistance in pathogenic bacteria has created the need for the development of novel therapeutic agents. Antimicrobial treatments for bacterial infections have been aimed to eradicate infections by inhibiting microbial growth. Various antibiotics such as penicillin and sulphonamides are either toxic or inhibitory to bacterial growth. Now, pharmaceutical companies attempt to overcome a challenging problem. Indiscriminate use of antibiotics has created antimicrobial resistance in pathogenic bacteria. Increasing numbers of bacteria have gained higher tolerance against conventional antibacterial agents including broad-spectrum antibiotics. Therefore, the development of novel therapeutics to fight against infections has been a tough issue. Attempts have been done to find an innovative solution. The use of therapeutics derived from plants has gained great attention in recent years. Several plant essential oils have been tested for their antimicrobial activities. In compare to synthetic molecules, plant essential oils are always safer and healthier. It is easier and cheaper to obtain them in compare to the synthetic ones. That is why our research group attempted to test the antimicrobial efficacy of a special essential oil combination. Although this combination is used in traditional medicine, any scientific research for the essential oils is present that support its therapeutic application and mechanism of action. The special combination of Olea europaea, Nigella sativa and Rosemarinus officinalis essential oils showed a great antimicrobial activity at the end of our experiments. However, in order to be able to use this combination in human, it must have nontoxic properties. In the current study, a special combination of Olea europaea, Nigella sativa and Rosemarinus officinalis essential oils was investigated for its cytotoxicity. The cell viability was measured by the MTS assay according to the manufacturer's instructions. After HaCat cells were incubated with the different concentrations of oil combination for 24, 48 and 72 hours, MTS reagent with growth medium was added to cells and absorbance was read at 490 nm with an ELISA plate reader. The results of the study showed that the combination is nontoxic at the concentration that it showed antimicrobial activity.

Poster 52

INFLUENCE OF PROGRAMMED CELL DEATH ON THE CELLULAR LIPID COMPOSITION

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The membrane phospholipid arachidonyl-phospatidylcholine (20:4-PC) oscillates during the cell cycle and counteracts proliferation by suppressing Akt (protein kinase B) membrane binding (1). Whether changes in the phospholipid composition are linked to Akt activation during apoptosis (programmed cell death) has not been investigated so far. Thus, we induced apoptosis in NIH-3T3 mouse fibroblasts through different mechanisms and looked for similarities in the cellular lipid profile during the early apoptotic stages (10 min to 48 h) using ultraperformance liquid chromatography-coupled ESI tandem mass spectrometry (UPLC-MS/MS). More than 100 species of phosphatidylcholines, -ethanolamines, -serines, -inositols, -gycerols and sphingomyelins were quantified. The total amount of phospholipid subclasses was either decreased or remained unaffected depending on apoptotic inducer and phospholipid subclass. Most remarkable was the time-dependent increase of polyunsaturated phospholipids (including 20:4-PC) relative to plamitoleate-containing species, which was evident for all apoptotic inducers tested. The accumulation of polyunsaturated phosphatidylcholine correlated with a decrease in cell proliferation but not yet cell viability. Future studies shall address whether the shift in the phospholipid composition during apoptosis modulates Akt activation and thus the onset of apoptosis.

(1) Koeberle A, et al. (2013) Arachidonoyl-phosphatidylcholine oscillates during the cell cycle and counteracts proliferation by suppressing Akt membrane binding. PNAS 110(7):2546-51.

Poster 53

LONG-TERM FRUCTOSE CONSUMPTION GENDER INDEPENDENTLY LEADS TO OBESITY

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Objective: Currently, fructose accounts for 10% of daily caloric intake in many developed countries. High-fructose consumption has been shown to cause metabolic disturbances. However, limited data is available on whether the influence of fructose is modified by gender. Therefore, in this study, we investigated whether fructose intake (10% beverage) gender dependently impacts body weight gain and plasma glucose, triglyceride, VLDL and HDL levels in rats.

Methods: Four week-old male and female Wistar rats were divided into two groups: control and fructose. Fructose was given to rats in drinking water (10%), in order to approach the approximate sugar concentrations in common soft drinks. Food and liquid intakes and body weights were monitored weekly for 24 weeks in male and female rats. Plasma glucose, triglyceride, VLDL and HDL levels were measured at the end of the feeding.

Results: In the control groups, average daily food intake of male rats was found to be higher than that of female rats. Consequently, the daily caloric intake in male rats (72,6 ± 2,9 kcal) was found to be higher than that of female rats (50,5 ± 2,9 kcal). Likewise, in fructose groups, daily caloric intake, due to food and liquid consumptions, in male rats (94,9 ± 4,2 kcal) was higher than that of female rats (60,1 ± 2,2 kcal). Furthermore, fructose consumption gender independently enhanced plasma glucose, triglyceride and VLDL levels. Fructose did not change HDL levels both in male and female rats.

Conclusion: Our results suggest that fructose consumption gender independently could enhance the body weights. The increases in the body weights show a good correlation with the increases of caloric intakes. Long-term fructose intake caused hyperglycemia, hypertriglyceridemia in both male and female rats. Female estrogen did not offer any advantage over metabolic parameters.

Keywords: Fructose, gender, obesity, triglyceride, glucose, HDL

Poster 54

IMPROVING EFFECT OF RESVERATROL ON FRUCTOSE-INDUCED OBESITY, OMENTAL ADIPOSITY AND FATTY LIVER

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Objective: The increased consumption of fructose may contribute to the worldwide epidemic of metabolic syndrome. In this study, we have examined whether fructose intake influences obesity, omental adiposity, and plasma and liver lipid levels in conjunction with insulin receptor (IR) and Akt expression in rats. Resveratrol was tested for its potential efficacy on changes induced by fructose.

Methods: Four week-old male Wistar rats in the study were divided into four groups: control resveratrol, fructose and resveratrol plus fructose. Fructose was given as 10% solutions in drinking water. Feeding for all rats was maintained with a standard diet with or without resveratrol for 24 weeks. Liver protein levels for IR and Akt were determined using Western blot analysis.

Results: Dietary fructose increased plasma insulin and triglyceride levels, omental weight and hepatic triglyceride content leading to obesity. Moreover, fructose consumption impaired hepatic expression levels of IR and Akt. Resveratrol restored the metabolic irregularities as well as IR and Akt down-regulation in rats fed with fructose.

Conclusion: Long-term fructose consumption caused hyperinsulinemia, obesity, omental adiposity and liver triglyceride accumulation. The metabolic dysfunction induced by fructose consumption positively correlates with the attenuation in hepatic IR/Akt expression. Resveratrol improved body and omental weight gains in fructose fed rats, which is attributable to its caloric restriction mimetic effect. Resveratrol implies regulation of insulin signaling pathway up-regulating IR and Akt expression. Dietary resveratrol supplementation could be useful in the alleviation of fructose-induced metabolic disturbances.

Keywords: Fructose, obesity, omental adiposity, fatty liver, IR, Akt, resveratrol

Poster 55

PSEUDOMONAS AERUGINOSA ERADICATES STAPHYLOCOCCUS AUREUS BY MANIPULATING THE HOST IMMUNITY: A ROLE FOR A SECRETED PLA2

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Cystic fibrosis (CF) is a lethal autosomal, recessive inherited disease that commonly affects Caucasians. This disease is due to mutation of CF transmembrane conductance regulator (CFTR) gene that encodes a protein channel in epithelial cells where it regulates the luminal secretion of chloride and water transport. Airways of CF patients are mainly colonized by Staphylococcus aureus (SA) while Pseudomonas aeruginosa (PA) predominates in adults. However, the mechanisms behind this infection switch are unclear. The type-IIA secreted phospholipase A2 (sPLA2-IIA) is a host enzyme endowed with bactericidal activity.
Here we showed that sPLA2-IIA levels increased in expectorations of CF patients in age-dependent manner. These levels were sufficient to kill SA with only marginal effects on PA strains. Killong of SA was due to selective hydrolysis of membrane phospholipid hydrolysis of this bacterium by sPLA2-IIA. Bronchial epithelial cells (BECs) are major cell source of this enzyme in CF patients. Both laboratory strains and PA isolates from CF patients induced sPLA2-IIA expression by BECs from CF patients. In animal model of lung infection, PA induced sPLA2-IIA production that favors SA killing. We suggest that sPLA2-IIA induction by PA contributes to SA eradication in CF airways. This highlights a new concept suggesting that a bacterium can eradicate another bacterium by manipulating the host immunity.

Poster 56

SIMILARITY OF LIPID LEVELS IN PEOPLE WITH CARDIO-VASCULAR DISEASES AND IN PEOPLE WHO WORK IN HAZARDOUS INDUSTRIES

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It is necessary to analyze the lipid spectrum to diagnose and monitor diseases of cardiovascular system. It is a statement that high cholesterol in the blood is one of the major causes of cardiovascular diseases. In developing countries that statement is never checked. It is because of lack of funds for lipid control or due to the absence of necessary equipment. So there are no systematic analysis of the lipid spectrum. Systematic check of lipid spectrum is not performed in people working in hazardous industries as well. Especially when working with harmful substances. Therefore, the lack of monitoring of lipid spectrum limits the possibility of primary prevention. On the second hand it is almost impossible to prevent the risk of a wide range of diseases, associated with xenobiotic contact.

We have analyzed the status of cholesterol in patients with cardiovascular problems in Lviv (Cardiac Group - (CG)), and people who work in hazardous industries (HIG).

We find changes of lipids in both groups. Cardiac group, as opposed to the stereotype, is not characterized only by increased levels of LDL or total cholesterol. There was also significant decline of triglycerides in this group. This means that the development of cardiac diseases may be accompanied not only by elevation of all lipid levels, but also with it\'s decline. It is interesting that the same effect on lipid status was in people working with xenobiotics in hazardous industries.

The negative impact of harmful factors on the lipid level is significant. Differences between the average levels of lipids in CG and HIG show that xenobiotics may have an impact not only on the cardiovascular system. So a constant influence of xenobiotics leads not only to atherosclerotic changes in vessels, but can also cause hipolipidemiy.

So it is important to count main components of the lipid spectrum when treating people in a CG. The same is about control of cholesterol in people from HIG. Also there is a reason to continue research about the impact of harmful factors in hazardous industries, as well as further studies about cholesterol status of people who have cardiovascular problems.

Poster 57

THE EFFECT OF mPGES-1 DELETION ON PLATELET FUNCTION IN MICE

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Background: Microsomal prostaglandin E synthase-1 (mPGES-1) is an inducible terminal synthase that generates PGE2 and is one of the key players in inflammation. Genetic deletion and pharmacological inhibition of mPGES-1 are protective in experimental models of inflammation. Platelets play essential roles in homeostasis and modulation of inflammatory processes, releasing a variety of cytokines, chemokines and lipid mediators. Activated platelets have been recognized as major players in autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, multiple sclerosis). Targeting mPGES-1 alters eicosanoid profiles and may potentially affect platelet function.

Objective: To investigate the effect of mPGES-1 deletion on platelet function in mice stimulated with LPS.

Methods: mPGES-1 WT and KO DBA mice were injected with 2µg LPS (n=6) or saline (n=5) i.p, for 24h. Mice were anesthetized and blood was slowly drawn by a syringe containing 100µl 3.8% sodium citrate from the heart and transferred to tubes containing 100µl sodium citrate to avoid platelet activation. Platelets were stained with anti-CD41 together with anti-CD62P (P-selectin) or anti-CD154 (CD40L) antibody and analyzed by whole blood flow cytometry. Platelet-leucocyte interaction was also investigated.

Results: LPS stimulation reduced the number of platelets in WT mice in contrast to KO. Moreover, LPS stimulation increased platelet activation (as assessed by CD62P, CD154 expression and platelet-leukocyte interaction) in both WT and KO. In addition, the degree of platelet activation (CD62P expression) as well as platelet-leukocyte interactions was higher in WT compared to KO. Surprisingly, CD40L expression on platelets showed high levels in KO compared to WT, however when CD40L expression was measured regardless of cell-origin CD40L was low in KO mice indicating low activation of inflammation.

Conclusions: mPGES-1 deletion affected platelets number and activation in LPS stimulated mice. The data suggest possible role of mPGES-1 in platelets function in inflammation.

Poster 58

QUANTIFICATION OF BIOACTIVE LIPIDS IN OSTEOARTHRITIS SYNOVIAL FLUID COMPARED TO CONTROLS

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Abstract : Osteoarthritis (OA) is an extremely common type of degenerative joint disease and is a major cause of chronic pain and disability. Changes to both the structure and local biochemical environment of the diseased joint contribute to both disease progression and associated pain. The aim of the present study was to determine the impact of knee OA on levels of bioactive lipids locally the joint versus systemically. Using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method (1) we have quantified levels of 25 bioactive lipids in synovial fluid (SF) and plasma collected from individuals with established OA, versus healthy aged match controls. 142 individuals (68 healthy controls and 74 individuals affected with knee OA) were recruited and plasma and urine collected. SF was collected from 71 affected knees of OA patients, 13 unaffected knees from OA patients and 44 control knees.
Plasma levels of 15-HETE and 8-HETE were significantly associated with knee OA, a lower concentration was correlated with a lower prevalence of knee OA. Levels of five bioactive lipids (11,12-DHET, 14,15-DHET, 16-HETE, LTB4 and PGD2) were increased in SF from OA knees, compared to healthy controls. For nominally significant lipids we estimated the association between healthy (K/L< 2) and OA knee within a patient (n=10). We considered only lipids that show the same direction as observed in the OA vs healthy control analysis. Only three (PGD2 and 11,12-DHET and 14,15-DHET) of the five bioactive lipids showed a consistent effect when the levels in SF were compared between the OA knee and the unaffected knee of OA patients (Table 1). Our data suggests that some markers of inflammation such as LTB4 may represent systemic effects, whilst others (PGD2 and 11,12-DHET and 14,15-DHET) may be very localized and OA specific.
(1) Wong A, Sagar DR, Ortori CA, Kendall DA, Chapman V, Barrett DA (2014). Simultaneous tissue profiling of eicosanoid and endocannabinoid lipid families in a rat model of osteoarthritis. J Lipid Res (in press).
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Poster 59

5-LIPOXYGENASE: A NOVEL TARGET FOR STEM CELL THERAPY IN ACUTE MYELOID LEUKEMIA

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Current therapeutic strategies, regardless of whether conventional cytotoxic or novel approaches to molecular therapy, often fail to eradicate cancer, because they are unable to efficiently target the cancer stem cell (CSC). Therefore, one of the major therapeutic challenges is to target the CSC. A key signaling pathway for the maintenance of CSC in many cancer entities is the Wnt-signaling pathway. Compounds which are able to inhibit Wnt-signaling have enormous therapeutic potential in the setting of maintenance therapy. Non-steroidal anti-inflammatory drugs (NSAIDs), developed as COX-1/2 inhibitors are efficient Wnt-signaling inhibitors. However, anticancer effects of NSAIDs seem to be independent of COX. Furthermore, at concentrations at which some NSAIDs exert inhibitory effects on Wnt-signaling and aberrant stem cell capacity, they inhibit not only COX but also 5-Lipoxygenase (5-LO). 5-LO has recently been shown to be indispensable for the maintenance of stem cells in a model of CML (chronic myeloid leukemia)-like disease. Therefore, we investigated in a model of AML (acute myeloid leukemia) the effects of genetic and pharmacological targeting of 5-LO on the leukemic stem cells.
Our findings show that pharmacological inhibition of 5-LO interferes with the aberrant replating efficiency and inhibit the short-term (ST) and long-term (LT) stem cell capacity of PML/RAR-expressing HSPCs. As revealed by inhibitor studies, reporter gene assays and the genetic disruption of 5-LO, Wnt and stem cell inhibition is mediated by the enzymatically inactive form of 5-LO which is able to co-localize with β-catenin and hinders its translocation into the nucleus.
These data establish 5-LO inhibitors as Wnt-inhibitors whose effects are not due to interruption of 5-LO-mediated lipid signaling rather than generation of a catalytically inactive form of 5-LO which acquires novel functions. Because leukemic stem cells are significantly involved in disease relapse after remission, eradication of these cells via pharmacological inhibition of 5-LO may represent as a novel clinical approach to treat AML patients.

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DEFICIENCY OF PROSTACYCLIN SYNTHASE REDUCES DIET-INDUCED OBESITY IN MICE

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Prostacylin (PGI2) synthase (PGIS) is the final committed enzyme in the metabolic pathway leading to PGI2 production. Although it has been suggested that PGI2 receptor IP regulates adipocyte differentiation and is involved in adiposity, the role of PGIS in adiposity is still not clear. In this study, we used PGIS-deficient mice to examine the involvement of PGIS in adiposity in vivo.
When fed high fat diet (HFD), obesity and weight gain was significantly lower in PGIS-deficient mice than wild-type (WT) mice. Epididymal fat mass and adipocyte size in the fat tissue were reduced in PGIS-deficient mice relative to WT mice. HFD-induced liver steatosis was also suppressed by PGIS deficiency. We next determined the amount of prostanoids in epididymal fat and found that prostaglandin (PG) E2 and PGF2alfa levels were increased in PGIS-deficient fat, whereas 6-ketoPGF1alfa, a PGI2 metabolite, was not detectable. Furthermore, we divided epididymal white adipose tissues derived from WT mice into adipocytes and stromal vascular fraction (SVF) and examined PGIS expression in these fractions. In SVF, PGIS mRNA expression was higher than adipocytes and increased in HFD-fed mice. Our immunohistochemical analysis revealed that PGIS was expressed in the vessels in stroma. Finally, to investigate the involvement of PGIS in adipocyte differentiation, mouse embryonic fibroblasts (MEF) were prepared from WT or PGIS-deficient mice and then differentiated into adipocytes in vitro. After 8 days of adipogenic induction, adipocyte differentiation in PGIS-deficient MEF was similar to that in WT MEF. We further found that a PGI2 analog, carbacyclin, significantly exacerebate adipogenesis of both WT and PGIS-deficient MEF.
These results indicated that PGIS deficiency reduced adiposity via suppression of PGI2 synthesis. In adipose tissues, PGIS-derived PGI2 from SVF might regulate adipogenesis.

Poster 61

DETERMINATION OF PCDD/FS AND DIOXIN-LIKE PCBS IN THE AMBIENT AIR OF THE CEMENT INDUSTRY IN SOUR EL GHOZLANE SUBURBAN ATMOSPHERE, ALGIERS, USING THE CALUX BIOASSAY AND THE SENSITIVE H1L7.5C1 MOUSE HEPATOMA CELL LINE

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Since the CALUX (Chemically Activated LUciferase gene eXpression) bioassay is a fast and inexpensive tool for the throughput analysis of dioxin-like compounds in a large number of samples and requires only small sample volumes.
In this study, a new method for the analysis of PCDD/Fs and dioxin-like PCBs in Ambient air of the cement industry in Sour El Ghozlane suburban atmosphere at a sampling point from March 2013 to April 2013. with the CALUX bioassay was developed, optimized and validated. The method consists of 4 steps: sampling, extraction, clean up and bioassay analysis.
To avoid the use of large amounts of toxic solvents, new techniques were used for filtration and extraction. During sampling, the airborne particulate matter was enriched onto PTFE filters by using a two medium volume samplers with or without a size-selective inlet for PM10 and TSP were used and each sampling period lasted approximately 24 h. and an Accelerated Solvent Extractor (ASE) was used instead of the traditional soxhlet extraction. After sampling and extraction the extract, clean up was done using a multi-layer silica gel column coupled to a carbon column. The PCDD/F and PCB fractions were finally analyzed with the H1L7.5c1 mouse hepatoma cell lines. The 24 h average concentrations of PCDD/F and PCB of Sour El Ghozlane suburban atmosphere were found in the range 4.76–165.76 CALUX BEQ pg/m3 and 28.63–800.14 CALUX BEQ pg/m3, respectively, in the sampling period. The limit of quantification was 1.4 CALUX BEQ pg/m3 for the PCBs and 5.6 CALUX BEQ pg/m3 for the PCDD/Fs, when using the new sensitive H1L7.5c1 cell line.

Poster 62

TURBULENT FLOW TECHNIQUE FOR SELECTIVE EXTRACTION OF DOCOSAHEXAENOIC ACID AND EICOSAPENTAENOIC ACID FROM HUMAN SERUM FOLLOWED BY ULTRA-FAST LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY ANALYSIS

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Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the major n-3 poly-unsaturated fatty acids (PUFAs) in fish oil. Dietary supplementation with n-3 PUFAs has recently become popular and their adequate intake during pregnancy, prematurely delivered newborns and early childhood is of clinical importance. The results of experimental and epidemiological investigations reveal that n-3 PUFAs, especially α-linolenic acid (ALA), eicosapentaenoic acid and docosahexaenoic acid may decrease the risk of cardiovascular diseases. Numerous reports have revealed that appropriate fetal development, including neuronal, retinal and immune function depends on EPA and DHA levels which are crucial also for prevention of preterm birth. Thus the supplementation of EPA and DHA is highly recommended during pregnancy although the optimal dosing and treatment strategies still need to be determined.
Developing rapid methods for measuring long-chain (n-3) poly-unsaturated fatty acid contents has been a crucial request. A turboflow procedure using TurboFlow HTLC column combined with Acclaim RSLC analytical column was developed for extraction, visualization, and quantification of DHA and EPA in human serum. The turboflow protocol was optimized and the best conditions of the loading solvent were 100% of methanol as the washing solvent, and 80:20% of acetonitrile/water as eluting solvents. The results indicated that in comparison to previously published methods, this strategy was effective and efficient in extraction, characterization, and determination of DHA and EPA in human serum. Circulating DHA and EPA serum levels in steady state were measured in prematurely delivered newborns. DHA and EPA can provide an important complement of lipid mediators.

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INHIBITION OF MPGES1 IN HUMAN VESSEL, ARGUMENT FOR CARDIOVASCULAR SAFE ALTERNATIVE TO COXIB

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Background and aim: Prostaglandin E2 (PGE2) is synthesized from arachidonic acid through the enzymatic activities of two cyclooxygenases (COX-1 and/or COX-2) and mostly microsomal prostaglandin E synthase-1 (mPGES-1). Inhibition of PGE2 by COX-2 inhibitors is effective in reducing inflammation but their cardiovascular side effects limit their use. Recently mPGES-1 inhibitors have been thought as a potential novel therapy, an alternative to COX-2 inhibitors. The aim of our study was to evaluate the effect of mPGES-1 inhibitor on human vascular tone under normal or inflammatory conditions.
Methods: Using organ bath experiments, contractions induced by norepinephrine (NE) were performed on fresh isolated human saphenous vein (SV) and also cultured (18h) in the presence (+Inflam) or absence (-Inflam) of both interleukin-1beta (IL-1β) and lipopolysaccharide (LPS). The maximal effect (Emax) expressed as % of KCl (40mM) initial contraction and sensitivity (pEC50) of the vessels to NE were calculated. In addition the vascular preparations could be incubated 30min without (Control) or with a mPGES1 inhibitor (Compound-3 (C3), 10µM; Leclerc *et al.,* 2013).
Results: In fresh SV preparations, incubation with C3 significantly attenuated vascular reactivity to NE (Control: Emax=130±3%, C3: Emax=97±3%, n=7) and pEC50 (Control: pEC50=6.6±0.1, C3: pEC50=6.0±0.1). These reductions were reversed after co-incubation with both C3 and, IP receptor antagonist Cay10441 (1µM, 30min). Under inflammatory conditions, the maximum contraction induced by NE and pEC50 induced by NE in human SV were decreased (-Inflam: Emax=147±22%, pEC50=6.52±0.17; +Inflam: Emax=101±13%, pEC50=6.16±0.25, n=3) In inflammatory condition, C3 significantly reduced vascular reactivity to NE (+Inflam and Control: Emax=106±7%, pEC50=6.29±0.21; +Inflam and C3: Emax=59±10%, pEC50=4.68±0.40, n=3).
Conclusion: Our results showed that mPGES1 inhibitor (C3) attenuated vascular tone induced by NE in both normal and inflammatory condition (more potently +Inflam ~1.8 fold). This reduction in vascular tone might be associated with increased PGI2 release probably due to an increase of PGH2 availability for prostacyclin synthases (PGIS). In contrary, it was shown that COX-2 inhibitors cause direct increase in vascular tone (Foudi *et al.,* 2009) and that could be related with their cardiovascular risks. mPGES1 inhibitors might been thought as a cardiovascular safe alternative to COX-2 inhibitors in the treatment of inflammatory diseases.

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Poster 64

INVESTIGATION OF COX-2 INHIBITION IN VITRO – A COMPARISON OF DIFFERENT TEST SYSTEMS

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Cyclooxygenase-2 (COX-2) is an enzyme of the arachidonic acid cascade converting arachidonic acid and other polyunsaturated fatty acids to the biologically active prostaglandins (PG). The expression of COX-2 is among others regulated by the pro-inflammatory transcription factor NF-kappaB. Activation of COX-2 is thought to cause pain, fever and inflammation by giving rise to high levels of PGs, especially PGE2. Moreover, PGE2 plays a key role in cancer promotion and progression. This makes COX-2 to one of today's most relevant pharmaceutical targets.
Several in vitro assays have been developed to determine the effect of pharmaceuticals or natural compounds on COX-2 activity. However, information on the comparability of the results from these assays and the predictability for effects on COX-2 activity in vivo is scarce.
In this study the potency of the pharmaceuticals celecoxib, indomethacin and dexamethasone were investigated in different test systems: (I) a cell free system utilizing recombinant COX-2, (II) the permanent human colon carcinoma cell line HCA-7 constitutively expressing COX-2 and (III) primary human monocytes which express COX-2 upon lipopolysaccharide stimulus.
PGE2 levels were quantified by means of LC-MS with online-solid-phase-extraction as automated sample preparation. This powerful analytical tool allows the sensitive quantitative analysis of PGE2, PGD2, and thromboxane B2, with a short analysis time of 7 minutes per sample. Moreover, concentrations were determined with good accuracy (89-113%) following direct injection of samples from COX-2 assays.
The comparison of the IC50 values determined with different test systems indicated that the effect on COX-2 activity highly depends on the used test system. For example, indomethacin showed an IC50 of 362 nM in the cell free assay and IC50 values of 583 nM in HCA-7 cells and 10.3 nM in primary monocytes. Thus an effective action on acute inflammation can be assumed, while the efficacy on reducing PGs in tumor cells seems to be low. Our results demonstrate that the selection of the appropriate test system is crucial to get meaningful results for biological questions regarding COX-2 inhibition. Moreover, the potency of COX-2 inhibitors shall only be compared within one test system using exactly the same assay conditions.

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INHIBITION OF SOLUBLE EPOXIDE HYDROLASE MODULATES INFLAMMATION AND AUTOPHAGY IN OBESE ADIPOSE TISSUE AND LIVER. ROLE FOR OMEGA-3 EPOXIDES.

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The enzyme soluble epoxide hydrolase (sEH) is an established therapeutic target in cardiovascular and inflammatory diseases. sEH limits tissue levels and availability of epoxy metabolites derived from cytochrome P-450 epoxygenases by converting these anti-inflammatory mediators into their inactive diols. In this study, we explored the metabolic effects of a sEH inhibitor (sEHi: trans-4-{4-[3-(4-trifluoromethoxy-phenyl)-ureido]-cyclohexyloxy}-benzoic acid) in the context of high tissue levels of omega-3 fatty acids. To address this without the need for a dietary supply, we used mice with transgenic expression of the fat-1 gene, which encodes an omega-3 desaturase capable of enriching tissues endogenously with omega-3 fatty acids. Obese fat-1 mice showed increased epoxygenase (CYP1A1, CYP2E1 and CYP2U1) and sEH expression in adipose tissue. Consistently, LC/ESI-MS/MS analysis identified increased omega-3 epoxygenase metabolites 17,18-epoxyeicosatetraenoic acid (17,18-EEQ) and 19,20-epoxydocosapentaenoic (19,20-EDP) levels in these mice. As compared to placebo, fat-1 mice receiving sEHi displayed a further reduction in adipocyte hypertrophy and total fat volume accompanied by increased interscapular brown adipose tissue (iBAT) volume, as monitored by magnetic resonance imaging (MRI). These mice also showed a more remarkable reduction in macrophage infiltration and collagen deposition in the adipose tissue. In addition, MRI spectroscopy revealed a more intense anti-steatotic action in obese fat-1 mice treated with sEHi. Notably, sEHi attenuated lipid peroxidation and protein expression of LC3-II in adipose tissue, while increasing this established marker of autophagy in the liver, a dual action that is interpreted as beneficial in lipid homeostasis and metabolic control. Conclusion: our findings confirm the beneficial effects of omega-3 fatty acids in obesity-related disorders and suggest a role for omega-3 epoxides in regulating inflamma
tion and autophagy in insulin-sensitive tissues.

Poster 66

ATHEROSCLEROTIC PLAQUES PRODUCE LTB4 WHICH CONTRIBUTES TO NEUTROPHIL RECRUITMENT.

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Leukotriene B4 is an inflammatory eicosanoid mediator generated by the 5-lipoxygenase (5-LO) in the arachidonic acid pathway. One of its main roles is to recruit and activate immune cells, in particular neutrophils. Numerous experimental and genetic studies have suggested that LTB4 could affect impact atherosclerosis; however the function of LTB4 as a chemotactic agent for neutrophils in this disease, and the resulting consequences on plaques, have never been assessed.

Using EIA, we quantified the production of LTB4, in atherosclerotic plaques of ApoE-/- mice subjected to a chow diet, a high fat diet, or treated with LPS. Only mice that had received LPS injections showed a significant production of LTB4 in their aortic plaques, indicating that an infectious state can activate the 5-LO signalling pathway in atherosclerosis. To determine whether this activation would lead to the attraction of neutrophils, we assessed the ability of labelled neutrophils to enter plaques in mice that had been injected with LPS to enhance the LTB4 production of the plaques. We compared neutrophil plaque invasion in mice with an impaired production of LTB4 (ApoE-/-5-LO-/-) to control mice (ApoE-/-5-LO+/+). Fluorescence microscopy analyses, confirmed by FLIM, showed that neutrophil entry was significantly decreased in plaques of ApoE-/-5-LO-/- mice. This result shows that LTB4 has a critical role in the chemotaxis of neutrophils to atherosclerotic plaques.

Our results evidence the role of LTB4 in neutrophil recruitment to atherosclerotic plaques. Further investigation is needed to explore the behaviour of recruited neutrophils in this inflammatory context and their potential impact on the plaque.

Poster 67

COMPARISON OF SOLID PHASE EXTRACTION PROTOCOLS FOR LC-MS BASED ANALYSIS OF OXYLIPINS

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Oxidation products of polyunsaturated fatty acids – oxylipins – are potent mediators of various physiological processes. In order to understand their biological roles, accurate analytical methods for oxylipins in complex matrices are required.

LC-MS is the technique of choice for quantitative oxylipin analysis. Powerful methods have been developed allowing the simultaneous analysis of a large number of lipid mediators.

Sample preparation is generally carried out by solid phase extraction (SPE). Some of the methods utilize mixed mode SPE phases, like the polymeric Waters-Oasis-HLB (Waters, Eschborn, Germany) or the silica based Bond Elut Certify II column (Agilent, Weilbronn, Germany) modified with non-polar octyl groups and a strong anion exchanger. Also non-polar polymeric phases like the Strata-X (Phenomenex, Aschaffenburg, Germany) or the octadecyl modified silica gel based Waters SepPak tC18 (Waters) are frequently used. Despite the importance of a high accuracy of the quantitative analysis for the understanding of oxylipin biology, only scarce data is available on the performance of the different SPE protocols.

The aim of the present study was to identify the best SPE method for the analysis of none esterified oxylipins in human plasma. For this purpose, we compared the performance of the five most frequently used SPE protocols for oxylipin analysis.

Recovery rates of 13 deuterated internal standards (IS) from different oxylipin classes spiked to pooled human EDTA plasma were determined. Moreover, extraction efficacies of oxylipins from plasma were investigated by comparing absolute peak areas and calculated concentrations. In order to understand if the apparent losses of IS occur during SPE, samples were spiked at different time points in the preparation procedure. Furthermore, we investigated ion suppression of the SPE extracts by post column infusion of a standard mix.

The tested protocols led to dramatic differences in the IS recoveries resulting in different plasma oxylipin patterns. Based on the correlation of extraction efficacy and ion suppression data, the obtained variances between protocols were mechanistically investigated and will be thoroughly discussed. Finally, the SPE protocol giving in our hands the best results for the analysis of oxylipins in human plasma is identified.

Poster 68

DESIGN AND SYNTHESIS OF NOVEL 2-MERCAPTO BENZOTHIAZOLE AND 1,2,3-TRIAZOLE CONJUGATES AS COX-2 INHIBITORS.

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Over the years several NSAIDs have been developed as COX 1 and COX 2 inhibitors to reduce the pain. NSAIDs inhibit COX enzymes, which play a key role in synthesizing prostaglandins from arachidonic acid that is released from the plasma membrane by the action of phospholipase A2. Due to the adverse effects caused by several compounds scientists are in search for compounds that directly targets COX-2, an enzyme responsible for inflammation and pain. Targeting selectivity for COX-2 reduces the risk of peptic ulceration. Benzothiazoles have been known for their diverse biological activities including anti-inflammatory agents and are part of several bio-active molecules. Keeping in view the biological potentials of benzo thiazoles and 1,2,3-triazoles a focused library of novel bis-heterocycles encompassing 2-mercapto benzothiazole and 1,2,3-triazoles were synthesized using click chemistry approach. The synthesized compounds have been tested for their anti-inflammatory activity by using biochemical cyclooxygenase (COX) activity assays and carrageenan-induced hind paw edema. Among the tested compounds, compound 4d demonstrated a potent selective COX-2 inhibition with COX-2/COX-1 ratio of 0.44. Results from carrageenan-induced hind paw edema showed that some of the compounds posses significant anti-inflammatory activity as compared to the standard drug Ibuprofen. The compounds showing significant activity were further subjected to anti-nociceptive activity by writhing test and they exhibited comparable activity with the standard Ibuprofen. Further ulcerogenic studies shows that none of these compounds causing gastric ulceration. All these compounds were further screened for their anti-tubercular activity against Mycobacterium tuberculosis H37Rv strain by broth microdilution assay method. Some of the compounds inhibited the growth of the H37Rv strain at concentrations of 8 μg/mL. These compounds have been further identified as bactericidal and are completely killing the microbes at 32-64 μg/mL concentrations. Molecular docking studies of the active compounds reveal that these compounds are targeting DprE1 and may act as DprE1 inhibitors. These results indirectly indicate that the compounds capable of inhibiting PGE 2 (COX 2) may also be explored against the PGE2 mediated deceases like cancer, tuberculosis etc.

Poster 69

AUROTHIOMALATE INHIBITS THE EXPRESSION OF mPGES-1 IN PRIMARY HUMAN CHONDROCYTES

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Microsomal PGE synthase-1 (mPGES-1) is a terminal enzyme in the production of prostaglandin E2 (PGE2), and its expression is upregulated during inflammation. mPGES-1 is considered as a potential drug target for the treatment of arthritis to reduce adverse effects related to the current non-steroidal anti-inflammatory drugs (NSAIDs). Our objective was to study the expression of mPGES-1 in primary human chondrocytes, and to investigate the effects of clinically used antirheumatic drugs on it.

Primary human chondrocytes were isolated from cartilage samples obtained from patients undergoing a total knee replacement surgery. Expression of mPGES-1 was studied by quantitative real-time PCR and Western blot analysis. Prostaglandin E2 levels were measured by enzyme-linked immunosorbent assay.

mPGES-1 expression in primary human chondrocytes was enhanced when the cells were exposed to interleukin-1beta; and mPGES-1 protein levels continued to increase up to the 96 hours' follow-up. Interestingly, aurothiomalate inhibited mPGES-1 expression and PGE2 production in a dose-dependent manner as did the anti-inflammatory steroid dexamethasone. Other disease-modifying antirheumatic drugs (DMARDs) studied, i.e. sulfasalazine, methotrexate and hydroxychloroquine did not alter mPGES-1 expression.

The results introduce aurothiomalate as the first and so far the only DMARD found to be able to inhibit mPGES-1 expression. The effect is likely involved in the mechanisms of action for this gold containing DMARD in rheumatic diseases. The results are implicated in the regulatory mechanisms of mPGES-1 that are under intensive research.

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EFFECTS OF SPHINGOSINE-1-PHOSPHATE ON THE CONTRACTILE REPONSIVENESS OF VASCULAR SMOOTH MUSCLE

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We aimed to examine the effect of S1P on the vascular tone and to elucidate the underlying signalling pathways. Experiments were conducted on thoracic aorta segments isolated from adult male wild type (WT), as well as S1P2, S1P3 receptor, eNOS and Galpha12/13 knock-out (KO) mice under isometric conditions with myography.
Firstly, the changes of the resting vascular tone were determined in the presence of S1P. Then the effect of incubation with S1P on K+-induced vasoconstrictions was examined. Furthermore, we determined the eNOS mediated vasorelaxation to acetylcholine in WT vessels before and after S1P treatment.
S1P (10 μM) induced weak contraction in WT segments, which was abolished by the Rho-kinase inhibitor Y-27632. The contractile effect of S1P was similar in S1P3-KO but was absent in S1P2-KO and Galpha12/13-KO vessels. Incubation with 10 microM S1P for 20 minutes enhanced the contractile effect of 20 mM K+ in WT segments. This potentiation was present in S1P3-KO but not in S1P2-KO and Galpha12/13-KO vessels. Interestingly, K+-induced contractions remained elevated even in the 3 hours following the removal of S1P in WT and S1P3-KO rings. This sustained potentiation was absent in S1P2-KO but not in Galpha12/13-KO vessels. The effect was also sensitive to co-incubation with Y-27632 but surprisingly, the potentiation developed after the removal of S1P and Y-27632. We noticed the opposite effect in eNOS-KO vessels, namely the potentiation developed after S1P incubation but disappeared after the second hour. In accordance, 20 min incubation with S1P decreased eNOS mediated vasorelaxation 2-3 hours after exposure, but not in the first hour. Vessels lacking both endothelium and Galpha12/13 showed no potentiation.
According to our results S1P significantly enhances the contractile responsiveness of the vascular smooth muscle. This effect persists for hours mediated in the early phase via the S1P2 receptor – Galpha12/13 – Rho-kinase signalling pathway which is followed by a permanent inhibition of eNOS via the S1P2 receptor. The augmented responsiveness may contribute to the development of vasospasm under pathophysiological conditions associated with enhanced S1P production.

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EFFECTS OF LYSOPHOSPHATIDIC ACID ON VASCULAR TONE

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Our aim was to investigate the vasoactive effect(s) of lysophosphatidic acid (LPA) in order to better understand its role in cardiovascular physiology. Thoracic (TA) and abdominal aorta (AA) segments were isolated from adult male wild type (WT) and knock out (KO) mice deficient in LPA1 or LPA2 receptors, endothelial NO synthase (eNOS), cyclooxygenase-1 (COX1) or thromboxane receptors (TP). Wire myography was used to measure the isometric tension changes of the vessels. The endothelium was mechanically removed in some segments. Expression of LPA receptors in freshly isolated mouse aortic endothelial cells (MAEC) and the vascular smooth muscle (VSM) were analyzed by quantitative real-time PCR.
The integrity of the endothelium determined the vasoactive effects of LPA: it induced relaxation in intact vessels (both TAs and AAs), whereas in the absence of endothelium or eNOS vasocontraction developed which was stronger in the AA. PCR experiments demonstrated the presence of mRNA encoding the 1, 2, 4, and 5 subtypes of LPA receptors in MAEC. In VSM LPA1 was predominantly expressed whereas LPA3 and LPA4 were expressed at lower levels. The LPA1–3 agonist VPC31143 mimicked the effects of LPA, while the LPA1,3 antagonist Ki16425 inhibited it. Likewise, LPA-induced vasorelaxation was missing in case of the genetic deletion of LPA1 but not that of LPA2. Inhibition of the protein kinase B/Akt pathway by wortmannin and MK-2206 had no effect on LPA-mediated vasorelaxation, while inhibition of phospholipase C gamma; by U73122 or edelfosine eliminated it. Surprisingly, inhibition of COX by indomethacin and genetic deletion of either COX1 or TP abolished LPA- and VPC31143-evoked vasocontraction in endothelium denuded AA segments.
Our results indicate that in intact vessels LPA induces LPA1- and PLC gamma;-dependent activation of eNOS and consequent relaxation of the VSM. In contrast, in the absence of endothelium or eNOS LPA induces vasoconstriction by activating VSM COX1 and autocrine/paracrine release of a prostanoid, most likely thromboxane A2. This latter effect of LPA may contribute to the altered vascular reactivity in pathophysiological states associated with endothelial dysfunction.

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IMPACT ON THE PRODUCT PROFILE OF HUMAN 5-LIPOXYGENASE AFTER INTERACTION WITH MYELOPEROXIDASE-DERIVED OXIDANTS

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The human 5-lipoxygenase oxidizes arachidonic acid to 5S-hydroperoxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-HpETE) and leukotriene (LT) A4. In neutrophils, LTA4 is further converted to the potent chemoattractant LTB4. These cells contain also the heme enzyme myeloperoxidase that produces in the presence of hydrogen peroxide several potent oxidants such as hypochlorous acid (HOCl) and monochloramine (NH2Cl). Myeloperoxidase metabolites are involved in pathogen defense and immune regulation. Here we addressed the following question: Do myeloperoxidase-derived oxidants affect the activity of 5-lipoxygenase and the product profile of this enzyme?
For this task recombinant 5-lipoxygenase, dialyzed against phosphate buffer, was incubated with increasing amounts of HOCl or NH2Cl. Afterwards arachidonic acid metabolites of 5-lipoxygenase were analyzed using C18-HPLC with spectrophotometric detection. The applied chromatographic separation enables the simultaneous detection of both the hydroperoxide and the hydroxide forms of oxidized arachidonic acid metabolites. The incubation of 5-lipoxygenase with HOCl or NH2Cl resulted in a significant decrease of 5S-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-HETE) and 6-trans-LTB4, the non-enzymatic hydrolysis product of LTA4. The 5-HpETE concentration was only slightly affected. Interestingly, the specificity of the hydroperoxidation reaction was changed. New oxidation products were detected at the C12 and C15 position of arachidonic acid. Thus, the impact of metabolites synthesized by 5-lipoxygenase drastically changed after incubation with HOCl or NH2Cl. 6-Trans-LTB4 compromised at least one third of these metabolites of unaffected 5-lipoxygenase in addition to 5-HpETE and 5-HETE. However, after modification of 5-lipoxygenase with myeloperoxidase-derived oxidants, 5-HpETE was the dominating metabolite, whereas 5-HETE and 6-trans-LTB4 were only of minor importance. Furthermore, the myeloperoxidase-hydrogen peroxide-chloride system caused a comparable modification of the product profile of the 5-lipoxygenase.
In summary, myeloperoxidase-derived oxidants changed the product profile of 5-lipoxygenase. Apparently, this was due to a modification of critical amino acid residues of the 5-lipoxygenase. Further work is necessary to assess the specific type and position of oxidation in 5-lipoxygenase and to specify whether this interaction between 5-lipoxygenase and myeloperoxidase-derived oxidants takes also place in stimulated neutrophils.

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5-LIPOXYGENASE BINDING TO NANODISCS: VISUALIZATION BY NATIVE GEL ELECTROPHORESIS AND NEGATIVE STAIN ELECTRON MICROSCOPY.

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Many proteins function in more or less transient complex formations on or across a membrane surface. Since long, liposomes and membrane fractions have been practical membrane mimetics in the study of such reactions. However, little is known about these reactions from a structural point of view and here the membranous environment provided by nanodiscs has become a useful tool.
These are circular fragments of phospholipid bilayer, encircled by two copies of membrane scaffold proteins (MSP) that are repeats of short, amphipathic-alpha-alfa-helices. The longer the MSP, the larger the diameter of the disc so that discs of a defined size can be made to match the size of a transmembrane or membrane embedded protein (-complex).
5-Lipoxygenase (5LO) has a central role in the biosynthesis of Leukotrienes from arachidonic acid (AA) originally stored in the nuclear membranes. In the initial steps a cytosolic calcium-ion-burst recruits the soluble protein 5LO to these membranes where a molecule of AA is presented to 5LO by the transmembrane protein Five-Lipoxygenase Activating Protein.
We have so far studied the calcium induced recruitment of 5LO onto nanodiscs by several methods like negative stain electron microscopy and native gel electrophoresis as well as compared the 5LO activity on nanodiscs with that on liposomes.

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LIPOXYGENASE INHIBITORS INTERFERE WITH PROSTAGLANDIN E2 TRANSPORT

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The 5-Lipoxygenase (5-LO) inhibitors AA-861, BWA4C, C06, CJ-13,610 and the FDA approved compound zileuton as well as the pan-LO inhibitor NDGA are frequently used to study the role of leukotrienes (LT) in models of inflammation and cancer. In recent years several publications could show that at least some of the anti-inflammatory and anti-carcinogenic properties of these compounds are of a secondary nature. Here we show that the 5-LO inhibitors investigated in this study inhibit prostaglandin E2 (PGE2) formation in supernatants of cytokine-stimulated cancer cell lines as well as colon cells constitutively expressing PGE2 in low micromolar concentrations. This effect was not due to suppression of 5-LO activity since enzyme expression was not detectable in Hela cells. In addition, AA-861, BWA4C, CJ-13,610 and zileuton dose-dependently blocked lipopolysaccharide triggered PG formation in human whole blood preparations. Further investigations revealed that inhibition of expression of enzymes involved in PGE2 synthesis was not part of the underlying mechanism. We also found catalytic activities of these enzymes such as arachidonic acid (AA) liberation, PGH2 and PGE2 formation were not impaired by the compounds. This is in contrast to recently published data stating that inhibition of PGE2 formation in murine monocytes by zileuton is due to interference with cPLA2 translocation. When intracellular PGE2 levels in treated Hela cells were analyzed, we found elevated concentrations of the eicosanoid in AA861, BWA4C, C06 and zileuton treated samples suggesting inhibition of PGE2 transport. The ATP-binding cassette transporter MRP4 is thought to facilitate PG export in humans and all cell lines used in this study expressed this protein. Therefore, we performed transport inhibition experiments in a MRP4 over expressing cell line (HEK293/4.63). Here, the LO inhibitors interfered with the export of known MRP4 substrates. Therefore, several major conclusions can be drawn from this study: (A) Inhibition of PGE2 release by 5-LO inhibitors seems to be a class effect affecting various compounds frequently used in pharmacological studies; (B) In vivo and in vitro studies investigating the role of LTs in models of inflammation and cancer have to be reassessed carefully due to possible prostaglandin inhibitory properties of the pharmacological tools used.

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EICOSAPENTAENOIC ACID DOWNREGULATES LIPOGENIC GENES AND UPREGULATES GENES ASSOCIATED WITH FATTY ACID OXIDATION AND MITOCHONDRIAL BIOGENESIS IN SUBCUTANEOUS ADIPOCYTES FROM OVERWEIGHT SUBJECTS

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Introduction: The n-3 polyunsaturated fatty acids (n-3 PUFAs) eicosapentaenoic (EPA) and docosahexaenoic (DHA) have been reported to have protective effects in obesity-linked metabolic disorders. Thus, n-3 PUFAs have been shown to regulate white adipose tissue (WAT) functions by ameliorating low-grade inflammation and the dysregulation of adipokine secretion associated with obesity. Moreover, n-3 PUFAs are able to improve WAT glucose and lipid metabolism as well as mitochondrial function in animal models of obesity and related disorders.
Objective: The main objective of the present study was to analyze the effects of EPA on the gene expression pattern of several pathways involved in lipid metabolism including lipogenesis, fatty acid oxidation as well as mitochondrial biogenesis in human adipocytes from overweight subjects.
Methods: Human subcutaneous preadipocytes from overweight females (BMI: 28.1-29.8 kg/m2) were differentiated according to manufacturer's procedures (Zen-Bio). Fully differentiated adipocytes were treated with EPA (100-200 microM) during 24 h. Changes in mRNA expression were investigated using RT-PCR. Mitochondrial content was evaluated using the mitochondria-specific dye MitoTracker® Green (Molecular Probes, Life Technologies Ltd, Paisley, UK).
Results: EPA treatment (200 microM) significantly downregulated the expression of lipogenic genes such as fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD-1), diacylglycerol O-acyltransferase (DGAT1 and DGAT2). Interestingly, EPA (100 microM) upregulates ($P<0.05$) the expression of genes involved in fatty acid oxidation such as carnitine palmitoyltransferase (CPT-1). Moreover, EPA (100 microM)-treated adipocytes showed a significant increase ($P<0.01$) in mitochondrial content, which was accompanied by a significant upregulation ($P<0.05$) of nuclear respiratory factor-1 (NRF-1), mitochondrial transcription factor A (TFAM) and cytochrome c oxidase IV (COXIV) mRNA levels. EPA treatment also promoted ($P<0.05$) the expression of sirtuin 1 (SIRT1) and the brown fat determination factor PR domain containing 16 (PRDM16).
Conclusions: Our data suggest that EPA might regulate adipocyte lipid content by reducing fat storage and by promoting fat oxidation and mitochondrial biogenesis in human overweight subcutaneous adipocytes, which could contribute to the claimed beneficial effects of n-3 PUFAs in obesity-related metabolic disorders.

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ACTIVATED COX/PGE2 PATHWAY IN A SUBSET OF HIGH-RISK NEUROBLASTOMA

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Neuroblastoma is a childhood tumor with aggressive high-risk subsets, e.g. 11q-deletion and MYCN-amplification, resulting in poor outcome even with extensive treatment. Therefore, it would be desirable to find new strategies for targeted therapy to increase survival and decrease the many side effects of chemotherapeutic drugs. Here we propose a role for prostaglandin E2 (PGE2), a pro-inflammatory lipid mediator in the pathogenesis of neuroblastoma. Levels of prostaglandins were assayed in 40 primary human neuroblastoma tumors by mass spectrometry (MS). The expression of COX-1, COX-2, mPGES-1, L-PGDS, H-PGDS and 15-PGDH were analyzed in eight tumors by immunohistochemistry (IHC). Expression of mPGES-1 was found in all analyzed tumors. Additionally, COX-1 was expressed in all tumors whereas COX-2 was only expressed in a few tumors. LC-MS/MS analysis of extracted lipids from tumor tissue showed elevated levels of PGE2 in a subset of high-risk tumors with 11q deletion.
These tumors also showed high expression of mPGES-1. In addition, the 11q-deleted tumors lacked expression of 15-PGDH, the enzyme responsible for metabolizing PGE2, further contributing to the high PGE2 levels.
Investigation of the tumor microenvironment uncovered an immunosuppressive milieu with abundant occurrence of CD163+ M2 polarized macrophages and CD11b+ myeloid-derived suppressor cells (MDSC). Cells expressing mPGES-1 was also found to express vimentin, fibroblast activation protein, alpha (FAP) and platelet-derived growth factor receptor beta (PDGFRbeta) indicating that fibroblasts are the cells responsible for PGE2 production in the tumor microenvironment.
Collectively, this indicates an involvement of prostaglandin E2 in the tumor microenvironment of neuroblastoma opening up new therapeutic strategies using existing anti-inflammatory drugs as NSAIDS, or by developing new family of compounds targeting mPGES-1 directly, in combination with cytotoxic drugs.

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STUDY ON RESPONSES OF CERAMIDE METABOLISM PATHWAY ENZYMES IN MICE LIVER TISSUE CULTURE TREATED WITH WITHAFERIN A AND WITHANOLIDE A.

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Ceramide play important roles in intracellular signaling involve in differentiation, proliferation and apoptosis. Withaferin A and Withanolide A are main bioactive compound that traditionally use to cure ulcer, rheumatism and leucoderama. In this investigation, we evaluated ceramide synthase, serine-palmitoyl transferase and dihydroceramide desaturase as anabolic pathway enzymes and ceramidase activity as anabolic enzyme in liver tissue culture treated with 0 to 120µg/kg. Results showed markedly significant inhibition on ceramidase activity (61%) at 80 to 120µg/kg of withaferin A and slightly increase (18%) on ceramide synthase and dihydroceramide desaturase (15%) activities with respect to control. There was no considerable effect on activity of serine-palmitoyl transferase in liver for both of these compounds. In addition, sphingosine level did not vary considerably in response to each compound. However, ceramide level increased in a dose dependent manner of withaferin A treatment and reached the highest level at 80 µg/kg exposure. On the other hand Withanolide A treatment did not elevated ceramide considerably as compared with control.
Our findings clarified the role of ceramide elevation in response to withaferin A in comparison to Withanolide A treatment that may involve in reported biological activities of this medicinal plant compound.
Key word: Withaferin A, ceramide synthesis, sphingosine, liver tissue

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REVEALING THE DIVERSITY AND NUMBER OF UNIQUE LIPID MOLECULAR SPECIES IN HUMAN PLATELETS

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Platelets play a central role in haemostasis, vascular integrity, angiogenesis and inflammation. How activated platelets regulate these processes is not fully understood, and may involve as yet undiscovered lipid signalling mediators. Here we report characterization of novel lipid species generated by thrombin-activated human platelets using an untargeted lipidomic approach with UPLC-MS (Orbitrap Elite). Lipid extracts from thrombin activated platelets were analysed in both positive and negative ion mode over m/z 100-1800. Data processing using in-house generated software, and statistical analysis revealed that resting human platelets contain 5245 unique lipid molecular species in at least 2 out of 3 genetically-unrelated donors. On thrombin activation, 759 lipid species were upregulated (>2-fold). Putative up-regulated lipids included eicosanoids (prostaglandins, thromboxane, HETEs etc.), docosanoids, mono- and diglycerides, lysophospholipids, acyl carnitines, oxidised phospholipids, ceramides and gangliosides. Many of these have never been described as generated by platelets before, and several have known potent signalling actions relevant to cancer and atherosclerosis. Over 75% of upregulated lipids were not in databases (HMDB, Metlin, LipidMaps & LipidHome) and potentially represent novel lipid families of relevance to human disease. Of particular interest are 43 fatty acids, 18 oxidised fatty acids and 110 oxidised phospholipids (total 171 lipid species), and temporal dynamics of their generation during thrombin activation will be presented. Their origin via cyclooxygenase (COX) or lipoxygenase (LOX) was investigated using aspirin inhibition and murine LOX-deficient platelets. In vivo COX-1 inhibition in human platelets with aspirin altered the majority of the thrombin upregulated (759) lipid species; with 537 downregulated, 75 further upregulated. Out of the 537 aspirin-inhibited lipid species, 471 were common in at least 2 out of 3 donors. These included both eicosanoids and docosanoids. The relative profile of 171 tentatively identified lipid species will be presented from thrombin-activated murine LOX-deficient platelets. This study for the first time presents a systems level overview of thrombin activated platelet lipid metabolism and shows diversity of novel and unknown lipid species in these important human cells.

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SIGNALING PATHWAYS OF THROMBOXANE RECEPTOR-MEDIATED VASOCONSTRICTION: MAJOR ROLE OF PHOSPHOLIPASE C EPSILON

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We aimed to elucidate the intracellular signaling pathways of the sustained vasoconstriction and hypertension induced by thromboxane A2 (TXA2) via stimulation of TP prostanoid receptors.
Isometric tension recording was performed in thoracic aortic segments (TAs) isolated from wild-type mice (WT) as well as from mice deficient in TP receptors (TP-KO), the alpha subunits of q/11 or 12/13 G-proteins (Galphaq/11-KO and Galpha12/13-KO) or PLC epsilon (PLCe-KO). Phosphoinositol-hydrolysis was monitored by measurement of 3H-inositol-phosphate (3H-InsP) formation in TAs. Changes of intracellular calcium levels in TAs and primary cultures of vascular smooth muscle cells (VSMC) were assessed by ratiometric measurement of Fura-2-AM fluorescence. Arterial blood pressure was determined in ketamine/xylazine anesthetized animals.
TAs from both Galphaq/11-KO and Galpha12/13-KO mice showed decreased contraction and intracellular calcium level elevation in response to the TP receptor agonist U-46619, which failed to induce any effect in TP-KO vessels. Interestingly, the U-46619 induced Ca2+ signal in Galphaq/11-KO VSMC persisted in Ca2+-free medium and was unaffected by the Rho-kinase (ROCK) inhibitor Y-27632, but almost completely abolished by the RhoA inhibitor TAT-C3. Since RhoA can activate reportedly PLC epsilon we further investigated this pathway. TAs from PLCe-KO mice showed significantly decreased vasoconstriction, 3H-InsP accumulation and intracellular calcium level elevation upon administration of U-46619 whereas the effects of the alpha1-adrenoreceptor agonist phenylephrine remained unchanged. In accordance, the hypertensive effect of U-46619 but not that of norepinephrine decreased in PLCe-KO as compared to WT mice.
Our results indicate that q/11 and 12/13 G-proteins are simultaneously involved in the mediation of TXA2-induced vasoconstriction. While the Gq/11-dependent effect is likely to be mediated by PLC beta, the Gq/11-independent pathway also involves phosphoinositol-hydrolysis and subsequent intracellular Ca2+ release in a RhoA-dependent, but ROCK-independent manner. Our results suggest that G12/13- and RhoA-mediated activation of PLC epsilon plays a major role in TXA2-induced vasoconstriction and hypertension.

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DP AND CRTH2 – TEAM PLAYERS IN COORDINATING THE RESPONSE TO PROSTAGLANDIN D2

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Prostaglandin D2 profoundly controls eosinophil effector functions during allergic reactions and is the ligand for two distinct G-protein coupled receptors, DP (D-type prostanoid receptor 1) and CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells). Although, CRTH2 agonists mimic several effects of PGD2, they cannot account for the full range of PGD2 mediated pro-inflammatory and immunmodulatory functions. This is further stressed by the inefficient outcomes of CRTH2 antagonists in clinical trials. Recently, we have shown that DP is crucial for efficient CRTH2-mediated Ca2+ signalling which demonstrates that DP and CRTH2 can influence each other's signalling properties1. Up to now, the role of DP in eosinophil biology and the particular cooperation between DP and CRTH2 is incompletely understood and might be the key to fully explain the effects of PGD2 on eosinophils in allergic diseases.

Reporter gene assays, Western blot and qRT-PCR were performed with heterologous HEK293 (human embryonic kidney cells) cell lines overexpressing DP and/or CRTH2. AnnexinV/PI co-staining, intracellular staining of pERK1/2, F-actin staining, shape change and adhesion experiments under flow were performed with freshly isolated human peripheral blood eosinophils.

The expression of DP is essential for PGD2-mediated activation of SRE (serum response element) and expression of the immediate early gene cFos in HEK293 overexpressing DP and/or CRTH2. The selective DP agonist BW245c enhanced eosinophil viability. Both, CRTH2 and DP contributed to cytoskeletal rearrangement of eosinophils while CRTH2 activation led to adhesion of eosinophils to fibronectin under flow conditions and ERK1/2 phosphorylation.

The data obtained demonstrate the significance of both receptors, DP and CRTH2, in the coordination of the functional response of eosinophils to PGD2. Our findings highlight that DP signalling regulates responses to PGD2 such as apoptosis, the transcriptional activation of SRE and expression of cFos. CRTH2 activation leads to pro-inflammatory effects – however, DP-activation may profoundly contribute to inflammation by enhancing the survival of eosinophils.
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Poster 81

CATALOGUING THE EFFECTS OF GENETIC VARIATIONS IN EICOSANOID RECEPTOR PROMOTER REGIONS

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Eicosanoids are crucial for many biological processes, including inflammation and the correct functioning of the immune system. They exert their function through binding to receptors, and the effects of a given eicosanoid can differ greatly depending on the receptor to which it binds. Thus, the correct expression of these receptors is crucial to cell physiology and the correct response to stimuli. Their importance is highlighted by their association with various pathologies.
In this work we investigate the effects of common mutation in the 5' upstream promoter regions of eicosanoid receptors, with the aim of producing a comprehensive catalogue characterising their effects in terms of transcription factor (TF) binding, effects on gene expression, and disease association.
We take SNPs upstream of the 5' start site of prostanoid, lipoxin, oxoeicosanoid and leukotriene receptors and look to see if they a) affect a TF binding motif b) map to a DNase hypersensitive region, c) map to a TF binding area according to ENCODE/ChIP-seq data, or d) are in linkage disequilibrium with known disease genes taken from the NHGRI catalogue of published GWAS, using the 1000 genome data.
We find a number of SNPs that show an important role in the regulation of gene expression, as shown by multiple layers of evidence. We also find SNPs in linkage disequilibrium with variants associated with various pathologies, including asthma, obesity and cancer.
More work is needed to establish the precise functional roles of these variants and to explain their link with disease. Such approaches should include high throughput epigenetic and transcriptomic screening in appropriate patient populations.

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FUNCTIONAL RECEPTORS FOR PROSTAGLANDIN E2 IN ISOLATED HUMAN SMALL AIRWAYS

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Introduction: Prostaglandin E2 (PGE2) may contract or relax human airways. The receptors involved in the different effects have not been conclusively determined. In addition, previous studies have focused on large airways, but small airways are particularly important in asthma. The aim of our study was to define the receptors mediating responses to PGE2 in isolated human small bronchi.

Methods: Macroscopically healthy human lung tissue was obtained from 84 patients undergoing lobectomy and placed immediately in ice-cold buffer. Within three hours of resection, bronchial tubes were identified, gently dissected clear, cut into intact rings with approximately 0.5-1 mm inner airway diameter and placed in separate culture plate wells containing DMEM to allow tissue equilibration. The next day, the rings were mounted in a tissue myograph and changes in smooth muscle force were measured isometrically.

Results: To identify the contractile receptor(s), experiments were performed in presence or absence of the EP1 receptor antagonist ONO-8130, EP3 receptor antagonist ONO-AE5-599 or TP receptor antagonist SQ-29,548. In preparations kept at baseline, PGE2 thus induced a concentration-dependent contraction (Emax: 77.0±1.5; pEC50: 4.8±0.1) that was virtually abolished by SQ-29,548 (Emax: 10.2±1.0; $p<0.05$) but left unaffected by the EP receptor antagonists.
In segments pre-contracted with histamine, PGE2 caused a concentration-dependent relaxation (Emin: 42.9±5.6; pEC50: 6.3±0.3). Pre-treatment with the EP4 receptor antagonist ONO-AE3-208 caused a rightward shift and inhibition of the response (Emin: 57.9±10.3; pEC50: 5.2±0.4) ($p<0.05$) whereas the EP2 receptor antagonist PF-04418948 had no effect. Likewise, the EP4 receptor agonist TCS 2510 concentration-dependently relaxed pre-contracted segments (Emin: 34.0±13.8; pEC50: 6.7±0.6) ($p<0.05$).
Whilst pre-treatment with the different EP receptor antagonists had no effects on contractions induced by anti-IgE, the TP receptor antagonist inhibited a significant component of this response which is dependent upon release of mast cell mediators. Moreover, pre-treatment with exogenous PGE2 dose-dependently inhibited the IgE-dependent contraction.

Discussion: We confirmed that PGE2 causes bronchorelaxation through the EP4 receptor and for the first time show this in small airways. In addition, the contractile effect of PGE2 is mediated by the TP receptor, and may contribute to antigen-induced airway obstruction. Exogenous PGE2 is a powerful inhibitor of the IgE-dependent contractions.

Poster 83

UNRAVELING HOW ENZYMES CAN USE BULKY RESIDUES TO DRIVE SITE-SELECTIVE C-H ACTIVATION: THE CASE OF MAMMALIAN LIPOXYGENASES CATALYZING ARACHIDONIC ACID OXIDATION.

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The regioselective activation of C-H bonds in complex molecules containing several of them is still an exciting challenge in chemistry. However, many enzymes catalyze these processes and much can be learnt from the way they do it. For example, each mammalian lipoxygenase isoenzyme abstracts a hydrogen atom from essentially a unique carbon position. In this paper we present a comprehensive QM/MM study of the hydrogen abstraction reaction from arachidonic acid (AA) catalyzed by rabbit 15-lipoxygenase (15-rLO). Most of the products of this reaction arise from the initial hydrogen abstraction from the carbon C13 of AA. Nevertheless, we have shown that 15-rLO seems able to catalyze not only the abstraction of H13, but also of H10. After having studied 20 of these hydrogen transfers, we have even concluded that the reaction mechanisms for both abstractions are identical (proton coupled electron transfer processes), with transition state structures matching their geometries around the shifting hydrogen. In spite of that similarity, the average potential energy barrier for the H13 abstractions is 4 kcal/mol higher than for the H10 abstractions, in good agreement with the experimental C13:C10 ratio of 97:3. We have found that a subtle steric hindrance by Leu597 and Ile663 is the main cause for that difference. Driving the strict regiospecificity exhibited by 15-rLO appears to be the essential function of the bulky side chains of those conserved residues, this way making possible the vital physiological role of 15-rLO and, probably, of all the mammalian lipoxygenase isoenzymes. The understanding of how Nature uses residues with the bulkiest aliphatic side chains to achieve the selective activation of C-H bonds can stimulate the design of efficient biocatalysts to that aim.

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THE ROLE OF ADIPOSE TISSUES IN THE DEVELOPMENT OF CORONARY ATHEROSCLEROSIS IN OBESE PATIENTS WITH ISCHEMIC HEART DISEASE IN CORRELATION WITH FATTY ACIDS, 11BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 1 AND GLUCOCORTICOID EXPRESSION

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Background: Visceral fat deposition and its associated atherogenic complications are mediated by glucocorticoids. Cardiac visceral fat comprises mediastinal adipose tissue (MAT) and epicardial adipose tissue (EAT), and MAT is a potential biomarker of risk for obese patients.
Aim: Our objective was to evaluate the role of EAT and MAT 11beta-hydroxysteroid dehydrogenase type 1(11beta-HSD-1) and glucocorticoid receptor (GCR) expression in comparison with subcutaneous adipose tissue (SAT) in the development of coronary atherosclerosis in obese patients with coronary artery disease (CAD) and to assess their correlations with CD68 and fatty acids from these tissues.
Methods and results: Expression of 11beta-HSD-1 and GCR was measured by qRT-PCR in EAT, MAT and SAT of thirty-one obese patients undergoing coronary artery bypass grafting due to CAD (obese CAD group) and sixteen obese patients without CAD undergoing heart valve surgery (controls). Stearidonic acid was significantly increased in EAT and MAT of the obese CAD group and arachidonic acid was significantly expressed in MAT of the obese male CAD group ($p < 0.05$). Whereas, significant difference was observed in n-6/n-3 ratio in EAT and MAT and palmitoleic acid, eicosatrienoic acid and n-3 in EAT in obese CAD patients. 11beta-HSD-1 and GCR expression in MAT were found to be significantly increased in the obese CAD group compared with controls ($p < 0.05$). In the obese CAD group, 11beta-HSD-1 and GCR mRNA levels were strongly correlated in MAT.
Conclusions: We report for the first time the increased expression of 11beta-HSD-1 and GCR in MAT compared with EAT and SAT, and also describe the interrelated effects of stearidonic acid, HOMA-IR, plasma cortisol and GCR mRNA levels, explaining 40.2% of the variance in 11beta-HSD-1 mRNA levels in MAT of obese CAD patients. These findings support the hypothesis that MAT contributes locally to the development of coronary atherosclerosis via glucocorticoid action.

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SPHINGOLIPIDS: CANDIDATE BIOMARKERS FOR DIAGNOSIS OF LIMITED SYSTEMIC SCLEROSIS AND POTENTIAL PROGNOSTIC BIOMARKER FOR TREATMENT OUTCOME OF PROSTACYCLIN INFUSIONS

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Systemic sclerosis (SSc) is a clinical heterogeneous disease with an unclear etiopathogenesis. It is characterized by small vessel vasculopathy, production of autoantibodies and excessive extracellular matrix production resulting in multi-organ fibrosis. Within this entity, secondary Raynaud phenomenon (2°RP) is a widespread symptom leading to a disturbed blood circulation of the fingers and toes. Currently there is no established biomarker for the diagnosis of early lcSSc and curative therapy.
In a cohort of 28 limited cutaneous SSc (lcSSc) patients we could confirm the findings of Tokumura et al. that sphingosine-1-phosphate (S1P) is elevated in serum (Tokumura, 2009) ($p \leq 0.001$, different lcSSc stages vs. healthy controls). In addition results from these sphingolipid measurements revealed elevated dihydro-S1P (dhS1P) levels in the serum of lcSSc patients ($p \leq 0.001$, different lcSSc stages vs. controls). Neither S1P nor dhS1P were elevated in our two control groups consisting of healthy individuals and patients with primary Raynaud phenomenon (1°RP), respectively. Correlation analysis of S1P and dhS1P versus a molecular fibrotic marker (cartilage oligomeric matrix protein (COMP)) revealed a correlation between these parameters indicating an involvement of sphingolipids in fibrosis during SSc. Based on our findings that S1P and dhS1P seemed to be candidate biomarkers for diagnosis of lcSSc we investigated the effect of prostacyclin infusions (Iloprost) give
n for five consecutive days to 10 lcSSc patients on S1P and dhS1P serum levels. Our data showed a significant increase in S1P ($p=0.0037$) and dhS1P ($p= 0.0204$) serum concentrations in lcSSc patients after Iloprost treatment. In contrast, COMP in the cohort was not changed significantly ($p=0.1153$), but interestingly two subgroups of lcSSc patients are identified after prostacyclin medication. Three patients exhibited increased COMP serum levels (increase: 38.74% ± 47.65%) while COMP concentrations decreased in the remaining seven patients (decrease: 81.52% ± 8.27%). At present it is not clear, how prostacyclin modulates serum S1P and dhS1P levels, and whether there will be a long-term benefit, especially on the progression of fibrosis on lcSSc patients. However, these data suggest an interdependence of sphingolipids, prostacyclin, and fibrotic changes in lcSSc. We are currently expanding this study to more lcSSc patients to challenge our hypothesis.

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