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and

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SIGNALLING BY FATTY ACID DERIVATIVES AND SPHINGOLIPIDS IN HEALTH AND DISEASE

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GENERAL INFORMATION

Instructions for using conference WLAN

If you are a member of an institution (e.g. a university), which is a member of the "eduroam" community, you should use the wireless network "eduroam". In most cases, you can use the wireless network eduroam in the same way you are used to connect to the wireless network at your home institution. Please use your account and the domain of your institution (e.g. account@uni-frankfurt.de, please refer to the login information provided by your institution).
If your institution (e.g. a university) is not a member of the "eduroam" community, you have to obtain a guest-account and a password at the Conference Office. Please choose the WLAN called “Flughafen” and enter user name and password. After authenticating successfully, you will have access to the Internet.

Poster sessions

CRC poster session on Wednesday, 12:10 - 14:10

Poster session I on Thursday, 12:10 - 14:10

Poster session II on Friday, 13:30 - 15:30

Poster mounting: before lecture start in the morning (08:30/09:00) of poster session day

Poster dismantling: directly after lectures finish in the evening on the same day

Lunch vouchers

Vouchers for lunch break will be provided (Wednesday – Friday). They are valid in *Mensa Pi x Gaumen* and *Cafeteria Level* (please refer to the map on last page for locations).

Conference dinner

The Conference dinner will take place at the *Casino Anbau West* on Campus Westend (Theodor-W.-Adorno-Platz 3, limited parking available, please use subway U8 from station *Uni Campus Riedberg* to station *Holzhausenstraße*). For detailed maps of Campus Riedberg and Campus Westend see last page.

VI European Workshop on Lipid Mediators (6EWLM)

Goethe University in Frankfurt am Main,

Otto Stern Zentrum, Lecture Hall H2, Campus Riedberg,
Ruth-Moufang-Str. 2, 60438 Frankfurt am Main

Frankfurt, Germany
September 27th-30th, 2016

Tuesday, September 27th

EDUCATIONAL SESSION

13.00 **Registration**

ANALYTICAL CHEMISTRY OF LIPID MEDIATORS

14.00 **Introduction (Per-Johan Jakobsson / Xavier Norel)**

14:10-15:00 **Giuseppe Astarita (Georgetown University, Washington DC, USA)**
Untargeted and targeted lipidomics approaches with a focus on sample preparation, chromatography and data analysis (40 min + 10 Q&A)

15:00-15:50 **David Touboul (CNRS, ICSN, Gif-sur-Yvette, France)**
Integration of lipidomics with other omics approaches (40 min + 10 Q&A)

15:50-16:30 ***Coffee Break***

16:30-17:20 **Rob Vreeken (Janssen Pharma, Belgium & Maastricht University, The Netherlands)**
Imaging mass spectrometry of lipids (40 min + 10 Q&A)

17:20-18:10 **Martin Giera (Leiden University Medical Center, The Netherlands)**
Analytical pitfalls and challenges in clinical metabolomics and lipidomics (40 min + 10 min Q&A)

Wednesday, September 28th

08:00-09:00 **Registration**

09:00-09:15 **Opening address by organizers – Otto Stern Zentrum, Campus Riedberg, Lecture Hall H2**

COLLABORATIVE RESEARCH CENTRE SESSIONS

Session A. LIPID MEDIATORS AND RESOLUTION OF INFLAMMATION

Chairpersons: Bernhard Brüne / Dieter Steinhilber (Goethe University Frankfurt)

09:15-09:45 **Hartmut Kühn (Charité Berlin, Germany)**
The role of ALOX15 in the biosynthesis of pro-resolving inflammatory mediators (25 +5 Q&A)

09:45-10:05 **Klaus Scholich (Goethe University Frankfurt, Germany)**
Platelet-derived thromboxane promotes proinflammatory macrophage phenotypes during inflammation (15 +5 Q&A)

10:05-10:40 ***Coffee Break***

10:40-11:00 **Dieter Steinhilber (Goethe University Frankfurt, Germany)**
5-Lipoxygenase and cancer – where are the links? (15 +5 Q&A)

11:00-11:20 ***Cecile Gladine (INRA, Human Nutrition Research Unit, France)**
Omega-3 fatty acid-derived oxylipins reduce inflammation response in human macrophages: putative mechanism through PPAR-gamma binding (15 +5 Q&A)

11:20-11:40 ***Andreea Ioan Facsinay (University Medical Center Leiden, The Netherlands)**
The omega-6 fatty acid adrenic acid acts as a pro-resolving mediator (15 +5 Q&A)

11:40-12:10 **Derek W Gilroy (University College London, UK)**
Resolution of acute inflammation and innate immune conditioning (25 +5 Q&A)

12:10-14:10 ***Lunch and CRC poster session***

Session B. SPHINGOLIPIDS

Chairpersons: Josef Pfeilschifter / Dagmar Meyer zu Heringdorf (Goethe University Frankfurt)

14:10-14:40 **Sibylle Schneider-Schaulies (University of Würzburg, Germany)**
Sphingomyelinases regulating T cell activation and modulation by a pathogen (25 +5 Q&A)

14:40-15:10 **Andrea Huwiler (University of Bern, Switzerland)**
The yin and yang of S1P in a fibrotic cell response (25 +5 Q&A)

15:10-15:30 **Georgios Grammatikos (Goethe University Frankfurt, Germany)**
Sphingolipids as potential biomarkers of hepatic fibrosis and hepatocellular carcinoma (15 +5 Q&A)

15:30-15:50 **Anja Schwiebs (Goethe University Frankfurt, Germany)**
Activation-Induced Cell Death of Dendritic Cells Is Dependent on Sphingosine Kinase 1 (15 +5 Q&A)

15:50-16:10 ***Robinson Daniel** **(Northwestern University Feinberg, Chicago, USA)**
Long chain fatty acids and their related oxylipins in preterm human milk during the first month of lactation. (15 +5 Q&A)

16:10-16:40 ***Coffee Break and Exhibition Visit***

Session C. CYTOCHROME P450 PATHWAYS AND SOLUBLE EPOXIDE HYDROLASE

Chairpersons: Ingrid Fleming / Eugen Proschak (Goethe University Frankfurt)

16:40-17:10 **John Imig (Medical College of Wisconsin, Milwaukee, USA)**
Soluble Epoxide Hydrolase & Novel Therapeutic Vistas (25 +5 Q&A)

17:10-17:40 **Ingrid Fleming (Institute for Vascular Signalling, Goethe University Frankfurt, Germany)**
Looking good? The soluble epoxide hydrolase in the retina (25 +5 Q&A)

17:40-18:00 **Eugen Proschak (Goethe University Frankfurt, Germany)**
Discovery of chemical tools for investigation of the phosphatase activity of soluble epoxide hydrolase (15 +5 Q&A)

18:00-18:20 ***Anne Konkel** **(OMEICOS Therapeutics GmbH, Berlin, Germany)**
Omega-3 epoxyeicosanoids – from bench to bedside (15 +5 Q&A)

18:20-21:00 **Welcome reception at Otto-Stern-Zentrum**

Thursday, September 29th

08:00-08:30 **Registration**

Plenary lecture

08:30-09:15 **Bruce Hammock (University of California Davis, USA)**
Epoxides of arachidonic acid and docosahexaenoic acid modulate inflammatory and neuropathic pain

Session 1. FAT TISSUE-DERIVED BIOACTIVE LIPIDS AND LIPID DROPLETS: PHYSIOLOGY AND PATHOPHYSIOLOGY / RESOLVING LIPID MEDIATORS

Chairpersons: Joan Claria (Hospital Clinic, Barcelona), Gökçe Topal (Istanbul University)

09:15-09:45 **Daniel Closa (CSIC, Barcelona, Spain)**
Halogenated lipids in the inflammatory processes; the case of acute pancreatitis (25 +5 Q&A)

09:45-10:15 **Enrique Claro (Universitat Autònoma de Barcelona, Barcelona, Spain)**
Biogenesis and catabolism of lipid droplets during stress (25 +5 Q&A)

10:15-10:50 ***Coffee Break***

10:50-11:10 ***Karsten Weylandt (Charité Berlin, Germany)**
Alox15-deficiency modulates colitis activity in murine colitis (15 +5 Q&A)

11:10-11:30 ***Trond Vidar Hansen (University of Oslo, Norway)**
The novel lipid mediator PD1n-3 DPA: Structural elucidation, biosynthesis, bioactions and total organic synthesis (15 +5 Q&A)

11:30-11:50 ***Minna Holopainen (Finnish Red Cross Blood Service, Helsinki, Finland)**
Remodeling of phospholipid membrane in mesenchymal stromal cells: impact of polyunsaturated fatty acid supplementation (15 +5 Q&A)

11:50-12:10 ***Valerie Urbach (Paris, France)**
Resolvin D1 reduces IL-8 secretion, improves alveolar macrophage activity, restores airway surface liquid height and normalizes nasal potential difference in cystic fibrosis (15 +5 Q&A)

12:10-14:10 ***Lunch and poster session I***

Session 2. PROSTAGLANDINS, LEUKOTRIENES AND n-3 PUFAs IN CANCER

Chairpersons: Gerard Bannenberg (GOED, Madrid) / Per Kogner (Karolinska Institute, Stockholm)

14:10-14:40 **Per Kogner (Karolinska Institutet, Stockholm, Sweden)**
Tumor promoting inflammation as therapeutic target in childhood cancer (25 +5 Q&A)

14:40-15:10 **Dipak Panigrahy (Beth Israel Hospital and Harvard Medical School, Boston, USA)**
Omega-3 polyunsaturated fatty acid-derived pro-resolving lipid mediators in cancer (25 +5 Q&A)

15:10-15:30 ***Nadine Rohwer (Charité, Berlin, Germany)**
Low dose aspirin treatment inhibits mouse colon tumorigenesis and modifies the eicosanoid metabolome (15 +5 Q&A)

15:30-15:50 ***Shuntaro Hara (School of Pharmacy, Tokyo, Japan)**
Role of prostaglandin terminal synthases PGIS and mPGES-1 in chemical-induced carcinogenesis (15 +5 Q&A)

15:50-16:10 ***Ulrike Garscha (Institute of Pharmacy, Jena, Germany)**
5-Lipoxygenase-activating protein (FLAP) rescues activity of 5-lipoxygenase mutations that delay nuclear membrane association and disrupt product formation (20 +5 Q&A)

16:10-16:40 ***Coffee Break and Exhibition Visit***

Session 3. PROTEO-METABOLO-LIPIDOMICS

Chairpersons: Nils Helge Schebb (University of Wuppertal) / Nicolas Flamand (Quebec)

16:40-17:10 **Nerea Ferreiros (pharmazentrum, Goethe University Frankfurt, Germany)**
Someone there? Handicaps for the detection of pro resolving lipid mediators (25 +5 Q&A)

17:10-17:40 **Stefan Offermanns (Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany)**
Physiological and pathophysiological roles of short chain fatty acid receptors (25 +5 Q&A)

17:40-18:00 ***Pei-an Betty Shih (University of California, San Diego, USA)**
Integrating multi-omics biomarkers and postprandial metabolism to develop personalized treatment for anorexia nervosa (15 +5 Q&A)

18:00-18:20 ***Andreas Koeberle (University of Jena, Germany)**
Functional lipidomics reveals role of phospholipid-based lipid mediators in vitamin A signaling (15 +5 Q&A)

20:00 **Conference Dinner at the Campus Westend, Goethe University Frankfurt, Theodor-W.-Adorno-Platz 3, 60323 Frankfurt**

Friday, September 30th

6th European Workshop on Lipid Mediators – Day 2

08:30-09:15 ***Opening lecture***

Bill Lands (Fellow, American Society for Nutrition, USA)
Relationship between essential fatty acid intake and lipid mediator biosynthesis and action

YOUNG INVESTIGATOR SESSION

Chairpersons: Jesús Balsinde (CSIC) / Joan Claria (Hospital Clinic, Barcelona)

09:15-09:27 **Cristina Lopez-Vicario (Esther Koplowitz Center, Barcelona, Spain)**
Bioactive ω-3-derived lipid mediators regulate white adipose tissue homeostasis and prevent obesity-induced inflammation and insulin resistance (10 +2 Q&A)

09:27-09:39 **Astrid Kahnt (Goethe University Frankfurt, Germany)**
Molecular mechanisms of lipoxin and resolvin biosynthesis (10 +2 Q&A)

09:39-09:51 **Gulsev Ozen (Istanbul University, Turkey)**
Role of PGE_2 and H_2S released by PVAT in the vascular tone of healthy or atherosclerotic human coronary vessels (10 +2 Q&A)

09:51-10:03 **Jessica Roos (Goethe University Frankfurt, Germany)**
Drug-mediated intracellular donation of nitric oxide suppresses 5-lipoxygenase product synthesis in vitro and in vivo by site-directed cysteine-nitrosylation of 5-lipoxygenase (10 +2 Q&A)

10:03-10:15 **Owein Guillemot-Legris (Louvain Drug Research Institute, Brussels, Belgium)**
High-fat diet feeding differentially affects the central nervous system inflammatory tone: involvement of bioactive lipids (10 +2 Q&A)

10:15-10:27 **Chabha Benyahia (Inserm, Paris, France)**
The reduced vasodilation of human pulmonary vessels is related to the down regulation of PGI_2 pathway (10 +2 Q&A)

10:27-10:39 **Simona Pace (University of Jena, Germany)**
Sex modulation of pro-inflammatory lipid mediator biosynthesis during acute inflammation (10 +2 Q&A)

10:39-10:51 **Sandra Gouveia-Figueira (Umeå University, Sweden)**
UPLC-ESI-MS/MS for comprehensive profiling of bioactive lipids in human lung lavage fluids after biodiesel exhaust exposure (10 +2 Q&A)

10:51-11:03 **Julia Esser-von Bieren (Techn. Universität and Helmholtz Zentrum, Munich, Germany)**
Age dictates a steroid resistant cascade of Wnt5a, transglutaminase-2 and leukotrienes in inflamed airways (10 +2 Q&A)

11:03-11:15 **Charlotte Rey (Université de Bordeaux, France)**
Resolvin D1 and E1 promote resolution of inflammation in microglial cells in vitro (10 +2 Q&A)

11:15-11:50 ***Coffee Break and Exhibition Visit***

Session 4. CYCLOOXYGENASE PATHWAY, PROSTACYCLIN AND PGE_2 SYNTHESIS IN THE CARDIOVASCULAR SYSTEM

Chairpersons: Sönmez Uydes Dogan (Istanbul University) / Xavier Norel (INSERM, Paris)

11:50-12:20 **Nicolas Flamand (Université Laval, Québec City, Canada)**
Regulation of human leukocyte functions by endocannabinoids and their metabolites (25 +5 Q&A)

12:20-12:50 **Akos Heinemann (University of Graz, Austria)**
Role of prostaglandins in leukocyte trafficking (25 +5 Q&A)

12:50-13:10 ***Yao Chengcan (University of Edinburgh, Ireland)**
Regulation of systemic inflammation by prostaglandin E_2 (15 +5 Q&A)

13:10-13:30 ***Zoltan Benyo (Semmelweis University Budapest, Hungary)**
Complex signaling pathways of thromboxane receptor mediated vascular smooth muscle contraction (15 +5 Q&A)

13:30-15:30 ***Lunch and Poster Session II***

Session 5. PHARMACEUTICALS BASED ON EICOSANOIDS / LIPID MEDIATOR PHARMACOLOGY AND RECEPTORS

Chairpersons: Per-Johan Jakobsson (Karolinska Institute Stockholm) / Nan Chiang (Boston)

15:30-16:00 **Brendan Whittle (William Harvey Research Institute, London, UK)**
Development of prostanoids as therapeutic agents for vascular disease (25 +5 Q&A)

16:00-16:30 **Nan Chiang (Brigham & Women's Hospital and Harvard Medical School, Boston, USA)**
Specialized Proresolving Mediators (SPM) as templates for resolution pharmacology (25 +5 Q&A)

16:30-16:50 ***Daniel Merk (Goethe University Frankfurt, Germany)**
Facing non-alcoholic steatohepatitis with FXR activation and sEH inhibition (15 +5 Q&A)

16:50-17:10 ***Johannes Hendrick von Hegedus (Leiden University Medical Center, The Netherlands)**
Chronic Toll-like receptor signalling induces an temporal switch towards a more resolving lipid profile in monocyte-derived macrophages (15 +5 Q&A)

17:10-17:30 ***Joan Raouf (Karolinska Institute, Stockholm, Sweden)**
mPGES-1 deletion increases prostacyclin and evades the elevated systemic ADMA associated with COX-2 inhibitors: relevance to cardiovascular safety of mPGES-1 (15 +5 Q&A)

17:30 ***Adjourn: Workshop closing address by organizers***

** indicates presentation selected from submitted abstracts, see p. 32 – 46 and p. 59 - 63*

Invited Speakers
COLLABORATIVE RESEARCH CENTER SESSIONS

THE ROLE OF ALOX15 IN THE BIOSYNTHESIS OF PRO-RESOLVING INFLAMMATORY MEDIATORS

H. KÜHN
Institute of Biochemistry, University Medicine Berlin - Charité, Chariteplatz 1, D-10117 Berlin, Germany.

Lipoxins (Lx) and resolvins (Rv) are anti-inflammatory and pro-resolving eicosanoids, which have been implicated in the resolution phase of acute inflammation. These compounds constitute trihydroxy derivatives of arachidonic acid and docosahexaenoic acid and are biosynthesized via a complex reaction cascade involving the catalytic activity of various lipoxygenating enzymes. Although the bioactivities of these mediators have been studied in great detail their biosynthetic mechanisms have less extensively been explored. In principal Lx and Rv can be synthesized via two different metabolic routes: i) The multiple oxygenase pathway, which involves the fatty acid oxygenase activity of various lipid peroxidizing enzymes (LOX-isoforms, aspirin treated COXII cytochrome P450 isoenzymes). ii) The epoxy leukotriene pathway, which involves both, the oxygenase and the epoxy leukotriene synthase activity of lipid perioxidizing enzymes. Although the relative contribution of the different lipid peroxidizing enzymes depends on the kind of inflammation and on the stage of inflammatory resolution, LOX-isoforms (ALOX5, ALOX15, ALOX15B, ALOX12) have been suggested to play a major role. To characterize the impact of various LOX-isoforms on Lx biosynthesis we quantified Lx-synthase activity of different enzyme combinations and found that a concerted action of ALOX5 and ALOX15 was most effective. In contrast, the combinations ALOX5/ALOX12 and ALOX5/ALOX15B were less efficient and only small amounts of LxB4 were formed when ALOX15 was used in the absence of ALOX5. 12-lipoxygenating ALOX15 orthologs (mouse, rat, pig) are less efficient in converting ALOX5 derived 5S-HETE and 5S,6R/S-DiHETE to lipoxin isomers when compared with 15-lipoxygenating ALOX15 orthologs (man, chimpanzee, orangutan). These data suggest that ALOX5 and ALOX15 may play a major role for the biosynthesis of Lx-isomers and that the biosynthesizing capacity of ALOX15 orthologs was improved during mammalian evolution, when 12-lipoxygenating ALOX15 orthologs developed to 15-lipoxygenating enzyme species. The improved Lx-synthesizing capacity of 15-lipoxygenating ALOX15 orthologs may considered as driving force for the evolutionary switch in positional specificity of ALOX15 orthologs.

PLATELET-DERIVED THROMBOXANE PROMOTES PROINFLAMMATORY MACROPHAGE PHENOTYPES DURING INFLAMMATION

S. Pierre, B. Linke, J. Suo, N. Tarighi, D. del Turco, D. Thomas, N. Ferreiros, D. Stegner, M. Sisignano, N. deBruin, R.M. Nüsing, T. Deller, B. Nieswandt, G. Geisslinger, K. SCHOLICH
Institut für Klinische Pharmakologie, Uniklinikum Frankfurt, Germany.

Platelets are well known for their role in hemostasis but are also increasingly recognized for their supporting role in innate immune responses. Here, we studied the role of platelets in the development of peripheral inflammation and found that platelets colocalize with macrophages in the inflamed tissue outside of blood vessels in different animal models for cutaneous inflammation. In macrophages isolated from paws during zymosan-induced inflammation treatment with collagen induced thromboxane synthesis through the platelet-expressed collagen receptor glycoprotein VI (GPVI). Deletion of GPVI or its downstream effector thromboxane A2 receptor (TP) reduced zymosan-induced mechanical allodynia without altering macrophage recruitment or formation of macrophage/platelet complexes. Instead, macrophages in inflamed paws of GPVI- and TP-deficient mice exhibited an increased expression of anti-inflammatory markers and synthesized less proinflammatory mediators (PGE_2 and IL6). TP expression on platelets was necessary to mediate increased PGE_2 and IL6 synthesis, while TP expression on macrophages was sufficient to decrease the expression of the anti-inflammatory macrophage marker CD206, showing that TP activation on platelets and macrophages regulate different aspects of macrophage activation.

Invited Speakers
COLLABORATIVE RESEARCH CENTER SESSIONS

5-LIPOXYGENASE AND CANCER – WHERE ARE THE LINKS?

D. STEINHILBER
Institute of Pharmaceutical Chemistry, Goethe University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany.

5-Lipoxygenase (5-LO) catalyzes the initial two steps in the biosynthesis of leukotrienes. Furthermore, the enzyme was shown to be involved in the development of certain types of cancer including different kinds of leukemia. We found that 5-LO promoter activity is induced by HDAC class I inhibitors which upregulate the histone mark H3K4me3 and we could identify the histone H3 lysine 4 trimethylase MLL as regulator of 5-LO promoter activity. Interestingly, the oncogenic MLL-AF4 fusion protein which is able to induce and maintain the onset of high-risk acute lymphoblastic leukemia MLL-AF4 was able to enhance 5-LO promoter activity by 47-fold. The aberrant promoter activation by MLL-AF4 could be attenuated by HDAC class I inhibitors by the activation of the endogenous MLL protein which then displaces the oncogenic MLL-AF4 from the ALOX5 promoter. Thus, HDAC class I inhibitors seem to switch 'inactive MLL' into 'active MLL' and overwrite the dominant functions deriving from MLL-AF4. Here we demonstrate for the first time by using a bona fide target gene of the MLL complex that the high constitutive oncogenic activity of MLL-AF4 can be diminished by class I HDACi.Another link between cancer development and 5-LO is provided by a genome wide screen for p53 target genes. ChIP-seq analysis revealed that 5-LO is a direct p53 target gene and that its expression is induced by genotoxic stress via actinomycin D or etoposide treatment in a p53-dependent manner. p53 binds to a specific binding site consisting of a complete p53 consensus-binding motif in intron G of the 5-LO gene. In addition, immunofluorescence and immunoprecipitation assays indicate the direct binding of 5-LO to p53 protein. Furthermore, we found that 5-LO can inhibit the transcriptional activity of p53 suggesting that 5-LO acts in a negative feedback loop to limit induction of p53 target genes. Taken together, the data indicate that 5-LO has another function besides leukotriene generation and that it is involved in the regulation of cell proliferation, differentiation and apoptosis by modulation of the p53 and Wnt pathways.

RESOLUTION TRIGGERS A PROLONGED PHASE OF PROSTANOID BIO-SYNTHESIS THAT MAINTAINS TOLERANCE AND PROTECTS AGAINST CHRONIC INFLAMMATION

D. W. GILROY
Division of Medicine University College London Rayne Building 5 University Street LONDON WC1E 6JF, UK.

I investigate the cells, soluble mediators and receptors that collectively help switch inflammation off, so-called inflammatory resolution. My overall hypothesis is that understanding how acute inflammation resolves will provide insight into the aetiology of chronic inflammatory diseases. In addition, identifying mediators and receptors essential for resolution will help develop drugs that will drive on-going inflammation down a pro-resolution pathway. However, recent work suggests that resolution of acute inflammation is not the end of immune responses to infection/injury but, that through cells of the mononuclear phagocyte system expressing arachidonic acid, docosahexaenoic acid and eicosapentaenoic acid metabolising enzymes resolution triggers a prolonged sequence of local events lasting weeks/months that helps maintain immune tolerance and prevent chronic inflammation. In particular, I will present data generated from both rodents and humans showing that once ac ute inflammation resolves infiltrating monocyte-derived macrophages, selectively expressing mPGES-1 and 2, generate highly elevated levels of prostaglandin E2 and thromboxane B2 that negatively control the uptake and presentation of inflammation-derived endogenous tissue antigens. We believe that this is a novel aspect of inflammation biology that represents an essential step in protecting tissues, long-term, from developing chronic inflammatory diseases. In my presentation, I will discuss the implication of these findings to human health and disease as well as drug discovery.

SPHINGOMYELINASES REGULATING T CELL ACTIVATION AND MODULATION BY A PATHOGEN

S. SCHNEIDER-SCHAULIES
Institute for Virology and Immunobiology, University of Wuerzburg, Germany.

Measles virus (MV) efficiently causes T cell suppression as reflected by a loss of proliferative responses and actin cytoskeletal rearrangements upon stimulation. Addressing the potential role of sphingolipid breakdown in this process, we found that MV contact sequentially activated neutral and acid sphingomyelinases in T cells in a, and that abrogation of NSM/ASM efficiently rescued MV-induced cytoskeletal paralysis and loss of spreading responses upon co-stimulation. CD3/CD28-costimulation causes transient activation of NSM2 activity of which as well as accumulation of ceramide is spatially confined to the lamellum and excluded from the center of the immune synapse. This was effectively perturbed upon MV pre-exposure indicating that aberrant activation of NSM2 takes part in MV induced T cell suppression. In fact, genetic ablation of the enzyme caused hyperresponsiveness of co-stimulated T cells suggesting that NSM2 might act as break in physiological T cell activation by dampening T cell responses. Surprisingly, however, signaling via CD3 required NSM activity, and NSM deficient T cells were highly dependent on co-stimulation indicating that efficient co-stimulation may rely on CD28 licensing by TCR signaling independently of NSM. Based on the observation that CD28 ligation alone promotes T cell activation if CD28-mediated ASM activation is prevented, we suggest that physiological co-stimulation involves both NSM activation through CD3 and ablation of CD28-triggered ASM activation.

THE YIN AND YANG OF S1P IN A FIBROTIC CELL RESPONSE

A. HUWILER
Institute of Pharmacology University of Bern Inselspital INO-F CH-3010 Bern, Germany.

Sphingosine-1-phosphate (S1P) is now appreciated as a key lipid mediator that mainly acts through five different high-affinity cell surface receptors (S1P1-5) and thereby contributes to various physiological and pathophysiological events such as angiogenesis, tumor growth and progression, and immune cell regulation. Accumulating evidence now also suggests that S1P is an important regulator of tissue fibrosis. There seems to be a Janus-faced behaviour of S1P as it mediates both pro- and anti-fibrotic effects depending on its site of action. From the extracellular side, via activation of S1P receptors, S1P promotes fibrotic processes, whereas intracellular S1P exerts an opposite effect and dampens a fibrotic reaction. Data will be shown that highlight the possibility for novel therapeutic strategies to treat fibrotic diseases.

SPHINGOLIPIDS AS POTENTIAL BIOMARKERS OF HEPATIC FIBROSIS AND HEPATOCELLULAR CARCINOMA

G. GRAMMATIKOS, N. Ferreiròs, A. Piiper, O. Waidmann, C. Sarrazin, S. Zeuzem, J.M. Pfeilschifter
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Pathophysiologic pathways underlying liver fibrosis and liver oncogenesis constitute an intriguing research topic because of the major burden of chronic liver diseases on global health. Recently, translational research approaches focus on the improvement of the non-invasive diagnosis and surveillance of hepatic fibrosis and hepatocellular carcinoma (HCC). Thereby sphingolipids (SLs) may constitute promising target metabolites since they regulate both fibrogenesis [1] as well as proliferation and apoptosis of tumours [2]. Driven by the rapid evolution of spectrometric methods, assessment of sphingolipid concentrations in serum has identified SLs as evident biomarkers of various diseases [3]. However, only limited data are so far available regarding the role of SLs as biomarkers of liver diseases. In own studies we observed significant variations of serum SLs in patients with severe liver fibrosis due to chronic viral hepatitis, with sphingosine and sphinganine being, both in univariate ($P<0.05$) as well as in multivariate analysis, significantly associated to severity of liver fibrosis in HCV-infected patients (odds ratio [OR]: 1.111; confidence interval [CI]: 1.028-1.202; $P=0.007$ and OR, 0.634; CI, 0.435-0.925; $P=0.018$, respectively) [4]. Furthermore, we identified a highly significant upregulation of long and very long chain ceramides (C16-C24) in the serum of patients with HCC as compared to patients with cirrhosis ($P<0.001$) [5]. Especially the diagnostic accuracy of C16-ceramide and sphingosine-1-phosphate (S1P), assessed by receiver operating curve (ROC) analysis, showed a higher area under the curve (AUC) value as compared to alpha fetoprotein (AFP) (0.999 and 0.985 versus 0.823, $P<0.001$ respectively). Our data demonstrate a tight interaction between variations in serum SL levels and progression of liver fibrosis as well as occurrence of HCC in a cirrhotic background. Thus, on the one hand sphingosine and sphinganine appear as promising novel biomarkers in chronic HCV infection and should be further evaluated within the noninvasive prediction of liver fibrosis while on the other hand C16-ceramide and S1P may serve as novel diagnostic markers for the identification of HCC in patients with liver diseases. Our data justify further investigations on the role of sphingolipids in hepatic fibrosis and HCC.

ACTIVATION-INDUCED CELL DEATH OF DENDRITIC CELLS IS DEPENDENT ON SPHINGOSINE KINASE 1

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Dendritic cells engage a central role for the direction of immune responses and are crucially involved in autoimmunity and cancerogenesis. Sphingosine 1-phosphate (S1P) is an immunomodulatory lipid mediator that is not only directing immune cell migration but apparently also regulates distinct functional aspects of innate and adaptive immune responses. In this study we examined the relation of sphingolipids to the function and survival of bone marrow-derived dendritic cells under long-term inflammatory stimulation. We observed that differentiated cells undergo activation-induced cell death (AICD) upon LPS stimulation with an increased metabolic activity shortly after stimulation, followed by a rapid activation of caspase 3 and subsequent augmented apoptosis. Importantly, we highlight a profound role of the S1P producing enzyme Sphk1 in secretion of inflammatory cytokines and survival of dendritic cells that might be mediated by a change in sphingolipid level as well as by a change in STAT3 expression. Cell growth during differentiation of Sphk1-deficient cells treated with the functional S1P receptor antagonist FTYP was reduced. Importantly, in dendritic cells we did not observe a compensatory regulation of Sphk2 mRNA in Sphk1-deficient cells. Instead, we discovered a massive increase in Sphk1 mRNA concentration upon long-term stimulation with LPS in wild type cells that might function as an attempt to rescue from inflammation-caused cell death. Taken together, in this investigation we describe details of a crucial involvement of sphingolipids and Sphk1 in AICD during long-term immunogenic activity of dendritic cells that might play an important role in autoimmunity and might explain the altered immune responses observed in many in vivo studies of Sphk1 modulation.

SOLUBLE EPOXIDE HYDROLASE & NOVEL THERAPEUTIC VISTAS

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Soluble epoxide hydrolase (SEH) converts biologically active epoxyeicosatrienoic acids (EETs) to their corresponding and diols. EETs have biological actions including vasodilation, natriureis, anti-inflammation, and anti-apoptosis that could combat a number of diseases. Soluble epoxide hydrolase inhibitors (SEHIs) have been developed and advanced to human clinical trials for the treatment of diabetes, hypertension, and chronic obstructive pulmonary disease. Experimental evidence during the development of SEHIs revealed unique interactions between SEH / EETs and cyclooxygenase-2 (COX-2) or peroxisome proliferator-activated receptor-gamma (PPAR-gamma). These unique interactions led to the development of novel bifunctional small molecules that combine SEHI with COX-2 inhibition or PPAR-gamma agonistic activity to uniquely modify the eicosanoid metabolome. Novel bifunctional SEHI/COX-2 inhibitors and SEHI/PPAR-gamma agonists are being tested animal models of disease for potential therapeutic value. We have determined that SEHI/COX-2 inhibitors and SEHI/PPAR-gamma agonists have therapeutic actions to treat type 2 diabetes and cardiometabolic disease. Accordingly, there is great promise for SEHIs or bifunctional SEHIs to treat human diseases.

LOOKING GOOD? THE SOLUBLE EPOXIDE HYDROLASE IN THE RETINA

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Cytochrome P450 epoxygenases generate bioactive lipid epoxides which can be further metabolized to supposedly less active diols by the soluble epoxide hydrolase (sEH). As the role of epoxides and diols in angiogenesis is unclear we compared retinal vasculature development in wild-type and sEH-/- mice. Deletion of the sEH significantly delayed angiogenesis, tip cell and filopodia formation, a phenomenon associated with activation of the Notch signalling pathway. In the retina the sEH was localized in Müller glia cells and Müller cell-specific sEH deletion reproduced the sEH-/- retinal phenotype. Lipid profiling revealed that sEH deletion decreased retinal and Müller cell levels of 19,20-dihydroxydocosapentaenoic acid (DHDP), a diol of docosahexenoic acid (DHA). 19,20-DHDP suppressed endothelial Notch signalling in vitro by via inhibition of the γ-secretase and the redistribution of presenilin 1 from lipid rafts. Moreover, 19,20-DHDP but not the parent epoxide, was able to rescue the defective angiogenesis in sEH-/- mice. Next we determined the expression of the sEH in retinas from different animals models of diabetes. We found that sEH activity and expression were increased in streptozocin-induced type 1 diabetes, high fat diet-induced type 2 diabetes as well as in a genetic model of type 1 diabetes (Ins2Akita mice). Moreover, retinal levels of 19,20-DHDP were markedly increase in Müller cells from diabetic retinas. To investigate the role of sEH and the sEH-derived diol 19,20-DHDP in diabetic retinopathy we studied the development of retinopathy in Ins2Akita mice. Retinopathy was characterized by an increase in vascular permeability associated with an irregular endothelial cell VE-cadherin organization and a reduced number of retinal pericytes. Long-term treatment (6 months) of Ins2Akita mice with an sEH-inhibitor reduced levels of 19,20-DHDP in the retina (LC-MS/MS) and attenuated vascular permeability (leakage of intravascular FITC-labelled BSA) at the same time as normalizing the VE-cadherin pattern. The latter findings were paralleled by an improved pericyte coverage. Overall, these data indicate that retinal angiogenesis is regulated by a novel form of neuroretina-vascular interaction i.e., via the sEH-dependent generation of a diol of DHA in Müller cells. Moreover, sEH inhibition may serve as a new therapeutic target for diabetic retinopathy.

DISCOVERY OF CHEMICAL TOOLS FOR INVESTIGATION OF THE PHOSPHATASE ACTIVITY OF SOLUBLE EPOXIDE HYDROLASE

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Soluble epoxide hydrolase (sEH) is a bifunctional enzyme which possesses an epoxide hydrolase and lipid phosphatase activity (sEH-P) at two distinct catalytic domains [1]. While the physiological role of the epoxide hydrolase domain is well-understood, the consequences of the phosphatase activity remain unclear. Thus, there is a need for a chemical tool to enable the investigation of sEH-P function in cell culture and in vivo. In the present study we describe the discovery and characterization of the first potent and selective sEH-P inhibitor. Therefore, bacterial expression of the recombinant N-terminal domain of sEH-P was established a high-throughput screening protocol using a sensitive and commercially available substrate fluorescein diphosphate was developed. Oxaprozin, an approved nonsteroidal anti-inflammatory drug, was identified as sEH-P inhibitor (IC50 = 11 μM) which did not impair hydrolase activity of sEH at 500 μM [2]. Subsequent derivatization of oxaprozin led to discovery of optimized compounds exhibiting inhibitory activities in nanomolar range. First structure of sEH-P in complex with an inhibitor was solved by X-ray crystallography at a resolution of 1.45 Å. Finally, the inhibitor was used to investigate the role of sEH-P in LPA and S1P signalling.

EPOXIDES OF ARACHIDONIC ACID AND DOCOSAHEXAENOIC ACID MODULATE INFLAMMATORY AND NEUROPATHIC PAIN

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It is an exciting time for those of us working in the field of epoxyfatty acids. These powerful chemical mediators appear to regulate a variety of diverse processes which in turn are often associated with positive effects on maintaining health and treating disease. We have increasing numbers of these oxylipin mediators available in pure form for research, analytical methods to monitor EpFA and their metabolites, and genetic and chemical probes to alter their biosynthesis and degradation. The plasma levels of these mediators appear to be useful biomarkers of health and multiple groups are exploring therapeutic interventions at the level of biosynthesis, degradation and action of the EpFA.

EETs are EpFA formed from arachidonic acid. They are degraded rapidly by the soluble epoxide hydrolase (sEH) and to a lesser extent by other EHs which vary with tissue. With EETs their titer depends both on the rate of biosynthesis by P450s and their degradation by the sEH. By inhibiting the sEH we can stabilize EETs and thus study their biology. An early observations was that sEH inhibitors reduced inflammation in a number of systems and they were not only more potent than NSAIDs, they synergized with NSAIDs in reducing inflammation. It seemed logical that they should also reduce inflammatory pain and they do. However, neuropathic pain is that unrelenting, unforgiving and often untreatable pain that comes from nerve damage. We did not expect that sEH inhibitors would work, but in fact they are far more potent that any drug currently on the market including gabapentin and Pregabalin. Thus they represent non NSAID, non-opioid analgesics avoiding side effects of ulceration, heart disease, and loss of coordination and cognition.

Our recent studies on neuropathic pain bring up some observations on EpFA that appear general to numerous biologies. One observation is that inducing the production of EpFA (for example by omeprazole) can be synergistic with sEHI in treating disease. A second observation that appears to hold across biologies ranging from cardiac hypertrophy to fibrosis is that an ω-3 enriched and/or ω-6 depleted diet will usually enhance the potency of sEH inhibitors. A third observation addresses an enigma in the field. Both sEH inhibitors and mimics of EpFA alter a variety of disease state ranging from diabetes to depression and gastrointestinal erosion. This makes the molecules seem more like magical potions than drug. Yet we have found that these disease states share disruption of at least one common biochemical axis: the disruption of mitochondrial function leading to reactive oxygen release which alters the endoplasmic reticulum stress response. Neuropathic pain is no exception with ER stress a clear mechanism.

We have EH inhibitors potent in the low picomolar range with high oral availability. Several groups are moving sEH inhibitors to the clinic, and we have received a NIH Blueprint Grant to do human clinical trials for neuropathic pain. So far we have found no serious side effects in vitro or in vivo, and dramatic improvement in inflammatory and neuropathic pain in dogs, cats, horses and rodents. In choice placement preference assays for pain the compounds show high levels of efficacy on diabetic pain and nerve constriction with no apparent dependence or behavioral changes. Thus at the very least we have powerful and selective tools to probe the P450 branch of the arachidonate cascade. In addition there is hope that we as a field will provide some new valuable ways to reduce suffering on man and companion animals.

HALOGENATED LIPIDS IN THE INFLAMMATORY PROCESSES; THE CASE OF ACUTE PANCREATITIS

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Fatty acid chlorhohydrins are a specific class of halogenated lipids generated from the oxidation of fatty acids by HOCl. During inflammatory processes, HOCl is generated by the action of the enzyme myelioperoxidase and several biological activities have been reported for these halogenated lipids, including toxicity to endothelial cells, induction of leukocyte-endothelial cell adhesion or lysis of red blood cells. In general, these effects are restricted to the site of inflammation, as occurs in the atheroma plaques, but in acute pancreatitis, generation of halogenated fatty acids is combined with high lipolytic activity due to the release of pancreatic enzymes. This fact results in an increase in circulating fatty acid chlorohydrins and contributes to the progression of the systemic inflammatory response associated to acute pancreatitis. Some of these halogenated lipids, in particular oleic acid chlorohydrin, also emerge as potential early prognostic markers in human patients.

BIOGENESIS AND CATABOLISM OF LIPID DROPLETS IN STRESS

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Lipid droplets (LD) are cytosolic organelles containing a core of triacylglycerols (TAG) and cholesterol esters, surrounded by a monolayer of amphipathic lipids and attached proteins. Cells can generate cytosolic lipid droplets under two different physiological conditions: either when growing in a rich, lipoprotein-containing medium, or when they undergo a severe stress leading to death. In the first case, the purpose of LD biogenesis seems logical: to store nutrients. However, regarding the second case, why would a dying cell synthesize energy-expensive TAG and pack them into LD?
Using complete nutrient deprivation of cells in culture as a simple model of stress, we have shown that LD are generated using TAG synthesized after recycling the cell's own phospholipids. We hypothesized that LD formed during stress could have a pro-survival value. In all cell types tested (human LN18 glioblastoma, HeLa cells, CHO and rat primary astrocytes), death was associated with LD depletion and was accelerated by blocking LD biogenesis. Complete nutrient deprivation also induced β-oxidation of fatty acids. Under these conditions, cell survival is strictly dependent on fatty acid catabolism, which in turn requires the presence of LD.

TUMOR PROMOTING INFLAMMATION AS THERAPEUTIC TARGET IN CHILDHOOD CANCER

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Cancer in children is less common than in adults, but despite recent advances in biological understanding and clinical treatment cancer is still the major cause of deaths among children in the high-income countries.
During recent years we, and others, have shown that tumor-promoting inflammation is important for tumor development, disease progression and metastatic spread as well as resistance to therapy not only in adult cancer, but also in childhood malignancies. In particular we have investigated embryonal tumors of the neural systems, neuroblastoma in the sympathetic nervous system, and medulloblastoma that is the most common malignant tumor of CNS. Epidemiological and clinical characterization together with innovative preclinical studies have provided basis for novel therapeutic intervention.
Eicosanoids, both prostaglandins, in particular PGE2, and leukotrienes are expressed and provide novel therapeutic targets both in medulloblastoma and neuroblastoma. Inhibitors in clinical use for non-malignant disorders show promising efficacy both as single therapies and in combinations. Recently cancer associated fibroblasts were shown to be the cells expressing tumor promoting PGE2 in neuroblastoma and specific mPGES1 inhibition decrease tumor growth as an option for novel specific treatment. Targeting mPGES-1 with a novel compound induced M1 polarization of macrophages, decreased CAFs and reduced angiogenesis significantly in treated tumors.
Treatment with low-dose aspirin inhibits tumor development, delays tumor outgrowth and decreases tumor-promoting inflammation by inhibiting regulatory cells of the innate immune system as well as immunosuppressive mediators in a highly aggressive transgenic neuroblastoma model. In follow-up studies we showed the importance to inhibit myeloid derived suppressor cells to enhance novel checkpoint inhibitor immunotherapy.
Since embryonal neural tumors express less docosahexanoic acid than the normal neural system we investigated DHA supplementation in vitro and in in vivo with promising anti-tumor effects both in neuroblastoma and medulloblastoma. We have just recently finished our phase-1 study of Omega-3 DHA and EPA to childhood cancer survivors. Based on these results we aim to go further with additional clinical studies. The aim is to enhance upfront therapy without adding toxicity and/or decrease the risk of relapse and increase quality of life with long-term maintenance treatment after finishing other means of therapy.

OMEGA-3 POLYUNSATURATED FATTY ACID-DERIVED PRO-RESOLVING LIPID MEDIATORS IN CANCER

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Cancer therapy reduces tumor burden by killing tumor cells, yet simultaneously creates tumor cell debris that may stimulate inflammation and tumor growth. Thus, standard cancer therapy is inherently a double-edged sword. We show that tumor cells killed by chemotherapy or targeted therapy (tumor cell debris) stimulated primary tumor growth when co-injected with a subthreshold (non-tumorigenic) inoculum of tumor cells by triggering the release of pro-inflammatory cytokines. Debris-stimulated tumors were suppressed by the anti-inflammatory and pro-resolving lipid autacoids, resolvin (Rv) D1, RvD2 or RvE1. These mediators specifically inhibited debris-stimulated cancer progression by enhancing endogenous clearance of debris via stimulation of macrophage phagocytosis. Resolvins counter-regulated the release of cytokines/chemokines by macrophages stimulated with cell debris. These results suggest that enhancing endogenous clearance of tumor cell debris is a new target that may complement cancer therapies.

SOMEONE THERE? HANDICAPS FOR THE DETECTION OF PRO RESOLVING LIPID MEDIATORS

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Pro-resolving lipid mediators are signal molecules locally synthesized during the acute inflammatory response. These compounds are involved in the active resolution of inflammation including the reestablishment of the homeostasis of the injured tissue to avoid the development of chronic inflammatory diseases.These molecules derive mainly from omega-3 fatty acids, docosahexaenoic and eicosapentaenoic acids, due to the action of different cyclooxygenase and lipooxigenase enzymes.
There are many handicaps for the determination of pro-resolving lipid mediators (lipoxins, resolvins, protectins and maresins) in biological samples under pathophysiological conditions: the low expected concentrations of the analytes in tissue and blood derivatives, their localization, the stability of the signaling molecules and the presence of several matrix components which strongly interfere the analytical determination of pro-resolving lipid mediators, among others.
To avoid false positive results during the determination of pro-resolving lipid mediators several approaches to enhance selectivity and sensitivity of the analytical procedure are required, such as solid phase extraction procedures, chromatographic separation using very selective stationary phases, the availability of stable-isotope-labeled internal standards or the use of the mass fragmentation pattern for identification of the analytes. Only the use of the most selective and sensitive analytical technologies can assure reliable results during the analysis of pro-resolving lipid mediators in biological matrices.

PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ROLES OF SHORT CHAIN FATTY ACID RECEPTORS

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The short-chain fatty acid receptors FFA2 (GPR43) and FFA3 (GPR41) are activated by acetate, propionate, and butyrate. These ligands are produced by bacteria in the gut. In addition, the body itself can in particular produce acetate, and acetate plasma levels have been shown to be increased e.g. in diabetic patients or during periods of starvation. FFA2 and FFA3 are both expressed by enteroendocrine cells and pancreatic beta-cells. In addition, FFA2 is found on immune cells and adipocytes, whereas FFA3 is expressed by some peripheral neurons. It has therefore been speculated that short chain fatty acid receptors are involved in the regulation of various body functions under different nutritional and metabolic conditions. The talk will summarize recent data on the role of FFA2 and FFA3 in the regulation of metabolic and immunological functions and discuss the potential pharmacological relevance of this receptor system.

RELATIONSHIP BETWEEN ESSENTIAL FATTY ACID INTAKE AND LIPID MEDIATOR BIOSYNTHESIS AND ACTION

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A chain of molecular events by which n-3 and n-6 essential fatty acids affect human health has two distinct segments:
1- formation and accumulation of 20- and 22-carbon highly unsaturated fatty acids (HUFA) from 18-carbon nutrients in foods;
2- release and conversion of tissue HUFA into potent lipid mediators that act on selective receptors and produce medical consequences.
A hyperbolic, saturable, competitive dynamic of ligand binding to metabolic enzymes and receptors produces non-linear dose-response interactions that need careful management when planning or interpreting nutrient-based interventions. The relatively indiscriminate actions of n-3 and n-6 acids in the first set of events allow the balance of n-3 and n-6 nutrients to determine the HUFA balance that accumulates in tissue phospholipids. However, some selective actions that occur in the second set of events can be more intense with n-6 than n-3 mediators and cause healthy physiology to shift toward pathophysiology.
In the absence of n-3 nutrients, dietary linoleate (18:2n-6) has a very narrow therapeutic window that is widened by n-3 nutrients. The known quantitative dynamics of competing n-3 and n-6 nutrients gives predictable outcomes for the balance of tissue HUFA and the predisposition to chronic inflammatory conditions made worse by n-6 eicosanoids. These predictable relationships allow the design of successful preventive nutrition protocols that confirm and extend epidemiological observations of benefits from dietary n-3 nutrients.

REGULATION OF HUMAN LEUKOCYTE FUNCTIONS BY ENDOCANNABINOIDS AND THEIR METABOLITES

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Endocannabinoids are bioactive lipids that have been implicated in many physiologic disorders, including obesity, metabolic syndrome, hepatic diseases, pain, and inflammation. Numerous studies have reported that mice lacking the CB2 receptor have an exacerbated inflammatory phenotype. This suggests that therapeutic strategies aiming at modulating CB2 signaling could be promising for the treatment of various inflammatory conditions. However, the immunomodulatory effects of endocannabinoids are numerous and are not always mediated by the cannabinoid receptors CB1 and CB2. This indicates that endocannabinoids also have additional mechanisms of action. The presence of an arachidonic acid (AA) molecule in the structure of some endocannabinoids make them susceptible to eicosanoid biosynthetic enzymes directly or indirectly after having been hydrolyzed into AA by endocannabinoid lipase. This presentation will summarize our current knowledge about the immunomodulatory roles of endocannabinoids as well as the metabolic pathways regulating those functions, with a focus on human leukocytes. We will present data regarding the expression of endocannabinoid hydrolases by human leukocytes, notably neutrophils, eosinophils, monocytes and alveolar macrophages, and the impact of endocannabinoids on their functional responses.

ROLE OF PROSTAGLANDINS IN LEUKOCYTE TRAFFICKING

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Prostaglandins (PG) are a family of arachidonic acid metabolites, including PGD_2, PGE_2 and PGI_2, which mutually govern a broad variety of biological function through distinct seven-transmembrane G protein-coupled receptors (GPCRs).

PGD_2, the major lipid metabolite released by activated mast cells, has emerged as a principal mediator of allergic and inflammatory diseases. PGD_2-driven cellular functions are mediated via two distinct receptors, D prostanoid receptor 1 (DP_1) and 2 (DP_2), also referred to as "chemoattractant receptor-homologous molecule expressed on T helper type 2 cell (Th2) cells (CRTH2)". Beside multiple immunoregulatory effects, PGD_2 interaction through eosinophilic DP_2 leads to migration-related events such as chemotaxis, adhesion and integrin expression *in vitro*. Moreover, the involvement of DP_2 in the emergence of tissue eosinophilia has been shown in models of allergic inflammation. Of note, DP_1 activity and DP_1/DP_2 heterodimerization are essential for the biological response to DP_2 activation. Moreover, disease-related down- or upregulation of one receptor seems to modify the other's signaling behavior. Accordingly, eosinophil DP receptor expression is altered in various patient cohorts. Thus, blocking the effect of PGD_2 represents a novel therapeutic approach for such indications.

Besides PGD_2, many immune responses are regulated by PGE_2 through activation of four distinct E-type prostanoid (EP1-4) receptors which show differential patterns of tissue distribution and multiple signaling pathways. Interestingly, activation of EP4 counteracts PGD_2 and effectively inhibits eosinophil chemotactic responses *in vitro*. PGE_2 was also shown to play a bronchoprotective role in patients with asthma. Therefore, activation of EP4 holds promise for treating airway inflammatory diseases such as eosinophilic asthma.

PGI_2, the major prostanoid released by activated endothelial cells, is thought to be anti-inflammatory in nature and regulates both the innate and adaptive immune system via the $G\alpha_s$ coupled IP receptor. PGI_2 mimetics have been successfully used in pulmonary arterial hypertension and cardiovascular diseases, but their importance as immunomodulatory agents has just recently been addressed. Mice deficient for IP show augmented allergic responses, IP activation abrogates the mobilization and locomotion of bone marrow eosinophils in guinea pigs and inhibits human eosinophil chemotaxis, adhesion, and transmigration. Thus, stable PGI_2 mimetics might protect against exaggerated recruitment of eosinophils to inflammatory sites.

DEVELOPMENT OF PROSTANOIDS AS THERAPEUTIC AGENTS FOR VASCULAR DISEASE

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Prostanoids, particularly of the E series, were considered as potential candidates for a number of vascular utilities, including peripheral vascular disease and Raynaud's syndrome, but their clinical use was somewhat limited or not fully proven. However, prostacyclin and its mimetics are now widely used to successfully treat severe pulmonary arterial hypertension (PAH), a highly proliferative, vascular remodelling and often fatal disease. Prostacyclin (or PGI2) itself was discovered as an unstable endogenous lipid mediator in 1976 at the Wellcome Research Laboratories. The first use of prostacyclin for the successful treatment of PAH was reported in the early 1980's, while the synthetic formulation of prostacyclin, epoprostenol (Flolan®) was launched in 1995 for this serious disease.
Soon after the discovery of prostacyclin, a search for a suitable synthetic stable analogue was initiated by a number of pharmaceutical companies, including Wellcome and Schering, the latter company developing iloprost. The project at Wellcome resulted, after 5 years and 750 compounds, in the choice of a tricyclic analogue, BW15AU for further pre-clinical development. After the closure of Wellcome, the compound was acquired by a then small start-up US company, United Therapeutics, renamed treprostinil and very rapidly developed for PAH, being approved within 5 years by the FDA for this disease. Various formulations of treprostinil for use by other routes of administration including inhalation and oral forms, have now become available for the treatment of serious PAH over the past 12 years since its approval. Iloprost is available as an intravenous or inhalation formulation for PAH treatment.
Activation of the prostanoid IP receptor contributes to some of the vascular actions of prostacyclin mimetics. More recently, a non-prostanoid that is considered to act specifically on the IP receptor, selexipag has undergone clinical studies, has been approved in the US and Europe and launched in the UK in July this year.We have focussed on the mechanisms underlying the substantial efficacy of the prostacyclins in PAH. This work has identified that multiple prostanoid receptors, not only the IP receptor, but the DP1 and EP2 receptor, can be activated by treprostinil and we have studied the distinct receptor activating profile of the different prostacyclins. We have now shown the broad beneficial profile of treprostinil acting on prostanoid receptors such as the EP2 receptors promoting key pharmacological actions on the vasculature, including the anti-proliferative properties in the pulmonary arterial vasculature, which could underlie its therapeutic value in PAH.

SPECIALIZED PRORESOLVING MEDIATORS AS TEMPLATES FOR RESOLUTION PHARMACOLOGY

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Specialized pro-resolving mediators (SPM; lipoxins, resolvins, protectins and maresins) are a superfamily of potent mediators that stimulate resolution of inflammation, are organ protective and activate tissue regeneration. They have proven to be checkpoint regulators controlling key steps in resolution. Among these SPM, resolvin (Rv)D2 is a potent immunoresolvent biosynthesized during active resolution that stereoselectively stimulates resolution of acute inflammation, controls polymicrobial sepsis and reduces pain. Here, using an unbiased G protein-coupled receptor-beta-arrestin-based screening and functional sensing systems, we identified a receptor for RvD2, namely GPR18/DRV2, that is expressed in human leukocytes including polymorphonuclear neutrophils (PMN), monocytes and macrophages. In human macrophages, RvD2-stimulated intracellular cyclic AMP was dependent on DRV2. RvD2-stimulated phagocytosis of E. coli and apoptotic PMN (efferocytosis) were enhanced by DRV2 overexpression and significantly reduced by shRNA knockdown. Specific binding of RvD2 to recombinant DRV2 was confirmed using a synthetic 3H-labeled-RvD2. Scatchard analysis gave a Kd~10nM consistent with RvD2 bioactive concentration range. In both E. coli and S. aureus infections, RvD2 enhanced phagocyte clearance of bacteria and accelerated resolution. In cecal ligation and puncture (CLP), RvD2 significantly increased survival (>50%), reduced hypothermia and bacterial titers. These actions of RvD2 in infections were abolished in DRV2 deficient mice. During PMN-mediated second organ injury, RvD2's protective actions were also significantly diminished in DRV2 deficient mice. Together, these results provide evidence for a novel RvD2-DRV2 resolution axis that enhances human and mouse phagocyte functions, illustrating endogenous resolution mechanisms in bacterial infections and intrinsic organ protection. Thus, SPM, their pathways and receptors provide new opportunities for the control of unwanted inflammation and infection enabling the potential of resolution pharmacology.

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ORAL SESSION

COLLABORATIVE RESEARCH CENTER

September 28th

(selected oral from submitted abstracts)

Selected Oral 1

OMEGA-3 FATTY ACID-DERIVED OXYLIPINS REDUCE INFLAMMATION RESPONSE IN HUMAN MACROPHAGES: PUTATIVE MECHANISM THROUGH PPAR-GAMMA BINDING

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The anti-inflammatory properties of omega 3 fatty acids have been largely demonstrated in vitro and in vivo but research gaps remain regarding the contribution of their oxygenated metabolites also called oxylipins. We aimed to investigate and compare the anti-inflammatory properties and potential mechanisms of action of different types of omega 3 fatty acid-derived oxylipins including (i) four DHA-derived oxylipins, i.e. Neuroprostanes (14-A4- and 4(RS)-4-F4t-NeuroP), Protectin DX (PDX) as well as Neuroprotectin D1 (NPD1/PD1), (ii) a n-3 DPA derived oxylipin i.e. 10S,17S-diH n-3 DPAEEZ, (iii) two phytoprostanes (16-B1-PhytoP and 9-L1-PhytoP) and their enantiomers and one phytofuran (ent-16-(RS)-epi-9-PhytoF). Human peripheral blood mononuclear cells were isolated from healthy donors by Ficoll density gradient centrifugation. Monocytes were differentiated into resting macrophages (RM) for 7 days. RM were exposed to the different types of oxylipins at 3 different doses (i.e. 0.1, 1 and 10 µM) during 30 min. The inflammatory response was then induced with LPS (100 ng/mL) for 6 hours.
Preliminary results of gene expression analysis (qPCR) show that IL-6, MCP-1, COX-2, TNF-alpha or CCL3 mRNA were significantly lower in macrophages pre-exposed to 10µM 14-A4-NeuroP (-84%, -57%, -29%, -41% and -23% respectively). Significant but less pronounced effects on IL-6 and MCP-1 were also observed with 10µM 4(RS)-4-F4t-NeuroP (-25% and -25% respectively). Reduced levels of TNF-alpha protein secretion (ELISA) were found in macrophages pre-exposed to 10µM 4(RS)-4-F4t-NeuroP (-12% $p<0.05$) while measurable but less pronounced effects were observed with 14-A4-NeuroP, PDX, PD1 or 10S,17S-diH n-3 DPAEEZ (-9%, -22%, -10% and -15% ns, respectively). Abundance and phosphorylation of IκB-alpha (Western Blot) suggest that 14-A4- and 4(RS)-4-F4t-NeuroPs could exert their anti-inflammatory effects through the inhibition of IκB-alpha phosphorylation. Finally, cotransfection of luciferase reporter vector with human PPAR-gamma expression vector performed in Cos-7 cells suggests that all tested oxylipins may act in part through PPAR-gamma. In conclusion, these results suggest that the anti-inflammatory properties of omega 3 fatty acids could be mediated, at least in part, by oxylipins, and bring new insights into their mechanism of action.

Selected Oral 2

THE OMEGA-6 FATTY ACID ADRENIC ACID ACTS AS A PRO-RESOLVING MEDIATOR

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The resolution phase of inflammation is crucial to prevent acute inflammation from becoming chronic. Resolution is an active process that involves specialized pro-resolving mediators (SPM), derived from omega-3 polyunsaturated fatty acids (PUFAs). The pro-resolving capacity of SPM has been established based on 1) their accumulation during the resolution phase of an acute inflammatory response, 2) the inhibition of neutrophil recruitment to inflammation and 3) their potential to enhance phagocytosis by macrophages. By characterizing the inflammatory response in the murine zymosan-induced peritonitis model, we unexpectedly found that the omega-6 PUFA adrenic acid (AdA) accumulated in the peritoneal exudate cells during resolution, suggestive for a possible pro-resolving function of AdA. We therefore investigated whether AdA displays pro-resolving functions in vitro. By using a HPLC-MS/MS screening platform, low micromolar concentrations of AdA were shown to block the formation of the potent neutrophil chemoattractant leukotriene B4 (LTB4) in human neutrophils. As a consequence, the supernatant of these AdA exposed neutrophils no longer induced neutrophil migration. Moreover, AdA blocked the release of arachidonic acid (AA) from triglycerides, suggesting an inhibitory effect on adipose triglyceride lipase, which catalyzes this process. Finally, AdA increased phagocytosis by THP-1 macrophages in a dose dependent manner. These data indicate that AdA could represent a novel pro-resolving mediator effectively blocking the production of LTB4 by neutrophils, thereby unveiling a novel pathway that could promote the resolution of inflammation.

Selected Oral 3

LONG CHAIN FATTY ACIDS AND THEIR RELATED OXYLIPINS IN PRETERM HUMAN MILK DURING THE FIRST MONTH OF LACTATION.

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The objective is to quantify fatty acid (FA) oxylipins in preterm human milk expressed during the first month of lactation.
Women who delivered prior to 32 weeks of gestation and intended to express breast milk for infant feedings were prospectively enrolled. Samples were collected at 1, 2 and 4 weeks of lactation. Clinical variables were abstracted from the medical record. Eighteen oxylipins were quantified via solid phase extraction and LC-MS (limit of quantification: 4.88-39pg). Analysis by GC-FID quantified FA precursors arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Reported concentrations are pg/ml of milk. Spearman correlations and log-transformed linear mixed effects models evaluated associations between FAs and related oxylipins.
Thirty women with a median (IQR) age of 33 (29-35) years were enrolled. Gestational age at delivery was 29 (27-30) weeks. Infant birth weight was 1235 (970-1470) grams. Forty one percent of women took DHA supplements during lactation. Representative oxylipin concentrations (min,max) classified by parent FA were: AA: TXB2 (0,65.4); LXA4 (0,22.7); LTB4 (0,59.2); 15-HETE (23.4,686.5); 12-HETE (13.8,574.1); 5-HETE (29.3,8700); DHA: RvD1 (0,12.1); RvD2 (0,67.6); PD1 (0,27.3); MaR1 (0,360.3); PDX (0,55.5); 17-HDOHE (21.6,2001); 14-HDOHE (6.5,1600); EPA: 18-HEPE (0,255.6). Oxylipin concentrations remained similar throughout lactation ($p>0.1$ for all). RvD1 and RvD2 were detectable in only 5% of all samples. DHA and AA concentrations declined during lactation ($p=0.03$ and $p=0.01$, respectively). DHA supplements increased milk DHA levels ($p<0.01$). Neuroprotectins (PD1 and PDX) were related to DHA levels at 4 weeks of lactation (rho=0.47, $p=0.01$; rho=0.43, $p=0.02$, respectively). Additionally, anti-inflammatory DHA metabolites 17-HDOHE and 14-HDOHE correlated with DHA levels at 2 (rho=0.70, $p=0.0001$; rho=0.62, $p=0.0003$, respectively) and 4 (rho=0.52, $p=0.005$; rho=0.54, $p=0.003$, respectively) weeks. Linear regression showed correlation between AA and TXB2 ($p=0.02$). Correlation coefficients were significant between EPA and 18-HEPE at all times ($p<0.04$).
DHA and AA decline in mother's own breast milk emphasizing the importance of supplementing these conditionally essential FAs in mothers and/or infants to maintain sufficient delivery during critical phases of infant growth and development. This is further supported by positive correlations between DHA levels and anti-inflammatory mediators and neuroprotectins.

References

1. Koeberle et al., (2013) Proc. Natl. Acad. Sci., 110, 2546.

Selected Oral 4

OMEGA-3 EPOXYEICOSANOIDS – FROM BENCH TO BEDSIDE

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The drug development program pursued by OMEICOS in collaboration with the MDC was stimulated by recent findings suggesting that the antiarrhythmic effects attributed to omega-3 polyunsaturated fatty acids (n-3 PUFAs) are actually mediated by highly potent n-3 PUFA-derived metabolites. Providing the biochemical starting point, we found that the omega-3 double bond of eicosapentaenoic acid is efficiently epoxidized by cytochrome P450 (CYP) enzymes to yield 17,18-epoxyeicosatetraenoic acid (17,18-EEQ) as the main metabolite. In line with these in vitro findings, 17,18-EEQ was detected as the predominant endogenous CYP-epoxyeicosanoid after EPA/DHA-supplementation in rodents and man. Providing the pathophysiological starting point, our studies with CYP2J2 transgenic as well as soluble epoxide hydrolase (sEH)-deficient mice revealed the capacity of the CYP-epoxygenase pathway to reduce the susceptibility to ventricular tachycardia (VT) and atrial fibrillation (AF). Based on these results, we hypothesized that 17,18-EEQ may function as a mediator of the antiarrhythmic effects of EPA. To test this hypothesis under in vivo conditions, we developed metabolically robust synthetic analogs of 17,18-EEQ with high oral bioavailability. As a first proof of concept, the current lead compound (OMT-28) was effective in ameliorating VT in a rat model of myocardial infarction and in reducing AF-inducibility after chronic ß-adrenergic stimulation in mice. Moreover, OMT-28 improved the post-ischemic functional recovery of isolated hearts and showed anti-inflammatory and anti-fibrotic effects in different animal models. Mechanistic studies were performed using spontaneously beating neonatal rat cardiomyocytes, where 17,18-EEQ and OMT-28 exert negative chronotropic effects with EC50 values in the low nanomolar range. Pharmacological intervention and phosphorylation studies showed that 17,18-EEQ and OMT-28 act via a Gi-protein coupled receptor that activates the PI3K/Akt/NO pathway and finally the sarcolemmal K(ATP) channel. Moreover, this pathway is obviously linked to components of calcium handling and thus protects against calcium-overload. Taken together, this unique mode of action appears promising for the treatment of cardiac disease. The development of OMT-28 is now in the late preclinical phase and after concluding the phase-1 clinical trial, our goal is to test the capacity of this novel drug candidate for treatment of patients suffering from AF, the most common arrhythmia in human.

ORAL SESSION

6th EUROPEAN WORKSHOP ON LIPID MEDIATORS

September 29th

(selected oral from submitted abstracts)

Selected Oral 1

ALOX15-DEFICIENCY MODULATES COLITIS ACTIVITY IN MURINE COLITIS

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The chronic inflammatory bowel diseases, Crohn's disease and Ulcerative colitis, are a significant healthcare problem in the western world. Previous studies in humans as well as in mice indicate that omega-3 polyunsaturated fatty acids (PUFA) can alleviate colitis, however the molecular mechanisms underlying this effect are still unclear. Different lipoxygenase isoforms have previously been implicated in inflammatory bowel disease and experiments with a putative ALOX15 inhibitor suggested an anti-inflammatory activity of Alox15 in dextran sodium sulfate (DSS)-induced mouse colitis. We employed Alox15-deficient mice to explore the role of systemic inactivation of the Alox15 gene in acute DSS- and TNBS-induced colitis. Furthermore, in order to assess the effect of omega-3 PUFA in the context of Alox15-deficiency we then used this model in combination with the well-established fat-1 mouse as model system for a high omega-3 PUFA tissue status.
We confirmed that heterozygous expression of the fat-1 gene (encoding a C. elegans n-3 fatty acid desaturase) led to protection from DSS and TNBS induced colitis as demonstrated by reduced body weight loss and significantly less colon shortening in the fat-1 group. This protective effect of fat-1 expression was suppressed in Alox15-deficient mice. Female Alox15-deficient mice with low omega-3 PUFA tissue levels robustly developed less severe inflammation DSS-colitis when compared with wildtype mice. This was associated with a significantly reduced increase in colon permeability when compared with wildtype mice and increased expression of tight-junction proteins. These data suggest a pro-inflammatory activity of Alox15 in DSS-induced acute colitis and this effect may be related to a higher expression of tight junction proteins.
Taken together, our results confirm that increasing the omega-3 PUFA tissue content protects from DSS and TNBS colitis, and indicate that knocking out Alox15 can block this effect. Also given the protective effect of Alox15-deficiency in female mice with low omega-3 PUFA tissue levels the results show that lipoxygenases play a central role in mediating anti-inflammatory effects. This might be due to changes in the oxylipin patterns observed in these mice. Pinpointing the effective oxylipins/eicosanoids in this context is the central aim of on-going analyses.

Selected Oral 2

THE NOVEL LIPID MEDIATOR PD1N-3 DPA: STRUCTURAL ELUCIDATION, BIOSYNTHESIS, BIOACTIONS AND TOTAL ORGANIC SYNTHESIS

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The resolvins, protectins and maresins are examples of lipid mediators families biosynthesized from the dietary n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) during the resolution phase of acute inflammation in animal models of self-limited inflammation. In 2013 Dalli, Colas and Serhan reported a new SPM that was coined PD1n-3 DPA.1 This novel C22 n-3 oxygenated SPM is biosynthesized from n-3 docosapentaenoic acid (n-3 DPA). In this presentation, the structural elucidation and the biosynthetic pathway, together with the potent anti-inflammatory and pro-resolving properties of PD1n-3 DPA, will be presented.2 The first total organic synthesis will briefly also be outlined. The results presented contribute new knowledge on the structure-function of the growing numbers of endogenous novel SPMs.

The authors declare no conflict of interests.

Referrences

1.Dalli, J.; Colas, R. A.; Serhan, C. N. (2013). Sci. Rep. 3, 1940, DOI: 10.1038/srep01940.
2.Aursnes, M.., Tungen, J. E., Vik, A., Colas, R., Cheng, C.-Y. C., Dalli, J., Serhan, C. N., Hansen, T. V. 2014. J. Nat. Prod. 77, 910.

Selected Oral 3

REMODELING OF PHOSPHOLIPID MEMBRANE IN MESENCHYMAL STROMAL CELLS: IMPACT OF POLYUNSATURATED FATTY ACID SUPPLEMENTATION

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Human mesenchymal stromal cells (hMSC) show great potential in cell therapy. The therapeutic response of hMSCs resembles the resolution phase of inflammation in which bioactive lipid mediators (LMs) play a key role. Remodeling of membrane phospholipids plays a vital part in the biosynthesis of these LMs. Significance of different phospholipid classes in the formation of LMs remains, however, unclear. Our research group has shown that the membrane composition has an impact on the immunomodulatory potential of hMSCs: a higher n-6/n-3 fatty acid (FA) ratio in phospholipids correlates with lower immunosuppressive capacity. According to our hypothesis, the remodeling of phospholipid profile of hMSCs and hMSC-derived extracellular vesicles (EVs) has an essential role in hMSC immunomodulation.
To investigate the remodeling kinetics of membrane phospholipids and membrane derived EVs after a supplementation of n-3 and n-6 polyunsaturated FAs (PUFAs).
hMSCs were supplemented with arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid for 2, 6, and 24 hours, and phospholipids analyzed with electrospray-ionization mass spectrometry (ESI-MS) and FAs with gas chromatography (GC). Remodeling enzyme expression was studied with QPCR. For the collection of EVs, hMSCs were first supplemented with PUFAs for 24 h in standard medium, after which for another 48 h in serum-free medium. hMSC-EVs were isolated by ultracentrifugation, and analyzed with nanoparticle tracking analysis and ESI-MS. .
ESI-MS results revealed that supplemented PUFAs incorporated to the cell membrane with different kinetics between phospholipid classes. Incorporation to phospholipids was detected already after 2 and 6 h supplementation but strongest incorporation was achieved after 24 h. Analysis of hMSC-EVs revealed that the remodeling of phospholipid composition was transmitted to hMSC-EVs. A more detailed analysis of FAs by GC and remodeling enzyme expression with QPCR are ongoing.
Our results shed light on the lipid remodeling pathways of hMSCs in response to altered PUFA conditions. Detected incorporation of supplemented PUFAs into the cell or EV membrane is likely to have profound effects on the function of these cells. Elucidating the underlying components of lipid metabolism is crucial in order to understand the immunosuppressive mechanisms of hMSCs, and eventually to promote their therapeutic potential.

Selected Oral 4

RESOLVIN D1 REDUCES IL-8 SECRETION, IMPROVES ALVEOLAR MACROPHAGE ACTIVITY, RESTORES AIRWAY SURFACE LIQUID HEIGHT AND NORMALIZES NASAL POTENTIAL DIFFERENCE IN CYSTIC FIBROSIS

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Cystic Fibrosis (CF) is caused by a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) which results in airway surface liquid (ASL) dehydration, impaired muco-ciliary clearance that favours chronic pulmonary infection and inflammation leading to progressive lung destruction. Resolvin D1 (RvD1) is a docosahexaenoic acid (DHA) derived lipid mediator which effects the resolution of inflammation. We report here for the first time, that RvD1 ameliorates three key components of CF lung disease pathogenesis in CF models; NFκB driven inflammation, macrophage mediated killing of P. aeruginosa, and ion and fluid airway epithelial transport. In vitro studies were performed on NuLi-1 (normal genotype) and CuFi-1 (homozygous for F508del mutation) human bronchial epithelial cell lines and primary cultures of CF bronchial epithelial cells grown under air-liquid interface as polarised, differentiated bronchial epithelia. In vivo studies were performed on homozygous F508del-CFTR mice (FVB/N) and their wild-type CFTR littermates. Cells or mice were treated with vehicle control or RvD1 (1nM-100nM). Boc2 (10μM) was used as ALX/FPR2 receptor antagonist. Apical IL-8 secretion was induced by TNFα and measured by ELISA. The airway surface Layer (ASL) was stained with Texas red®-dextran and imaged using live-cell confocal fluorescence microscopy. Regulation of airway epithelial transport was investigated by the measurement of nasal transepithelial potential difference. RvD1 significantly reduced IL-8 secretion following TNFα mediated NFκB activation via preservation of IκB integrity in CuFi-1 bronchial epithelial cells. In addition, RvD1 enhanced the phagocytic capacity of primary CF alveolar macrophages and increased their ability to kill engulfed P. aeruginosa. Furthermore, RvD1 elevated airway surface liquid height in Primary CF and CuFi-1 bronchial epithelial cell models. This effect involved ALX/FPR2 receptor stimulation and was prevented by intracellular calcium chelation. RvD1 had no significant effect on ASL height in non-CF NuLi-1 cells, but rescued ASL height loss induced by CFTR inhibition. Finally, RvD1 restored the baseline nasal potential difference resulting from a decrease in the amiloride-sensitive Na+ absorption and a stimulation of Cl- secretion in CF mice. In contrast, RvD1 did not produce significant changes on nasal potential difference in non-CF mice. These findings recommend the further development of RvD1 as a candidate for the treatment of CF lung disease.

Selected Oral 5

LOW DOSE ASPIRIN TREATMENT INHIBITS MOUSE COLON TUMORIGENESIS AND MODIFIES THE EICOSANOID METABOLOME

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Although early detection and treatment of colorectal cancer (CRC) have improved in recent years, it remains a significant healthcare problem with high morbidity and mortality. Epidemiological and preclinical studies have shown that long-term intake of low dose aspirin reduces the risk of CRC, however the molecular mechanism of this protection is still largely unclear. The aim of this study was to investigate the efficacy of low dose aspirin treatment in a mouse colon tumor model, including evaluation of the mode of action with an emphasis on lipid mediators. For tumor induction, C57BL/6J mice were treated with the carcinogen azoxymethane (AOM, 10 mg/kg) once i.p. followed by three 5-day cycles (at 14 day intervals) of dextran sodium sulphate (DSS, 2 %) in drinking water. Aspirin treatment (25 mg/kg/day) was started concomitant with AOM administration and continued for 12 weeks. An initial pharmcokinetic/pharmacodynamic (PK/PD) study in healthy mice indicated that aspirin at 25 mg/kg/day given via drinking water had a similar PD effect on COX-1 activity (assessed as plasma TXB2) as 5-day low dose aspirin treatment (100 mg/day) in healthy human volunteers.
Aspirin significantly reduced tumor burden in three independent experiments. Evaluation of systemic plasma TXB2 revealed a significant reduction, indicating reduced platelet activation. Furthermore, aspirin-induced reduction in tumorigenesis was accompanied by strongly diminished inflammatory activity, a significant reduction in CD31-positive microvessel density and a trend towards increased apoptosis. Quantification of prostanoids and other oxylipins by liquid chromatography-mass spectrometry confirmed a significant decrease of TXB2 and pro-inflammatory PGE2 in both normal and tumor tissues of aspirin-treated mice. Regarding the acetylated COX-2 pathway, aspirin treatment did not result in significant changes in 15-HETE, 18-HEPE and 17-HDHA, known as precursors of aspirin-triggered lipoxins, resolvins and protectins. Similarly, levels of epoxy metabolites, produced by cytochrome P450, remained unchanged by aspirin.
The results confirm low dose aspirin as an effective anti-tumor treatment in this established model of colon carcinogenesis. The influence of aspirin on intestinal inflammation (including PGE2 synthesis) and platelet activation as well as on tumor cell apoptosis and tumor angiogenesis might all contribute to the beneficial effects seen in this model.

Selected Oral 6

ROLE OF PROSTAGLANDIN TERMINAL SYNTHASES PGIS AND MPGES-1 IN CHEMICAL-INDUCED CARCINOGENESIS

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Prostacyclin synthase (PGIS) and microsomal prostaglandin E synthase-1 (mPGES-1) are prostaglandin (PG) terminal synthases that function downstream of inducible cyclooxygenase (COX-2) in the PGI_2 and PGE_2 biosynthetic pathway, respectively. To reveal crosstalk between these PG terminal synthases, we have investigated phenotypes of these individual or double-knockout mice, and found that PGIS and mPGES-1 cooperatively facilitated inflammatory reactions. Recently, we further found that gene deletion of PGIS and mPGES-1 had opposite effects on azoxymethane-induced colon carcinogenesis. However, the crosstalk between these enzymes in chemical-induced carcinogenesis in the other tissues has not been fully elucidated.

In the present study, we first investigated the involvement of these two terminal enzymes in two-stage skin carcinogenesis model, in which 7,12-dimethylbez[a] anthracene (DMBA) was used as an initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as a promoter. Topical application of DMBA and TPA induced papillomas in almost all of the treated wild-type (WT) mice, but mPGES-1 deficiency significantly reduced the tumor incidence at 20 weeks of tumor induction. Double gene deletion of PGIS and mPGES-1 restored a degree of skin carcinogenesis in mPGES-1-deficient mice, but only deletion of PGIS gene did not affect skin carcinogenesis. We next examined the role of PGIS 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 increased by PGIS gene deletion. Malignant potential of bladder tumors in BBN-treated PGIS-deficient mice was greater than those in WT mice. These results suggested that PGIS-derived PGI_2 suppresses chemical-induced carcinogenesis, while mPGES-1-derived PGE_2 exacerbates it. The balance of these prostanoids might be critical for cancer progression.

Selected Oral 7

5-LIPOXYGENASE-ACTIVATING PROTEIN (FLAP) RESCUES ACTIVITY OF 5-LIPOXYGENASE MUTATIONS THAT DELAY NUCLEAR MEMBRANE ASSOCIATION AND DISRUPT PRODUCT FORMATION

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Leukotrienes (LT) are pro-inflammatory lipid mediators that are formed from arachidonic acid (AA) via the 5-lipoxygenase (5-LOX) pathway and are pronounced in asthma, allergy and cardiovascular diseases. AA is converted by 5-LOX first to 5(S)-hydroperoxyeicosatetraenoic acid (5-HPETE) and subsequently to LTA4. In cellulo, 5-LOX receives its substrate from the membrane-embedded 5-LOX-activating protein (FLAP) for product formation, and inhibition of FLAP or genetic knock-down blocks LT formation. Beside the absolute necessity of FLAP for 5-LOX activity in the cell, the crystal structure of 5-LOX revealed an active site that is concealed by two residues, F177 and Y181 referred to as "FY-cork". We examined the influence of these residues on 5-LOX activity, substrate access, membrane binding, and interaction with FLAP in intact HEK293 cells expressing 5-LOX in the absence and presence of FLAP. Uncapping the 5-LOX active site by mutation of F177 and/or Y181 to alanin e (5-LOX-F177A, 5-LOX-Y181A, 5-LOX-F177/Y181A) resulted in delayed and diminished 5-LOX membrane association in A23187-stimulated cells. Additionally, for 5-LOX-F177A and 5-LOX-F177/Y181A, the formation of 5-LOX products was dramatically reduced relative to 5-LOX-wild-type (wt). Strikingly, co-expression of FLAP in A23187-activated HEK293 cells effectively restored formation of 5-H(p)ETE by these same 5-LOX mutants (≈ 60-70% 5-LOX-wt levels) but not of LTA4 hydrolysis products. Substitution of Y181 by less bulkier residues as phenylalanine or alanine, allows dioxygenation at carbon 5 and generated 5-H(p)ETE at levels comparable to 5-LOX-wt but mainly prevented LT formation. Again, co-expression of FLAP partially restored LTA4 hydrolysis product formation by 5-LOX-Y181A. Together, the data suggest that amino acids obstructing access to the active site are essential for membrane association, and protein-lipid as well as protein-protein (5-LOX/FLAP) interaction promote LT formation in the cell.

Selected Oral 8

INTEGRATING MULTI-OMICS BIOMARKERS AND POSTPRANDIAL METABOLISM TO DEVELOP PERSONALIZED TREATMENT FOR ANOREXIA NERVOSA

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Anorexia Nervosa (AN) is a serious mental illness characterized by emaciation, an intense fear of gaining weight despite being underweight, and distorted body image. Few treatments reverse the core symptoms in AN such as food aversion. Consequently, AN has a chronic and relapsing course and the highest death rate of any psychiatric illness. The hypothesis of this paper is that the pathogenesis and psychopathophysiology of AN can be better elucidated combining longitudinal phenotyping with multiple "omics" techniques, including exon sequencing, proteomics, lipidomic, and metabolomics.
This paper summarizes the key findings of a series of pilot studies in AN as well as study design, recruitment strategies, methods for biomarker and postprandial metabolism investigations, and planned outcomes in our NIH-funded multidisciplinary study.
Exon sequencing data was analyzed in 1205 AN and 1948 controls. Metabolomics, lipidomics, and proteomics data were collected in two independent convenience samples consist of 75 subject with eating disorders and 61 age-matched healthy controls. Study participants were female and the mean age was 22.9 (4.9 [SD]) years. These exploratory analyses indicated that Epoxide hydrolase 2 (EPHX2) genetic variation was significantly associated with AN and that activity of epoxide hydrolase (sEH) was elevated in AN compared to controls. The polyunsaturated fatty acid (PUFA) and eicosanoids data revealed that Cytochromes P450 pathway was implicated in AN, and AN displayed a dysregulated baseline and postprandial metabolism of sEH-dependent omega-3 and omega-6 PUFA epoxides. These data suggest that dietary factors contribute to the burden of EPHX2-associated AN susceptibility and may affect disease outcomes.
The primary aim of our newly-funded NIH study is to establish EPHX2 multi-omics biomarkers, and test whether sEH-associated postprandial metabolism increases AN risk and affects treatment outcome through an ω-6 rich breakfast challenge. Participants will include 100 ill AN patients, 100 recovered AN patients, and 100 age- and race-matched healthy women. These data will allow us to investigate: 1) how genetic and dietary factors independently and synergistically contribute to AN risk and progression, and 2) if clinical severity and treatment response in AN are affected by the magnitude of sEH dysregulation and resulting proinflammatory eicosanoids. Results of our study will 1) identify clinically relevant biomarkers, 2) unravel mechanistic functions of sEH, and 3) delineate contributory roles of dietary PUFAs and Cytochrome P450 pathway eicosanoids for the purpose of developing novel AN treatments and improving disease prognosis.

Selected Oral 9

FUNCTIONAL LIPIDOMICS REVEALS ROLE OF PHOSPHOLIPID-BASED LIPID MEDIATORS IN VITAMIN A SIGNALING

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Functional lipidomics combines comprehensive lipid profiling with target identification and lead discovery to reveal novel strategies for pharmacological intervention. Using this approach, we found that phosphatidylcholines with polyunsaturated fatty acids (PUFA-PC) oscillate during the cell cycle. PUFA-PC suppresses cell proliferation by inhibiting membrane binding and thus activation of the survival kinase Akt [1] and is apparently part of a feed-forward mechanism of apoptosis. By screening a library of nutrients, natural products and drugs for selective effects on the phospholipidome and lipid mediator production, we identified PUFA-PC as critical mediator for the long-term regulation of Akt by vitamin A. The pleiotropic effects of retinol (vitamin A) on adult physiology and embryonic development are mediated through the active metabolite all-trans retinoic acid (RA). Bound to retinoic acid receptors (RARs), RA controls transcription but also fine-tunes the expression of RA target genes by activating kinases such as Akt. The mechanisms for long-term regulation of Akt by vitamin A are incompletely understood. We have shown by lipidomic profiling that retinol and RA deplete NIH-3T3 fibroblasts from phosphatidylcholines (PC) with polyunsaturated fatty acids, in particular linoleic acid (18:2), and concomitantly induce long-term Akt activation. Moreover, we found that the cellular ratio of 18:2-PC determines the activation state of Akt, and ascribed the effects of vitamin A on lipid composition and Akt signaling to retinoic acid X receptor (RXR) activation by using selective agonists. Administration of vitamin A to mice decreased 18:2-PC levels in brain (but not in other tissues) and in parallel enhanced basal Akt activation, which was attributed to astrocytes rather than to neurons in dissociated hippocampal cultures. Our study reveals how vitamin A is likely to regulate long-term Akt signaling: binding to nuclear receptors, modulating the membrane lipid composition and subsequently enhancing Akt membrane translocation and thus activation. We anticipate this cascade to be key for brain homeostasis in light of the well-established roles of vitamin A, polyunsaturated fatty acids as well as Akt for brain physiology.

YOUNG SESSION

6th EUROPEAN WORKSHOP ON LIPID MEDIATORS

September 30th

Oral YS1

BIOACTIVE OMEGA-3-DERIVED LIPID MEDIATORS REGULATE WHITE ADIPOSE TISSUE HOMEOSTASIS AND PREVENT OBESITY-INDUCED INFLAMMATION AND INSULIN RESISTANCE

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Lipotoxicity, low-grade inflammation, impaired insulin sensitivity, defective autophagy and exacerbated endoplasmic reticulum (ER) stress are key sequela of obesity. In the current study, we explored whether bioactive lipid mediators derived from omega-3 polyunsaturated fatty acids can restore adipose tissue homeostasis in obesity. By means of LC-MS/MS lipidomic analysis we identified the cytochrome P450-derived omega-3 epoxides 19,20-EDP and 17,18-EEQ (epoxydocosapentaenoic and epoxyeicosatetraenoic acid, respectively) as the most abundant omega-3-derived lipid mediators in adipose tissue from fat-1 mice, which are endogenously enriched with omega-3s since the embryonic stage. These epoxides reduced the emergence of ER stress, as shown by the attenuation of IRE-1a and eif2a phosphorylation, and returned to homeostasis the autophagic process, as estimated by Atg7, Atg12-Atg5 and LC3-II expression, in adipocytes challenged with palmitate, which is a well established insulin resistant and lipotoxic model. Moreover, omega-3-derived epoxides stimulated glucose uptake and induced a concentration-dependent up-regulation of insulin receptor substrate-1 (IRS-1) and glucose transporter-type 4 (GLUT-4) in adipocytes incubated with palmitate, indicating an improvement in insulin sensitivity. Similar actions were seen with 17(S)-HDHA, which is generated from DHA through the 15-lipoxygenase activity and serves as the precursor of the specialized pro-resolving mediators (i.e. D-series resolvins and protectins). These mediators still offered an additional benefit on inducing macrophage phagocytosis in a cell-based fluorescent assay, corroborating the potentiality of omega-3-derived lipid mediators to promote the resolution of inflammation. Taken together, these findings identify the ability of bioactive omega-3-derived lipid mediators to ameliorate obesity-induced metabolic complications in insulin-sensitive tissues.

Oral YS2

MOLECULAR MECHANISMS OF LIPOXIN AND RESOLVIN BIOSYNTHESIS

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The resolution of inflammation is an active process controlled by endogenous specialized pro-resolving lipid mediators (SPM) which are formed by various immune cells from polyunsaturated fatty acids during the course of an acute inflammatory response by the concerted action of different lipoxygenases, cyclooxygenase-2 and cytochrome P450 enzymes. Lipoxins and resolvins represent two members of this still growing family of SPM. However, the molecular mechanisms underlying cellular lipoxin and resolvin biosynthesis in humans are not completely understood. But this knowledge is of great importance for the development of pro-resolutionary pharmacotherapies as well as a better comprehension of potential resolution-toxic effects induced by already existing anti-inflammatory therapies. Therefore, we aimed to investigate lipoxin and D-type resolvin formation in primary immune cells such as neutrophils, macrophages, platelets and monocytic cell lines in detail. We found that various enzymes known to be involved in the biosynthesis of pro-inflammatory leukotrienes such as FLAP, cPLA2alpha, LTA4 hydrolase and LTC4 synthase also influence transcellular lipoxin and resolvin formation [1]. In addition, we studied differently polarized and stimulated macrophages as single cell model for lipoxin and resolvin biosynthesis. Here, polarisation and activation considerably rearranged the enzymatic layout of the cells leading to substantial changes in lipid mediator patterns.

References

1. Lehmann, C. et al. (2015) FASEB J. 29(12):5029-43.

Oral YS3

ROLE OF PGE2 AND H2S RELEASED BY PVAT IN THE VASCULAR TONE OF HEALTHY OR ATHEROSCLEROTIC HUMAN CORONARY VESSELS

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The perivascular adipose tissue (PVAT) releases a variety of factors that affect vascular function. However, the effects of PVAT in atherosclerosis are largely unknown. Prostaglandin E_2 (PGE_2) and hydrogen sulfide (H_2S) released by PVAT could be involved in pathogenesis of atherosclerosis and also regulation of vascular tone. We aimed to determine the role of PVAT in vascular tone of healthy or atherosclerotic human coronary vessels (HCV). The involvement of PGE_2 and H_2S in this effect has been also determined.

HCV were dissected after heart transplantation for non-ischemic cardiomyopathy or myocardial ischemia, respectively. Their healthy or atherosclerotic state was determined. Using an organ bath system, dose-response curves were established with PGE_2 receptor (EP-receptors) agonists on HCV preparations with or without PVAT. These preparations could be pre-treated with EP receptor antagonists or an inhibitor of the enzyme responsible for H_2S synthesis (CSE). A thromboxane receptor antagonist was present throughout the PGE_2 protocols. The contractions were expressed as percent of the contraction induced by KCl. The release of H_2S and the density of CSE were measured using polarographic sensor and western blot methods, respectively. EP3-receptor mRNA was determined in HCV and PVAT by RT-PCR.

In HCV without PVAT, PGE_2 and EP3-agonists induced contraction in a concentration-dependent manner. The maximal contraction induced by PGE_2 was significantly inhibited by a selective EP3 receptor antagonist (control: Emax=133±17% versus treated: Emax=77±15%, n=5-13), while EP1-antagonists were ineffective in healthy HCV. Similar result was obtained in atherosclerotic HCV. In the presence of PVAT, the contractile response to PGE_2 was significantly reduced only in healthy HCV, this reduction was abolished after incubation with the EP3 receptor antagonist or CSE inhibitor. The EP3-receptor mRNA and CSE expression were detected in HCV and PVAT. H_2S production from PVAT was significantly decreased after EP3-receptor antagonist.

Our results demonstrate that PGE_2 induces HCV contraction via the activation of EP3-receptor expressed in vascular wall. Moreover, PGE_2 could stimulate the release of a PVAT-vasorelaxant factor (H_2S) via EP3-receptor present in PVAT. This vasorelaxant effect is abolished in atherosclerotic HCV and could be involved in pathogenesis of atherosclerosis.

Oral YS4

DRUG-MEDIATED INTRACELLULAR DONATION OF NITRIC OXIDE SUPPRESSES 5-LIPOXYGENASE PRODUCT SYNTHESIS IN VITRO AND IN VIVO BY SITE-DIRECTED CYSTEINE-NITROSYLATION OF 5-LIPOXYGENASE

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The 5-lipoxygenase (5-LO) is the key enzyme of leukotriene biosynthesis and is critically involved in a number of inflammatory diseases such as arthritis, gout, bronchial asthma, atherosclerosis and cancer. Considerable efforts have been made in past to develop safe and efficient 5-LO inhibitors. However, many compounds failed under in vivo conditions either due to adverse side effects or a loss of efficacy due to increased oxidative state and/or phosphorylation of 5-LO in inflamed tissues. Currently, the only approved 5-LO inhibitor is the iron chelator zileuton. However, poor efficacy, unfavourable dosage regimes as well as possible hepatic toxicity limit the clinical use of zileuton. In the present study we demonstrate for the first time that clinically relevant concentrations of nitric oxide (NO) releasing non-steroidal anti-inflammatory drugs and other agents releasing NO intracellularly suppress 5-LO product synthesis in human isolated granulocytes by direct S -nitrosylation of 5-LO at the catalytically important cysteines 416 and 418. Furthermore, suppression of 5-LO product synthesis was observed in ionophore-stimulated human whole blood as well in an animal model of pulmonary inflammation. These results provide a novel mechanistic strategy for the development of a new class of anti-leukotriene drugs, which may be able to overcome the limitations of previously developed 5-LO inhibitors and may allow sustained and efficient suppression of leukotriene biosynthesis under in vivo conditions.

Oral YS5

HIGH-FAT DIET FEEDING DIFFERENTIALLY AFFECTS THE CENTRAL NERVOUS SYSTEM INFLAMMATORY TONE: INVOLVEMENT OF BIOACTIVE LIPIDS

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Obesity and its associated disorders are becoming a major health issue in many countries. The resulting low-grade inflammation not only affects the periphery but also the central nervous system (CNS) and will increase the incidence of CNS pathologies such as Alzheimer's disease, stroke or dementia. The impact of a high fat diet (HFD) on the CNS was well characterized with regards to the hypothalamus where it is associated with the activation of glial cells and an increased inflammatory tone further leading to both leptin and insulin resistance. However much less is known about the repercussions of a HFD on the other regions of the CNS in terms of inflammation. Lipids are recognized as central mediators involved in the onset, development and resolution of inflammation. Obesity alters the endogenous levels of several bioactive lipids such as ceramides, phosphatidylcholines and endocannabinoids. In turn, some bioactive lipids exert either pro- or anti-inflammatory effects during obesity. For instance, ceramides exert pro-inflammatory effects in the liver and progressively lead to insulin resistance. Conversely, n−3 polyunsaturated fatty acids show beneficial effects by counteracting HFD-induced adipose tissue inflammation. Still, the potential involvement of other lipids needs to be addressed to better characterize the inflammatory tone deriving from obesity. We set out to characterize, at multiple time-points (from 1 to 16 weeks) and in two different CNS regions (cerebellum and cortex), the inflammatory tone induced by a HFD. Our results clearly suggest region-dependent as well as time-dependent adaptations of the CNS to the HFD. The differences in inflammatory tone between the two regions considered seem to involve astrocytes. Furthermore, we performed a large-scale lipid screening coupled to ex-vivo testing that enabled us to identify three classes of lipids – phosphatidylinositols, phosphatidylethanolamines, and lysophosphatidylcholines – as well as palmitoylethanolamide, as potentially responsible for the difference in inflammatory tone. This study demonstrates that the inflammation induced by a HFD does not similarly affect distinct regions of the central nervous system. Moreover, the lipids identified and tested ex-vivo showed interesting anti-inflammatory properties and should be further studied to better characterize their activity and their role in controlling obesity-derived inflammation in the central nervous system.

Oral YS6

THE REDUCED VASODILATION OF HUMAN PULMONARY VESSELS IS RELATED TO THE DOWN REGULATION OF PGI2 PATHWAY: CORRELATIONS BETWEEN HUMAN PULMONARY ARTERIES AND VEINS

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Prostaglandin I_2 (PGI_2) pathway is involved in human pulmonary hypertension (PH) pathogenesis (1,2, 3) and PGI_2-mimetics are widely used in the clinical management of PH patients. The role and relationship between pulmonary veins (HPV) and arteries (HPA) in the PH pathophysiology are not documented in vitro.
In this study we have investigated:
1/ The in vitro effects of PGI_2-mimetics (iloprost, treprostinil, beraprost, MRE269) on the vascular tone of pulmonary vessels derived from PH (group 3) patients who didn't receive any PGI_2-mimetics before surgery.
2/ The density of prostanoid receptors (EP4, EP2, IP and DP) involved in these responses and of the enzyme responsible for PGI_2 synthesis (PGIS)
3/ The biological and physiological links existing between HPA and HPV.
We have used an organ bath system to measure the vascular tone of HPA and HPV derived from control and PH patients. The different preparations were stimulated by PGI_2-mimetics and pharmacological studies were performed with different antagonists. The expression of PGIS and prostanoid receptors were analyzed by Western blot and real-time-PCR in vascular homogenates. Linear regression and correlation have been used to determine the relationship between HPA and HPV.
The relaxations induced by treprostinil or PGI_2 are significantly reduced in PH veins and arteries. This effect is in relation with a decreased expression of IP and DP receptors in PH vessels. The expression of IP, DP, EP2 and PGIS are positively correlated in arterial and venous preparations derived from the same patient. Furthermore, relaxations (Emax, pEC50) induced by IP agonists (iloprost, treprostinil) are also correlated in these preparations.
This study suggests that the decreased expression of the IP receptors is part of the pathophysiology of PH. It is not related to degradation of the IP receptor due to PGI_2-mimetic treatments before surgery. Together, these results demonstrate an important role of veins in PH pathophysiology due to the similarity of biology and physiology processes between the HPA and HPV.

References

1. Christman BW. et al.,(1992). N Engl J Med. 9;327(2):70-5.
2. Tuder RM. et al. (1999)..Am J Respir Crit Care Med. Pathol. 159(6):1925-32.
3. Falcetti E. et al., (2010). Am J Respir Crit Care Med. 182(9):1161-70.

Oral YS7

SEX MODULATION OF PRO-INFLAMMATORY LIPID MEDIATOR BIOSYNTHESIS DURING ACUTE INFLAMMATION

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Leukotrienes (LTs) and prostaglandins (PGs) are lipid mediators produced from arachidonic acid (AA) that are involved in several inflammatory disorders characterized by a sex bias (i.e. asthma). Here, we report about a sex bias in the biosynthesis of LTs and PGs. Starting from in vitro sex differences in LT biosynthesis in human neutrophils and monocytes due to suppression by androgens, we have investigated the role of sex in LT biosynthesis in zymosan-induced peritonitis in mice, a model of acute inflammation, where LTs play pivotal roles. Upon zymosan injection, higher vascular permeability and cell influx in the peritoneum was evident for female mice, accompanied by significantly higher LT levels in the peritoneal exudates. Orchidectomy of male mice increased the levels of LTs in the exudates compared to sham-treated animals. On the cellular level (resident peritoneal macrophages), these sex differences can be explained by disparities in the subcellular localization of 5-lipoxygenase (5-LO) with lower LT production in male cells. Intriguingly, opposite sex differences were observed for PG biosynthesis in zymosan-induced peritonitis, with higher PG formation in males. While no sex differences in PG levels were observed in the early phase (< 4 hrs), higher production of PGE_2 was evident in exudates of male mice in the late phase of the response (4 - 8 hrs), seemingly due to PG production by infiltrating neutrophils. Note that these sex differences in LT and PG biosynthesis were obvious also in carrageenan-induced pleurisy in rats. Thus, after intrapleural carrageenan injection, higher levels of PGE_2 were found in pleural exudates of male rats (6 - 8 hrs), where inflammation is sustained by neutrophils. Accordingly, isolated neutrophils from human blood of males synthesized higher amounts of PGE_2 upon ionophore stimulation as compared to neutrophils from female blood. Of interest, blockade of LT synthesis in isolated neutrophils as well as in carrageenan-induced pleurisy by MK886 abolished the sex differences in PGE_2 synthesis, suggesting that elevated PGE_2 synthesis in males might be due to lower LT formation. Conclusively, our data demonstrate that sex is an important variable in the biosynthesis of pro-inflammatory eicosanoids with consequences for the inflammatory response.

Oral YS8

UPLC-ESI-MS/MS FOR COMPREHENSIVE PROFILING OF BIOACTIVE LIPIDS IN HUMAN LUNG LAVAGE FLUIDS AFTER BIODIESEL EXHAUST EXPOSURE

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The pulmonary inflammatory cascade involves production of pro- and anti-inflammatory cytokines, chemokines and other mediators, resulting in an influx of inflammatory cells. Key molecules in this process include bioactive lipid mediators (i.e. oxylipins and endocannabinoids) with relevant roles in initiation, propagation, and resolution of inflammation. In order to investigate the responsiveness, bioactive lipid mediators derived from several fatty acid precursors through the cytochrome P450 (CYP), lipoxygenase (LOX), and cyclooxygenase (COX) enzymatic pathways, were quantified in lung lavage fluids from humans exposed to biodiesel exhaust emissions.
Lipidomics profiling using our previously validated UPLC-ESI-MS/MS protocol was performed on lung lavage samples from a human exposure chamber study where healthy subjects (N=15) were exposed on two separate occasions for 1 hour each to biodiesel exhaust and filtered air in a controlled fashion..Bronchial wash (BW) and bronchoalveolar lavage (BAL) were collected in sequence at 6 hours post-exposure. The UPLC-ESI-MS/MS was used in multiple reaction monitoring mode to quantify 15 endocannabinoids and 42 oxylipins in BW and BAL.
The BAL levels were 0.27 – 413 pM (oxylipins), and 0.37 – 181 pM (endocananbinoids and N-acylethanolamines). The BW levels were 0.39 – 626 pM (oxylipins) and 7.4 – 344 pM (endocananbinoids and N-acylethanolamines). Of the quantified fatty acid metabolites, nine were responsive to biodiesel exhaust exposure, six in BAL and three in BW. Of these, elevated levels of 12,13-DiHOME, 13-HODE, and PGE2 in BAL reached Bonferroni-corrected significance in response to biodiesel exhaust exposure. This also constitutes the first report of a comprehensive profiling of endocannabinoids in human lung fluids.
Our previously validated UPLC-ESI-MS/MS method for bioactive lipid profiling was successfully used in the first study reporting on responsiveness of 12,13-DiHOME, 13-HODE, and PGE2 in human lung lavage fluids following biodiesel exhaust exposure.

Oral YS9

AGE DICTATES A STEROID RESISTANT CASCADE OF WNT5A, TRANSGLUTAMINASE-2 AND LEUKOTRIENES IN INFLAMED AIRWAYS

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Airway remodeling is a detrimental and refractory process showing age-dependent clinical manifestations, which are mechanistically undefined. The leukotriene (LT) and wingless/ integrase (Wnt) pathways have been implicated in remodeling, but age-specific expression profiles and common regulators remained elusive.
We sought to study the activation of the LT and Wnt pathways during allergic airway inflammation induced early or late after birth and to address regulatory mechanisms and clinical relevance in normal human bronchial epithelial cells (NHBEs) and nasal polyp tissues. Mice were sensitized with house dust mite allergens (HDM) from day 3, 15 or 60 after birth. Remodeling factors in murine bronchoalveolar lavage fluid (BALF), lung or human nasal polyp tissues were analyzed by westernblot, immunoassays or histology. Regulatory mechanisms were studied in cytokine/HDM- stimulated NHBEs and macrophages.
BALF LT levels in HDM-sensitized mice were increased in neonatal and adult, but reduced in juvenile mice. Lungs of neonatally-sensitized mice showed increased 5-lipoxygenase levels, whilst adult mice expressed more secretory phospholipase A2 (sPLA2-X), Wnt5a and transglutaminase 2 (TGM2). Older mice showed co-localization of Wnt5a and LT enzymes in the epithelium, a pattern also observed in human nasal polyps. IL-4 promoted epithelial Wnt5a secretion, which upregulated macrophage TGM2 expression and TGM2 inhibition in turn reduced LT release. Finally, TGM2, sPLA2-X and LT enzymes in NHBEs and nasal polyps were refractory to corticosteroids.
Our findings reveal age differences in LT and Wnt pathways during airway inflammation and identify a steroid-resistant cascade of epithelial remodeling factors, which may represent a therapeutic target for airway remodeling.

Oral YS10

RESOLVIN D1 AND E1 PROMOTE RESOLUTION OF INFLAMMATION IN MICROGLIAL CELLS IN VITRO

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Sustained inflammation in the brain together with microglia activation can lead to neuronal damage. Hence limiting brain inflammation and activation of microglia is a real therapeutic strategy for inflammatory disease. Specialized pro-resolving mediators (SPM) derived from n-3 long chain polyunsaturated fatty acids (n-3 PUFA), such as resolvin D1 (RvD1) and resolvin E1 (RvE1), emerge as key regulators in physiologic pathways since they actively turn off the systemic inflammatory response. They underlie most of the beneficial effects attributed to their precursors in a number of peripheral inflammatory models. The mechanisms underlying these effects are still unknown and the involvement of SPM in the resolution of inflammation has been poorly described in the central nervous system, especially in microglial cells. We thus evaluated the anti-inflammatory activities of RvD1 and RvE1 in microglial cells in vitro. BV-2 cells were pre-incubated with RvD1 or RvE1 before lipopolysaccharide (LPS) treatment. RvD1 and RvE1 both decreased LPS-induced proinflammatory cytokines (TNF-α, IL-6 and IL-1β) gene expression and modulate microglial phenotype, suggesting their proresolutive activity in microglia. However, the mechanisms involved are distinct as RvE1 regulates NFκB signaling pathway and RvD1 regulates miRNAs expression. Our data deepen our comprehension of the beneficial effects of n-3 PUFAs via novel pro-resolving lipid derivates that stimulates the return to tissue homeostasis in the central nervous system. Overall, our findings highlight the possibility to exploit the beneficial effect of RvD1 and RvE1 in vivo. These results supported that stimulation of the resolution of inflammation would become a new strategy for brain inflammatory disease therapy.

ORAL SESSION

6th EUROPEAN WORKSHOP ON LIPID MEDIATORS

September 30th

Selected Oral 1

REGULATION OF SYSTEMIC INFLAMMATION BY PROSTAGLANDIN E2

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Systemic inflammation, resulted from the massive release of pro-inflammatory molecules into the circulatory system, is a major risk factor for severe illness, but the precise mechanisms underlying its control are not fully understood. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat acute inflammation (e.g., pain/fever) by inhibiting synthesis of prostaglandins, but NSAIDs have also severe adverse effects (e.g., the best-known gastrointestinal bleeding) and NSAID use during evolving bacterial infection is associated with more critical illness. We have observed that prostaglandin E_2 (PGE_2), through its receptor EP4, was down-regulated in human systemic inflammatory disease. Supporting this finding, mice with inhibited PGE_2 synthesis developed systemic inflammation characterized by enhanced cytokine storm, splenomegaly, accumulation of neutrophils and low-grade of hepatitis. The systemic inflammation was associated with disruption of gut barrier gene expression and translocation of gut bacteria, which can be prevented by treatment with EP4 agonists or antibiotics. We have further demonstrated that PGE_2-EP4 signaling acts directly on type 3 innate lymphoid cells (ILC3s), promoting their homeostasis and proliferation and driving them to produce IL-22 in the intestine. While exogenous IL-22 protected against intestinal barrier damage and inflammation, disruption of the ILC3–IL-22 axis impairs PGE_2-mediated inhibition of systemic inflammation. In summary, the ILC3–IL-22 axis is essential in protecting against gut barrier dysfunction, enabling PGE_2-EP4 signaling to impede intestinal inflammation as well as systemic inflammation.

References

1. Duffin R., et al (2016) Science 351,1333-1338.

Selected Oral 2

COMPLEX SIGNALING PATHWAYS OF THROMBOXANE RECEPTOR MEDIATED VASCULAR SMOOTH MUSCLE CONTRACTION

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Prostanoids can induce sustained vascular smooth muscle (VSM) contraction via activation of TP thromboxane receptors. We aimed to identify the heterotrimeric G-proteins and elucidate the intracellular signaling pathways mediating this vasoactive effect.
Isometric tension recording was performed in thoracic aortic segments (TAs) isolated from wild-type (WT) or phospholipase C epsilon knock out (PLCepsilon-KO) mice as well as from mice deficient in G alphaq/11- or G alpha12/13-proteins in the smooth muscle (SM-Gq/11-KO and SM-G12/13-KO) (1). Intracellular calcium levels [Ca^{2+}]i were assessed by ratiometric measurement of FURA-2AM fluorenscence. Blood pressure was determined in the femoral artery of anesthetized mice.
Low concentrations (1-30 nM) of the TP agonist U-46619 induced moderate vasoconstriction which remained unaltered in SM-Gq/11-KO but disappeared in SM-G12/13-KO vessels or in the presence of the Rho-kinase (ROCK) inhibitor Y-27632. In contrast, higher concentrations of U-46619 induced sustained vasoconstriction, which could be inhibited by either Gq/11- or G12/13-deletion, indicating the involvement of both pathways in mediating the effect. Surprisingly, SM-Gq/11-KO VSM, which was completely unresponsive to phenylephrine, showed a significant [Ca2+]i signal for U-46619, which persisted in Ca^{2+}-free medium, and was unaffected by Y-27632, but was almost completely abolished by the RhoA inhibitor TAT-C3. TAs of mice deficient in the RhoA-sensitive PLCepsilon (2) showed attenuated vasoconstriction and decreased VSM [Ca^{2+}]i signal upon administration of U-46619, as compared to WT controls. In accordance, the hypertensive effect of U-46619 but not that of norepinephrine was strongly reduced in PLCepsilon-KO mice. In addition, PLCepsilon-KO mice developed attenuated hypertension in response to nitric oxide synthase blockade.
Weak activation of TPs appears to increase the vascular tone and reactivity exclusively via G12/13-RhoA-ROCK signaling, whereas strong TP stimulation activates both the Gq/11- and the G12/13 pathways and induces a sustained intracellular Ca^{2+} release via a RhoA-dependent, but ROCK-independent activation of PLCepsilon. This unique signaling mechanism is likely to be responsible for spastic vasoconstrictions induced by overactivation of TPs and contributes to the hypertension in NO deficiency.

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References

1. Wirth A et. al., (2008) Nat Med. 14: 64-68, 2008
2. Smrcka AV et . al. (2012) Cell Signal. 24: 1333-1343.

Selected Oral 3

FACING NON-ALCOHOLIC STEATOHEPATITIS WITH FXR ACTIVATION AND SEH INHIBITION

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Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) arising from western diet and lifestyle evolve as serious health burden with alarming incidence[1]. NAFLD and NASH are characterized by accumulation of fat in liver subsequently causing inflammation and fibrosis and are strongly associated with the metabolic syndrome [1]. Although the high prevalence of NAFLD and NASH elicited intensive research for novel treatment options there is still no satisfying pharmacological therapy [2]. Several molecular targets have been identified as potentially suitable for NAFLD/NASH treatment. Promising clinical data has been reported for elafibranor [3], an agonist of the peroxisome proliferator-activated receptors (PPAR) α and δ as well as for obeticholic acid [4] which activates the farnesoid X receptor (FXR). Additionally, the inhibition of a number of enzymes including stearyl-CoA desaturase 1 (SCD1) [5] and soluble epoxide hydrolase (sEH) [6,7] proved effective in treating NASH in vivo. In light of the multifactorial nature of NASH, modulation of more than one target might provide a superior therapeutic effect. Especially, combination of FXR activation that revealed anti-steatotic and anti-fibrotic effects in clinical trials with inhibition of sEH generating anti-inflammatory effects promises synergistic activity. The nuclear receptor FXR acts as intracellular bile acid sensor and liver protector. Its activation has various beneficial metabolic effects and via induction of small heterodimer partner (SHP) as well as sterol regulatory element binding protein 1c (SREBP1c) reduces liver fat content [8] sEH is an enzyme of the arachidonic acid cascade located in the CYP pathway and catalyzes the degradation of anti-inflammatory epoxyeicosatrienoic acids (EETs) to dihydroxyeicosatrienoic acids (DHETs). Therefore, sEH inhibition hinders EET degradation and has anti-inflammatory properties [7]. To exploit the concept of dual FXR/sEH modulation for NASH treatment we developed dual agents with partial FXR agonistic and sEH inhibitory potency. Initially, we merged known pharmacophores [9,10] for both targets to generate our lead compound that exhibited moderate FXR activation and sEH inhibition at 50 μM in vitro. By systematic exploration of the structure-activity relationship (SAR) of the compound class on both targets, we optimized the potency for partial FXR activation and sEH inhibition to low nanomolar values and finally used this knowledge to generate compounds with the desired dual activity and well-balanced nanomolar potency. The most promising compounds were intensively trialed in vitro for FXR target gene induction, selectivity, metabolic stability and toxicity. The compounds revealed a favorable profile and pilot in vivo data is encouraging. In summary, we report the first class of dual FXR agonists/sEH inhibitors and based on favorable in vitro and in vivo properties, further exploration of the concept and the compound class is warranted.

References:
1. Rinella, M., (2015) JAMA 313(22): 2263–73.
2. Gawrieh, S.; Chalasani, N., (2015) Semin. Liver Dis. 35(3): 338–48.
3. Ratziu, V. et al. (2016) Gastroenterology 150(5): 1147–1159.
4. Neuschwander-Tetri, B. et al. (2014) Lancet , 385(9972): 956–65.
5. Zhang, Z. et al., (2014) J. Med. Chem , 57(12): 5039–56.
6. Liu, Y. et al., (2012) PLoS One 7(6): e39165.
7. He, J. et al. (2016) J. Diabetes 8(3): 305–13.
8. Arab, J. et al., (2016) Hepatology , doi:10.1002/hep.28709.
9. Merk, D. et al, (2014) J. Med. Chem. 57(19): 8035–55.
10. Blöcher, R. et al., (2016) J. Med. Chem., 59(1): 61–81.

Selected Oral 4

CHRONIC TOLL-LIKE RECEPTOR SIGNALLING INDUCES AN TEMPORAL SWITCH TOWARDS A MORE RESOLVING LIPID PROFILE IN MONOCYTE-DERIVED MACROPHAGES

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Specialized pro-resolving lipid mediators (SPM) are enzymatically synthesised from omega-3 and omega-6 polyunsaturated fatty acids and counter-regulate inflammation while promoting resolution. Previous data suggest a temporal lipid-mediator profile switch during experimental acute inflammation, from pro-inflammatory lipid mediators in the beginning of inflammation towards SPM during the resolution phase of inflammation. However, the cells and receptors involved in this lipid-mediator switch remain unknown. We hypothesize that the initiation of resolution is orchestrated by tissue-resident cells in response to a persistent inflammatory stimulus.
Macrophages are resident cells present in various tissues. During inflammation, they are exposed to danger signals including like Toll-like receptor (TLR) ligands. Therefore, we set out to investigate whether the duration of TLR signalling can influence the synthesis of pro-inflammatory or pro-resolving lipid mediators. To this end, we stimulated monocyte-derived macrophages with a TLR stimulus and examined changes in their lipid profile over time.
Using targeted lipidomics (LC-MS/MS), we were able to measure sixty analytes, including SPM and their precursors. Although we did not detect SPM at any timepoint, our data indicate that the syntheses of cyclooxygenase (COX) and 15-lipoxygenase (15-LOX) metabolites have a different temporal regulation in macrophages. An increase in COX derived prostaglandins was detected in the first six hours, while after 48 hours an increase in 15-LOX SPM precursors 15-HETE and 17-HDHA could be observed. Interestingly 15-HETE a product of omega-3 PUFA's shows an additional increase in the first six hours that was not observed for 17-HDHA a product of omega-3 PUFA's indicating that not only the enzymes, but also the availability of omega-3 and omega-6 PUFA's is regulated in a temporal fashion. To assess if lipid mediators correspond to the levels of enzymes that synthesize these lipids mRNA levels were measured (Q-PCR). COX expression sharply increased in the first six hours of TLR stimulation and 15-LOX shows a steady increase between six hours and 24 hours. These data were in line with the lipidomics data.These data suggest that chronic stimulation of tissue resident cells results into a lipid-switch that could provide the signal for the initiation of resolution.

Selected Oral 5

MPGES-1 DELETION INCREASES PROSTACYCLIN AND EVADES THE ELEVATED SYSTEMIC ADMA ASSOCIATED WITH COX-2 INHIBITORS: RELEVANCE TO CARDIOVASCULAR SAFETY OF MPGES-1 INHIBITORS

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* NSK, JR and BA-S contributed equally to this study.
# P-JJ and JAM contributed equally to this study

Cardiovascular side effects caused by non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit cyclooxygenase 2 (COX-2) activity, are a global health issue preventing development of new drugs that target prostaglandins for inflammation and cancer therapy. Microsomal prostaglandin E synthase-1 (mPGES-1) inhibitors encapsulate all of the therapeutic promise of NSAIDs with the potential of reduced side effects. However, unfounded and untested concerns over their cardiovascular toxicity remain. Here we have profiled mPGES-1 in renal and vascular pathways that reflect what we know of NSAID cardiovascular toxicity; specifically, these are the COX product prostacyclin, which is cardio-protective and the endogenous eNOS inhibitor ADMA, which is cardio-toxic.
Deletion of mPGES-1 reduced vascular PGE2 formation but increased plasma levels of prostacyclin. In the kidney, mPGES-1 and COX-2 were compartmentalized to the renal cortex and renal medulla respectively. In vivo, COX-2 inhibition altered renal medullary expression of genes associated with production (Prmt1) and metabolism (Agxt2) of ADMA, resulting in significantly elevated plasma ADMA levels. These changes were mirrored in mice lacking prostacyclin synthase (PGIS), but in contrast, deletion of mPGES-1 had no effect on the ADMA pathway. Vascular NO responses, a readout of ADMA activity, were actually improved by mPGES-1 deletion consistent with a preserved ADMA pathway coupled with the loss of constrictor PGE_2 responses. PGIS but not mPGES-1 mediates the cardiovascular protective functions of COX-2 on the renal ADMA pathway. These data should provide renewed confidence in the development of selective inhibitors of mPGES-1 as safer alternatives to NSAIDs for inflammation, pain and cancer.

POSTER SESSION

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Poster 1

DEXAMETHASONE DOWNREGULATES SPHINGOSINE 1-PHOSPHATE (S1P) RECEPTOR 1 EXPRESSION, WHICH IN TURN INHIBITS S1P-INDUCED MESANGIAL CELL MIGRATION.

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Sphingosine 1-phosphate (S1P) is generated by sphingosine kinase (Sphk)-1 and -2 and acts mainly as an extracellular ligand at five specific G protein-coupled receptors, denoted S1P1-5. After activation, S1P receptors regulate important processes in the progression of renal diseases, such as mesangial cell migration and survival. Previously, we showed that dexamethasone enhances Sphk1 activity and S1P formation which protected mesangial cells from stress-induced apoptosis [1]. Here we demonstrate that dexamethasone treatment lowered S1P1 mRNA and protein expression levels in rat mesangial cells measured by TaqMan® and Western blot analyses. This effect was abolished in the presence of the glucocorticoid receptor antagonist RU-486. In addition, in vivo studies showed that dexamethasone downregulated S1P1 expression in glomeruli isolated from C57BL/6 mice treated with dexamethasone (10 mg/kg body weight). Functionally, we identified S1P1 as a key player mediating S1P-induced mesangial cell migration. Using Boyden Chamber assays, we could show that dexamethasone treatment significantly lowered S1P-induced migration of mesangial cells. This effect was again reversed in the presence of RU-486. In summary, we suggest that dexamethasone inhibits S1P-induced mesangial cell migration via downregulation of S1P1. Overall, these results demonstrate that dexamethasone has functional important effects on sphingolipid metabolism and action in renal mesangial cells [2].

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References:

1. Förster, A. et al. (2010) Kidney Int. 77: 870-879.
2. Koch, A. et al. (2015) Biol. Chem. 396: 803-812.

Poster 2

APOPTOTIC CANCER CELLS SUPPRESS 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 such as 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-hydroperoxyeicosatetraenoic acid to the unstable leukotriene A4 with the notion that tumor- promoting functions are attributed to its products. To understand the role of 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. Expression and activity of 5-LO in TAMs were markedly reduced upon co-culture with cancer cells. Also in primary TAMs from murine breast tumors, 5-LO was marginally expressed when compared to bone marrow-derived macrophages. Downregulation of 5-LO was dependent on tumor cell death and the direct contact between macrophages and cancer cells. This was observed in co-cultures as well as 3D human spheroid models, where 5-LO negative macrophages co-localized with apoptotic tumor cells. Stability of the 5-LO mRNA was unchanged, rather a transcriptional silencing of 5-LO expression occurred. Further experiments address the transcriptional alterations leading to 5-LO suppression in TAM, and analyze functional consequences.

Poster 3

OXIDIZED LIPIDS IN OXALIPLATIN-INDUCED NEUROPATHIC PAIN

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Oxaliplatin is one of the most effective drugs as first-line treatment for common cancers like colorectal cancer. However, it causes severe peripheral neuropathic pain which is the major dose-limiting side effect and frequently leads to temporal delay or discontinuation of cancer treatment in patients. Currently, there is no available pharmacological treatment for chemotherapy-induced peripheral neuropathy (CIPN) and the pathological mechanisms involved in CIPN are not well understood. Accumulating evidence points toward a contribution of oxidized lipid mediators which may play an important role in onset and manifestation of CIPN.Here we investigate the synthesis of oxidized lipids from different oxygenation pathways in a mouse model of oxaliplatin-induced peripheral neuropathic pain. We perform lipid profiling from nociceptive tissues such as dorsal root ganglia (DRG), peripheral nerves and the spinal dorsal horn and investigate their effects in calcium-imaging and in vitro assays as well as in vivo behaviour experiments. In our screens we identify several oxidized lipids and characterize their effects on sensitization processes of sensory neurons and related ion channels and on neurogenic inflammation. Finally, we suggest that blocking the synthesis of these lipid mediators or modulating their target receptors may be a useful approach for the development of novel analgesics or neuroprotective strategies, particularly for the treatment of chemotherapy-induced peripheral neuropathic pain.

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Poster 4

DEVELOPMENT OF A REVERSED PHASE CHIRAL LC-MS/MS METHOD FOR THE DETERMINATION OF EPOXY-EICOSANOID ENANTIOMERS

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Stereospecificity is an important feature of all steps leading to the formation and action as well as storage and degradation of bioactive monoepoxides derived from long-chain polyunsaturated fatty acids. Currently developed strategies of targeted lipidomics allow the quantification of all regioisomeric epoxides derived from ARA (epoxyeicosatrienoic acids; EETs), EPA (epoxyeicosatetraenoic acids; EEQs), and DHA (epoxydocosapentaenoic acids; EDPs), but are unable to differentiate between the corresponding R,S-and S,R-enantiomers. Contrary, classical chiral-phase HPLC methods are available for the resolution and preparation of epoxyeicosanoid enantiomers; however, due to the use of non-polar solvent systems, they are not compatible with mass spectrometry-based high sensitive detection. To overcome these problems, we tested the capacity of several novel chiral phases to resolve epoxyeicosanoid enantiomers under reversed phase conditions. Among the chiral-phases tested (Lux Amylose-1, Lux Amylose-2, Lux Cellulose-3 and ChiralPak-AS), Amylose-2 gave an excellent resolution of various monohydroxy compounds (HETEs, HEPEs and HDHAs), but partially failed in sufficiently resolving several of the monoepoxides such as 17,18-EEQ and 19,20-EDP. Cellulose-3 was superior in yielding baseline resolution of almost all enantiomeric monoepoxides derived from ARA, EPA, and DHA (except 11,12-EET and 11,12-EEQ) using a simple methanol/water gradient in the presence of 0.05 % acetic acid. Moreover, the polar solvent system allowed detecting single enantiomers with high sensitivity using electrospray ionization triple quad tandem mass spectrometry (ESI-MS/MS). Providing a first example of application for the Amylose-2-MS/MS system, we confirmed the almost exclusive formation of 12(S)-HETE and 12(S)-HEPE in human whole blood samples after Ca2+-ionophore stimulation. Using the Cellulose-3-MS/MS system, we characterized the complex mixture of epoxyeicosanoid enantiomers in liver samples of wildtype and soluble epoxide hydrolase (sEH) KO mice. In line with the enantioselectivity of the sEH, sEH-KO mice showed a specific accumulation of the R,S-enantiomers of 14,15-EET, 17,18-EEQ, and 19,20-EDP. Taken together, we suggest that reversed phase chiral LC-MS/MS is a suitable tool for detecting and quantifying the enantiomers of epoxy- as well as monohydroxy-eicosanoids in biological samples.

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Poster 5

THE ROLE OF CYP2C44 / SEH IN TUMOUR FORMATION AND METASTASIS

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Polyunsaturated fatty acids (PUFA) are metabolized to epoxides by cytochrome P450 (CYP) enzymes and then further metabolized to diols by the soluble epoxide hydrolase (sEH). The deletion of the sEH combined with supra-physiological arachidonic acid epoxide levels have been linked with tumour formation and metastasis but the role of endogenously generated epoxides and diols is not well understood. To clarify the situation we set out to study tumour formation and metastasis in mouse models with genetically modulated PUFA epoxide/diol status.
sEH-/- and Cyp2c44-/- mice were crossed with polyoma middle T oncoprotein (PyMT+/-) mice and maintained under standard conditions for 20 weeks. There was a significant increase in primary tumour numbers, and tumour burden in PyMTxsEH-/- and PyMTxCyp2c44-/- mice than in the PyMT group. Moreover, primary tumours from Cyp2c44-/--deficient mice demonstrated higher expression of LYVE1 while the sEH-/- deficient tumours demonstrated higher CD31 levels versus the PyMT mice. Also lung and lymph metastasis were significantly increased in PyMTxCyp2c44-/- mice. PUFA profiling of primary tumours revealed markedly higher levels of 9,10- and 12,13-epoxyoctadecenoic acid (EpOME)/dihydroxyoctadecenoic acid (DiHOME) in the PyMTxsEH-/- group while 10,11-epoxydocosapentaenoic acid (EDP), 16,17-EDP and 11,12-epoxyeicosatrienoic acid (EET) and 14,15-EET levels were elevated in PyMTxCyp2c44-/- tumours. An in vitro confrontation assay of murine embryonic stem cells co-cultured with tumour cells revealed that EpOME promoted angiogenesis while DiHOME promoted lymphangiogenesis which fits well with the tumour phenotypes. Similarly the EDPs that were elevated in the Cyp2c44-/- deficient tumours, elicited lymphangiogenesis in vitro which correlated with the weighting towards lymph development in vivo and enhanced lymph node and lung metastasis.
PUFA epoxides and diols play a determinant role in the specification of angiogenesis versus lymphangiogenesis and, as a consequence, metastatic potential.

Poster 6

INVOLVEMENT OF PGE2, REGULATING MMP-1 AND MMP-2, IN ENDOTHELIAL DYSFUNCTION AMONG OBESE WOMEN.

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Obesity is one of the biggest epidemic health problems in the world and has been considered as a major risk factor for cardiovascular diseases accompanied by endothelial dysfunction. In this study, we aimed to investigate the role of prostaglandin E_2 (PGE_2) in regulation of endothelial function and also matrix metalloproteinase (MMP) release in obesity.
Endothelial function was explored in 53 obese subjects without co morbid factors and 49 non-obese subjects (control) by assessing the forearm microvascular cutaneous vasoreactivity using Laser Doppler Flowmetry coupled with iontophoresis. Data were expressed as cutaneous vascular conductance (CVC) which represents the ratio between the cutaneous blood flow and mean arterial pressure (MAP) values, to take into account variations in blood pressure between subjects. Basal CVC values and after stimulation with acetylcholine (ACh) (peak ACh-CVC and delta ACh-CVC) were significantly lower in obese subjects than in controls. Conversely, peak local skin heating (LSH)-CVC and delta LSH-CVC were similar between groups. These results indicate that endothelium-dependent, but not endothelium independent vasodilation is impaired in obese subjects. We further, measured the plasma levels of MMP-1, MMP-2, TIMP-1 (tissue inhibitor of metalloproteinase), TIMP-2, PGE_2 and prostacyclin (PGI_2) by ELISA. PGE_2 levels is significantly increased in obese subjects versus controls. In controls, PGE_2 levels was positively correlated with basal CVC ($r = 0.60$, $P = 0.005$).). In obese subjects, positive correlation was also detected between PGE_2 levels and MAP or PGI_2 levels ($r = 0.36$, $P = 0.035$ and $r = 0.60$, $P = 0.002$, respectively) while it was negatively correlated with MMP-2/TIMP-2 ratio ($r = -0.38$, $P = 0.024$). In addition, only in obese women but not in obese man a significant increase in PGE_2 was correlated with an increase in MMP-1 and MMP-1/TIMP-1 ratio ($r = 0.66$, $P = 0.018$; $r = 0.61$, $P = 0.035$, respectively).
These results suggest that increased levels of PGE_2 could be involved in endothelial dysfunction observed in obese subjects. Regulation of MMP by PGE_2 observed in only obese women could be linked to sex hormone.

Keywords : PGE_2, PGI_2, MMP-1, MMP-2, Endothelial Dysfunction, Obesity.

Poster 7

PHOSPHATIDYLSERINE ETHER LIPIDS AS NOVEL TUMOR-ENRICHED ANTIGENS FOR TYPE II NKT CELLS

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Control of tumor growth by adaptive immune cells requires the recognition of tumor antigens. Beside an altered proteome that provides peptide neo-antigens, tumors show an altered lipidome, supplying novel or overexpressed lipid antigens that are recognized by natural killer T cells (NKT cells). NKT cells are a heterogeneous population of immunoregulatory lymphocytes that recognize foreign as well as self-lipids that are presented on the surface of antigen-presenting cells (APC) via CD1d molecules. Whereas type I NKT cells mainly recognize glycosphingolipids and induce protective immunity, type II NKT cells recognize a variety of lipid antigens and suppress immunity. We therefore hypothesized the presence of type II NKT antigens in tumors that limit anti-tumor immunity. To identify such tumor-enriched lipid antigens we performed a comparative mass spectrometric screening of CD1d-binding lipids from autochthonous mouse mammary tumors and normal mammary glands. Of 31 C D1d-bound lipids that were enriched in mammary tumors compared to normal tissue a surprisingly high proportion were ether lipids, most of them exhibiting a serine headgroup. These phosphatidylserine (PS) ether lipids showed the highest overabundance in murine mammary carcinomas. Accordingly, PS synthesis pathways are overexpressed in breast cancer. Especially enhanced expression of PS-synthase 1 (PTDSS1) correlates with a poor prognosis for breast cancer patients. By using PS-loaded CD1d tetramers, a new subset of type II NKT cells recognizing the serine headgroup was identified. Such NKT cells recognizing PS ether lipids showed an intrinsically reduced inflammatory potential. Analysis of PTDSS1-knockdown tumors grown in syngeneic mice confirmed a role for PS in anti-tumor immunity. In conclusion, we provide evidence of the existence of tumor-enriched NKT cell antigens that impact tumor development by affecting anti-tumor immunity.

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Poster 8

SPHINGOSINE-1-PHOSPHATE INDUCES LIPOCALIN-2 IN MACROPHAGES TO PROMOTE TUMOR LYMPHANGIOGENESIS

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Macrophages affect all hallmarks of cancer, provide a direct growth support to cancer cells, and recruit new blood vessels. Little is known about the interaction of cancer cells with infiltrating monocytes or tumor-derived signals that re-program macrophages towards a pro-tumorigenic phenotype. Lipocalin-2 (Lcn-2) is a member of the lipocalin superfamiliy that transports small lipophilic ligands and essential factors such as iron. Lcn-2 is up-regulated in a number of pathological conditions, such as inflammation and cancer and has been defined as a pro-survival factor for macrophages as well as cancer cells. We provide evidence towards the potential role of Lcn-2 in cancer progression by promoting tumor lymphangiogenesis. Lcn-2 is secreted by primary human macrophages, activated with tumor cell supernatants downstream of the S1P-S1PR1-STAT3 axis and supports pro-tumorigenic macrophage polarization, i.e. promoting tumor lymphangiogenesis.

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Poster 9

CHARACTERIZATION AND CELLULAR LOCALIZATION OF PROTEIN ISOFORMS OF HUMAN 5-LIPOXYGENASE

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Human 5-lipoxygenase (5-LO) initiates the leukotriene (LT) biosynthesis in two steps. First the arachidonic acid is oxidized into 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HpETE) and decay to the 5-HETE. Second, 5-HpETE is converted to the instable epoxide leukotriene A4 (LTA4) which is metabolized to LTB4 by the LTA4 hydrolase or to the cysteine containing leukotrienes LTC4, D4 and E4 by the LTC4 synthase. LTs play an important role in many diseases like asthma bronchiale, atherosclerosis and in many types of cancer [1-3]. The 5-LO is expressed in many cell types like neutrophils, basophils, eosinophils, mast cells, macrophages/monocytes, B- and T-lymphocytes, whereas platelets are 5-LO negative [4-7]. Ann-Kathrin Häfner et al. could show that 5-LO is able to form dimers [8]. Recently, we were able to identify novel splice variants on mRNA level in B-cells and T-cells named delta 4 and delta p12. Boudreau et al. found the isoform d13 in BL41-E95A cells on mRNA level, too [9]. We investigated the isoforms on mRNA and protein level. For investigation on mRNA level, we screened different cell lines with PCR. HEK293T cells were stable integrated by sleeping beauty method and we could show that the WT is localized in the nucleus, whereas the isoforms are located in the cytosol. After activity assays of stable transfected cells, there was no inhibition of the 5-LO activity. Mutation on phosphorylation sites of the 5-LO WT show an influence of the localization in the cell, which could be altered after treatment with different stimuli.

References:

1. Samuelsson, B. et al. (1987) Science 237: 1171-1176.
2. Funk, C. D. (2001) Science 294: 1871-1875.
3. Shimizu, T. et al. (1986) PNAS 83: 4175-4179.
4. Steinhilber, D. (1994) Pharm. Acta Helv. 69: 3-14.
5. Brock, T. G. (2005) J Cell Biochem, 96 (6): 1203-1211.
6. Cook-Moreau et al. (2007) Immunology 122: 157-166.
7. Allain, E. P. (2015) PLoS One 10 (7): 1-17.
8. Häfner, A. K. et al. (2011) Biol Chem, 392: 1097-1111.
9. Boudreau, L. H. et al. (2011) FASEB 25: 1097-1105.

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Poster 10

REGULATION OF LEUKOTRIENE BIOSYNTHESIS BY SPHINGOSINE-1-PHOSPHATE

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Human 5-lipoxygenase (5-LO) is the key enzyme in the biosynthesis of leukotrienes, which are mediators of pro-inflammatory and immune modulatory responses [1]. Since 5-LO is involved in the pathogenesis of atherosclerosis, asthma and several types of cancer, the pharmacological inhibition of the 5-LO enzyme activity offers a possible strategy for the treatment of these diseases. The bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P) is an important lipid mediator, which is involved in a number of biological processes like immune cell trafficking, immune cell proliferation and regulation of permeability of the vascular system [2]. However, S1P is also implicated in pathophysiological processes including cancer and atherosclerosis.
Here we demonstrate for the first time, that S1P is able to reduce leukotriene biosynthesis in intact granulocytes from human donors by inhibition of the 5-LO enzyme activity. A concentration-dependent inhibition of the formation of the 5-LO products leukotriene B4 and 5-HETE with an IC50 value of 1 µM was observed in intact granulocytes, whereas no inhibition was seen in crude cell fractionations or with recombinant purified 5-LO enzyme. Further experiments addressing the mode of action indicate a calcium-dependent irreversible inactivation of 5-LO enzyme by S1P via binding to cell surface G-protein coupled receptors.
Agonist studies revealed an inhibition of leukotriene biosynthesis in intact granulocytes by receptor agonists targeting S1P-R4, suggesting a S1P-R4 mediated inactivation of 5-LO enzyme by S1P. Additional analysis of the S1P receptor mRNA expression in granulocytes confirmed these findings. Finally, inhibition of 5-LO product formation was observed in a lipopolysaccharide (LPS)-induced murine lung inflammation model after intraperitoneal injection of S1P accompanied by suppression of infiltration of macrophages into bronchoalveolar lavage fluid. In conclusion, we could show for the first time that S1P is a novel endogenous regulator of leukotriene biosynthesis providing a potential explanation for some of the physiological and pathophysiological effects of S1P in cellular processes as well as diseases.
This work was funded by the German Research Foundation (DFG-Sonderforschungsbereich SFB-1039), the Else-Kröner-Fresenius-Foundation (EKFS) and the Graduate School TRIP (Translational Research Innovation–Pharma). TJM is recipient of a Heisenberg-fellowship from the German Research Foundation (DFG-MA-5825/2-1).
References:
1. Radmark et al. (2015) BBA 1851(4): 331.
2. Maceyka et al. (2012) Trends Cell Biol, 22(1): 50.

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Poster 11

CHIRAL CHROMATOGRAPHY AS SELECTIVE TOOL FOR ANALYSIS OF LIPID MEDIATORS INVOLVED IN INFLAMMATION

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Lipid mediators play an important role as biological messengers involved in specific cellular responses. Endogenously derived from different fatty acids such as arachidonic, docosahexaenoic and eicosapentaenoic acid many mediators are built at their intended site of action via cyclooxygenases 1 and 2 and lipoxygenases 5, 12 and 15. Prostaglandins featuring both pro- and anti-inflammatory properties as well as the pro-resolving mediators lipoxins, resolvins, protectins and maresins and their hydroxyeicosatetraenoic acid precursors have been analyzed in spiked complex biological matrices such as plasma or urine. Due to interferences with other endogenous molecules present in biological material, a solid phase extraction method using an octadecyl-modified silicagel cartridge was carried out. A sensitive and selective analytical method using liquid chromatography-tandem mass spectrometry has been developed for the simultaneous quantification of prostaglandins D_2, E_2, $F_{2\alpha}$, J_2 and 6-keto-$F_{1\alpha}$, thromboxanes B_2 and 11-dh-B2, lipoxins A5 and B4, lipoxin A4 and 6-epi-lipoxin A4, resolvins D1 and D2 as well as aspirin-triggered lipoxin A4 and resolvin D1, 7(S)-maresin, protectins DX and (neuro-)protectin D1, 17(S)- and 17(R)-hydroxy-docosahexaenoic, hydroxyeicosatetraenoic acids 5(S), 12(S), 15(S), 15(R) and 19 and docosahexaenoic acid. The required chromatographic resolution has been achieved using a chiral Lux Amylose-1 column run in gradient elution mode with water and acetonitrile / methanol. All analytes are fully separated in a runtime of 24 minutes. The mass spectrometer was operated in negative ionization mode with three m/z transitions for each analyte and isotopically labeled stable derivatives were used for quantification purposes. Identification of the analytes was based on both the retention time of the chromatographic peaks corresponding to standards and internal standards and MS/MS fragmentation. The developed method was tested for precision and accuracy and allows to establish lower limits of quantification in the range of 0.2-0.5 ng / mL per sample for the studied analytes. Stability of the analytes in the studied matrices after 5 h at room temperature and 24 h at 5° C has been demonstrated.

Poster 12

DESIGN AND EVALUATION OF DUAL INHIBITORS OF THE SOLUBLE EPOXIDE HYDROLASE (SEH) AND LEUKOTRIENE A4 HYDROLASE (LTA4H)

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The enzymes soluble epoxide hydrolase (sEH) and leukotriene A4 hydrolase (LTA4H) are involved in two of the three major metabolic pathways of the arachidonic acid (AA) cascade and both enzymes transform epoxide-containing lipids into diols. The resulting bioactive lipids mediate several biological effects such as inflammation and immune response. Therefore, inhibition of these enzymes would be beneficial for the treatment of inflammation. [1] Recent studies showed that an inhibition of a single pathway within the AA cascade led to a shunt into another pathway. [2] Concerning this crosstalk an inhibition of more than one pathway is advantageous, reduces side-effects and increases efficacy.[3] Based on these results the concept of dual inhibitors was used.[4] Here we present design and synthesis of dual inhibitors of the sEH and LTA4H. The inhibitory potential was determined in fluorescence-based assays while structure-based design was employed to optimize the inhibitory potency.

References

1. S. H. Hwang, et al., (2013) Curr. Med. Chem., 20, 1783–1799.
2. O. Jung, et al., (2010) PloS one 2010, 5, e11979.
3. a) J.-Y. Liu, et al., (2010) Biochem. Pharmacol. 79, 880–887;
b) S. H. Hwang, et al., (2011) J. Med. Chem. 54, 3037–3050.
4. R. Morphy and Z. Rankovic, (2016) J. Med. Chem., 49, 4961–4970.

Poster 13

1-DEOXYSPHINGOLIPIDS (DEOXYSL) ARE ATYPICAL SPHINGOLIPIDS THAT ARE ELEVATED IN THE PLASMA OF PATIENTS WITH TYPE 2 DIABETES AND HEREDITARY SENSORY NEUROPATHY TYPE 1 (HSAN1).

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Clinically, diabetic sensory neuropathy and HSAN1 are very similar, suggesting the involvement of deoxySLs in the pathology of both diseases. However, very little is known about the cell biology of these lipids and the underlying pathomechanism. We synthesized an alkyne analogue of 1-deoxysphinganine (doxSA), the metabolic precursor of all deoxySLs, and used it to study the metabolism and localization of deoxySLs. Our results indicate that the metabolism of these lipids is restricted to only some lipid species and that they are not converted to canonical sphingolipids or fatty acids. Furthermore, exogenously added alkyne-doxSA was trafficked to mitochondria and interfered with normal mitochondrial morphology in fibroblasts and adult mouse dorsal root ganglia neurons. Swelling and irregular distribution of the mitochondria preceded axonal degeneration in cultured neurons. Our findings therefore indicate that a local enrichment of deoxySLs in mitochondria could contribute to the mitotoxicity seen in diabetic neuropathy and HSAN1.

Poster 14

ELUCIDATING NOVEL METABOLIC PATHWAYS OF THE CYTOTOXIC 1-DEOXYSPHINGOLIPIDS

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Serine palmitoyltransferase (SPT), which catalyzes the first step in the de novo synthesis of sphingolipids, typically condenses serine and palmitoyl-CoA. Under certain conditions SPT can also use alanine, resulting in the cytotoxic atypical 1-deoxysphingolipids (1-deoxySLs). Elevated 1-deoxySLs cause the inherited neuropathy HSAN1; they have also been found to be increased in patients with metabolic syndrome and type 2 diabetes. Diabetic sensory polyneuropathy is clinically very similar to HSAN1, which suggests that 1-deoxySLs may also be implicated in its pathology. 1-DeoxySLs are missing the C1-hydroxyl group of canonical sphingolipids which is necessary for their degradation, and therefore they are widely assumed to be "dead-end" metabolites. Here we investigated the metabolic pathway of 1-deoxySLs in order to understand and potentially control their buildup. We used high-resolution high accuracy mass spectrometry and metabolic profiling workflows to identify novel downstream 1-deoxySL metabolites. The formation of these novel 1-deoxySL metabolites was modulated using specific chemical inhibitors and inducers, as well gene overexpression. In this manner we identified 8 novel 1-deoxySL metabolites which were singly or doubly hydroxylated, or further desaturated, forming 3 branches of a downstream metabolic pathway. We elucidated the order of the metabolic pathway by treating cells with each of the individually purified metabolites. Furthermore, we found that inhibition or induction of the CYP4F enzyme subfamily prevented or increased the formation of these downstream metabolites, respectively. Overexpression of a specific Cyp4f gene also significantly increased the formation of two of the metabolites. While toxic 1-deoxySLs are not metabolized by the canonical pathway, we showed for the first time that they are in fact further metabolized by CYP4F enzymes. We identified 8 novel 1-deoxySL metabolites and their metabolic order. A number of reports have suggested that this enzyme subfamily is down-regulated in mouse models of obesity and fatty liver disease. Therefore, in the future this metabolic pathway may be exploited as a novel therapeutic target to reduce 1-deoxySL levels in metabolic syndrome and type 2 diabetes patients.

Poster 15

FINGOLIMOD BUT NOT FINGOLIMOD-PHOSPHATE INHIBITS IL-33 INDUCED IFN-GAMMA FORMATION IN CD8+ AND IFN-BETA TREATMENT INCREASES CERAMIDES IN PLASMA OF MULTIPLE SCLEROSIS PATIENTS

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A significant proportion of multiple sclerosis (MS) patients respond to treatment with fingolimod (FTY720) or recombinant interferon-beta (IFN-beta). Following phosphorylation by sphingosine kinase 2, FTY720-P acts as a partial sphingosine-1-phosphate receptor agonist and internalizes sphingosine-1-phosphate-receptors, thereby preventing central memory T cell sequestration. IFN-beta performs regulatory effects on the immune system and e.g. increases the number of regulatory T cells. Because IL-33 plays a central role in MS, as it has been detected in human MS plaques in situ, we were interested, whether FTY720 might also affect the IL-33 induced T cell activation. Additionally, Fingolimod specifically targets sphingosine-1-phosphate receptors, therefore it was also interesting to analyze weather fingolimod or other therapies influence sphingolipid plasma concentrations. To address this questions, first in a mouse system, we used primary murine CD8+ T cells and analyzed FTY720/-P effects on IL-33-induced cytokines. Additionally, serum- and plasma samples from MS patients were assessed and concentrations of 15 bioactive sphingolipids were determined by LC-MS-MS. Fingolimod-, IFN-beta and natalizumab-treated patients were compared to untreated patients or healthy controls (n= 8 – 16 per group). IL-33 co-activated CD8+ T cells, especially by increasing IFN-gamma formation in vitro. Interestingly, FTY720 but not FTY720-P was able to inhibit this IL-33 induced IFN-gamma formation, even independently from its intracellular phosphorylation by SphK2, as seen with SphK2-/- splenocytes. Instead we found that FTY720 interacted with the SET/protein phosphatase 2A pathway. IFN-beta treated MS patients revealed increased concentrations of sphingolipids in plasma, especially chain-length specific ceramides. This data, adding detailed immunological understanding of therapeutic fingolimod and IFN-beta effects, might facilitate clinical decision making during MS treatment.

Poster 16

THE INFLUENCE OF ANTI-ESTROGENS ON THE SPHINGOLIPID METABOLISM IN UDP-GLUCOSE CERAMIDE GLUCOSYLTRANSFERASE (UGCG) OVEREXPRESSING BREAST CANCER CELLS

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The UDP-glucose ceramide glucosyltransferase (UGCG) is a golgi apparatus located membrane protein, which transfers UDP-glucose to ceramide resulting in glucosylceramide (GlcCer) generation. GlcCer is an important membrane component, which maintains the water permeability barrier of the skin, but a discussion arised whether or not GlcCer are also regulators of cell signaling cascades or even signaling molecules in physiological and pathophysiological processes. Alterations in UGCG expression and GlcCer levels are linked to several pathophysiological processes like diabetes, disorders of skin, cancer and is also associated with multi drug resistance (MDR) development in several cancer types. Treatment of breast cancer with the anti-estrogen tamoxifen can lead to tamoxifen-resistance of the cancer cells, but studies also showed a tamoxifen-dependent blocking of UGCG and subsequently abrogated MDR. The assumption is, that fine tuning of the ceramide metabolism leads to abrogated MDR, that underlies the importance of investigating the interaction between anti-estrogens and the sphingolipid metabolism, but only a few studies embrace this topic. We focus on the question how the anti-estrogen fulvestrant, which leads to less side effects as tamoxifen, has an impact on the sphingolipid metabolism. In first studies we could show that the UGCG and other enzymes of the sphingolipid metabolism are affected by the anti-estrogen fulvestrant and that this effect is crucial for the cell fate.

Poster 17

PRO-INFLAMMATORY OBESITY IN AGED CANNABINOID-2 RECEPTOR DEFICIENT MICE

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Cannabinoid-1 receptor signaling increases the rewarding effects of food intake and promotes the growth of adipocytes, whereas CB2 possibly opposes these pro-obesity effects by silencing the activated immune cells that are key drivers of the metabolic syndrome. Pro- and anti-orexigenic cannabimimetic signaling may become unbalanced with age because of alterations of the immune and endocannabinoid system. To specifically address the role of CB2 for age-associated obesity we analyzed metabolic, cardiovascular, immune and neuronal functions in 1.2-1.8 year old CB2-/- and control mice, fed with a standard diet and assessed effects of the CB2 agonist, HU308 during high fat diet in 12-16 week old mice. The CB2-/- mice were obese with hypertrophy of visceral fat, immune cell polarization towards pro-inflammatory sub-populations in fat and liver and hypertension, as well as increased mortality despite normal blood glucose. They also developed stronger paw inflammation and a premature loss of transient receptor potential responsiveness in primary sensory neurons, a phenomenon typical for small fiber disease. The CB2 agonist HU308 prevented HFD-evoked hypertension, reduced HFD-evoked polarization of adipose tissue macrophages towards the M1-like pro-inflammatory type and reduced HFD-evoked nociceptive hypersensitivity but had no effect on weight gain. CB2 agonists may fortify CB2-mediated anti-obesity signaling without the risk of anti-CB1 mediated depression that caused the failure of rimonabant.

Poster 18

EVOLUTIONARY ALTERATION OF REACTION SPECIFICITY OF MAMMALIAN ALOX15 ORTHOLOGS IS AIMED AT OPTIMIZING THE BIOSYNTHETIC CAPACITY FOR ANTIINFLAMMATORY LIPOXINS

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ALOX15 orthologs have been implicated in maturational degradation of intracellular organelles and in the biosynthesis of pro-resolving eicosanoids. On the basis of currently available experimental data we hypothesized that lower mammals (mice, rats, pigs) express 12-lipoxygenating ALOX15 orthologs. In contrast, 15-lipoxygenating isoforms are found in higher primates (orangutans, men) and these results suggest a targeted evolution of ALOX15 specificity. To test this hypothesis we first cloned and characterized ALOX15 orthologs of selected catarrhini representing different stages of late primate evolution. Here we found that higher primates (H. sapiens, shimpanzees) express 15-lipoxygenating orthologs. In contrast, lower primates (baboons, macaca) express 12-lipoxygenating enzymes. Gibbons, which are flanked in evolution by macacas (12-lipoxygenating ALOX15) and orangutans (15-lipoxygenating ALOX15), express an ALOX15 ortholog with pronounced dual specificity. To explore the driving force for this specificity alterations we quantified the lipoxin synthase activity of 12- (M. mulatta, M. musculus, R.norwegicus, S. scrofa, humIle418Ala) and 15-lipoxygenating (H. sapiens, P. troglodytes, P. pygmaeus, O. cuniculus, ratPhe353A) ALOX15 variants and found that, when normalized to their arachidonic acid oxygenase activities, the lipoxin synthase activity of 15-lipoxygenating ALOX15 variants were more than 5-fold higher ($p<0.01$). Comparative MD simulations and QM/MM calculations indicated that for 15-lipoxygenating rabbit ALOX15 ortholog the energy barrier for C13-hydrogen abstraction (15-lipoxygenation) was 4.1 kcal/mol lower than for arachidonic acid 12-lipoxygenation. In contrast, for the 12-lipoxygenating Ile418Ala mutant the energy barrier for 15-lipoxygenation was 2.5 kcal/mol higher than for 12-lipoxygenation. Taken together our data suggest a targeted evolution of ALOX15 specificity, which is aimed at optimizing the biosynthetic capacity for pro-resolving lipoxins.

Poster 19

LYSOPHOSPHATIDIC ACID SIGNALING IN THE SENSATION OF ITCH

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Lysophosphatidic acids (LPAs) are bioactive lipids, produced extracellularly via phospholipase D (PLD, autotaxin) or PLA pathways and intracellularly via GPATs. They signal through specific G-protein coupled receptors (LPAR1-5) and thereby contribute to the regulation of angiogenesis, immune responses, and neuronal development. In particular, central LPAs modulate the fine tuning of glutamate signaling through atypical postsynaptic LPARs, which act as scavengers (Prg1, 2 = LPP4, 5). Here, we asked if the LPA-glutamate route modifies nociception and itch, which crucially depend on glutamate signaling strength. We used a number of loss-of-function and pharmacologic approaches including Prg1 -/- and LPAR2-/- mice in combination with nociceptive and itch models and found the strongest phenotype for itch: Injections of both formalin (nociception) and histamine (itch) elicited an increase of LPAs in the mouse thalamus, hypothalamus and somatosensory cortex. LPAR2 and Prg1 are the predominant LPA receptors at these sites and knockout of Prg1 or LPAR2 resulted in intensified scratching behavior compared with wildtype mice, particularly for non-histaminergic, chloroquine-evoked itch. LPAR2 deficient mice additionally showed increased scratching behavior for Bam 8-22 evoked itch, which specifically activates Mas related G-protein coupled receptors responsible for non-histaminergic itch. In addition, LPAR2-/- mice were scratching spontaneously and had stronger nociceptive responses than wildtype mice. LPAR2 agonist or antagonist treatment failed to recapitulate the phenotype of LPAR2-/- mice likely because the drugs do not pass the blood brain barrier. Instead, inhibition of autotaxin reduced scratching behavior associated with reductions of plasma LPAs with however, in part opposing effects on central LPAs. Overall, the data suggest that central LPAs via LPAR2 have inhibitory functions in the context of non-histaminergic and spontaneous itch.

Poster 20

CHARACTERIZATION OF CHOLESTEROL HOMEOSTASIS IN SPHINGOSINE-1-PHOSPHATE LYASE-DEFICIENT FIBROBLASTS

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Sphingosine-1-phosphate (S1P) is involved in the regulation of cell growth, survival, migration and adhesion. It is formed by sphingosine kinases and degraded by phosphatases and S1P lyase. Mice that lack S1P lyase are characterized by the accumulation of S1P and sphingosine in their cells and tissues, and by lymphopenia, generalized inflammation, multiple organ damage, and a strongly reduced life span. On the other hand, embryonic fibroblasts from S1P lyase-deficient mice (Sgpl1-/--MEFs) are resistant to chemotherapy-induced apoptosis, in part due to an upregulation of multidrug transporters of the ATP-binding cassette (ABC) transporter family. Interestingly, S1P lyase-deficient mice have elevated plasma levels of cholesterol and triglycerides, while suffering from strongly reduced body fat. The aim of the present study was to analyze the link between S1P lyase deficiency and altered cholesterol homeostasis using Sgpl1-/--MEFs. In Sgpl1-/--MEFs, total cholesterol content measured with Amplex Red was not altered when the cells were kept in serum-free medium, but was elevated when the cells were grown in the presence of 10 % FCS. Furthermore, the uptake of [3H]cholesterol into Sgpl1-/--MEFs was enhanced in the presence of 10 % FCS, but not significantly altered in the presence of 1 % serum albumin. In agreement, the low-density lipoprotein (LDL) receptor was upregulated in Sgpl1-/--MEFs. The release of [3H]cholesterol was not altered, although the transporter involved in reverse cholesterol transport, ABCA1, was upregulated on the mRNA and protein level. The expression of both the LDL receptor and ABCA1 is regulated by the transcription factor, sterol regulatory element-binding protein (SREBP)-2. In agreement, we observed an enhanced proteolytic activation of SREBP-2 in Sgpl1-/--MEFs. On the other hand, the protein expression of HMG-CoA reductase was decreased in Sgpl1-/--MEFs. Finally, staining of cellular cholesterol with filipin and confocal laser scanning microscopy revealed a disturbed subcellular distribution of cholesterol in Sgpl1-/--MEFs. It is concluded that both the decreased expression of HMG-CoA reductase and the disturbed intracellular distribution of cholesterol lead to activation of SREBP-2, which in turn by induction of the LDL receptor leads to increased cholesterol uptake in Sgpl1-/--MEFs.

Poster 21

THE SOLUBLE EPOXIDE HYDROLASE DETERMINES AND CHOLESTEROL HOMEOSTASIS BY REGULATING AMPK AND SREBP

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Cytochrome P450 (CYP) enzymes generate bioactive epoxides from polyunsaturated fatty acids, some of which have been attributed anti-hypertensive, anti-inflammatory and insulin-sensitizing properties. The soluble epoxide hydrolase (sEH) metabolizes fatty acid epoxides to the corresponding diols, thereby limiting their beneficial effects. Although levels of CYP and sEH enzymes are high in the liver, little is known about the actions of the sEH or its substrates and products on hepatic function. In hepatocytes from sEH-/- mice, the activity of the AMP-activated protein kinase (AMPK); which phosphorylates and inactivates the HMG CoA reductase (HMGCR), was significantly increased. AMPK activation also attenuated the expression and maturation of the sterol-response element binding protein (SREBP) which plays a pivotal role in lipid- and cholesterol-homeostasis. In sEH-/- livers as well as in isolated hepatocytes the expression of HMGCR and other SREBP down-stream targets such as the fatty acid synthase and the LDL-receptor were also down regulated. In wild-type mice fed a high fat diet over 20 weeks, hepatic sEH expression was elevated and accompanied by pronounced increase in hepatic lipid accumulation and cholesterol levels. Surprisingly, sEH-/- mice receiving the same diet developed more severe liver steatosis and higher serum levels of alanine-aminotransferase. These findings indicated that the sEH-/- mice may demonstrate defects in the handling of dietary cholesterol. Indeed, a cluster of genes/proteins (liver gene array and plasma proteomics) involved in cholesterol homeostasis were differentially regulated in wild-type versus sEH-/- littermates, including the microsomal triglyceride transfer protein as well as apolipoproteins ApoB100 and ApoE that are responsible for cholesterol transport. Thus, the sEH is an important determinant of hepatic glucose consumption and endogenous cholesterol synthesis as well as cholesterol transport. These observations have implications for putative sEH inhibitor therapy which should not be considered without dietary control of cholesterol intake.

Poster 22

SPHINGOSINE KINASE 2 DEFICIENCY INCREASES PROLIFERATION AND MIGRATION OF RENAL MOUSE MESANGIAL CELLS

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Both of the sphingosine kinase (SK) subtypes SK-1 and SK-2 catalyze the production of the bioactive lipid molecule sphingosine-1-phosphate (S1P). However, the subtype-specific cellular functions are largely unknown. In this study, we investigated the cellular function of SK-2 in primary mouse renal mesangial cells (mMC) from wild-type C57BL/6 or SK-2 knockout (SK2ko) mice. We found that SK2ko cells displayed a significantly higher proliferative and migratory activity when compared to wild-type cells, with concomitant increased cellular activities of the classical extracellular signal regulated kinase (ERK) and PI3K/Akt cascades, and of the small G protein RhoA. Furthermore, we detected an upregulation of SK-1 protein and S1P3 receptor mRNA expression in SK-2ko cells. The MEK inhibitor U0126 and the S1P1/3 receptor antagonist VPC23019 blocked the increased migration of SK-2ko cells. Additionally, S1P3ko mesangial cells showed a reduced proliferative behavior and reduced migration rate upon S1P stimulation, suggesting a crucial involvement of the S1P3 receptor. In summary, our data demonstrate that SK–2 exerts suppressive effects on cell growth and migration in renal mesangial cells, and that therapeutic targeting of SKs for treating proliferative kidney diseases requires subtype-selective inhibitors.

Poster 23

ROLE OF SPHINGOSINE 1-PHOSPHATE RECEPTOR 5 (S1P5) FOR THE DEVELOPMENT OF BLEOMYCIN-INDUCED SKIN FIBROSIS.

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Systemic sclerosis (SSc) is a rare and clinical heterogeneous autoimmune disease with an unclear pathogenesis. It is characterized by inflammation and autoimmunity, resulting in progressive fibrosis of the skin and various internal organs. According to our working hypothesis, in fibrogenesis an initial vascular injury leads to activation of endothelium and release of mediators which promote differentiation of myofibroblasts, ultimatively responsible for fibrotic remodeling. Possibly, activated myofibroblasts are directly derived from endothelial cells (EC) that have acquired a mesenchymal phenotype through a transition process, called endothelial to mesenchymal transition (EndMT). The extent of involvement of EndMT in development and progression of fibrotic disorders is still under debate. EC are a possible source of the sphingolipid sphingosine 1-phosphate (S1P), which is elevated in sera of SSc patients and has been implicated in the induction of a myofibroblastic phenotype through trans-activating TGF-beta signaling. Moreover, S1P may be causatively related to abnormalities described in vasculature, immune system and connective tissues of SSc patients. Effects of S1P are mediated by binding to the five membrane bound G-protein coupled receptors S1P1 to 5. Recently, for human mesangial cells it has been shown that the S1P5 receptor is required for TGF-beta2 –induced expression of connective tissue growth factor CTGF, a key player in the onset of fibrosis.To investigate the role of S1P and especially S1P5 in the development of SSc, the well-described bleomycin-induced mouse model of scleroderma was applied. Herein C57BL/6 (n=20) and S1P5 -/- mice (n=20), were injected subcutaneously (s.c.) with either bleomycin or PBS on 5 days a week for a time period of 2 or 4 weeks. A preliminary assessment of these in vivo samples comparing the fibrotic changes, revealed interesting differences which may suggest an intermediary function of S1P in early fibrogenesis.

Poster 24

HYPOXIA POTENTIATES PALMITATE-INDUCED PRO-INFLAMMATORY ACTIVATION OF PRIMARY HUMAN MACROPHAGES

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Pro-inflammatory cytokines secreted by adipose tissue macrophages (ATMs) contribute to chronic low grade inflammation and obesity-induced insulin resistance. Recent studies have shown that adipose tissue hypoxia promotes an inflammatory phenotype in ATMs. However, our understanding of how hypoxia modulates the response of ATMs to free fatty acids (FFAs) within obese adipose tissue is limited. We examined the effects of hypoxia (1% O_2) on pro-inflammatory responses of human monocyte-derived macrophages to the saturated fatty acid, palmitate. Compared to normoxia, hypoxia significantly increased palmitate-induced mRNA expression and protein secretion of IL-6 and IL-1beta. Whereas palmitate-induced endoplasmic reticulum stress and nuclear factor-kappaB pathway activation were not enhanced by hypoxia, hypoxia increased activation of JNK and p38 mitogen-activated protein kinase signaling in palmitate-treated cells. Inhibition of JNK blocked hypoxic induction of pro-inflammatory cytokine expression, whereas knockdown of hypoxia-induced transcription factors (HIF) HIF-1alpha and HIF-2alpha alone or in combination failed to reduce IL-6 and only modestly reduced IL-1beta gene expression in palmitate-treated hypoxic macrophages. Enhanced pro-inflammatory cytokine production and JNK activity under hypoxia were prevented by inhibiting reactive oxygen species generation. In addition, silencing of dual-specificity phosphatase 16 increased normoxic levels of IL-6 and IL-1beta and reduced the hypoxic potentiation in palmitate-treated macrophages. The secretome of hypoxic palmitate-treated macrophages promoted IL-6 and macrophage chemoattractant protein-1 expression in primary human adipocytes, which was sensitive to macrophage JNK inhibition. Our results reveal that the coexistence of hypoxia along with FFA exacerbates macrophage-mediated inflammation.

Poster 25

GENE SILENCING OF MCP-1 BY THE ENDOCANNABINOID ANANDAMIDE DECREASES THE RECRUITMENT OF MONOCYTES

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The endocannabinoid system has been implicated in the regulation of stress, emotional behavior and by reducing the infarct size of the heart as well as blood pressure. Endocannabinoids are thought to be cardioprotective. We therefor set out to identify the impact of those bioactive lipids on the inflamed vasculature.
Anadamide (AEA) is the ethanolamide of arachidonic acid and is one of the best characterized endocannabinoids. Pretreatment of murine aortic segments, murine lung endothelial cells and human aortic smooth muscle cells (hAoSMCs) with AEA decreases the IL1β- and TNFα-induced induction of monocyte chemotactic protein 1 (MCP-1) on mRNA and protein level. In line with this, supernatant of hAoSMCs stimulated with AEA attenuated monocyte migration. These effects were not mediated by PPAR or by the cannabinoid receptor CB2. Importantly, NF-κB translocation into the nucleus was not affected by AEA. To identify the underlying mechanism, ChIP experiments were performed, which revealed that AEA epigenetically silenced the MCP-1 gene but not ICAM-1, by reducing the active chromatin mark H3K4me3 and histone acetylation. Consistently, NF-κB and Pol II recruitment was reduced to the MCP-1 transcription start site.
AEA reduces the inflammatory response of the vasculature by epigenetic silencing of specific inflammatory genes.

Poster 26

AOM/DSS-MEDIATED ULCERATIVE COLITIS IS AUGMENTED IN CERAMIDE SYNTHASE 2 NULL MICE DUE TO ELEVATED MIGRATION OF T-CELLS.

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Ulcerative Colitis (UC) is an inflammatory bowel disease (IBD) located in the colon of patients of different ages. The gut lumen, a highly inflammatory milieu, is defined by a single layer of colonic epithelium. The cause of UC is still unknown, however the destruction of the colonic barrier function and subsequent activation as well as deregulation of various immune cells are known to form the most common pathological conditions of UC. The imbalance of CD4 positive T-cells hereby plays an important role.
Here we investigated the severe inflammation in ceramide synthase 2 (CerS2) knockout mice after chronical DSS-treatment. We performed FACS analysis of the invading immune cells and utilized LC-MS/MS for profiling of ceramides of $CerS2^{-/-}$ mice after DSS treatment. We furthermore investigated the expression levels of ceramides and used immunohistochemical analysis of the inflamed tissue.
We have hereby observed an increased onset of DSS-induced UC in $CerS2^{-/-}$ mice and identified a decreased synthesis of ceramides next to disrupted layers of colonic epithelium in the gut lumen. Interestingly, we have found a strong alteration of immune cell invasion after chronically DSS-treatment in mice lacking the CerS2.
Finally, we suggest that the absence of CerS2 leads to a changed ceramides expression pattern and thereby induces an alteration of immune response during UC in the colon with the consequence of an increased inflammatory onset and intensity.

This work was supported by the DFG (German Research Association) grant SFB1039 TP B05 and TP Z01

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Poster 27

SPHINGOSINE-1-PHOSPHATE ENHANCES ALPHA$_1$-ADRENORECEPTOR-MEDIATED VASOCONSTRICTION

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Sphingosine-1-phosphate (S1P) is a sphingolipid mediator known to influence a wide range of biological processes including vascular functions via its five G-protein-coupled receptors ($S1P_{1-5}$). In our present experiments we intended to examine the vasoactive effects of S1P. Our aim was to evaluate its capability to alter the basal vascular tone and to influence vasoconstriction mediated by alpha$_1$-adrenoreceptors.
Segments of the thoracic aorta were isolated from adult male wild type (WT) and knockout (KO) mice deficient in $S1P_1$ or $S1P_2$, or $S1P_3$ receptors. Isometric tension of the segments was measured via myography. Effects of S1P and the α_1-agonist vasoconstrictor phenylephrine (PE) were tested. Changes in tension were normalized to vasoconstriction evoked by 124 mM K^+.
Administration of 10 μM S1P - that is in the concentration range of human serum - did not cause significant change in the resting vascular tone. Following a 20 min incubation of the segments with 10 μM S1P however, the EC_{50} of PE-induced vasoconstriction decreased from 145±1 nM to 80±1 nM, while E_{max} increased from 114±3% to 133±3% in WT vessels. Similar enhancement of the vascular reactivity was detected in $S1P_1$- and $S1P_3$ KO segments. In $S1P_2$ KO vessels, however, this phenomenon was absent. The modulation of PE-induced vasoconstriction by S1P was also abolished by simultaneous administration of the Rho-kinase inhibitor Y27632 (2 μM).
In further experiments we aimed to evaluate the duration of the S1P-induced enhancement of α_1-adrenoreceptor-mediated vasoconstriction. Therefore, 80 nM PE was applied repeatedly every 20 minutes, following a 20 min long exposure of the vessels to 10 μM S1P. Vasoconstrictor responses remained enhanced during the following 3 hours in WT segments, whereas this increase could not be detected in $S1P_2$ KO vessels.
Although S1P does not directly modify resting vascular tone by itself, yet it significantly enhances alpha$_1$-adrenoreceptor-mediated vasoconstriction. The $S1P_2$ receptor - Rho-kinase pathway is responsible for this effect. The sustained enhancement of vascular reactivity detected underlines the potential pathophysiological significance of the phenomenon.

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POSTER SESSION I (No: 1-31)
POSTER SESSION II (No: 32-61)

6th EUROPEAN WORKSHOP ON LIPID MEDIATORS

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Poster Session
6th EUROPEAN WORKSHOP ON LIPID MEDIATORS
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Poster 1

THE PROSTANOID EP2 RECEPTOR IS A KEY ANTI-PROLIFERATIVE TARGET FOR THE PROSTACYCLIN ANALOGUE, TREPROSTINIL IN SMOOTH MUSCLE CELLS AND FIBROBLASTS DERIVED FROM PATIENTS WITH PULMONARY ARTERIAL HYPERTENSION

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Prostacyclins remain the gold standard treatment for severe pulmonary arterial hypertension (PAH), a hyper-proliferative, vascular remodelling disease. The prostacyclin receptor (IP) is considered the classical pharmacological target for prostacyclins, though treprostinil is a uniquely potent activator of EP2 receptors. The aim was to assess the relative contribution of IP and EP2 receptors in mediating treprostinil effects on pulmonary artery smooth muscle cell (PASMC) and fibroblast proliferation. Responses were compared to MRE-269, the active metabolite of selexipag, a recently approved non prostanoid IP agonist.
Distal PASMCs from PAH patients were transfected with Smartpool siRNAs (Dharmacon) directed against the PTGER2 gene. In all experiments, human cells were stimulated for 4 days with serum-containing media in the presence of agonists ± EP2 antagonist (1microM PF-04418948), IP receptor antagonist (IPRA; 1microM RO-1138452) or siRNAs (3pmol) to measure cAMP (ELISA) and proliferation (MTS and cell counting). Real time qPCR determined the relative expression of EP2 and IP receptors in growing cells.
The antiproliferative effects of treprostinil, but not MRE-269, were significantly reversed ($P<0.001$, $n=4$) by EP2 siRNAs in PASMCs. Moreover, cAMP generated by butaprost (EP2 agonist) or treprostinil was fully or partially reversed by EP2 siRNA treatment ($P <0.01$), respectively. In fibroblasts isolated from PAH lungs, treprostinil was more efficacious at inhibiting proliferation compared to MRE-269 (54% versus 19% at 10microM; $P<0.001$). In both cell types, PF-04418948 reversed treprostinil but not MRE-269 responses, while RO-1138452 had the opposite effect. In control and PAH fibroblasts, IP receptor mRNA expression was weak, resulting in a high EP2/IP ratio (40 and 60, respectively). In control PASMCs, EP2 and IP were equivalent and the ratio rose to 114 in PAH cells.
In PAH, EP2 receptors are robustly expressed in smooth muscle cells and fibroblasts and substantially contribute to the antiproliferative effects of treprostinil. The prominent role of EP2 in PAH might be explained by enhanced EP2 expression concomitant with either low IP expression or a down-regulation of this receptor. The distinct pharmacological and functional differences between treprostinil and MRE-269 have important clinical implications, and EP2 receptors should be considered an important therapeutic target for vascular remodelling in PAH.

Funded by a research grant from United Therapeutics Corp.

Poster 2

ENDOTOXEMIA INDUCES PRODUCTION OF LTB4, NEUTROPHIL INFILTRATION AND COLLAGENOLYSIS IN ATHEROSCLEROTIC PLAQUES.

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Atherosclerosis is nowadays considered as being driven by immune responses. Polymorphonuclear neutrophils (PMNs) have just begun to emerge as a potential actor in atherosclerosis, but the factors involved in their recruitment and their activation in plaques have yet to be elucidated. Leukotriene B4, an eicosanoid mediator generated by the 5-lipoxygenase (5-LO,) is a powerful PMN chemoattractant. Experimental1 and genetic studies2 have suggested that LTB4 could play a role in atherosclerosis, but its chemotactic properties in this disease have not been assessed yet. We aim to determine whether plaques can produce LTB4 and whether it can recruit and activate PMNs which could later alter plaques. Using EIA, we quantified LTB4 production by aortic plaques from 50 week-old ApoE-/- and ApoE+/+ mice treated or not with LPS. To assess PMN recruitment in the plaque, we quantified PMNs in mice treated with LPS by flow cytometry. The effect of LPS treatment on plaques was determined by in situ zymography for proteolytic activities and by measurement of collagen content using PSR for plaque stability.
LPS treatment increased the content of LTB4 in atherosclerotic aortic tissues but not in healthy aorta. The percentage of PMNs amongst live leukocytes increased from 10%±1 to 45%±5.1, $p<0.05$ in aortic plaques after LPS treatment. Treatment with LPS increased significantly the proteolytic activities for collagen type I and type IV (respectively 1.9 and 3.1 -fold increase, $p<0.01$ and $p<0.001$) and altered plaque stability by decreasing its global content in collagen (from 73.04%±4 to 44%±4.7 of total plaque area, $p<0.001$).
LTB4 was able to recruit PMNs to atherosclerosis plaques during LPS endotoxemia. Once PMNs were inside the plaque, they increased plaque proteolytic activity for extracellular matrix components suggesting they release the content of their granules and they also contribute to alter plaque stability by decreasing its content in collagen. This study suggests that PMNs might contribute to plaque rupture, but further investigation is needed to confirm the potential impact of PMNs on plaques.

References:

1.Bäck, M. (2009). Cardiovasc. Drugs Ther. 23, 41–48.
2.Helgadottir, A. et al. (2006). Nat. Genet. 38, 68–74.

Keywords: LTB4 , atherosclerosis, PMNs

Poster 3

DIFFERENT DERIVATIZATION APPROACHES TO IMPROVE THE MASSPECTROMETRIC ANALYSIS OF OXYLIPINS

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Oxylipins are oxidation products of polyunsaturated fatty acids (PUFA) such as arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DPA). They are formed in the human organism in the ARA cascade by enzymatic pathways as well as autoxidation. Many of the resulting products act as potent lipid mediators and are involved in the regulation of different physiological processes such as the modulation of inflammation or blood coagluation. Interestingly, several oxylipins show opposing effects. For example, several hydroxylated ARA derivates act pro inflammatory, while the corresponding EPA oxylipins have anti-inflammatory functions. The biological function of several oxylipins is only poorly understood. Analysis of oxylipins in biological matrices is currently carried out by liquid chromatographic separation coupled to mass spectrometry (LC MS). Ionization is generally carried out by electrospray ionization in negative mode (ESI-). The ESI-MS signal is strongly affected by ion-suppression/enhancement by matrix compounds which hampers quantification. Moreover, the use of an acidic eluent for sufficient reversed phase LC separation compromises sensitivity. In this work, we compared different derivatization/ionization techniques for LC-MS analysis of hydroxy-PUFA: Derivatization with the permanently charged AMPP (4 [Aminomethyl] -phenyl pyridinium chloride) and ESI(+)-MS detection (Bollinger et al, 2010 Anal. Chem); derivatization with pentafluorobenzyl bromide and MS detection following electron capture ionization (APCI) and direct ESI(-)-MS detection. The results are discussed with respect to selectivity and sensitivity gained with the different methods.

Poster 4

PARKINSON'S DISEASE ASSOCIATED PAIN: THE ROLE OF DERANGED BIOACTIVE LIPIDS

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Parkinson's disease (PD) is often associated with chronic musculoskeletal and/or neuropathic pain. However, the mechanisms are not well understood. We have previously shown that polymorphisms in fatty acid amide hydrolase (FAAH), which metabolizes endogenous cannabinoids including anandamide and other ethanolamide endocannabinoids (eCBs), were associated with an enhanced risk for PD-related pain. Thus, a dysfunction of the endocannabinoid system may contribute to the pathogenesis of PD associated pain.
We chose a translational approach to test the hypothesis that bioactive lipid signaling is deranged in PD and analyzed profiles of bioactive lipids and sensory functions in PD patients and PD mice, using double mutant Pink1-/- & Synuclein-(Snca) knockin mice, which spontaneously develop motor symptoms of the disease on aging. PD patients and PD mice had seriously reduced plasma and tissue (mice) levels of eCBs, including anandamide, and patients carrying the FAAH polymorphism had increased levels of PGE2. Further, we found reductions of lysophosphatidic acids and increased ceramides. The lipid dysbalances in mice manifested before onset of serious motor function deficits and still "healthy" PD mice had sensory dysfunctions, namely a loss of thermal sensitivity of the hind limbs. Similarly, quantitative sensory testing (QSR) in PD patients revealed a loss of thermal sensitivity of the lower limb but hypersensitivity of the upper limbs. Hence, the mice replicate part of the human sensory-motor-lipid pathology and represent a valuable model to study the putative causal relationship of deranged bioactive lipids in PD and PD-associated pain and to develop treatments to reset the lipid balance.

Poster 5

LIQUID-CHROMATOGRAPHY-QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY AS A TOOL FOR BIOMARKER RESEARCH IN LIPIDOMICS

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Early diagnosis is very important for the treatment of several diseases. Thus, a way to identify diagnostic biomarkers is required. Our aim is to develop a qualitative untargeted lipidomic approach using reversed-phase liquid-chromatography coupled with quadrupole-time-of-flight mass spectrometry (QTOF) to search for potential biomarkers in complex biological samples.
For this purpose, a simple and unspecific sample preparation procedure has been chosen. After liquid-liquid extraction of plasma or tissue, extracts are injected into the chromatographic system. In the case of complex biological samples larger chromatographic separations are often required to enhance resolution power and avoid missing analytes, decreasing ion suppression effects and coelution. Using ultrahigh-performance liquid chromatography with a C18 1.8 µm, 2.1x50 mm column we are able to isolate with an adequate separation the most common lipid species in a 30 minute run. The QTOF analyser supplies the required mass resolution of less than 5 ppm and the fragmentation patterns necessary for the unequivocally identification of lipid species with the same molecular mass but different molecular structures. The robustness of the developed system with retention time shifts under 0.05 min and adequate mass calibration assure the low variability of the obtained information need ed to successfully perform multivariate data analysis. Measurements in both positive and negative ionization modes combined with MS/MS information and high mass accuracy allow for identification of many lipid species belonging to several families including PC, LPC, SM, PE, LPE, PI, TG and Ceramides in biological samples. Hereby we present a widely applicable and consistent method for biomarker discovery research in the field of lipidomics.

Poster 6

ALTERATIONS IN THROMBOTIC PARAMETERS AND URINARY SECRETION OF PROSTACYCLIN AND THROMBOXANE A2 METABOLITES IN HEALTHY AND ZYMOSAN-INJECTED RATS DURING LONG TERM ADMINISTRATION OF NON-SELECTIVE AND SELECTIVE CYCLOOXYGENASE INHIBITORS

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It is well-known that non-steroidal anti-inflammatory drugs (NSAIDs) have prominent gastrointestinal side effects, which prompted the development of selective inhibitors of cyclooxygenase-2, the coxibs. However, increased cardiovascular side effects related to coxib usage was reported. Most accepted mechanism for this side effects is the decrease in prostacyclin (PGI_2) without any change in thromboxane A_2 (TxA_2).
In order to reveal the mechanism of occlusive cardiovascular events caused by cycloocygenase (COX) inhibition; coxibs, etoricoxib (3 and 30 mg/kg/day) and celecoxib (3 and 30 mg/kg/day), COX-1 selective inhibitor valeryl salicylate (40 mg/kg/day), aspirin (3 and 100 mg/kg/day) and naproxen (15 mg/kg/day) were administered to healthy rats and to rats with zymosan-induced chronic inflammatory disease for 31 days. Daily urine was collected for determination of 2,3-dinor-6-keto-PGF1alpha and 11-dehydroTxB_2 by EIA method. At the 31st day ex-vivo, serum TxB_2 and plasma PGE_2 production after COX stimulation were measured. "Platelet Function Assay-100" (PFA-100) and $FeCl_3$-induced thrombus tests were performed. Etoricoxib 30mg/kg/day group had a reduction in both PFA-100 and thrombus formation time while etoricoxib 3 mg/kg/day group had zero non-closure rate in both tests at healthy rats. In both zymosan-injected celecoxib groups there was a reduction in PFA-100 time indicating platelet hyper-reactivity. In healthy rats, etoricoxib and celecoxib (30 mg/kg/day), naproxen and aspirin (100 mg/kg/day) caused a decrease in PGE_2 production, while all the drugs caused a decrease in TxB_2 production. In zymosan-injected groups, production of both eicosanoids decreased in all drug groups. In healthy rats, urinary level of 11-dehydro TxB_2 decreased at naproxen, valeryl salicylate and aspirin, 3 and 100 mg/kg/day groups. Urinary level of 2,3-dinor-6-keto-PGF1alpha decreased at etoricoxib and celecoxib (30 mg/kg/day) and aspirin (100 mg/kg/day) groups. After zymosan injection, urinary 11-dehydro TxB_2 level increased fivefold and from that point showed a more prominent decrease at celecoxib 30 mg/kg/day, naproxen and both aspirin groups while the level of 2,3-dinor-6-keto-PGF1alpha remained unchanged at all drug groups. According to our study, the PGI_2/TxB_2 hypothesis for the cardiovascular side effects of long term NSAID administration is not valid. Further investigations are mandatory for determining the exact mechanism and the patients at risk for this valuable drug group.

This study was supported by TUBİTAK (Scientific and Technologic Research Council of Turkey (Project No: 101S102).

Poster 7

REGULATORY MECHANISMS OF CHEMOTACTIC FACTOR(S) RELEASE BY INTRACELLULAR PHOSPHOLIPASE A2 IN IL-1B-STIMULATED RAT FIBROBLASTS.

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Chemokines are secreted proteins that regulate cell migration and are involved in inflammatory and immune responses. Our aim in this study is to define the functional crosstalk between the lipid signaling and chemokine signaling. Here, we provide evidence that the induction of some chemokines is regulated by calcium-independent phospholipase A2β (iPLA2β) in IL-1β-stimulated rat fibroblastic 3Y1 cells. Treatment of 3Y1 cells with IL-1β elicits increased release of chemotactic factor(s) into culture medium in a time-dependent manner. Inhibitor studies showed that an intracellular PLA2 inhibitor arachidonoyl trifluoromethyl ketone (AACOCF3), but not a cyclooxygenase inhibitor indomethacin, attenuated the release of chemotactic factor(s). The chemotactic activity was inactivated by either treatments of heat or proteinase K, suggesting this chemotactic factor(s) is a proteinaceous factor(s). We purified the chemotactic factor(s) from the conditioned medium of IL-1β-stimulated 3Y1 cell using a heparin column and then identified several chemokines, including monocyte chemoattractant protein (Mcp-1). Furthermore, the inducible expression of Mcp-1 was significantly attenuated by a pretreatment of AACOCF3. Gene silencing using siRNA revealed that the induction of Mcp-1 and its accompanying chemotactic activity were attenuated by knockdown of calcium-independent PLA2β (iPLA2β). Additionally, transcriptional activation of nuclear factor of activated T cell (NFAT), but not NF-κB, by IL-1β stimulation was attenuated by AACOCF3. Knockdown of NFATc4, not other NFAT isoforms, markedly attenuated cytokine-induced Mcp-1 expression. Collectively, these results indicated that iPLA2β plays the roles in IL-1β-induced chemokine expression, in part, via NFATc4 signaling.

Poster 8

12/15-LIPOXYGENASE AS A THERAPEUTIC TARGET IN DIABETIC RETINOPATHY

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Diabetic retinopathy (DR) is a major neurovascular complication in diabetic patients. Microvascular dysfunction in DR includes leukostasis, hyperpermeability and finally retinal neovascularization. The prevalence of diabetes is significantly growing, especially among working age people representing an increasing socioeconomic burden to deal with in the near future. This provides an imperative to prevent the development of DR particularly the current therapies have several limitations and primarily target the end-stages of the disease when significant retinal dysfunction has already ensued. Bioactive lipid metabolites derived from 12/15-lipoxygenase (12/15-LO) are attracting great interest as disease-related molecules with little attention in the context of early DR. 12- and 15-hydroxyeicosatetraenoic acids (HETEs) are arachidonic acid derived eicosanoids that produced by 12/15-LO and induce angiogenic properties of endothelial cells. We evaluated the changes in the levels of 12/15-LO-derived eicosanoids in human retinal endothelial cells (HRECs) subjected to hyperglycemia, retina of experimental diabetic mice and vitreous samples of diabetic patients using the LC/MS. In addition, the direct effect of 12- or 15-HETE on the function of HRECs was tested by studying the tube formation, leukocyte adhesion and permeability. To investigate the potential mechanism that governs the effect of 12- or 15-HETE on the function of HRECs, we studied the changes in VEGF signaling. Our experiments demonstrated significant increase in 12- and 15-HETEs in HRECs subjected to hyperglycemia and also in retina of experimental diabetic mice as well as in vitreous of patients with DR. Treatment of HRECs with 15-HETE induced capillary formation, leukostasis and hyperpermeability. Although there was no changes in the levels of VEGF, we noticed significant increase in the levels of phosphorylated VEGF-R2. Contrary to HRECs, treatment of rat Muller cells with 12-HETE induced upregulation of VEGF. Our results suggest that HETEs derived from retinal endothelial cell under high glucose treatment might act on VEGF signaling through paracrine loop by increasing production of VEGF from glial cells, and through autocrine loop that activates VEGF-R2 in retinal endothelial cells. The underlying mechanism that links 12/15-LO-derived eicosanoids to the activation of VEGF signaling in DR needs further investigation.

Poster 9

EFFECTS OF N-ACYLETHANOLAMINE-HYDROLYZING ACID AMIDASE INHIBITION ON ALVEOLAR MACROPHAGE ACTIVATION AND LUNG INFLAMMATION

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N-acylethanolamines (NAEs) are endogenous bioactive lipids linked to the endocannabinoid system. They are implicated in numerous biological processes including anxiety, pain and inflammation. Some NAEs, such as the anti-inflammatory compound N-palmitoylethanolamine (PEA) and the endocannabinoid N-arachidonoylethanolamine (AEA), have been more studied than others, however other single NAE species can also reduce LPS-induced macrophage activation.
The levels of NAEs and therefore their actions can be controlled by modulating the activity of their two hydrolytic enzymes, fatty acid amide hydrolase (FAAH) and N-acylethanolamine acid amidase (NAAA). Given that much more is known about FAAH, we decided to investigate whether NAAA inhibition could increase NAE levels and thus decrease macrophage activation. We then wanted to investigate the effects of NAAA inhibition in primary alveolar macrophages and see if NAAA inhibition could be an interesting strategy to decrease lung inflammation in vivo.
J774 macrophages and murine alveolar macrophages were activated by incubation with lipopolysaccharides (LPS–100ng/mL) for 8 hours. The NAAA inhibitor (AM9053) or vehicle were added one hour prior to LPS. mRNA expression of pro-inflammatory cytokines was measured by RT-qPCR. Levels of pro-inflammatory cytokines were measured by ELISA in the medium of cells. NAEs and PGD2 were quantified by HPLC-MS. In vivo, inflammation was induced in C57Bl/6 mice by i.p. administration of LPS, during 10 days. The NAAA inhibitor was administered i.p. 8 hours after the last LPS administration (i.e. 2 hours prior to sacrifice of the mice). mRNA expression of pro-inflammatory cytokines was then measured by RT-qPCR in inflamed lungs.Our data show that the NAAA inhibitor AM9053 increases NAEs levels in LPS-activated J774 macrophages and reduces macrophage activation. Similarly to what we observed in the J774 cell line, NAAA inhibition also decreases LPS-induced activation of murine alveolar macrophages recovered from bronchoalveolar lavages. Moreover, administration of AM9053 to mice with chronic LPS-induced inflammation exerts anti-inflammatory effects in the lung.
This work highlights the beneficial effects of NAEs and NAAA inhibition on macrophage activation and shows a protective effect of NAAA inhibition in vivo on lung inflammation. Therefore, these data support NAAA inhibition as an interesting anti-inflammatory strategy.

Poster 10

PARASITE-DRIVEN EICOSANOID REPROGRAMMING IN MYELOID CELLS - A MECHANISM FOR IMMUNE EVASION AND REPAIR?

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In contrast to the well-defined roles of lipid mediators in allergy, much less is known about their role during parasitic infection. Using a mouse model of intestinal helminth infection, we observed that large numbers of 12/15-lipoxygenase (12/15-LO) expressing cells infiltrated parasite-induced lesions in the small intestine. This was associated with a pronounced increase in the production of 12/15-LO metabolites (12-HETE, 15-HETE) in small intestinal tissue, whilst 5-LO products remained close to the detection limit. Making use of eosinophil lineage ablated (ΔdblGATA) mice we further showed that eosinophils were the major source of 12/15-LO metabolites in the intestine. Despite intact protective immunity in the absence of eosinophils, we observed a striking defect in the capacity of eosinophil deficient mice to degrade the debris of dead parasites within the intestinal mucosa. In human myeloid cells (eosinophils, neutrophils and macrophages) products from different parasitic nematodes differentially modulated the production of eicosanoids involved in granulocyte recruitment, tissue repair and mucus production. Taken together, our data suggest that parasites may evade host immunity and initiate repair by reverting the eicosanoid profile of innate host cells.

Poster 11

THE SUBSTRATE SELECTIVE COX2- INHIBITOR R-FLURBIPROFEN INCREASES LPS-INDUCED INFLAMMATION IN MICE

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Prostanoids are well described mediators of the cardinal signs of inflammation. Their synthesis is controlled by cyclooxygenases (COX) and especially in inflammatory conditions by COX-2 activity. Therefore, inhibiting the COX enzymes and the subsequent synthesis of prostanoids is a commonly used anti-inflammatory strategy in several conditions, as evidenced by the widespread use of non-steroidal anti-inflammatory drugs (NSAIDs). Profens (e.g. ibuprofen ketoprofen, naproxen) is one class of NSAIDs that potently inhibits COXs. Profens are chiral molecules for which the (S)-enantiomers are described as the active enantiomers blocking arachidonic acid oxidation and prostaglandin production. In this context, the (R)-enantiomers have been largely considered as inactive. However, (R)-profens have been recently described as able to interact with COX-2 and to selectively block the metabolism of particular substrates resulting in the concept of substrate-selective COX-2 inhibitors. Indeed, it has been known for a decade that 2-arachidonoylglycerol (2-AG), a major endocannabinoid, is a substrate of COX-2 thanks to its arachidonoyl moiety. The oxygenation of 2-AG by COX-2 leads to the formation of glyceryl esters of prostaglandins which, although considered as bioactive lipids, remain poorly characterized so far. Among the (R)-profens, (R)-flurbiprofen is able to inhibit COX-2 in a substrate-selective manner (IC_{50} = 0.08 microM) and was shown to have effects in vivo on experimental autoimmune encephalomyelitis. These elements support the interest of further studying the effect of (R)-profens in inflammatory settings. Here we wanted to study, in several tissues, the effect of R-flurbiprofen on the acute systemic inflammation induced by lipopolysaccharides (LPS) administration. We show that (R)-flurbiprofen given to mice receiving LPS (300 microg/kg, ip, for 4 hours) has marked pro-inflammatory properties in peripheral (e.g. lung, spleen, liver) and central (e.g. cerebellum) tissues. Indeed, the LPS-induced mRNA expression of pro-inflammatory markers, such as IL-1beta and MCP-1, was further increased by the administration of (R)-flurbiprofen. Further work will help understand the mechanisms underlying these observations.

Poster 12

ALTERATION IN CONTENT OF RAT BRAIN CHROMATIN NEUTRAL LIPIDS UNDER THE ACTION OF CISPLATIN

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It is well known, that nuclear membrane is the basic place of nuclear lipids localization, however 10% of them is obtained in chromatin fraction. Chromatin lipids play important role during the cell proliferation, differentiation and apoptosis as well as in regulation of DNA replication and transcription. These regulatory effects of lipids exhibit concentration depending character or disposition. Taking into consideration that DNA molecule is the primary pharmacological target for cisplatin action, one may imagine that chromatin bounded lipids are participated in realization of antineoplastic and cytostatic effects of drug.
The cisplatin in vivo effect on rat brain chromatin neutral lipids content was investigated. The alteration in total neutral lipids content as well as the changes of quantity of neutral lipid individual fractions in brain chromatin preparations after the cisplatin action were established. The obtained results demonstrated the deep and multiform transformation of lipid metabolism in chromatin fraction caused by cisplatin. The possible role of rat brain chromatin bound lipids in realization of cisplatin antitumor effects was discussed.

Poster 13

HIGH-THROUGHPUT QUANTITATIVE ANALYSIS OF LIPID MEDIATORS FOR PLATELET ACTIVATION

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Platelets are the central building block of coagulation and homeostasis and their aggregation process is regulated by a network of signal transduction pathways. Pathway markers, which are part of lipid mediators, are generated from the oxidation of polyunsaturated fatty acids (arachidonic acid and eicosapentaenoic acid). Previous studies presented that prostaglandins, one class of mediators, are potent inhibitors of platelet aggregation, while 12-HETE showed both platelet pro- and anti-aggregate activity. This study is aiming to provide a rapid quantification for the main lipid mediators and to investigate the roles and contribution of these bioactive lipid mediators in platelet activation and function. Platelets of mice were treated with multiple activation approaches by thrombin, or collagen related peptide, or the combination of both. After platelet activation, both the pellets and supernatants of each condition were analyzed for a comprehensive profile of mediat or distribution. In conclusion, we established a reliable and rapid approach to analyze a wide range of mediators. The workflow includes liquid-liquid extraction, high performance liquid chromatography-tandem mass spectrometry, and high-throughput data analysis.

Poster 14

Ω-3 AND Ω-6 FATTY ACID SUPPLEMENTS HAVE DIFFERENT EFFECTS ON MOBILIZATION OF INTRACELLULAR CALCIUM IN MAST CELLS

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Intracellular Ca^{2+} signals are among the major factors involved in signal transduction pathways evoking exocytosis. The fusion of an exocytotic vesicle with the plasma membrane and subsequent extrusion of the vesicle's content is regulated by an acute rise in the concentration of intracellular Ca^{2+}. Elevation of intracellular Ca^{2+} concentration is dependent either on Ca^{2+} influx from the extracellular space through the plasma membrane or on Ca^{2+} release from intracellular stores. The aim of the present work was to investigate the modulatory effect of ω -3 and ω -6 fatty acids on intracellular mobilization of Ca^{2+} in mast cells under resting and stimulation conditions.
The canine mastocytoma cell line C2 was supplemented for 8 days with 20 µM linolenic acid (LNA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA) or arachidonic acid (AA). Ca^{2+} response was monitored using the fluorescent Ca^{2+} indicator Fura-2 AM. Cells were loaded with Fura-2 AM and incubated at 37 °C for 45 min in a water bath with gentle shaking. After repeated washing the loaded cell were resuspended in Ca^{2+} free solution containing 0.3 mM EGTA to chelate excess extracelluar Ca^{2+}. During incubation at 37°C in quartz cuvette with a magnetic stirrer the change in the fluorescence ratio was measured at emission wavelength of 510 nm and excitation wavelength of 340 nm. Exocytosis was stimulated by addition of the mast cell-degranulating peptide mastoparan.
In resting cells the ω-6 fatty acids LA and AA increased the cytosolic Ca^{2+} concentration. The ω-3 (α-LNA, EPA and DHA) had no effect on the Ca^{2+} levels. Stimulation of C2 cells with mastoparan promotes a Ca^{2+} signal in a dose-dependent manner. After stimulation of C2 cells with 10 µM mastoparan, LA and AA supplementation induced significantly more Ca^{2+} release from the internal stores than ω-3 PUFA. We conclude that in the absence of extracellular Ca2+ ω-6 PUFA booster Ca^{2+} release from internal stores, indicating that ω-6 PUFA may increase depolarization of mitochondrial and endoplasmic reticulum membranes after stimulation.

Poster 15

DEVELOPMENT OF SMART CELL-FREE AND CELL-BASED ASSAY SYSTEMS FOR INVESTIGATION OF LEUKOTRIENE C4 SYNTHASE (LTC4S) ACTIVITY AND EVALUATION OF INHIBITORS

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Cysteinyl-leukotrienes (cys-LT) are powerful pro-inflammatory mediators that cause bronchoconstriction in anaphylaxis and asthma. They are formed by 5-lipoxygenase (5-LOX) from arachidonic acid (AA) yielding the unstable leukotriene A4 (LTA4) that is subsequently conjugated with glutathione (GSH) by LTC4 synthase (LTC4S), an integral membrane protein that belongs to the superfamily of membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG), to form LTC4. The tripeptide side chain of LTC4 is then cleaved in two successive steps to form LTD4 and LTE4. These cys-LTs are recognized by GPCRs. Cys-LT receptor antagonists as well as LTC4S inhibitors have been developed, but only the former have reached the market. One reason might be the high structural homology of LTC4S to related enzymes of the MAPEG family. One the other hand a convenient test systems to identify potential LTC4S inhibitors was missing due to instability of exogenously added LTA4 as substrate. The main purpose of our work was to establish cell-free and cell-based assay systems based on in situ-generated LTA4 that allow studying LTC4S activity and investigating LTC4S inhibitors. Co-incubations of microsomes from HEK293 cells stably expressing LTC4S together with isolated 5-LOX efficiently converted exogenous AA to LTC4 (~1.3 µg/200 µg protein). Stimulation of HEK293 cells co-expressing 5-LOX and LTC4S with Ca-ionophore A23187 and 20 µM AA leads to a strong LTC4 formation (~ 250 ng/106 cells). MK-886 consistently inhibited LTC4 formation in the assay types (IC50 = 3.2 and 3.1 µM, respectively) and we successfully confirmed TK04a as potent LTC4S inhibitor in these assay systems (IC50 = 17 and 300 nM, respectively). Further, we demonstrated transcellular LTC4 biosynthesis between human neutrophils or 5-LOX-expressing HEK293 cells that produce LTA4 from AA and HEK293 cells expressing LTC4S that transform LTA4 to LTC4. Summarizing, we established cell-free and cell-based HEK 293 systems for evaluating LTC4S inhibitors. Our assay approaches are advantageous as the substrate LTA4 is generated in situ and are suitable for studying enzymatic functionality of LTC4S including site-directed mutations.

Poster 16

OMEGA 3 PUFA COMBINED WITH GRAPE PROCYANIDINS INDUCE LIPIDS PROFILE AND LIPID MEDIATOR REMODELLING DEPENDING ON THE DIETARY BACKGROUND: MODULATION OF DESATURASES, CYCLOOXYGENASES AND GLUTATION PEROXIDASE

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Marine ω-3 PUFAs and polyphenols are well stablished for their benefits in the prevention/palliation of chronic inflammation, oxidative stress and metabolic disorders related with CVD and diabetes. However, their combined effects are not well documented. In addition, the increase of oxidizable substrates in oils enriched in DPA/DHA may deregulate the redox balance limiting the extension of the health benefits. We have hypothesized that grape procyanidins may ameliorate oxidative damages of PUFAs when added together and thus, the combination may enhance their own properties. Therefore, we studied the effects of the combined consumption of both food bioactive compounds through the modulation of fatty acids profiling and the production of lipid mediators in Wistar rats fed high fat high sucrose diets (HFHS) used as an animal model prone to develop metabolic disorders and chronic cellular inflammation. Rats fed standard diets were used as control.
Results suggested that fish oils favored the production of lipid mediators derived from the activity of 12/15 lypoxygenases on ω-3 PUFAs, enhanced glutathione peroxidase activity, modulated the synthesis of ARA pro-inflammatory lipid mediators through the cycloxygenase pathways and down-regulated the synthesis de novo of ARA leaded by Δ5 desaturase. Such effects were highly independent of the dietary context. Grape procyanidins showed a clear anti-oxidative effect on protection proteins from post-traductional carbonylation reducing lipid peroxidation, and enhancing antioxidant enzymes, their effects on inflammation were dependent of the dietary background. Opposite to the findings in rats fed with standard diets, procyanidins up-regulated COX pathways towards ω-6 pro-inflammtory lipid mediators and decreased the detoxification of ω-3 hydroperoxides in the HFHS background. Results were in highly agreement with chemico clinical biomarkers of infllamation and oxidative envir onment in animals. As a result, a synergistic effect between fish oils and procyanidins was stablish in the standard diet in terms of improving inflamation and oxidative stress. However, in the HFHS diets, fish oils seem to be the driving force of the positive effects. In both dietary cases, the modulation of the production of lipid mediators together to the modulation of desaturase activities seem to be the key of the beneficial effects.

Poster 17

LIPID MEDIATORS IN ENDURANCE EXERCISE

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Physical activity is considered as an effective therapy for many diseases. A meta-analysis concerning cardiovascular diseases showed that a standardized exercise program is more effective in secondary prevention of stroke than anticoagulant therapy and as effective as drug therapy in secondary prevention of coronary heart disease. However, the molecular mechanisms underlying these and many other positive health effects of exercise are not fully understood. Lipids, which were mainly recognised as "fuels" mobilised during exercise to maintain energy levels, are increasingly discussed for a much more differentiated role in exercise. The aim of this clinical study was to investigate the effect of a short-term endurance exercise in healthy volunteers on the plasma levels of different lipid mediators, particularly with focus on metabolites of arachidonic acid (AA). Therefore, several components of the AA metabolic cascade were assessed, starting from soluble phospholipase A (sPLA) as lipid liberating enzyme, linoleic acid as precursor and metabolites like dihydroxyeicosatrienoic acids (DHETs) and hydroxyeicosatetraenoic acids (HETEs). These lipids are particularly important because they play an important role in inflammation, immune response and even vascular tone. Analyses of plasma samples at different time points after endurance exercise revealed a distinct modulation of the different parameters of the AA cascade in a time-dependent manner after exercise which was not observed in the control group. In summary, a single bout of endurance exercise leads to modulation of lipid mediator signalling which is an interesting aspect that may support a better understanding of the molecular mechanisms of health benefits through exercise and may lead to new therapeutic options.

Poster 18

REGULATION OF OXIDATIVE STRESS BY MIRNA-328 IS MODULATED BY 5-LIPOXYGENASE

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Recently, we identified miR-328 as RNA decoy to hnRNP E2, a post-transcriptional regulator in monocytes. During myeloid cell differentiation, miR-328 expression is increased and antagonizes the inhibitory activity of hnRNP E2. This leads to an increased expression of hnRNP E2 target genes, increased ROS production and CD11b-mediated monocytic adhesion and migration. One representative gene regulated by the hnRNP E2/miR-328 balance is S100A9, an important protein for leukocyte functions which is mainly expressed by monocytes. It interacts with the NADPH oxidase complex to increase ROS production in myeloid cells which in turn contributes to inflammation and differentiation of monocytes. In general, the regulation of miRNA expression depends mainly on miRNA-processing generating the mature and functional miRNA form. Recent studies demonstrated that the endoribonuclease Dicer interacts with different proteins to influence miRNA-processing. For example, it has been shown that the Dicer C-terminus functions as a 5-lipoxygenase (5-LO)-binding domain. 5-LO catalyzes the two initial steps in the biosynthesis of leukotrienes, a group of inflammatory lipid mediators derived from arachidonic acid. The 5-LO/Dicer interaction leads to an increased catalytic 5-LO and Dicer processing activity. Here in this study we could show that 5-LO directly interacts with Dicer to modulate miRNA-328 processing in monocytes which contributes directly to oxidative stress and inflammation.

Poster 19

TARGETED PROFILING OF EICOSANOIDS IN THE PROGRESSION OF METASTATIC BREAST CANCER IN MICE

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Eicosanoids are lipid mediators synthesized from polyunsaturated fatty acids (PUFAs) involved in diverse physiological and pathophysiological processes. Moreover, eicosanoids seem to have a significant impact on tumor progression and generation of pre-metastatic niche, however their role is not fully understood. In the present work we profile changes in selected eicosanoids generated from arachidonic acid (AA) via cyclooxygenases (COXs), lipoxygenases (LOXs) and cytochrome P450 enzymes pathways in murine model of metastatic breast cancer in relation to the development of endothelial dysfunction assessed on the basis of alternations in NO metabolites and generation of reactive oxygen species (ROS).
4T1 tumor cells suspended in 0.05 mL of Hanks Balanced Salt Solution were orthotopically inoculated into the right mammary fat pad of Balb/c female mice. The tumor progression was evaluated in every week throughout the experimental period using electronic caliper. Plasma and urine samples were collected 1, 2, 3, 4 and 5 weeks after cancer cells injection.
The measurements of selected prostanoids, hydroxyeicosatetraenoic (HETEs) and epoxyeicosatrienoic (EETs) acids were performed in plasma, urine and lungs samples using UFLC Nexera system (Shimadzu) combined with the triple quadrupole mass spectrometer QTrap 5500 (ABSciex). The highest concentration of PGE_2 and PGD_2 in plasma was observed 2 weeks after cancer cells inoculation suggesting that among others mainly these prostanoids are implicated in the early tumor progression and development of metastatic niche microenvironment. Moreover, the biosynthesis 12(S)-HETE was increased at 2 and 4 week of tumor progression suggestive of intensification of angiogenesis probably caused by the activation of VEGF pathway. Additionally, the concentration of urinary metabolite of TXA2 was also elevated throughout the experimental period what suggest the activation of platelets or systemic inflammation. Taking together, there are pronounced changes in eicosanoids profile associated with the early progression of metastasis and late cancer-related inflammation. Further studies are warranted to elucidate which of these changes are specific to the in pre-mestastatic niche formation in the lung and which to the development of systemic cancer-related inflammation.

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Poster 20

MARESIN 1 MODULATES HYPOXIA-INDUCED AND LIPOTOXIC ER STRESS IN PRIMARY HEPATOCYTES

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Perturbation of the endoplasmic reticulum (ER) results in an evolutionarily conserved adaptive stress response called the unfolded protein response (UPR) leading to increased transcription of genes coding for inflammatory factors, chaperones and autophagy. ER stress and activation of UPR are common findings in metabolic diseases including obesity-induced non-alcoholic fatty liver disease (NAFLD). In this disease, stimuli such as hypoxia and accumulation of fatty acids (lipotoxicity) disturb hepatic protein folding and activate UPR, and if ER stress is not resolved, cellular functions deteriorate, often leading to liver cell death. Recently, a novel genus of specialized pro-resolving mediators (SPM) including resolvin and maresin families, have been described to ameliorate the inflammatory process by expediting its resolution. In the current study we explored whether SPM can modulate hepatic ER stress. Obese mice with NAFLD showed a remarkable hepatic up-regulation of CA-9 and HIF-1α and increased phosphorylation of eif2α and JNK, which are well established markers of hypoxia and ER stress, respectively. Primary hepatocytes cultured under hypoxic conditions showed an induction of ER stress sensors (i.e. pIRE1α, peif2α, CHOP and ATF3) and autophagy markers. Among the different SPM, maresin 1 (MaR1) was the most effective in reducing ER stress and autophagy markers Moreover, increasing concentrations of MaR1 prevented hepatocyte cell death. In addition, primary hepatocytes cultured under lipotoxic conditions showed an induction of markers of ER stress and autophagy, effects that were reversed by pre-treatment of these cells with MaR1. On the other hand, in experiments in precission-cut liver slices (ex vivo model), MaR1 prevented the hypoxia-induced up-regulation of TNFα and IL-1β. Interestingly, MaR1-treated Kupffer cells growing in a lipotoxic environment showed a remarkable improvement in their phagocytic capacity. Taken together, these results demonstrate that MaR1 is able to reduce ER stress and to re-establish the autophagic flux in hepatocytes preventing liver cell death and the progression of obesity-induced NAFLD.

Poster 21

INVESTIGATION OF 11R-LIPOXYGENASE QUATERNARY STRUCTURE

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Lipoxygenases (LOXs) are polyunsaturated fatty acid peroxidising enzymes that catalyse the key reactions in the formation of several bioactive lipid mediators in mammals such as leukotrienes, hepoxilins and lipoxins. Although LOXs have widely been considered to function as monomeric enzymes, several recent studies implicate that quaternary LOX complexes exist, and may carry an allosteric role. The arachidonate 11R-LOX from the arctic coral Gersemia fruticosa is a stable dimer. The catalysis of most LOXs is generally induced by cofactor Ca (2+) and the presence of a lipid bilayer, whereas 11R-LOX is completely dependent on these two factors. Binding of Ca (2+) induces enzyme localisation to the lipid membrane, where it can acquire its fatty acid substrate. The crystal structure of 11R-LOX has been solved, yet the dimer assembly, and whether it contributes to the allosteric regulation of this enzyme still remains unclear. We investigated the quaternary structure of 11R-LOX by small-angle X-ray scattering, chemical cross-linking coupled with mass-spectrometry, and site-directed mutagenesis in order to identify the dimer interface, and assess its role in catalysis. Here we present our current findings, and discuss the applicability of these results to other LOXs.

Poster 22

TGF-BETA/SMAD SIGNALLING MODULATES MLL AND MLL-AF4 MEDIATED 5-LIPOXYGENASE PROMOTER ACTIVATION

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5-lipoxygenase (5-LO) catalyzes the initial two steps in the biosynthesis of leukotrienes from arachidonic acid. Leukotrienes represent a group of eicosanoids which are implicated in various pathological processes such as inflammation and cancerogenesis. Previous studies demonstrated the major role of calcitriol and TGFβ in the upregulation of 5-LO gene expression during myeloid cell differentiation. Until now, no induction of 5-LO promoter by either TGFβ or calcitriol could be detected for reporter gene constructs containing the 5-LO core promoter region. Here, we investigated the role of TGFβ/Smad signalling on 5-LO promoter activation performing electrophoretic mobility shift assay (EMSA), chromatin immunoprecipitation (ChIP) and reporter gene assays. Mutation studies revealed two functional Smad binding elements (SBEs) in the proximal part of the 5-LO promoter which significantly induce 5-LO promoter activity. Since 5-LO gene expression has been linked with mixed lineage leukemia (MLL) which is characterized by the presence of MLL fusion proteins (e.g. MLL-AF4) that are the results of chromosomal translocations affecting the MLL gene at 11q23. Thus, we investigated the influence of TGFβ/Smads modulating MLL and MLL-AF4 mediated 5-LO promoter activation. We demonstrated that TGFβ/Smads can enhance MLL-mediated 5-LO promoter activation, whereas MLL-AF4-induced aberrant promoter activation is significantly reduced by TGFβ and Smad3/4. siRNA-mediated knockdown of MLL-interaction partners revealed that the transcription factors Menin and Sp1 are essential for MLL-AF4 mediated 5-LO induction. Our data suggest that Smad3/4 interact with MLL via Menin which then increases 5-LO promoter activity. Furthermore, our data suggest that induction of 5-LO gene expression by TGFβ/Smads is at least in part due to stimulation of transcript initiation.

Poster 23

SIGNALING PATHWAYS OF LYSOPHOSPHATIDIC ACID ELICITED VASOCONSTRICTION

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Lysophosphatidic acid (LPA) is a bioactive lysophospholipid with numerous biological actions. However, its definitive effect on the vascular tone has remained yet elusive. We have reported that LPA induces endothelium-dependent vasorelaxation via LPA1 receptors, phospholipase C, and nitric oxide (1). Removal of the endothelium abolished this effect and converted it to vasoconstriction. Here we describe the signaling pathways mediating this constrictor effect.
Aortic segments were isolated from wild type (WT) and knockout (KO) mice deficient in LPA1 or LPA2 receptors, cyclooxygenase-1 (COX1) or the thromboxane prostanoid receptor (TP). Vessels of mice subjected to smooth muscle specific deletion of Galphaq/11 or Galpha12/13 were also tested. Isometric tension of endothelium-denuded vascular segments was measured via myography. Expression of LPA receptors in aortic vascular smooth muscle (VSM) was analyzed by qPCR. Thromboxane A_2 (TXA_2) release from aortae was measured by TXB_2 ELISA.
LPA1, LPA2, LPA4 and LPA6 transcripts were abundantly detectable in VSM. LPA elicited dose-dependent vasoconstriction in endothelium-denuded vessels. The LPA1-3 agonist VPC31143 mimicked, whereas the LPA1-3 antagonist Ki16425 inhibited the contraction. Lack of LPA1 but not that of LPA2 abolished VPC31143-induced vasoconstriction. Genetic deletion of Galphaq/11 or Galpha12/13 as well as inhibition of Galphai/o by pertussis toxin (PTX) attenuated the vasoconstriction. Galphai/o signaling can be coupled to phospholipase A2 and COX1 activation, thus the potential involvement of autocrine/paracrine TXA_2 release in the LPA1-mediated vasoconstriction was tested. Vessels deficient in COX1 or TP showed diminished vasoconstrictor responses to VPC31143. Furthermore, VPC31143 increased TXA_2 production in WT and TP KO but not in LPA1 KO, COX1 KO or PTX-treated WT vessels.
The direct effect of LPA on VSM is vasoconstriction, which opposes the endothelium-dependent vasodilation. Interestingly, both effects are mediated by LPA1, which, in VSM via Gi/o, facilitates COX1-mediated autocrine/paracrine TXA_2 release. Subsequent activation of VSM TP causes vasoconstriction involving Galphaq/11 and Galpha12/13 proteins. We propose that in case of endothelial damage the interaction between the LPA/LPA1 and TXA_2/TP pathways may represent a vicious circle between platelet activation and vasoconstriction.

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References

1. Ruisanchez, Dancs, Kerék, el al., (2014). FASEB Journal 28: 880-890.

Poster 24

FORMATION OF 18-HYDROXY-EICOSAPENTAENOIC ACID BY HUMAN CYTOCHROME P450 ISOFORMS

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Omega-3 polyunsaturated fatty acids (n-3 PUFA) are the source of anti-inflammatory metabolites. Among them, the so called specialized pro-resolving metabolites (SPM) are involved in the resolution of inflammation. SPMs include the eicosapentaenoic acid (EPA)- and docosahexaenoic acid (DHA)-derived resolvins, maresines and protectins. The biosynthetic pathway of SPMs is only partly understood. 18-HEPE (18-hydroxy-eicosapentaenoic acid), a metabolite increasingly formed upon dietary n-3 PUFA supplementation, is thought to be an intermediate in the conversion of EPA to resolvin E; however, the mechanisms of 18-HEPE formation remained unclear. In vitro 18-HEPE can be formed by autoxidation, (aspirin-) acetylated COX-2 and bacterial Cytochrome P450 (CYP) enzymes. Human CYP enzymes producing 18-HEPE have not been identified yet.
Aim of this study was to analyze the capacity of human CYP enzymes to generate 18-HEPE. A panel of recombinant human CYP enzymes ("supersomes" containing the individual isoform reconstituted with NADPH-CYP reductase) was used: CYP1A1, CYP2C8, CYP2C9, CYP2D6*1, CYP2E1, CYP2J2, CYP2S1, and CYP3A4. The reaction mixtures contained 10 pmol CYP, 10 µM EPA, and either 1 mM NADPH or 400 µM cumene hydroperoxide (CHP) as co-substrate. Incubations without co-substrate served as negative control. The formation of EPA metabolites was quantified by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).
18-HEPE was clearly detectable after incubating EPA with CYP1A1, CYP2C8, CYP3A4, and CYP2S1. However, all these enzymes preferentially produced 17,18-epoxyeicosatetraenoic acid (17,18-EEQ), and 19-HEPE, whereas 18-HEPE was generated as a minor product only . For example CYP1A1 generated 17,18-EEQ, 19-HEPE, and 18-HEPE in a ratio of about 1:1:0.2.. Some of the human CYP enzymes are able to produce 18-HEPE as a side product when metabolizing EPA. Considering the low rate of CYP-catalyzed 18-HEPE formation, the actual contribution of this pathway to in vivo 18-HEPE formation remains to be elucidated.

Poster 25

SUPPRESSION OF NEUROBLASTOMA GROWTH BY TARGETING MPGES-1 EXPRESSING CAFS IN THE TUMOR MICROENVIRONMENT

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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. The neuroblastoma promoting microenvironment contains infiltrating cancer associated fibroblasts (CAFs) expressing prostaglandin E synthase-1 (mPGES-1), responsible for prostaglandin E_2 (PGE_2) synthesis. PGE_2 regulates tumor inflammation and immune suppression, angiogenesis, tumor progression and therapy resistance. We propose that targeting the PGE_2 production in CAFs in the neuroblastoma tumor microenvironment will reduce tumor progression. To elucidate if mPGES-1 and PGE_2 contribute to tumor progression two neuroblastoma mouse models were utilized, the transgenic MYCN-driven model and a xenograft model using an 11q-deleted cell line. In the transgenic model, starting from the age of 4.5 weeks, the mPGES-1 inhibitor, Compound III (C3), was administered with daily i.p. injections. In the xenograft model, treatment was started either from tumor take or from the time of tumor cells inoculation. Early initiated treatment with C3 delayed tumor development with approximately 50% in the xenograft model (median 38 vs. 25.5 days until tumor take). Also, growth of established xenografts and transgenic tumors was significantly inhibited compared to tumors in untreated animals ($p=0.02$ and $p=0.003$ respectively). In the fully immune competent transgenic mouse model, pharmacological mPGES-1 inhibition led to reduced angiogenesis, a shift in polarization of macrophages towards anti-tumorigenic M1 macrophages and reduced expression of the CAF marker PDGFRbeta. This indicate that inhibition of mPGES-1 reduce tumor progression by targeting tumor-promoting inflammation and suppression of anti-tumor immunity mediated by PGE_2. We conclude that treatment targeting non-malignant cells in the microenvironment of neuroblastoma may constitute a novel clinical therapeutic approach that deserves further development to facilitate clinical implementation.

Poster 26

THE MELLEOLIDE DEHYDROARMILLYLORSELLINATE (DAO) FROM HONEY MUSHROOM INHIBITS 5-LIPOXYGENASE AND DECREASES EICOSANOID BIOSYNTHESIS IN LEUKOCYTES

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Eicosanoids play distinguished roles in the regulation of inflammatory processes and may increase or attenuate inflammatory responses, with major impact on cell proliferation, cell migration, phagocytosis and cytokine production. The fatty acid arachidonic acid (AA) is the precursor of eicosanoids that include leukotrienes (LTs), prostaglandins (PGs), thromboxanes (TXs), lipoxins (LXs) and other hydroxylated AA derivatives. AA is stored in membrane phospholipids and is released by cytosolic phospholipase A2 (cPLA2) upon stimulation. Three different enzymes metabolize liberated AA can be metabolized by three different enzymatic pathways into eicosanoids: lipoxygenases (LO), cyclooxygenases (COX) and cytochromes P450 (CYP 450). Here we present the melloelide dehydroarmillylorsellinate (DAO) as a potent inhibitor of eicosanoid biosynthesis. Melleolides are structurally unique and bioactive natural products of the basidiomycete genus Armillaria. We find that besides antifungal and cytotoxic effects (1), DAO may also exhibit anti-inflammatory properties as it effectively inhibits 5-LO activity. We observed a strong suppression of 5-LO product formation in Ca-ionophore-stimulated human neutrophils by DAO (IC50 = 0.3 μM). This inhibitory effect by DAO was irreversible (demonstrated by wash-out experiments) and supplementation of neutrophils with exogenous AA impaired the potency of DAO (IC50= 2 μM). Moreover, DAO inhibited 5-LO activity in a cell-free assay with lower effectiveness (IC50= 3 μM). Using an UPLC-MS/MS-based lipidomics approach we determined the effects of DAO on overall eicosanoid formation in Ca-ionophore-activated human neutrophils and monocytes. DAO essentially suppressed all monitored. Again, the inhibitory potency of DAO was impaired when exogenous AA was added to the leukocytes, suggesting that DAO may inhibit other key enzymes along the AA pathway like cPLA2. Interestingly, when exogenous AA was provided an increase of PGs and 12-/15-LO products was evident by DAO treatment. In summary, the natural product DAO is a direct inhibitor of 5-LO that in addition exhibits a broad activity spectrum on eicosanoid biosynthesis.

References

1. Bohnert M.,et al. (2014) Bioorg Med Chem , 22(15):3856-61.

Poster 27

INHIBITION OF STEAROYL-COA DESATURASE 1 AFFECTS PALMITATE-DERIVED AUTOPHAGY AND LIPID DROPLET HOMEOSTASIS IN PANCREATIC BETA CELLS

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Type 2 diabetes mellitus (T2DM) along with obesity are defined as the major threats of the present global public health. Even though peripheral tissues suffer detrimental impairment due to obesity-associated insulin resistance, it is the pancreatic beta cell failure that is the critical feature of T2DM occurrence. Elevated level of free fatty acids (FAs) which is often accompanied by obesity, is one of the crucial factors which may lead to the compromised beta cell function and loss in T2DM. The way in which elevated levels of free saturated or unsaturated FAs contribute to progressive beta cell failure remains incompletely understood. Stearoyl-CoA desaturase 1 (SCD1), a key regulatory enzyme in biosynthesis of monounsaturated FAs, was shown to play an important role in regulation of beta cell function. A growing body of evidence indicates reciprocal communication between autophagic pathways, apoptosis, and intracellular lipids. In current work, we determine whether SCD1 activity is engaged in palmitate-induced pancreatic beta cell autophagy. We report augmented apoptosis and diminished autophagy upon cotreatment of INS-1E cells with palmitate and an SCD1 inhibitor. Moreover, we show that additional treatment of the cells with monensin, an inhibitor of autophagy at the step of fusion, exacerbates palmitate-induced apoptosis. Accordingly, diminished SCD1 activity affected the accumulation, composition, and saturation status of cellular membrane phospholipids, neutral lipids, as well as the protein and lipid content of lipid droplets. Such an effect was accompanied by aberrant endoplasmic reticulum stress, mitochondrial injury, and decreases in insulin secretion and cell proliferation. Our findings propose a novel mechanism where the inhibition of SCD1 activity affected autophagy at the step of autophagosome-lysosome fusion due to perturbations in cellular membrane integrity and impaired lipid droplet homeostasis, thus leading to an aberrant stress response and beta cell failure.

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Poster 28

EFFECT OF MNA ON THE INTRACELLULAR TRANSPORT OF AA UNDER FLOW AND STATIC CONDITIONS

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Arachidonic acid (AA) is part of phospholipids in cell membranes and stored in lipid droplets. It is well-known modulator of inflammation that is synthesized and released by living cells in response to various factors and it may play a role as the substrate for various lipid mediators. It was demonstrated that membrane protein FAT/CD36 was involved in transport of free fatty acid by endothelial cells (Kerkhoff et al, 2015) and that AA uptake was regulated by cationic compounds such as MNA (Majzner et al., 2015). In this study, we characterized in more details the effect of MNA on AA transport in relation to endothelial function as well as the mechanism of the uptake of FFA by endothelial cells. Cultured human umbilical vein cell line, (EA.hy926) and human aortic endothelial cells (HAEC) were incubated in supplemented endothelial cell growth medium (DMEM or EGM-2MV) under static (30min, 4h, 24h) and flow conditions (4h,24h; shear stress 15dyne/cm2) in a 37 °C, 5% CO2/95% air, humidified cell culture incubator. Cells during incubations were stimulated with arachidonic acid–d8 (AAd8) or MNA together with AAd8 (AAd8, 25uM; MNA, 25uM), the inhibition of AA uptake by floretin. The measurement of AAd8 and MNA uptake were taken in extracted lipids (with a mixture of hexane: 2-propanol) and medium after incubations by HPLC methods and calculated according to the protein contents or the amount of cells. The release of 6-ketoPGF1α in medium and extracted cells were also determined after incubations. To investigate lipid droplets accumulation in cells the fluorescence staining with Lipidtox was used. An Olympus ScanΛR automated fluorescence microscope was used to collect the fluorescence images which were captured using a DAPI filter for Hoechst 33342 (targeting DNA in the cell nucleus; and FITC filter for Lipidtox (targeting lipid droplets).Taken together, our study confirmed that MNA facilitates intracellular transport of AA in static and flow conditions as time and dose dependent response. Furthermore AA stimulation with or without MNA resulted in lipid droplets formation but also modulated endothelial function. Interestingly, we showed that FAT (CD36) is rather not involved in the response to AA±MNA.

Poster 29

ANTIARRHYTHMIC AND CARDIOPROTECTIVE PROPERTIES OF A SYNTHETIC 17,18-EEQ ANALOG

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17,18-epoxyeicosatetraenoic acid (17,18-EEQ) has been identified as the predominant CYP epoxygenase metabolite upregulated upon dietary EPA/DHA supplementation in rodents and man. We hypothesized that 17,18-EEQ may function as a mediator of the antiarrhythmic and cardioprotective effects attributed to the parental omega-3 fatty acids. To test this hypothesis, we analyzed the mechanism of action of 17,18-EEQ in cardiomyocytes and developed metabolically robust synthetic analogs for proof of concept studies in animal models. In vitro studies were performed in spontaneously beating neonatal rat cardiomyocytes. In this model, 17,18-EEQ and its synthetic analog OMT-28 exerted negative chronotropic effects with EC50 values of 6.2 and 1.8 nM. Pertussis toxin abolished these effects, indicating that 17,18-EEQ and OMT-28 act via a Gi-protein coupled receptor. The subsequent signalling pathway required PI3K, Akt and eNOS as revealed by respective pharmacological inhibitions with Wortmannin and L-NAME. 17,18-EEQ and OMT-28-induced Akt phosphorylation occurred at Ser473 and Thr308. Other essential components involved were the HMR1098-inhibitable sarcolemmal and 5-HD-inhibitable mitochondrial K(ATP) channels. Moreover, phosphorylation of the SERCA regulating phospholamban was significantly increased at Ser16. Taken together, these results suggest that 17,18-EEQ and OMT-28 mediate a complex signalling pathway that modulates the electrical excitability, calcium handling and mitochondrial function in cardiomyocytes. To further investigate the cardioprotective effects of OMT-28, we conducted ischemia/reperfusion studies in isolated mouse hearts. Perfusion with 100 nM OMT-28 significantly improved the post-ischemic functional recovery from 18±7 (vehicle control) to 59±10 % as characterized 40 min after reperfusion. In vivo studies to proof the antiarrhythmic potential of OMT-28 were performed in a mouse model, where the animals were rendered susceptible to atrial fibrillation (AF) by chronic ß-adrenergic stimulation (40mg/kg/d of isoproterenol via osmotic minipumps over 14 days). In this model, OMT-28 dose-dependently reduced AF-inducibility by programmed electrical stimulation. A single i.v. bolus of 0.625 mg/kg OMT-28 was sufficient to increase the ratio of animals with no AF from 19 to 73%. In conclusion, these in vitro and in vivo studies show that 17,18-EEQ and its synthetic analog OMT-28 induce a unique signalling pathway in cardiomocytes that has the potential to protect the heart against ischemia-reperfusion injury and arrhythmia.

Poster 30

POTENT SUPPRESSION OF 5-LIPOXYGENASE ACTIVITY IN VITRO AND IN VIVO BY THE NOVEL INHIBITOR RF22C

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Leukotrienes (LTs) are pro-inflammatory lipid mediators derived from arachidonic acid (AA) through the activity of 5-lipoxygenase (5-LO) with key roles in the pathophysiology of several immune/inflammatory disorders (i.e. asthma, allergic rhinitis). Thus, targeting 5-LO represents a suitable strategy to develop new anti-inflammatory drugs. Here, we provide a pharmacological characterization of the novel 1,2-benzoquinone RF-22c on the inhibition of the 5-LO enzyme, with focus on the cellular regulation of 5-LO and efficiency in animal models of acute inflammation related to LTs. The compound resulted 50-fold more potent in inhibiting LT biosynthesis than the reference compound zileuton, a 5-LO inhibitor that has been approved for the treatment of asthma. The suppression of LT formation was more efficient in intact cells ($IC_{50} \geq 22$ nM) as compared with the cell-free assays ($IC_{50} \geq 130$ nM). The inhibitory effect was specific and reversible as supported by the docking data and the wash-out experiment. None of the cofactors involved in 5-LO activation (Ca^{2+} mobilization, ERK phosphorylation) were affected by RF-22c and also not the 5-LO/FLAP interaction. Interestingly, RF-22c selectively inhibited 5-LO enzyme and was ineffective against 12/15-LOs and cyclooxygenases (COX-1/2). Moreover, no anti-oxidant properties were observed RF-22c failed to suppress ROS formation in PMA-activated neutrophils. In line with the 5-LO inhibitory activity in intact cells, RF-22c (0.1 mg/kg; i.p.) showed a pronounced effectiveness in vivo, impairing: a) bronchoconstriction in ovalbumin-sensitized mice after challenge with acetylcholine; b) exudate formation in carrageenan-induced paw edema; c) leukocyte afflux upon zymosan-induced air pouch. Importantly, no immediate hepatotoxicity was evident upon RF-22c treatment of the mice, as supported by the lack of the increase of serum liver transaminases. Conclusively, RF-22c potently suppresses 5-LO activity in v ivo and in vitro with promising potential for further preclinical investigations.

Poster 31

STRUCTURAL AND FUNCTIONAL ANALYSIS OF CALCIUM ION MEDIATED BINDING OF 5-LIPOXYGENASE TO NANODISCS

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An important step in the production of inflammatory mediators of the leukotriene family is the Ca^{2+} mediated recruitment of 5-Lipoxygenase (5LO) to nuclear membranes. To study this reaction in vitro, the natural membrane mimicking environment of nanodiscs was used. Nanodiscs with 10.5 nm inner diameter were made with the lipid POPC and membrane scaffolding protein MSP1E3D1. Monomeric and dimeric 5LO were investigated. Monomeric 5LO mixed with Ca2+ and nanodiscs are shown to form stable complexes that 1) produce the expected leukotriene products from arachidonic acid and 2) can be, for the first time, visualised by native gel electrophoresis and negative stain transmission electron microscopy and 3) show a highest ratio of two 5LO per nanodisc. We interpret this as one 5LO on each side of the disc. The dimer of 5LO is visualised by negative stain transmission electron microscopy and is shown to not bind to nanodiscs. This study shows the advantages of nanodiscs to o btain basic structural information as well as functional information of a complex between a monotopic membrane protein and the membrane.

References

1. Kumar, R.B. et. al. (2016), PLoS One 11(3): e0152116.

Poster 32

THE RELAXANT EFFECT OF SOME PROSTANOIDS ON RAT CORPUS CAVERNOSUM AND THEIR INTERACTION WITH NITRIC OXIDE /CGMP PATHWAY

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Prostaglandins play an important role in penile erection. It has been revealed that, in corpus cavernosum, PGF2alpha and TXA_2 induce contraction, while PGE_1 and PGE_2 induce relaxation and pro-erectile effect. However, there is no enough data concerning their interaction with nitric oxide/cGMP pathway and whether their combination with PDE-5 inhibitors could be useful in management of erectile dysfunction. The current study aims at exploring further details about the effect of selected prostaglandins including; L902688 (selective EP4 agonist), alprostadil (synthetic PGE_1) and iloprost (PGI_2 analogue) on corpus cavernosum and their interaction with nitric oxide/cGMP pathway.
Tension studies using isolated rat corpus cavernosum strips were conducted. Results are expressed as mean± SEM and derived from 5-7 rats. All applicable international and institutional guidelines for the care and use of animals were followed.
All tested compounds; L902688 (1 nM-10 microM), alprostadil (1 nM-10 microM) and iloprost (1 nM-1 microM) evoked concentration- dependent relaxation of phenylephrine- precontracted strips. The maximal relaxation induced by L902688 was 58±4% with pEC50 equals to 7.30±0.15, while the maximal relaxation for iloprost was 34±5% and 24±2% for alprostadil. In combination with sildenafil (1 microM), a significant potentiation of the relaxing effect of alprostadil and to a lesser extent with iloprost was observed. While the relaxant effect of L902688 was not significantly potentiated in presence of sildenafil.
The most potent relaxant of rat corpus cavernosum among the tested compounds was L902688 while iloprost and alprostadil were less effective as relaxants. With the knowledge that alprostadil has been successfully used for treatment of erectile dysfunction, iloprost and L902688 - which were more effective than alprostadil in relaxing corpus cavernosum in vitro - could be very promising in clinical practice. However, the relaxing effect of L902688 was not potentiated in presence of sildenafil which suggests that its mechanism does not involve cross talking with nitric oxide/cGMP pathway. On the other hand, the mechanism of action for both iloprost and alprostadil may interact with nitric oxide/cGMP pathway which worth further investigation to determine whether their combination with sildenafil could be helpful in management of erectile dysfunction.

Poster 33

THE REGULATION OF INSULIN SENSITIVITY IN SKELETAL MUSCLE IS ASSOCIATED WITH THE CROSSTALK BETWEEN 2-ARACHIDONOYLGLYCEROL AND STEAROYL-COA DESATURASE-1.

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The Endocannabinoid system (ECS) consists of endogenously synthesized, lipid derived mediators that bind to cannabinoid receptors and activate the signaling cascade. There is increasing number of evidences that the endocannabinoid system takes a major role in the regulation of energy metabolism, including lipogenesis in adipose tissue and liver and glucose uptake into skeletal muscle. Moreover, in obesity, ECS becomes overactivated and might be involved in the pathogenesis of type 2 diabetes. One of the most abundant endocannabinoids is 2-Arachidonoyloglicerol (2-AG). Others have shown that the key components of the ECS are present in human and rodent skeletal muscles. However, it is still unknown what is the role of endogenously synthesized 2-AG in the modulation of insulin sensitivity in skeletal muscle. In our studies we showed that mice injected with the monoacylglycerol lipase inhibitor (JZL184) are characterized by better insulin sensitivity, which is accompanied with significantly lower weight gain and reduced fasting glucose level when compared to control. In Gastrocnemius higher level of monoacylglycerols, including 2-AG, leads to an increase in free fatty acids content and changes in proteins involved in lipid metabolism, including AMP-activated protein kinase, hormone sensitive lipase as well as fatty acid synthase. Moreover, in skeletal muscle of mice injected with JZL184 we also found higher expression and activity of stearoyl-CoA desaturase-1 (SCD1), which is the key enzyme involved in fatty acid metabolism. Interestingly, transgenic mice with muscle specific overexpression of SCD1 (SCD1Tg) have significantly higher level of diacylglycerol lipase beta in Gastrocnemius, which might be correlated with increased synthesis of 2-AG. However, we did not observe this effect in SCD1 Tg mice after high fat diet. Injection of SCD1 Tg mice after high fat diet with JZL184 leads to improved insulin sensitivity, changes in lipid metabolism and decrease in desaturation index, suggesting that endogenously synthesized 2-AG might play an important role in enhancing insulin action by changing lipid metabolism in skeletal muscle.

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Poster 34

GLUTAMINYL CYLASE (QC) INHIBITION IN A MOUSE PERITONITIS MODEL EFFECTS EOSINOPHIL AND MACROPHAGE RECRUITMENT AND LEVELS OF RESOLUTION MOLECULES.

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Inflammation is a natural process which starts, increases and stops. The end signaling is an active process and involves many cellular and molecular factors. Immuno-inflammatory cells issued and recruited from the blood stream are key components for the control of inflammation. QCs catalyzing the formation of pyroglutamate from glutamine at the N-terminus of proteins. This is an important step in the maturation of chemokines, e.g. MCPs, which generates the fully active and stable peptides. QC-inhibitors inhibit the formation of pyroglutaminyl-chemokines. Furthermore, in an acute inflammatory mouse model (thioglycollate induce peritonitis) QC-inhibition reduces macrophage invasion into the peritoneum.
Objective of this experiment was to analyze the effect of QC-inhibition on cell recruitment and resolution molecules during acute inflammation, specifically addressing an impact on either inhibition of inflammation and/or its active resolution.
Mice with thioglycollate induced peritonitis were treated with QC inhibitor PQ912 (bid, orally) and peritoneal lavages were sampled up to 72h after induction. Immune cell populations in the lavage were sorted by cytofluorimetry. Levels of cyto-/chemokines were determined by Luminex technology and profiling of bioactive lipids involved in the control of inflammation by LC-MS/MS. Thioglycollate induces an infiltration of cells and an increase of bioactive mediators with peak levels at 24 to 48h after stimulation. PQ912 treatment shows an increase of eosinophils and inhibition of macrophages infiltration into the peritoneum at 24 and/or 48h after stimulation. A number of pro- (MCP-1, KC, IP-10,) and anti-inflammatory (IL-10) cyto-/chemokines show temporary (24h) higher levels under treatment. Notable treatment effects on lipid mediators were: decrease of the pro-inflammatory PGE2 and TBX2, no impairment of lipoxins, RvD1 and 7(R)-MAR1 (at 24h). and an increase of RvD2.

Poster 35

INTACT GLYCOSYLPHOSPHATIDYLINOSITOL PROTEIN ANCHORS AS BIOMARKERS FOR TYPE 2 DIABETES

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Glycosylphosphatidylinositol-anchored proteins (GPI-APs) in complex with phospholipids and cholesterol have been shown to be released from the surface of metabolically relevant mammalian cells through non-classical secretory mechanisms in response to metabolic stress as is prevalent during type 2 diabetes. Nevertheless, the presence of intact GPI-APs in body fluids of stressed people has not been studied so far, possibly due to conceptual (reductionistic and causal thinking) and technological limitations. To overcome these hurdles, biosensors based on surface acoustic waves (SAW) were used for the detection and biophysical characterization of GPI-APs in complex with phospholipids and cholesterol. Any interaction of those complexes with the gold surface of biosensor chips will result in changes in the shape of the SAW, altering both their frequency and amplitude and thereby reflecting changes in mass loading and biophysical properties. Preliminary data indicate that signatures recorded in course of successive association and dissociation of intact GPI-APs to the chip surface via specific interaction with bacterial alpha-toxin (for detection of GPI-APs) and annexin (for detection of phospholipids) display significant differences between plasma of diabetic, insulin-resistant and normal rats. Albeit SAW biosensing per se does not enable the delineation of the nature of GPI-APs in complex with phospholipids and cholesterol contained in serum, the SAW signatures will be characteristic for the overall contents and physicochemical characteristics of all intact GPI-APs released from (metabolically) stressed cells as summation signals, signatures, symbols or “Gestalten” of high informative value. First human data are currently being generated.

References

1.Yanez-Mo M et al., (2015) . J Extracell Vesicles 4: 27066-http://dx.doi/10.3402/jev.v4.27066.
2.Müller G et al., (2011). Cellular Signaling 23: 1207-1223.
3.Müller G et al., (2012) . Am J Mol Biol 2: 187-209.
4.Müller G. (2012). Diabetes Metab Syndr Obes 5: 247-282.
5.Shao et al., (2015). Nature Commun 6:6999 doi: 10.1038/ncomms7999.

Poster 36

EFFICACY OF ALKALINE HYDROLYSIS FOR THE LIBERATION OF ESTERIFIED OXYLIPINS

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Eicosanoids and other oxylipins are potent lipid mediators which are involved in the regulation of several physiological functions such as inflammation. It is believed that they act predominantly in their free, i.e. non-esterified, form. However, a major portion of oxylipins is found as esters in triglycerides or polar lipids.
Only little information is available on the biological activity of these esterified oxylipins. A first important step towards a better understanding of their biological role would be a systematic comparison of the changes in the pattern of free vs. esterified mediators induced by pharmacological intervention or dietary supplementation, and during onset and progression of diseases. While several LC-MS based methods for the detection of free oxylipins have been developed [Willenberg et al., Anal Bioanal Chem, 2015; Ostermann et al., Anal Bioanal Chem, 2015], the optimal procedure for the detection of esterified oxylipins is controversial [Willenberg et al., Anal Bioanal Chem, 2015]. Usually the sum of free and esterified oxylipins is quantified following base hydrolysis of the sample. However, used protocols differ considerably with respect to sample pre-treatment, base concentration and incubation temperature as well as time.
On the poster we demonstrate, that direct alkaline hydrolysis of human plasma results in poor recovery rates of spiked isotopically labeled oxylipins used as internal standards and may hence lead to a massive overestimation of the analytes' concentration in the sample. Starting from this direct hydrolysis we systematically investigated the factors influencing the liberation of oxylipins from lipids and their quantification by LC-MS analysis. Moreover, we compared the efficiency of described hydrolysis procedures and deduce an efficient strategy for the extraction and hydrolysis of esterified oxylipins in plasma.

Poster 37

COMPARISON OF THE GLUCURONIDATION OF N-3 AND N-6 DIHYDROXYLATED POLYUNSATURATED FATTY ACIDS

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Oxylipins are oxidation products of polyunsaturated fatty acids (PUFAs), which are formed from n-6 PUFA such as arachidonic acid (ARA) and n-3 PUFAs such as eicosapentaenoic (EPA) acid or docosahexaenoic acid (DHA) by three different enzymatic pathways or non-enzymatic autoxidation. Biological active oxylipins mediate inflammation processes, platelet aggregation or vasoconstriction among other functions and are thus important for the regulation of the physiological homeostasis. Cytochrome P450 monooxygenases convert PUFA to epoxy fatty acids, which are degraded by the soluble epoxide hydrolase yielding dihydroxy-PUFAs. Little is known about the degradation and elimination of dihydroxy-PUFAs. Therefore this study investigates the metabolic fate of these oxylipins. We focused on the analysis of the conjugation with glucuronic acid in order to understand how the rate of elimination influences the biological levels of dihydroxy-PUFAs.
Dihydroxy fatty acids from all relevant n3-PUFA and n6-PUFA were synthesized and their regioisomers isolated. Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (ARA), docosapentaenoic acid (DPA) and alpha-linolenic acid (ALA) were epoxygenated using meta-chlorperbenzoic acid. The resulting mixtures of epoxy-PUFA regioisomers were separated by means of semi-preparative reverse phase liquid chromatography (LC). Finally, the isolated epoxides were opened by an acid catalysed hydrolysis. The purity of the products was checked by RP-LC-ESI(-)-MS. The glucuronidation was investigated utilizing microsomes from kidney and liver tissues. Following incubation, the substrates and products were separated by liquid-liquid extraction and the glucuronides were quantified as free dihydroxy-PUFAs after cleavage by β-glucuronidase.
On the poster the conjugation kinetics (KM and vmax) of EPA and DHA and ARA are compared and discussed with respect to the rate of elimination of these oxylipines from human body. This information is of high biological relevance, because the different conjugation rate could strongly influence the circulating levels of dihydroxy-PUFA and thus leads to different physiological effects of the structurally similar oxylipins.

Poster 38

INHIBITION OF CYTOCHROME-P450 DEPENDENT FORMATION OF HYDROXY AND EPOXY FATTY ACIDS BY FOOD POLYPHENOLS

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Oxylipins are oxidation products of polyunsaturated fatty acids such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DPA) and are formed via three enzymatic pathways or autoxidation. In the human body oxylipins mediate several functions, such as the regulation of pain and inflammation. AA and other PUFA are converted to epoxy- and hydroxy-PUFA in the third enzymatic pathway of arachidonic acid cascade by cytochrome-P450 monooxygenases (P450). Several of these products are potent lipid mediators regulating vascular, renal and cardiac functions. While the products of ω-hydroxylation of AA (such as 20-HETE) are vasoconstrictors, the products of epoxidation (EETs) act vasodilatory. It is well described that several food polyphenols are potent inhibitors for P450 dependent (phase 1) drug metabolism leading to side effects of pharmaceuticals. The impact of food ingredients on P450-derived oxylipins however, have not been investigated so far. Therefore, the aim of this project is to characterize the effect of food polyphenols on P450 dependent oxylipin formation. Inhibition of the third branch of the AA cascade was investigated in microsomal incubations using microsomes from different tissues as well as individual P450 enzymes (supersomes) under optimized conditions. The formation of hydroxy, epoxy and dihydroxy fatty acids was quantitatively analysed by means of RP-LC-ESI (-)-MS/MS following liquid/liquid-extraction. On the poster the effect of several food polyphenols on oxylipin formation is demonstrated and compared to potent P450 inhibitors.

Poster 39

DIFFERENTIAL COMPLEX LIPID PLASMA PROFILES REVEAL PERTURBATIONS IN PREECLAMPSIA

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Preeclampsia, the onset of hypertension and proteinuria at ≥20 weeks of gestation, is a leading cause of maternal perinatal morbidity and mortality. Despite significant recent efforts to define biomarkers to improve the ability of clinicians to predict, diagnose and manage preeclampsia, none perform well enough to have broad clinical utility. Preeclampsia is known to affect lipid metabolic pathways, e.g., abnormally elevated triglycerides, total cholesterol and low density lipoprotein cholesterol have been found to be associated with preeclampsia. We quantified the plasma lipidome from women with preeclampsia or preterm labor using the Lipidyzer TM Platform (Sciex). Lipid profile abnormalities were found in the triacylglycerides species (TAGs) and diacylglycerides species (DAGs) as well as some novel markers found to increase in Preeclampsia.
Plasma from women with sPE (gestational age 25-37 weeks) were analyzed. Gestational age-matched plasma samples from women with preterm birth (PTL) were used as controls. Applying internal standards (Avanti Polar Lipids), plasma was extracted using a modified Bligh/Dyer protocol. The Lipidyzer TM Platform (SCIEX) was used for targeted profiling of approximately 1100 lipid molecular species from 13 different lipid classes for comprehensive coverage of the plasma lipidome. This strategy allowed for: i) quantitative results for each lipid class as a sum of individual species; ii) mole percent composition obtained computationally from lipid molecular species data; and iii) accurate lipid species compositions.
We analyzed over one thousand lipid molecular species; coefficient of variations (CVs) for all species was < 0.06%, highlighting excellent reproducibility of the assay. Plasma from women with severe preeclampsia primarily showed differences in the TAG and DAG species as compared with preterm labor controls. The data were probed for pathway interactions and statistical analyses were conducted in Surveyor Web Tools (Metabolon, USA). A novel set of lipid molecular species were found to increase significantly, namely PC(16:0/14:1), LPC(18:3), CE(18:0), TAG50:5-FA20:5 and CE(20:1). A subset of these markers which increase and the TAG and DAG molecular species which decreased could be used to validate these potential markers in parallel sample cohorts to better predict, diagnose and manage preeclampsia.

Poster 40

LIPOXIN A4 STIMULATES HBD2 SECRETION AND DELAYS THE COLONISATION OF CYSTIC FIBROSIS BRONCHIAL EPITHELIUM BY PSEUDOMONAS AERUGINOSA

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Cystic Fibrosis (CF) lung disease is characterised by defective Cl^- transport and increased Na^+ absorption across the airway epithelium. This leads to dehydration of the airway surface liquid (ASL) layer leading to a persistent bacterial infection, mainly induced by Pseudomonas aeruginosa and Staphylococcus aureus, and inflammation. Lipoxin A4 (LXA4) belongs to a class of newly identified lipid mediators playing a key role in the resolution of inflammation. In addition, it has been reported to be abnormally produced in CF (Karp et al., 2004, Ringholz et al., 2014). We have previously shown that LXA4 restores fluid transport in CF bronchial epithelia by inhibition of the epithelial Na+ channel (ENaC) and stimulation of calcium-activated Cl- secretion resulting in an increased airway surface liquid height (Bonnans et al, 2003; Verriere et al, 2012; Al-Alawi et al, 2014; Higgins et al, 2014). In this study, we investigated if LXA4 also exerts a major impact on bacterial infection by P. aeruginosa. By performing real time RT-PCR and Western blot experiments, we found that LXA4 (1 nM) stimulated the expression and the secretion of the antimicrobial peptide human beta-defensin-2 (hBD-2) by bronchial epithelial cells. In addition, measurements of extracellular pH with the fluorescent dye pHrodo Reddextran revealed restauration of apical extracellular pH in CF airway epithelia by LXA4 to a range that favors hBD-2 bactericidal activity. Finally, by measuring the colony forming units of P. aeruginosa, we found that LXA4 inhibits P. aeruginosa growth in the apical liquid of CF airway epithelial cells in culture and also reduces adhesion of P. aeruginosa to the cells. In conclusion, LXA4 plays a major role in the innate immune response of the bronchial epithelium by increasing the production of hBD-2, by restoring apical pH of bronchial epithelial cells and by reducing growth and adhesion of P. aeruginosa to CF bronchial epithelial cells.

Poster 41

LIPID BIOMARKERS FOR ACUTE LEUKEMIA SCREENING

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At the present time, it is considered that instead of screening for a single cancer related biomarkers it may be more productive to screen for core pathways similarly deregulated in most cancers including leukemia. While the alterations in the cell plasma membrane (PM) lipids metabolism have been increasingly recognized as a hallmark of cancer and the importance of lipid-based regulation of various cell bio-systems is well established but their role in hematological malignancies is not studied in detail. Our research aiming at the relationship between immune cell function and leukemia development first led to the determination of possible alterations in common characteristics of lipid modification mechanisms in the PM of human peripheral blood mononuclear cells (MNC) crude population in acute lymphoblastic (ALL) and myeloblastic (AML) forms of leukemia. We investigated the processes of different exogenous fatty acids (FA) early (5 sec) and long term (60 min) incorporation into the lysophosphatidylcholine (LPC) fraction of blood MNC obtained from patients with ALL and AML compared to healthy people. Possible disturbances in endogenous activities of enzyme systems involved in LPC generation/utilization mechanisms in the purified PM fraction of MNC were also investigated. The data obtained provide evidence for elevated level of diverse FA-containing LPCs in MNC of ALL and AML patients compared to norm. We have also shown that in MNC PM-associated and Ca^{2+}-dependent activities of some phospholipases detected in norm were completely inhibited in both forms of leukemia. Simultaneously, statistically significant higher activity of enzymes responsible for LPC deacylation and synthesis was also detected in leukemia compared to norm.To summarize, data obtained indicate deregulation of LPC homeostasis in blood MNC crude population in both ALL and AML. Thus, we conclude that the disturbances revealed are common characteristics for these types of malignancy studied and can be used as new and valuable tools for the hematological cancer screening program.

Poster 42

ALTERATIONS IN PERIPHERAL BLOOD MONONUCLEAR CELLS PLASMA MEMBRANE PHOSPHOLIPIDS IN PROSTATE AND BLADDER CANCERS

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The involvement of cell plasma membrane (PM) lipids in the regulatory mechanisms of various important membrane-associated processes is well documented. These compounds by quick and reversible changes in their composition and structure (microdomain) organization respond rapidly to different environmental perturbations, especially leading to the pathologies. It was hypothesized by us that in such intricate system diseases, as human tumors, alterations in PM lipid homeostasis in the peripheral blood mononuclear cells (PBMC) may possibly represent some information useful for detection and assessment of cancers.
The aim of this study was to investigate the quantitative changes in the phospholipid (PL) content of PBMC PM fractions in healthy volunteers and patients with prostate (PC) and bladder cancer (BC). Using the contemporary lipidomic approaches we have identified abnormal changes in certain PL-amount in PBMC of PC and BC patients compared to healthy individuals. Data obtained indicate the existence of perturbations in the PM lyso- and aminophospholipid homeostasis and might be involved in the development of cancer. In contrast to the absolute amounts, the relative amounts of examined PLs in PC and BC cell PM were not changed in comparison to the control. Thus, in pathological conditions the optimal relations of various PLs are mainly maintained in the membrane, which is crucial for preserving the structural and functional integrity of the cells.
We conclude that quantitative alterations of PLs have been associated to diseases and specific PLs may be involved in the onset and evolution of these cancers. These data will be useful as a starting point to define possible PLs as prospective biomarkers as well as for discovery of new personalized modes for disease treatment.

Poster 43

DEVELOPMENT OF BENZOTHIAZOLES AS DUAL 5-LIPOXYGENASE AND MICROSOMAL PROSTAGLANDIN E2 SYNTHASE-1 INHIBITORS

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Prostaglandins (PGs) and leukotrienes (LTs) are powerful bioactive lipid mediators that have a large number of biological actions in the human body [1, 2]. The common precursor of PGs and LTs is arachidonic acid (AA). The 5-lipoxygenase (5-LO) and the microsomal prostaglandin E2 synthase-1 (mPGES-1) are both enzymes within the arachidonic acid cascade. 5-LO is the initial enzyme which catalyzes the conversion of AA to the corresponding LTs; whereas the mPGES-1 is responsible for the transformation of PGH2 into PGE2 which is one of the most prominent mediators of inflammation, pain and fever. A valuable pharmacological approach for anti-inflammatory therapy is the dual inhibition of 5 LO and mPGES-1. In contrast to the traditional NSAIDs the dual inhibition of PGs and LTs might be superior over single interference with PGs in terms of anti-inflammatory effectiveness as well as regarding reduced side effects [3]. In the post area of selective COX-2 inhibitors differ ent approaches for dual inhibition of PGs and LTs have been pursued, like dual COX/LO, dual COX-2/LTA4-Hydrolase or dual 5-LO/mPGES-1 inhibitors [4, 5]. Within the dual 5-LO/mPGES-1 inhibitors the pirinixic acid derivatives are the most advanced one. However pirinixic acid derivatives are well known compounds with many various biological activities especially PPARalpha and PPARgamma activation [6]. Therefore, in this series we replaced the central scaffold of the pirinixic acid, the chlorinated pyrimidine core, by a benzothiazole, which was identified by a virtual screening approach [7].
Here, we present the synthesis and in vitro pharmacological characterization of the benzothiazole derivatives and we were able to identify compounds, which are about equally potent to the most potent pirinixic acid derivatives.

References

1. Funk, C. D. (2001) Science 294 (5548): 1871−1875.
2. Samuelsson, B., Morgenstern, R., Jakobsson, P. J. (2007) Pharmacol Rev. 59(3): 207–224.
3. Koeberle, A., Werz, O. (2009) Curr. Med. Chem., 16(32): 4274–4296.
4. Celotti, F., Laufer, S. (2001) Pharmacol Res. 43(5): 429-436.
5. Hanke, T. et al. (2013) J Med Chem. 56(22): 9031-9044.
6. Merk, D. et al. (2015) Future Med Chem. 7(12): 1597-1616.
7. Waltenberger, B. et al. (2011) J. Med. Chem., 54(9): 3163–3174.

Poster 44

ANANDAMIDE AND PALMITOYLETHANOLMIDE LEVELS AND METABOLISM IN T84 CELLS EXPOSED TO INTERFERON-γ

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The cytokine interferon-gamma (IFN-gamma) is an important mediator in inflammatory disorders of the gut, where disruption of the mucosal barrier is a key event. Human T84 colonic epithelial cells, which grow to confluence as a polarised monolayer, are a useful in vitro model of the intestinal epithelium. In this model, IFN-gamma produces an increased permeability without causing cell necrosis. Given the potentially beneficial role(s) played by endocannabinoids and related N-acylethanolamines (NAEs) in inflammatory intestinal disorders, we have investigated these systems in T84 cells exposed to IFN-gamma. In untreated T84 cells, 2-AG, AEA, PEA, OEA and SEA could be detected in extracts of cells+medium. Treatment of the cells with IFN-gamma (100 U/mL) significantly increased the concentrations of all five lipids in the extracts. In contrast, levels of linoleic acid-derived oxylipins and arachidonic acid derivatives TXB2 and 5-, 11-, 12- and 15-HETE were not significantly affected. Untreated T84 cells hydrolysed both AEA and PEA. The hydrolysis of AEA was inhibited by the FAAH inhibitor URB597 (1 μM) while the hydrolysis of PEA was inhibited both by URB597 and the NAAA inhibitor pentadecylamine (30μM). FAAH and NAAA mRNA levels were measured in T84 cells using qPCR. IFN-gamma produced a small (12%) decrease in FAAH mRNA levels. In contrast, NAAA levels were increased by 33%. Permeability of the cells were assessed using TEER measurements. Under control conditions, TEER values ~1000 were seen for the T84 cells cultured in transwells, indicative of a strong barrier function. The cells also showed a very low permeability to apical [14C]sucrose and [14C]mannitol. IFN-gamma treatment produced, as expected, a ~20% decrease in TEER and an increase in IL-8 and TNF-alpha production. Concomitant incubation with either 10 μM PEA, 1 μM URB597 or 30 μM pentadecylamine did not block the effect of IFN-gamma treatment on the observed TEER or cytokine production. It is concluded that in T84 cells, endocannabinoid and related NAE levels are increased in response to IFN-gamma treatment; that the cells express both FAAH and NAAA; but that addition of either PEA or blockade of these enzymes is insufficient to counter the deleterious effects of IFN-gamma treatment.

Poster 45

5,6-Δ-DHTL, A STABLE METABOLITE OF ARACHIDONIC ACID, IS AN EDHF REGULATED BY PARAOXONASE 1 IN THE MODULATION OF MICROVASCULAR DILATION

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Paraoxonase 1 (PON1) is an antiatherogenic high density lipoprotein-associated lactonase. Recent findings revealed that PON1 knockout (PON1KO) mice have low blood pressure, which is inversely correlated with the level of 5,6-epoxyeicosatrienoic acid (5,6-EET), a cytochrome P450-derived arachidonic acid metabolite. 5,6-EET is an endothelium-derived hyperpolarizing factor (EDHF) that causes arterial dilation. Under physiological conditions, it is unstable, transforming to the δ-lactone 5,6-δ-DHTL, which has been shown to be degraded by PON1. Our results reveal that the 5,6-δ-DHTL lactone isomer is also a potential EDHF, as it mediates vasodilation of resistance arteries and evokes endothelial hyperpolarization mediated by increased cytosolic Ca^{2+} influx and Ca-dependent potassium efflux. We thus hypothesize that PON1 regulates the level and function of lactone-containing metabolites that are potential EDHFs, thereby modulating vascular resistance.
5,6-δ-DHTL induced an increase in intracellular Ca^{2+} ($[Ca^{2+}]i$) in native human umbilical vein endothelial cells (HUVEC) in a dose-dependent manner. The 5,6-δ-DHTL-triggered $[Ca^{2+}]i$ elevation was reduced by IP3 and ryanodine antagonists but not by membrane TRPV4 antagonists, and was mainly NO-independent, as evidenced by its 20% reduction in the presence of L-NAME (eNOS inhibitor) and its insensitivity to the NO-scavenger CPTIO. 5,6-δ-DHTL also induced endothelial hyperpolarization, which was abolished by the inhibition of small and intermediate conductance K_{Ca} channels (by apamin and charybidotoxin, respectively). In isolated mice and human microvessels, endothelial-dependent dilation in response to increasing doses of 5,6-δ-DHTL was observed, while this dilation was abolished in denuded vessels. The 5,6-δ-DHTL-dependent vasodilation in PON1KO mice was higher (41%) than in C57BL wild type (24%) and was attenuated by 28% after incubation with rPON1.
5,6-δ-DHTL is a potential EDHF that dilates microvessels through an endothelial-dependent mechanism and is regulated by the PON1 enzyme.

Poster 46

QUANTIFICATION OF EPOXYEICOSATRIENOIC AND DIHYDROXYEICOSATRIENOIC ACID REGIOISOMERS IN HUMAN PLASMA: ANALYTICAL VALIDATION AND APPLICATION TO THE STUDY OF ENDOTHELIAL FUNCTION

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Epoxyeicosatrienoic acids (EETs) are endothelium-derived vasodilating lipid mediators metabolized into the less potent dihydroxyeicosatrienoic acids (DHETs) by soluble epoxyde hydrolase (sEH). We aimed to develop a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method to quantify EETs and DHETs in human plasma and monitored their levels during vascular endothelial stimulation.
Plasma samples were collected from 14 healthy volunteers and 5 hypertensive patients at baseline and during radial artery endothelium-dependent flow-mediated dilatation induced by hand skin heating, and spiked with 11,12-DHETd11 and 14,15-EETd8 as internal standards. Lipids were then extracted by a modified Bligh and Dyer method and saponified to release bound EETs and DHETs. Samples are purified by a second liquid-liquid extraction after reacidification and analyzed by HPLC-MS/MS. The assay allowed to identify (±)8(9)-epoxy-5Z,11Z,14Z-eicosatrienoic acid (8,9-EET); (±)11(12)-epoxy-5Z,8Z,14Z-eicosatrienoic acid (11,12-EET); (±)14(15)-epoxy-5Z,8Z,11Z-eicosatrienoic acid (14,15-EET); (±)8,9-dihydroxy-5Z,11Z,14Z-eicosatrienoic acid (8,9-DHET); (±)11,12-dihydroxy-5Z,8Z,14Z-eicosatrienoic acid (11,12-DHET); (±)14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid (14,15-DHET). (±)5(6)-epoxy-5Z,11Z,14Z-eicosatrienoic acid (5,6-EET) was virtually undetectable due to its chemical instability. The limits of quantification were 0.25 ng/mL and 0.5 ng/mL for DHETs and EETs respectively. Intra- and inter-assay variations ranged from 1.6% to 13.2%. In healthy subjects, heating induced a similar increase in mean (SD) levels of 8,9-EET (10.6±3.7 to 12.2±3.7 ng/mL), 11,12-EET (4.6±1.6 to 5.7±1.8 ng/mL) and 14,15-EET (4.8±2.1 to 5.9±2.4 ng/mL), and an increase in total DHET levels (1.53±0.54 to 1.79±0.73 ng/mL). The magnitude of this increase was correlated with the magnitude of the increase in the flow stimulation ($r=0.57$, $P=0.03$). We also observed a reduction in 14,15-DHET-to-14,15-EET ratio ($P=0.04$) during heating, suggesting a decrease in sEH activity. In contrast, no change in EET or DHET levels was observed in hypertensive patients during heating. Moreover, baseline 14,15-DHET-to-14,15-EET ratio was higher in hypertensive patients than in healthy subjects and did not decrease during heating.
We validated a sensitive HPLC-MS/MS method for measuring simultaneously plasma EET and DHET regioisomers in human plasma. Thus, EET regioisomers are released to a similar extent during endothelial stimulation in conduit arteries of healthy subjects but not in hypertensive patients, and both a decreased EETs production and increased EETs degradation to DHETs are involved in this alteration in EET bioavailability.

Poster 47

EFFECT OF EXTRA VIRGIN OLIVE OIL POLYPHENOLS ON ARACHIDONIC ACID CASCADE IN HUMAN INTESTINAL EPITHELIAL CELLS

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Extra virgin olive oil (EVOO) consumption is associated with protection from high prevalence diseases such as cardiovascular disease, inflammatory chronic processes and cancer. Oxidative stress and arachidonic acid (AA) cascade activation have a pivotal role in these pathological processes and beneficial effects of polyphenols can be related with its actions on redox state and eicosanoid production. The aim of this study was to examine the effect of representative EVOO polyphenols, tyrosol and hydroxytyrosol, on AA release and the synthesis of the main AA cascade metabolites. We use human intestinal epithelial Caco-2 cell cultures as experimental model and a versatile and reliable analytical LC-MS/MS methodology for the simultaneous analysis of multiple AA cascade metabolites. Our results show that both EVOO polyphenols (0.1-10 μM) were able to inhibit AA release induced by fetal bovine serum (10 %), H_2O_2 (1 mM), TNFα + IFNα (50 ng/ml) in a concentration manner. The findings also show that tyrosol and hydroxytyrosol (1 μM) reduced significantly the synthesis of AA metabolites such as prostaglandin E2, leukotriene B4, 5-, 12-, 15-hydroxyeicosatetraenoic acids induced by fetal bovine serum, H2O2 or cytokines (TNFα+IFNα) as well as the production of 13-hydroxyoctadecanoic acid induced by these stimuli. Thus, our findings demonstrated that representative EVOO polyphenols can regulate AA release and eicosanoid synthesis by cyclooxygenase and lipoxygenase pathways, events involved in the pathogenesis of several diseases of high prevalence and with an important inflammatory component.

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Poster 48

THE IMPACT OF G-PROTEIN COUPLED ESTROGEN RECEPTOR 1 (GPER1) ON CERAMIDE SYNTHASE (CERS) REGULATION

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Ceramides are a group of sphingolipids that play an important role in regulation of proliferation and apoptosis and are associated with pathophysiological processes like cancer development. Ceramide is generated de novo in the endoplasmic reticulum by six ceramide synthase (CerS) isoforms. In breast cancer cells CerS2, -4, -5 and -6 are expressed. Estrogen can modulate signaling cascades leading to transcriptional regulation of pro-proliferative genes in a multitude of possibilities. Here, we focus on the G-protein coupled estrogen receptor 1 (GPER1), which is activated by several ligands and activates various protein-kinase cascades leading to rapid changes in the level of second messengers. In previous work we showed that GPER1 mediates CerS2, -4, -5 and -6 transcription, but the signaling cascade was unclear. In addition, the sub-cellular localization of GPER1 is still discussed controversially. To examine how GPER1 affects CerS expression in breast cancer cells we generated a GPER1 stable MCF-7 cell line (MCF-7/GPER1 and MCF-7/ptarget is the empty vector control) and analyzed the sub-cellular localization. Investigating whether the effect of binding of several ligands on GPER1 and subsequently altered CerS expression is related to transcriptional effects, we performed luciferase reporter gene assays. Additionally, we determined the mRNA expression levels of CerS2, -4, -5 and -6 to evaluate differences in the expression between MCF-7/ptarget and MCF-7/GPER1 cells. These data could provide new insights on the role of GPER1 and its related pathways to find out whether it may represent a novel biomarker for aggressiveness of diseases or a new therapy target for breast cancer treatment.

Poster 49

PROTECTIVE ROLE OF OLEIC ACID ON ATHEROSCLEROTIC PROCESS IN NIACIN-TREATED MICE WITH METABOLIC SYNDROME

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Niacin, a member of the vitamin B family, is well known for its functions in the treatment and prevention of atherosclerosis due to beneficial plasma lipid modulation. Herein, we aimed the niacin divergent effect modulated by dietary fats in atherosclerotic lesion of mice with metabolic syndrome (MetS).
Niacin-treated MetS mice (Lepob/obLDLR-/-) were fed low-fat low-cholesterol diet (LFLCD) or three high-fat low-cholesterol diets (HFLCDs) of different fatty acid compositions (saturated fatty acids, SFAs; monounsaturated fatty acids, MUFAs; and polyunsaturated fatty acids, PUFAs). Body weight, serum cardiometabolic parameters, circulating monocyte activation, atherosclerotic size and composition lesion, and macrophage polarization-related genes from aortic roots were evaluated. HFLCD-SFAs administration increased insulin, triglycerides and pro-inflammatory cytokines in serum compared to HFLCD-MUFAs or HFLCD-PUFAs. The incremented lesion size, macrophage accumulation, M1 phenotype and classical monocyte activation was higher in mice fed HFLCD-SFAs, whereas those fed HFLCD-MUFAs or HFLCD-PUFAs showed a reduction of macrophage content, and to induce M2 polarization in aortic roots.
Our data suggest a differential role between unsaturated fatty acids and saturated fatty acids in a mouse model of niacin-treated MetS and support the concept of the cardioprotector role of oleic acid as the main lipid component of olive oil, contributing to an ameliorated atherosclerotic process and plaque stability.

Poster 50

ACUTE AND SHORT-TERM EFFECTS OF DIETARY FATTY ACIDS ON OSTECLASTOGENESIS VIA RANKL/RANK/OPG SYSTEM

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Postprandial state and dietary fats are directly linked with chronic diseases. We hypothesized that dietary fats may have both chronic and acute effects on health status by modulating osteoclast differentiation and activation in a fatty acid-dependent manner
In healthy subjects, a fat-enriched meal increased plasma levels of the RANKL/OPG ratio (SFAs > MUFAs = MUFAs+omega-3 PUFAs) in the postprandial state. Postprandial TRL-SFAs enhanced TRAP activity and the expression of osteoclast marker genes (TRAP, OSCAR, RANK, and CATHK) while downregulated the expression of OPG gene in human monocyte-derived osteoclasts. These effects were not observed with MUFA-enriched postprandial TRLs. Moreover, postprandial TRL-SFAs increased the release of osteoclastogenic cytokines (TNF-α, IL-1ß, and IL-6) meanwhile TRL-MUFAs and TRL-PUFAs increased the release of anti-osteoclastogenic cytokines (IL-4 and IL-10) in the medium of human monocyte-derived osteoclasts. In addition, Lepob/obLDLR-/- mice on an SFA-enriched diet had a greater atheroma plaque size, calcification, and RANKL/CATHK expression in aortic root than mice on MUFA-enriched diets, the latters increasing OPG expression in aortic roots.
These exciting findings open new opportunities for developing nutritional strategies with olive oil as the principal dietary source of MUFAs, notably oleic acid, to prevent development and progression of osteoclast-related diseases.

Poster 51

POTENTIAL ROLE OF PATHOGEN LIPOXYGENASE FOR PSEUDOMONAS AERUGINOSA INFECTIONS

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In mammals, lipoxygenases (LOXs) play important roles in the biosynthesis of pro- and anti-inflammatory mediators, but little is known about the functionality of pro-caryotic LOXs. Pseudomonas aeruginosa (PA) is a common opportunistic pathogen causing life-threatening infections in immuno-compromised patients due to its high intrinsic antibiotic drug resistance. PA is one of the rare bacterial species that express a secreted LOX (PA-LOX). This study was aimed at exploring the structural and functional properties of PA-LOX and its role in PA infections. For this purpose the enzyme was first overexpressed in E.coli, purified to homogeneity and characterized with respect to its protein-chemical (molecular weight, isoelectric point, iron content, crystal structure) and enzymatic properties (turnover rate, substrate and product specificity, pH-profile, activation energy, 14,15-leukotriene synthase activity). Experiments with stereospecifically labelled linoleic acid indicated that the formation of 13S-HpODE involved the abstraction of the proS-hydrogen from C11 of the fatty acid backbone. Thus, the two major elementary reactions of lipoxygenation (hydrogen abstraction, oxygen insertion) proceed antarafacially. The enzyme does not follow the triad concept of LOX specificity but Ala-420-Gly exchange resulted in a shift of arachidonic acid oxygenation from 15S- to dominant 11R-oxygenation. The crystal structure of PA-LOX (1.48Å) revealed that the substrate-binding pocket is bifurcated cavity containing a phospholipid molecule. The X-ray structure of the Ala-420-Gly mutant suggested an altered path of intra-enzyme oxygen diffusion, which explains the observed changes in reaction specificity. The presence of a phospholipid molecule inside the active site prompted us to explore the phospholipid and biomembrane oxygenase activity of PA-LOX. Here we found that PA-LOX was capable of oxidizing phospholipids forming specific hydroperoxides. When reacting with intact cells the enzyme cause cell lysis and these alterations were related to enzyme-catalyzed oxygenation of membrane phospholipids. Lipidomic analysis indicated that after long-term (24 h) in vitro incubations of the purified enzyme with red blood cells more than 50% of the polyenoic fatty acid containing membrane lipids were oxygenated. We are currently exploring whether patients with PA sepsis may suffer from increased hemolysis and whether treatment with PA-LOX inhibitors might constitute a suitable strategy to prevent this complication.

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DEVELOPMENT OF A TARGETED LC-ESI(-)-MS/MS METHOD FOR THE PARALLEL QUANTIFICATION OF 27 ISOPROSTANES AND 8 ISOFURANS

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Oxidative metabolites from polyunsaturated fatty acids (PUFA), i.e. oxylipins, are formed in mammals in the arachidonic acid (AA) cascade by three enzymatic pathways and autoxidation. While enzymatic conversion of PUFA results in stereoselective products such as prostaglandins and leukotrienes, autoxidation leads via radical reactions – among other products – to a vast number of regio- and stereoisomeric prostaglandin-like isoprostanes (IsoP) and isofuranes (IsoF) (Galano et al. (2015) Biochim Biophys Acta). For example, 64 isomers of the different IsoP are formed from AA comprising 4 regioisomers each with 16 diastereomers. One class of IsoP, the F2-IsoP, are common biomarkers for oxidative stress in vivo, and are associated with cardiovascular and inflammatory diseases. However, little is known about the overall pattern of IsoP and IsoF from AA and other PUFA formed in the human body and its modulation by diet and drugs. In order to investigate the prostaglandin-like autoxidation products as oxidative status marker in vivo, a comprehensive set of IsoP and IsoF needs to be analyzed. For this purpose we developed a LC-ESI(-)-MS/MS method allowing the parallel quantification of 27 IsoP and 8 IsoF derived from 6 different PUFA (ALA, AA, EPA, AdA, DPA, DHA) within 12 minutes. The chromatographic separation was carried out on a RP-C18 column (2.1 x 150 mm, 1.8 µm) yielding narrow peaks with an average width at half maximum of 3.3 to 4.2 sec. Detection was carried out on a triple quadrupole (QqQ) mass spectrometer operating in selected reaction monitoring mode (SRM) allowing the selective detection of regioisomers. The limit of detection (LOD) ranged between 0.1 and 1 nM and the lower limit of quantification (LLOQ) between 0.25 and 2 nM. For the quantification, two deuterated and two odd-chain internal standards are used. The method can also be integrated in an established targeted metabolomics platform for the detection of enzymatically formed oxylipins (Ostermann et al. (2015) Anal Bioanal Chem) allowing the separation and quantification of more than 150 oxylipins within 30.5 min. On the poster the performance of the method and its application on biological samples are presented.

Poster 53

TOTAL SYNTHESIS OF A DIHADA ISOMER, A PUTATIVE ENDOGENOUS ANALOG OF NEUROPROTECTIN D1 DERIVED FROM ADRENIC ACID

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The neuroprotectin D1 (NPD1) is a bioactive endogenous dihydroxylated and non-cyclic metabolite of docosahexaenoic acid (DHA, C22:6 n-3). Interestingly, it is involved in the termination program of acute inflammation at nanomolar doses. It is biosynthesized through a 15-lipoxygenase catalyzed oxygenation of one of its cis,cis-1,4-pentadiene moieties followed by an enzymatic epoxidation and hydrolysis leading to a rearrangement of the polyenic structure. Since adrenic acid (AdA, C22:4 n-6, present in myelin) also contains several cis,cis-1,4-pentadiene moieties, we believe that AdA is susceptible to lipoxygenases attacks too. Thus, on the basis of the mechanisms suggested for the biosynthesis of protectins, we wonder whether AdA is converted in vivo into its corresponding NPD1 and aspirin-triggered analogs. Thus, we decided to carry out the chemical synthesis of these putative novel NPD1 analogs in order to provide samples for their detection in enflamed tissues and investigation on their biological properties. A successful stereoselective synthesis of an optically active 10,17-diHAdA isomer containing a Z,E,E-triene flanked by two hydroxyl groups is presented. The strategy features Wittig olefinations, alkyne preparation, hydrometalations, Sonogashira cross coupling reactions. With this molecule in hands, besides its existence in vivo, we envision showing evidence of the influence of the lateral chains (length, number of double bonds) on the potent beneficial effects reported to date for NPD1 and its aspirin triggered isomer e.g. the anti-inflammatory and resolving effects, but also healing and neuroprotection.

Poster 54

REACTION SPECIFICITY OF 12- AND 15-LIPOXYGENATING ALOX15 ORTHOLOGS WITH DIFFERENT POLYENOIC FATTY ACIDS

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Polyunsaturated fatty acids are converted to hydroperoxides by lipoxygenases (LOX). The nomenclature of LOX is based on their regio-specificity for the conversion of arachidonic acid (AA): The human 15-lipoxygenase-1 (ALOX15) produces mainly 15-hydroperoxyeicosatetraenoic acid from AA, which can be reduced to 15-hydroxyeicosatetraenoic acid (15-HETE). In humans 12 HETE mainly results from the catalytic activity of ALOX12 and/or ALOX12B. However, the regio-selectivity of AA oxygenation of ALOX15 orthologs varies between species: 15-lipoxygenating orthologs of ALOX15 are found in higher primates (such as men, chimpanzee, orangutan), whereas 12-lipoxygenating isoforms are expressed in lower mammals (mice, rats, pigs). Little is known about the product specificity of ALOX15 orthologs with other substrates, e.g. eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA). Here, we compared the product pattern of 15-lipoxygenating ALOX15 orthologs (human, rabbit, chimpanzee, orangutan), 12-lipoxygenating ALOX15 orthologs (mouse, rat, pig, rhesus monkeys) as well as human ALOX15B and human platelet-type ALOX12 for the conversion of AA, EPA and DHA. Recombinant enzymes were incubated with each of the substrates (100 μM) for 4 min. Following peroxide reduction and acidification, lipids were extracted with ethyl acetate. The product pattern was analyzed by liquid-chromatography-mass-spectrometry. We found that AA-15-lipoxygenating ALOX15 orthologs convert EPA mainly to n-6 (15-HEPE) hydroperoxides. Variable amounts of n-9 oxygenation products (12-HEPE) were formed as minor side products. With DHA the product specificity of AA-15-lipoxygenating ALOX15 orthologs is more variable giving rise to a mixture of n-9 (14-HDHA) and n-6 (17-HDHA) hydroperoxides. For some orthologs 14-HDHA (orangutan) is dominating, whereas 17-HDHA prevails for other orthologs (rabbits). AA-12-lipoxygenating ALOX15 orthologs convert EPA dominantly to 12-HEPE (pig and macaca ALOX15), whereas rat and mouse orthologs surprisingly produce 15-HEPE as major oxygenation product. With DHA pig, macaca and mouse ALOX15 mainly produce 14-HDHA, whereas the rat ortholog forms a mixture of 14- and 17-HDHA. Human ALOX15B converts EPA almost exclusively to 15-HEPE and DHA to 17-HDHA. In contrast, human ALOX12 converts EPA and DHA to 12-HEPE and 14-HDHA. Taken together one can conclude that the reaction specificity of AA-15- and AA-12-lipoxygenating ALOX15 orthologs is variable and depends on the species and on the structure of the fatty acid substrate.

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LIPID METABOLITES IN HUMAN COLON MUCOSA, ADENOMA AND CARCINOMA TISSUES

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An elevation of prostaglandin E_2 (PGE_2) levels, a product of arachidonic acid (AA, 20:4 n-6) and cyclooxygenases 2 (COX2), is a hallmark in colorectal carcinoma (CRC). While omega-6 polyunsaturated fatty acid (n-6 PUFA) metabolites have mostly pro-inflammatory properties, anti-inflammatory effects are generally attributed to n-3 PUFA metabolites. Recent studies indicated an increase of n-6 PUFA levels and a decrease of n-3 PUFA levels in CRC. A downregulation of the 15-lipoxygenase (LOX) pathway of the arachidonic acid (AA, 20:4 n-6) cascade has also been described in CRC cells. The role of 15-LOX in the context of carcinogenesis in the colon is still controversial. Several studies postulated tumor suppressing properties of 15-LOX while other investigations suggest pro-inflammatory roles of 15-LOX and its metabolites. We investigated lipid profile changes in human colorectal carcinoma and adenoma tissue biopsies, focusing on n-6 and n-3 PUFA metabolites as well as on different branches of the AA cascade. Human colon adenoma tissue showed significant increased levels of AA derived 15-hydroxyeicosatetraenoic acid (15-HETE) compared to healthy tissue. Levels of the eicosapentaenoic acid (EPA, 20:5 n-3) derived 15- hydroxyeicosapentaenoic acid (15-HEPE) and the docosahexaenoic acid (DHA, 22:6 n-3) derived 17-hydroxydocosahexaenoic acid (17-HDHA), also increased significantly in adenoma tissue. Human colon carcinoma tissue also showed a tendency towards increased levels of 15-HETE, 15-HEPE and 17-HDHA.

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IN VIVO ASSESSMENT OF THE SOLUBLE EPOXIDE HYDROLASE INHIBITORY EFFECT OF SORAFENIB IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Hepatocellular Carcinoma (HCC) is the third leading cause of cancer death globally. Incidences in the United States and Europe are increasing. In most instances the disease is diagnosed in advanced stages when potentially curative treatments, such as surgical resection and locoreregional procedures are no longer possible. Systemic treatment options for advanced HCC are very limited. The standard therapy in this case is the multikinase inhibitor Sorafenib. One of the most important targets contributing to the anti-tumor effect of Sorafenib is the vascular endothelial growth factor (VEGF) receptor. Due to Sorafenib-induced inhibition of tumor angiogenesis and tumor-cell proliferation survival in patients with advanced HCC can be prolonged by nearly 3 months.
Moreover Sorafenib is an inhibitor of the soluble epoxide hydrolase (sEH). sEH catalyses the conversion of epoxides derived from long-chain polyunsaturated fatty acids such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) into their corresponding diols. Inhibition of sEH thus causes stabilization of these epoxides. Experimental studies in mice have shown that an increase in omega-3 DHA-derived 19,20-epoxydocosapentaenoic acid (19,20-EDP) in plasma and tumor, obtained by adding low dose of sEH inhibitors, is associated with a decrease of tumor growth by inhibition of tumor angiogenesis and reduced cell invasion.
In contrast, epoxyeicosatrienoic acids (EETs), epoxy metabolites from the omega-6 AA, have been implicated in tumor growth promotion: A number of experimental studies have shown that the inhibition of sEH leads to an increase in 14,15-EET, which promotes tumor growth and metastasis by cell invasion. The aim of this pilot study was to assess the effect of sorafenib treatment on the presence of epoxy lipid metabolites in blood from sorafenib-treated patients with HCC. Lipidomics technology was employed to analyse oxylipins and particularly epoxides and their corresponding dioles in blood samples from HCC patients.

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EFFECT OF DIFFERENT LIPID APHERESIS METHODS ON PLASMA OXYLIPIN LEVELS

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Lipoprotein–apheresis can reliably reduce low-density-lipoprotein (LDL) cholesterol and triglycerides in patients with atherosclerotic disease and treatment-refractory hypercholesterolemia or elevated lipoprotein (a). As we report elsewhere, different apheresis methods significantly influence the composition of omega-6 and omega-3 polyunsaturated fatty acids (n-6 PUFA and n-3 PUFA) in the plasma. Thus lipid apheresis may also interfere in the production of PUFA derived highly bioactive metabolites, the so-called oxylipins. The present study analyzed the oxylipin profiles in the plasma of 42 patients with hyperlipidemia treated by one of the three different apheresis methods in a direct pre- and post-therapy measurement. By using tandem liquid chromatography mass spectrometric methods (LC-MS/MS) we were able to simultaneously analyze broad oxylipin profiles of different PUFA. In all three lipoprotein apheresis methods we detected a significantly increased formation of various oxylipins derived from different pathways. In particular 12-HETE, a product of 12-lipooxygenase (12-LOX) derived from arachidonic acid, increased to up to seven times of the pre–apheresis values. Furthermore, concentrations of metabolites of the cyclooxygenase (COX) as well as 5- and 12-LOX pathways increased by approximately 50% as compared to the pre-apheresis concentrations. In summary, all apheresis approaches significantly influence the formation lipid mediators in the plasma. Increased concentrations of COX and LOX derived oxylipins indicate activation and mediator formation due to apheresis, including potentially pro-inflammatory oxylipins arising from the omega-6-PUFA arachidonic acid.

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IN VITRO CHARACTERIZATION OF 5-LIPOXYGENASE INHIBITION BY 2-AMINOTHIAZOLES

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Excessive inflammation is a hallmark of many chronic diseases [1]. In inflammatory diseases like asthma, allergic rhinitis, rheumatoid arthritis, atherosclerosis and certain types of cancer, 5-lipoxygenase (5-LO) represents a key target for drug therapy [2]. Previously, structure-activity relationships originated aminothiazole-comprising inhibitors with high potency in 5-LO relevant in vitro assays (ST-1853, ST-1906 IC50 = 50 nM in isolated intact polymorphonuclear leucocytes) [3]. Yet, the molecular mechanism of action is only poorly understood. In order to elucidate the molecular modes of 5-LO inhibition and to broaden the pharmacological profile, we conducted mechanistic in vitro studies with recombinant proteins as well as intact cell assays. In this study we assessed possible reactivity of the compound class in terms of redox activity and covalent binding. Furthermore, we could depict structural motifs that prevent covalent modifications of recombinant 5-LO, as well as pronounced phase II metabolism. Moreover the high potency in intact cells could be retained in various physiologically relevant in vitro whole blood assays. Therefore, we could approve the lead likeness of ST-1853 and ST-1906 for further development of this novel class of 5-LO inhibitors.

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References

1. Han J., Ulevitch R.J. (2005) Nat. Immunol., 6(12): 1198–1205.
2. Peters-Golden M., Henderson W.R. (2007) N. Engl. J. Med. 357(18): 1841-1854.
3. Kretschmer SBM, Woltersdorf S, et al. (2016) Future Med. Chem. 8(2):149–64.

Poster 59

TARGETING PROSTAGLANDIN E2 PRODUCTION IN MEDULLOBLASTOMA

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Prostaglandin E_2 (PGE_2) is a key lipid mediator of tumor progression. Apart from promoting angiogenesis, PGE_2 is crucial in maintaining the immuno-suppressive tumor microenvironment. Drugs targeting cyclooxygenase (COX)-1/2 have successfully reduced tumor growth in several cancer models, including the most common malignant pediatric brain tumor medulloblastoma, and have entered several study protocols. However, these drugs are associated with adverse long-term effects. The aim of this study is to investigate the inhibition of microsomal PGE synthase-1 (mPGES-1) as a therapeutic strategy for medulloblastoma. We use the selective mPGES-1 inhibitor Compound III (C3).
Human medulloblastoma cell lines (DAOY, D283, and D425) were treated with C3, NS-398 (selective COX-2 inhibitor), or diclofenac (dual COX-1/2 inhibitor) in the presence or absence of pro-inflammatory IL-1β. Prostaglandins in supernatants were extracted and quantified with LC-MS/MS. Cell toxicity and clone formation were determined with cell viability assay and clonogenic assay, respectively.
The increased production of PGE_2 by IL-1β was completely blocked when cells were co-treated with 10 μM C3, and we did not observe any shunting towards other prostanoids. Both C3 and diclofenac showed dose-dependent cell toxicity in the micromolar range. Diclofenac significantly reduced clone formation at 50 μM but not at 10 μM, while C3 was able to reduce clone formation even at non-toxic 1 μM. While NS-398 blocked induced PGE2 production, it was not cell toxic nor able to reduce clonogenic formation.
We investigate the effect of selective inhibition of mPGES-1 or COX-1/2 on medulloblastoma growth. We aim to further characterize the mPGES-1 inhibitor and compare to selective COX-2 inhibitor in preclinical medulloblastoma models. Specific inhibition of PGE_2 production, rather than general inhibition of prostanoid production, would potentially prove useful as a complement to current medulloblastoma treatments.

Poster 60

REGIOCONTROLLED SYNTHESES OF FAHFAS AND LC-MS/MS DIFFERENTIATION OF REGIOISOMERS

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It has recently been reported that branched fatty acid esters of hydroxyl-fatty acids, named FAHFAs, may serve as biomarkers for insulin resistance and type-2 diabetes risk (Yore et al., Cell, 2014, 318-332). FAHFAs were detected in mammalian adipose tissue, in blood plasma but also in rodent and human food. While saturated fatty acids are usually associated with negative impacts on human health, the regioisomers 5-PAHSA and 9-PAHSA, two branched FAHFAs comprised of palmitic acid (PA) and hydroxystearic acid (HSA) with the ester bond located at position 5 and 9 respectively, exert a positive systemic effect on glucose metabolism. The synthetic 9-PAHSA also showed anti-inflammatory effects. Interestingly, PAHSA levels were shown to be highly correlated with insulin sensitivity in humans. The aim of our study was to detect and quantify the presence of FAHFAs in different fluids using synthetic internal standards.The branched ester bond may be located at diverse positions in the lipid chain. Thus, in addition to the numerous combinations of fatty acids (FA) and hydroxyl-fatty acids (HFA), a large number of regioisomers may be present in vivo. The poster will depict:
1) A flexible and efficient chemical synthesis of branched FAHFA, allowing access to a large number of family members and regioisomers. 2) A preliminary study for detection of these branched FAHFAs in plasma by LC-MS/MS analyses that successfully differentiated regioisomers.

Poster 61

CHARACTERISATION OF OXIDISED PHOSPHOLIPIDS GENERATED IN HUMAN ERYTHROCYTES BY 15-LIPOXYGENASE FROM PSEUDOMONAS AERUGINOSA

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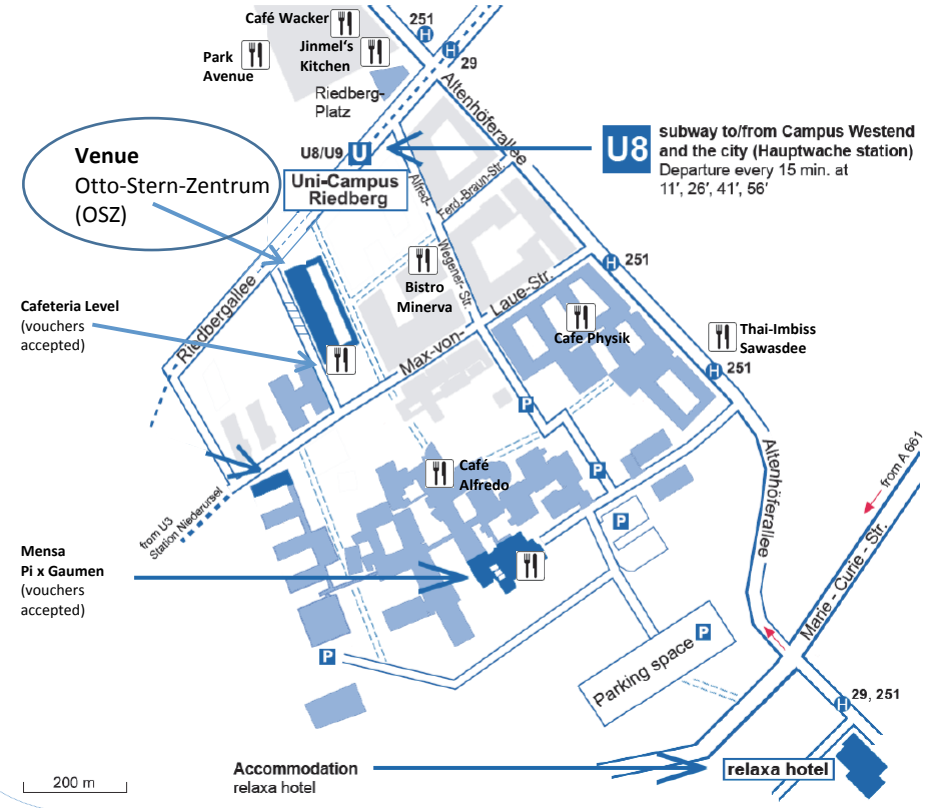
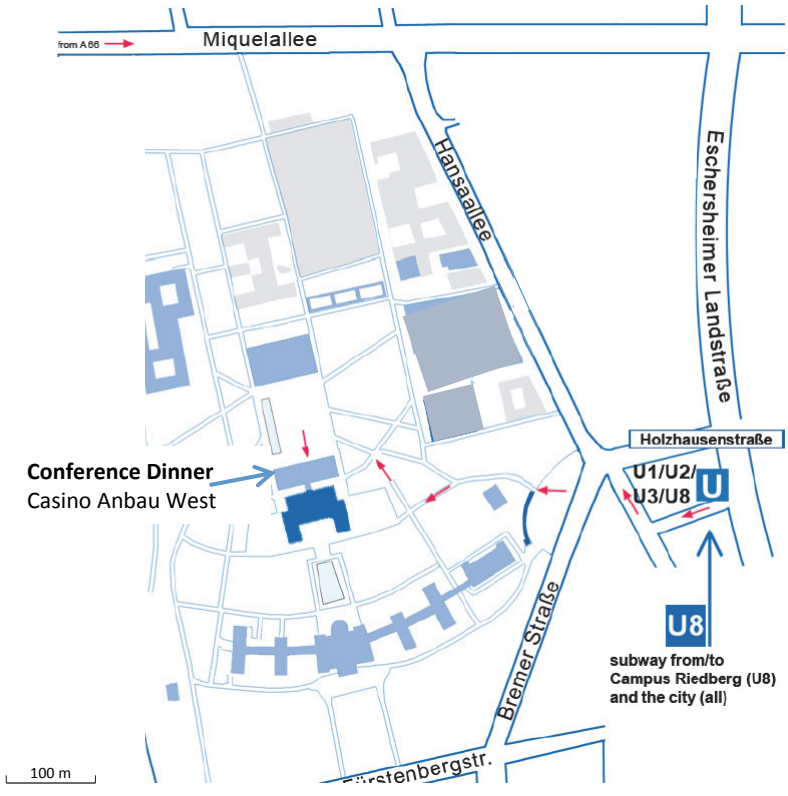
Pseudomonas aeruginosa (PA) is a medically significant Gram negative bacterial pathogen that expresses a unique secreted lipoxygenase (LOX), which oxygenates free arachidonic acid, predominantly to 15S-hydro(pero)xyeicosatetraenoic acid (15S-H(p)ETE). It binds phospholipids (PLs) at its active site and physically interacts with lipid vesicles. However, direct proof for the oxygenation of biomembrane PLs is lacking. Herein, we reveal, using a lipidomic approach, generation of numerous LOX-derived oxidised phospholipids (OxPLs), following in vitro incubation (12 - 24 hour) of intact human red blood cells (RBCs) with purified PA-LOX. Precursor scanning of red cell extracts for native PLs and oxidized fatty acids, followed by multiple reaction monitoring (MRM) and MS/MS of candidate species revealed a wealth of complex structures. These comprised over 30 different phosphatidylethoanolamines (PE) and phosphatidylcholine (PC) containing oxidized moieties that included15- hydroxyeicosatetraenoic acid (15-HETE), hydroxydocosahexaenoic acid (HDOHE), 15-ketoeicosatetraenoic acid (KETE) and hydroxyoctadecadienoic acids (HODEs) as well as masses consistent with oxo-valeroyl, oxo-glutaryl and oxo-nonanyl-containing PLs. Significant depletion of arachidonic, linoleic or oleic acid-containing PLs was observed, including for PE, PC, phosphatidylserine (PS) and phosphatidylinositol (PI). This change in the structural composition of the lipid bilayer of RBCs by PA-LOX may perturb membrane dynamics by altering barrier function and contribute to haemolysis, already known to be mediated by this enzyme. Furthermore, esterified eicosanoids and related oxPL are known regulators of diverse immune processes including coagulation, Toll-Like receptor signalling and of neutrophil anti-bacterial activities. Their generation by LOX oxidation of biomembranes during Pseudomonas infection could contribute to host innate immune responses.

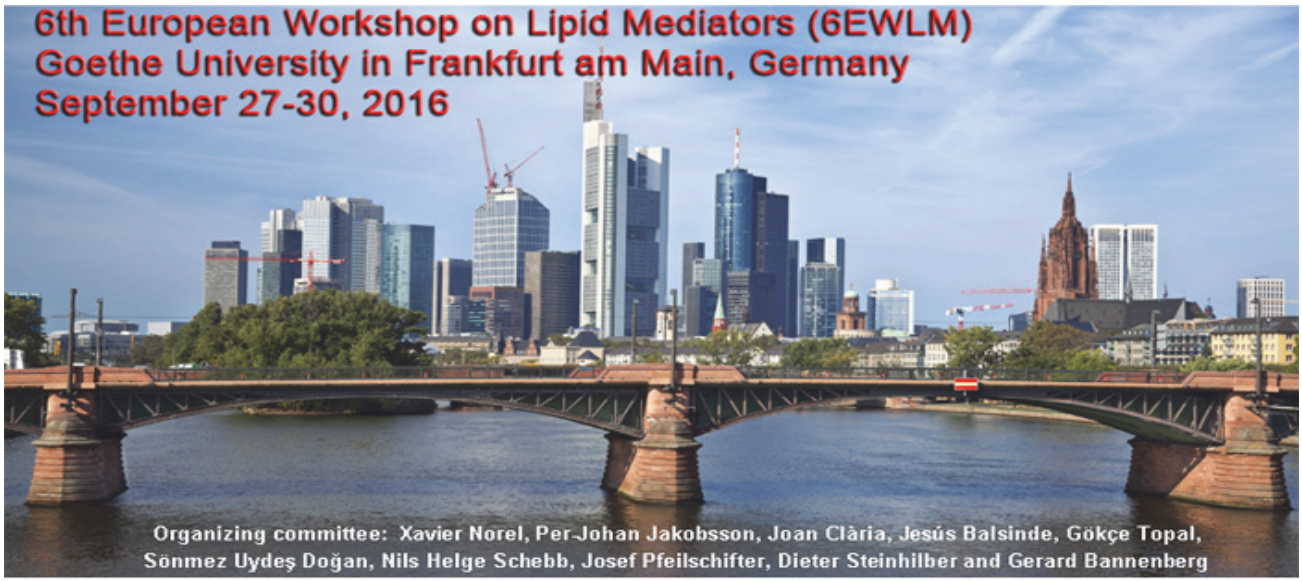
LOCATION OF CONFERENCE VENUE AND CONFERENCE DINNER

Campus Riedberg



Campus Westend





http://workshop-lipid.eu