Book of Abstracts

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Brussels, September 12-14, 2018

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Please do not hesitate to contact the local symposium team if you have any question, proposal or request during the meeting and during your stay in Brussels.

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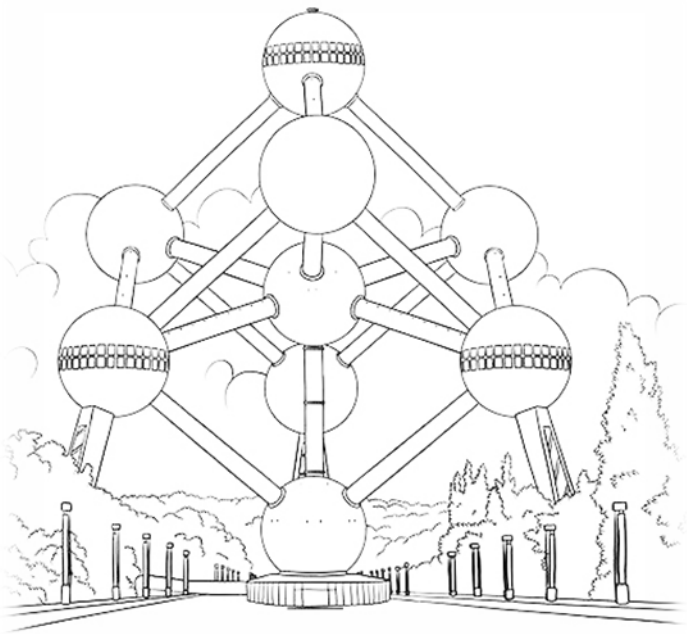


General Information

Venue and meeting location (Please see campus map at the end of this abstract book)

The meeting takes place on the health sciences campus of the Université catholique de Louvain in Brussels. Lectures are held in the **Auditorium F**, located within the "*Auditoires centraux*" building. Coffee breaks and poster sessions will be at the "*Bibliothèque*".
Lunches will be at the "Martin V" restaurant.

Lectures and Posters

Please mind the allocated time slots for lectures, including questions. Please load your talk on the computer prior to the start of your session. If you need help, do not hesitate to contact one of the organizers.
Posters will be on display for the duration of the meeting (at the "*Bibliothèque*"). Please put your poster up on arrival and registration or during the first coffee break at the latest.
Posters of speakers in the "Young Investigator Session" should be put up on Friday 14th.

Awards

Thanks to our sponsors, three awardees will be selected from the "Young Investigator" session. Three young scientists working in the field of inflammation resolution will be selected for a poster award sponsored by Ambiotis.

Gala dinner

The "Gala dinner" will take place at « Hof ter Musschen », 2 Avenue E. Mounier, 1200 Woluwe-Saint-Lambert. The venue can be reached following a short walk (10 minutes) from the meeting venue (a map can be found at the end of this abstract book).

Internet access

If your university is a partner in the Eduroam project, you can connect to the "eduroam" Wi-Fi by using the same login that you use at your own institution. During connection, you will be asked to enter the username and password provided by your institution.

If you do not have "eduroam" access, temporary Wi-Fi access is possible, you have to obtain a guest account and password at the registration desk.

Photos will be taken during the meeting and posted on the EWLM website later on. If you do not wish to have your picture published, please send an email (with an ID photo) to brussels2018@workshop-lipid.eu.

Wednesday, September 12

EDUCATIONAL SESSION

13:00-14:00 Registration. *Coffee*.

14:00-14:15 **Welcome** **Nils Helge Schebb** (University Wuppertal, Germany)
Per-Johan Jakobsson (Karolinska Institutet, Sweden)

14:15-15:00 **Jesmond Dalli** (William Harvey Research Institute, UK)
Profiling the resolution metabolome: considerations and methodologies

15:00-15:45 **Dieter Steinhilber** (Goethe University, Germany)
5-lipoxygenase in the formation of leukotrienes and SPM

15:45-16:15 *Coffee Break*

16:15-17:00 **Michael Spedding** (The University of Edinburgh, UK)
How lipids and metabolism are key to human evolution and disease, with an example in neurodegeneration (ALS)

17:00-17:45 **Heinfried H. Radeke** (Goethe University, Germany)
Sphingolipids and the immune system

18:00 ***Welcome reception***

Thursday, September 13

08:00-08:55 *Registration. Coffee.*

08:55-09:00 ***Morning address by organizers***

09:00-09:45 ***Opening plenary lecture***
Timothy Hla (Children's Hospital, Boston)
Biology of S1P signaling

SESSION 1. LIPID MEDIATOR RECEPTORS AND SIGNALING

Chairpersons: Xavier Norel *(INSERM U1148, France)*
Per-Johan Jakobsson *(Karolinska Institutet, Sweden)*

09:45-10:15 Dieter Steinhilber (Goethe University, Germany)
The 5-lipoxygenase pathway in cancer

10:15-10:45 Marina Ziche (University of Siena, Italy)
Prostaglandins in cancer progression

10:45-11:15 *Coffee Break: Poster session and Exhibition visit*

11:15-11:40 ***Yukihiko Sugimoto*** *(Kumamoto University, Japan)*
Stimulation and modulation of inflammatory mast cell responses by prostaglandin receptors

11:40-12:05 ***Amira Senbel*** *(Alexandria University, Egypt)*
Contraction induced by EP1 receptors activation in rat detrusor muscle is counteracted by the NO/cGMP pathway

12:05-12:30 ***Karin Larsson*** *(Karolinska Institutet, Sweden)*
Selective inhibition of PGE2 enhances the cytotoxic effect of doxorubicin and vincristine in multicellular tumor spheroids

12:30-14:10 *Lunch*

SESSION 2. LIPID MEDIATORS IN IMMUNE AND INFLAMMATORY RESPONSES

Chairpersons: Chengcan Yao (University of Edinburgh, UK)
Mireille Alhouayek (Université catholique de Louvain, Belgium)

14:10-14:40 Renger Witkamp (Wageningen University, The Netherlands)
From fish to cannabis – modulation of inflammation by n-3 PUFAs

14:40-15:10 Jon Lampa (Karolinska Institute, Sweden)
Role of prostaglandins in neuro-immune regulation

15:10-15:35 ***Shuntaro Hara*** *(Showa University, Japan)*
Regulation of Inflammatory Prostaglandin Synthesis by Acyl-CoA Synthetase ACSL4

15:35-16:00 ***Karsten Weylandt*** *(Brandenburg Medical School, Germany)*
ALOX15-deficiency: a two-edged sword in colitis

16:00-16:25 ***Romain Colas*** *(Queen Mary University of London, UK)*
Cerebrospinal Fluid Pro-resolving Mediators Correlate with Disease Severity and Outcome in Adults with Tuberculosis Meningitis

16:25-17:10 *Coffee Break: Poster session and Exhibition visit*

SESSION 3. LIPID MEDIATORS IN VASCULAR AND METABOLIC DISEASES

Chairpersons: Xavier Norel (INSERM U1148, France)
Joan Clària (Hospital Clínic-IDIBAPS, Spain)

17:10-17:40 Jean-Luc Cracowsky (INSERM UMR 1042 , Grenoble, France)
Targeting the prostacyclin pathway: beyond pulmonary arterial hypertension

17:40-18:10 Jane Mitchell (Imperial College London, UK)
COX-2 and prostanoid pathways in the cardiorenal system: mechanisms and biomarkers

18:10-18:22 ***Hasanga Manik Purage*** *(INSERM, U1148, Paris, France)*
PGI_2 induced relaxations overcome the PGE_2 induced vasoconstrictions in human coronary artery

18:23-18:35 ***Zsuzsanna Miklos*** (*Semmelweis University, Hungary)*
Lysophosphatidic acid induces strong vasoconstriction in the coronaries via multiple signalling pathways

18:35-19:00 ***Sven-Christian Pawelzik*** *(Karolinska University Hospital Solna , Sweden)*
Transcriptomic and lipidomic profiling of human heart valves reveal a specific prostaglandin E2 signature in calcific aortic stenosis

19:00 **Closing Remarks for Day 1**

20:00 *Gala dinner*

Friday, September 14

8:30-9:15 ***Plenary lecture***

Thierry Durand (University of Montpellier, France)
Isoprostanes, Phytoprostanes and Neuroprostanes: Biomarkers and Bioactive Lipids

YOUNG INVESTIGATOR SESSION

Chairpersons: Chengcan Yao (Edinburgh University, UK)
Gerard Bannenberg (Global Organization for EPA and DHA omega-3s)

9:15 – 9:23 Emanuela Talamonti (Stockholm University, Sweden)
Impairment of DHA synthesis alters the expression of neuronal plasticity markers and the brain inflammatory status in mice

9.24 – 9.32 Irina Alecu (University of Ottawa, Canada)
The secreted pro- and anti-inflammatory lipidomes of M1 and M2 macrophages

9.33 – 9.41 Gülsev Özen (Istanbul University, Turkey)
Investigation of PGE_2-MMP cross-talk in human vascular preparations, their attached PVAT and plasma in obese and non-obese patients

9.42 – 9.50 Nicholas Don-Doncow (Lund University, Sweden)
Inhibition of sphingosine-1-phospate generation reduces blood pressure levels by attenuating inflammation in an experimental model of hypertension

9.51 – 9.59 Ester Pagano (University of Naples Federico II, Italy)
The NAAA inhibitor AM9053 attenuates experimental colon carcinogenesis

10.00–10.08 Priit Eek (Tallinn University of Technology, Estonia)
Structure and Regulation of Arachidonate 11R-Lipoxygenase

10.09–10:17 Mary Walker (William Harvey Research Institute, United Kingdom)
13-series resolvins mediate the leukocyte-platelet actions of atorvastatin and pravastatin in inflammatory arthritis

10:18–10:26 Claudia Cristiano (University of Naples Federico II, Italy)
Role of palmitoylethanolamide in a mouse model of autism spectrum disorder: counteracting central and peripheral inflammation

10:27–10:35 Kimberley Pistorius (William Harvey Research Institute, United Kingdom)
PDn-3 DPA pathway regulates human monocyte differentiation and macrophage function

10:36–10:44 Jana Gerstmeier (Friedrich-Schiller-University, Germany)
Exotoxins from S. aureus are potent and selective stimulators of 15-lipoxygenase-1-derived SPM in human M2 macrophages to resolve inflammation

10:45–10:53 Heba Abd Elmoneim (INSERM U1148, France)
Targeting AMPK can augment prostacyclin production from human lungs

10:54–11:02 Eva Jarc (Jožef Stefan Institute, Slovenia)
Lipid droplets integrate metabolic and signalling pathways and are indispensable for the cellular stress response in breast cancer cells

11:03–11.11 Silke Thul (Karolinska Institutet, Sweden)
Resolution of inflammation through the lipoxin and ALX/FPR2 receptor pathway protects against abdominal aortic aneurysms

11:12-11:45 *Coffee Break: Poster session and Exhibition visit*

SESSION 4. ENDOCANNABINOIDS AND CYP METABOLITES

Chairpersons: Giulio Muccioli (Université catholique de Louvain, Belgium)
Joan Clària (Hospital Clínic-IDIBAPS, Spain)

11:45-12:15 Christopher Fowler (Umea University, Sweden)
The endocannabinoid system in cancer - beneficial or damaging?

12:15-12:45 Sophie Lotersztajn (INSERM UMR 1149, Paris, France)
The endocannabinoid system in the liver: novel perspectives

12:45-13:10 ***Mireille Alhouayek*** *(Université catholique de Louvain, Belgium)*
Prostaglandin D2-glycerol ester, a COX-2 metabolite of 2-arachidonoylglycerol, decreases inflammatory pain in mice

13:10-13:35 ***Wen Hui Wang*** *(New York Medical College, USA)*
Eicosaepoxytrienoic acid metabolites (EET) regulate kidney sodium transport

13:35-15:00 *Lunch*

15:00-15:30 *Awards ceremony*

SESSION 5. LIPIDOMICS AND ADVANCES IN FATTY ACIDS AND THEIR DERIVATIVES

Chairpersons: Nils Helge Schebb (University Wuppertal, Germany)
Gerard Bannenberg (Global Organization for EPA and DHA omega-3s)

15:30-16:00 Jesmond Dalli (William Harvey Research Institute, UK)
Functional Lipid Mediator Profiling in Establishing Disease Etiopathogenesis

16:00-16:30 William Griffiths (Swansea University, UK)
Lipidomics, Oxysterols and Lipid Mediators

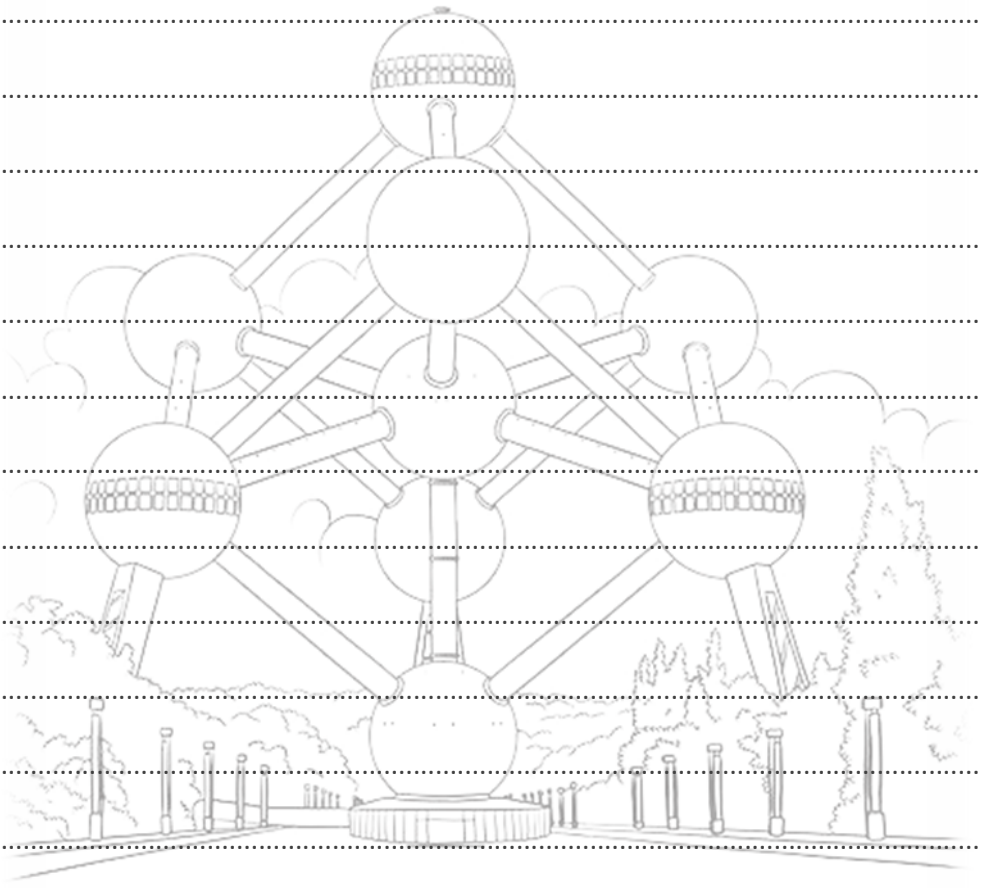
16:30-16:55 ***Esther Titos*** *(Hospital Clínic-IDIBAPS, Barcelona, Spain)*
Plasma lipidomic profiling and adipose tissue kinomics link arachidonic acid derived metabolites to MAP kinase activation in human obesity

16:55-17:20 ***Stefanie Liening*** *(Friedrich-Schiller-University of Jena, Germany)*
Development of a liquid chromatography coupled mass spectrometry analysis of glutathione conjugates of polyunsaturated fatty acids

17:20-17:45 ***Cristina Lopez-Vicario*** *(Hospital Clínic-IDIBAPS, Barcelona, Spain)*
Leukocytes from obese individuals exhibit an impaired SPM biosynthetic signature

17:45 *Adjourn: Workshop closing address by organizers*

(Coffee and beverages for last stayers)



PROFILING THE RESOLUTION METABOLOME: CONSIDERATIONS AND METHODOLOGIES

JESMOND DALLI

William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, EC1M 6BQ UK.

Gaining insights into mechanisms that control immunity is central in the quest to gain insights into the etiopathology of many of the diseases that afflict modern societies. It is now well appreciated that a novel group of autacoids termed as specialized proresolving mediators, which are enzymatically produced from essential fatty acids, orchestrate the immune response promoting the termination of inflammation as well as tissue repair and regeneration. Solid-phase extraction coupled with liquid chromatography tandem mass spectrometry provides a robust and sensitive approach for the identification and quantitation of specialized pro-resolving mediators (lipoxins, resolvins, protectins, and maresins), their pathway markers and the classic eicosanoids. We will review the methodologies employed for the extraction of these mediators from biological systems the procedures followed for their identification and quantitation as well as examples of the utility of the methodology in understanding disease processes.

5-LIPOXYGENASE IN THE FORMATION OF LEUKOTRIENES AND SPM

DIETER STEINHILBER

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

5-Lipoxygenase (5-LO) is involved in the biosynthesis of proinflammatory leukotrienes and of specialized proresolving mediators (SPM). 5-LO expression is upregulated during myeloid cell maturation and during macrophage M1 polarization but is downregulated during the shift into the anti-inflammatory M2-type. Polarization to M2 macrophages also induces 15-LO expression which leads to a concomitant shift in the lipid mediator pattern.

5-LO is a heavily regulated enzyme. Its cellular activity depends on multiple factors such as calcium, phosphatidylcholine-containing lipids, ATP, diacylglycerols and the phosphorylation status of the protein. 5-LO can be phosphorylated by MK2, ERKs which is considered to contribute to its activation whereas phosphorylation by PKA inhibits its activity. In-vitro, additional phosphorylation sites recognized by tyrosine kinases have been identified. Furthermore, the enzyme can shuttle between the cytosol and the nucleus and cell stimulation leads to the translocation of the enzyme from the soluble compartment (cytosolic or nuclear) to the nuclear membrane where it interacts with FLAP which provides arachidonic acid for conversion to 5-HpETE or LTA4. Interestingly, oxygenated fatty acids such as 12- or 15-HETE are poor substrates for purified 5-LO and several reports have shown that FLAP strongly stimulates the conversion of oxygenated fatty acids by 5-LO. The data suggest that cellular leukotriene as well as SPM formation by 5-LO is FLAP-dependent. The lecture gives an overview about the regulation of cellular 5-LO activity and the role of 5-LO in SPM formation.

HOW LIPIDS AND METABOLISM ARE KEY TO HUMAN EVOLUTION AND DISEASE, WITH AN EXAMPLE IN NEURODEGENERATION (ALS)

MICHAEL SPEDDING

Spedding Research Solutions SAS, 6 Rue Ampere, Le Vesinet, France

The IUPHAR/BPS guidetopharmacology.org is a freely available knowledgebase on all drug targets quality-controlled by 90 subcommittees of 860 expert scientists for NC-IUPHAR. However, the explosion of potential molecular targets for drugs has not been accompanied by marked clinical success in the therapies in psychiatric and neurological disorders. In many cases, drug discovery programs underestimate sources of complexity and the number of experimental variables, which are difficult to transpose from lab to lab. For example, recent human evolution differentiated us from other non-human primates by an increase in brain and muscle metabolism to run, which is not taken into account in drug discovery: lipid metabolism is critical. I will show how metabolomic and transcriptomic analysis of 'impossible diseases' such as amyotrophic lateral sclerosis (ALS) and Parkinson's disease can yield new therapeutic targets, based on lipid metabolism, and how animal models and patient samples can be used to find potential new therapies. Thus lipid metabolism can be critical for the process of drug discovery and development, but with differences depending on genetic and environmental impact.

SPHINGOLIPIDS AND THE IMMUNE SYSTEM

HEINFRIED H. RADEKE

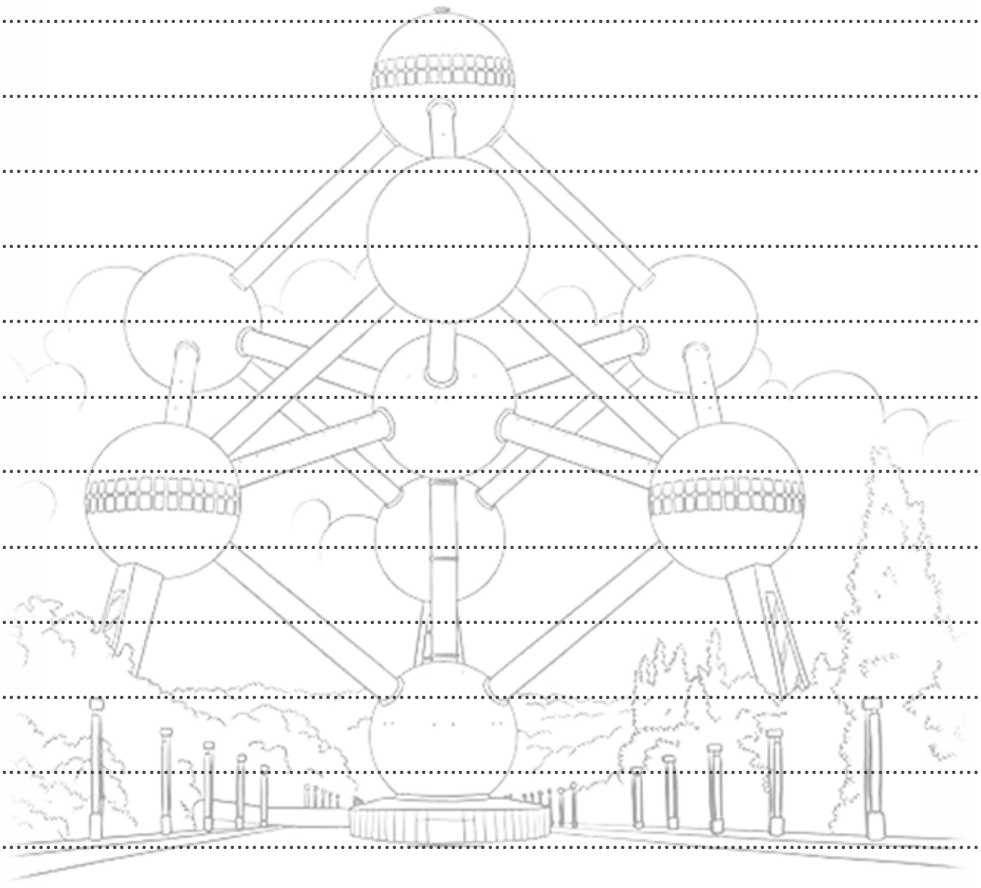
Pharmazentrum frankfurt/ZAFES, Institute of Pharmacology and Toxicology, Hospital of the Goethe University, D-60590 Frankfurt (Main), Germany

Sphingolipids have made their way from basic lipid mediators ascribed to cellular functions like survival, proliferation, and migration to important immune modulators and targets of clinical relevance in autoimmunity and chronic inflammation, e.g. fingolimod, ozanimod, etc., covered by several recent reviews. Currently, their function in carcinogenesis starts to be exploited in first clinical relevant trials.

Initially concepts of therapeutic targets of S1P were predominantly based on just one of the five S1P receptors (S1P1). Meanwhile, more detailed knowledge gathered recently about the other four S1P receptors 2-5 (S1P2-5) unraveled a vastly more complex picture of S1Ps actions and possibly, new therapeutic immune modulatory applications. In this presentation, these newly defined actions will be covered in some detail rather than the "S1P1-dependent lymphocyte sequestering effect". In addition, new findings of immune relevance regarding sphingolipid enzymes and transporters will be included and are briefly addressed.

New concepts of the spatial organization of adaptive immune cells, central memory versus local, tissue resident memory lymphocytes, are arising in the field of immunology and clearly challenging the textbook concepts. Their meaning for the concepts of SphL and S1P immune function and subsequent possible new therapeutic targets will be discussed.

In the end, immune cell type- and differentiation status-dependent more specific functions of S1P via its receptors beyond S1P1 and of the enzymes and transporters of the SphL pathway, vastly extend the scope of therapeutic options towards viral infection, post-ischemic tissue injuries, fibrotic diseases and carcinogenesis.



OPENING PLENARY LECTURE

BIOLOGY OF S1P SIGNALING

TIMOTHY HLA

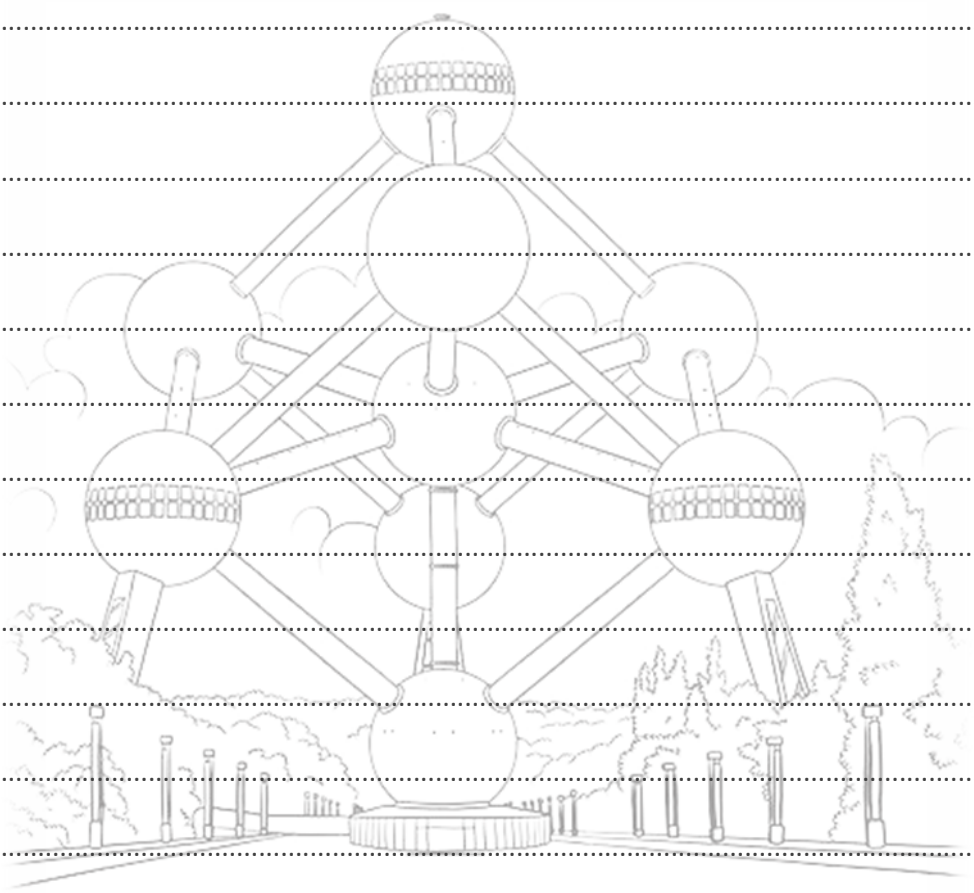
Vascular Biology Program, Boston Children's Hospital, Department of Surgery, Harvard Medical School

Sphingosine 1-phosphate (S1P) is a simple lysophospholipid produced from the metabolism of sphingomyelin. Our laboratory cloned the first S1P receptor as an orphan G protein-coupled receptor (GPCR) from vascular endothelial cells in 1990 and de-orphaned in 1998. We now know that five distinct GPCRs (S1P1-5) that are widely expressed mediate most of the actions of this lysophospholipid mediator. Some of the well-studied actions of S1P include its essential roles in vascular development, immune cell trafficking and neuronal development. Indeed, the first S1P receptor inhibitory drug, Fingolimod, is now approved as an oral medication in the treatment of multiple sclerosis. Novel S1P receptor-based therapeutics are being developed to control additional autoimmune diseases such as ulcerative colitis and psoriasis.

We recently discovered that the majority (~65%) of plasma S1P is chaperoned by HDL-bound Apolipoprotein M. Our recent studies suggest that chaperones impart specific biological functions to S1P.

In the vascular system, S1P works together with angiogenic factors such as VEGF-A to regulate early vascular development. Indeed, RBC release of S1P is essential for embryonic development. S1P receptors 1,2 and 3, which has distinct as well as overlapping signaling pathways, cooperate to regulate vascular development. However, postnatally, S1P receptors in the endothelium regulate vascular homeostasis. Dysregulation of S1P receptors influence vascular disease in mouse models of atherosclerosis, pathologic angiogenesis, etc. HDL-bound S1P acts as a biased agonist to suppress vascular inflammation and restore endothelial function. HDL-S1P-dependent signalling via the endothelial S1P receptor-1 is needed for liver regeneration after partial hepatectomy and suppression of fibrosis after liver injury. Thus, S1P signalling is modulated by chaperones and receptors in both physiological and pathological contexts in a wide variety of organ systems.

The work was funded by grants from the NHLBI, NCI and the Fondation Leducq transatlantic network program



Invited Speaker

THE 5-LIPOXYGENASE PATHWAY IN CANCER

DIETER STEINHILBER

Goethe University Frankfurt, Institute of Pharmaceutical Chemistry, Max-von-Laue-Str. 9, 60438 Frankfurt, 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. Previous studies with 5-LO inhibitors revealed that certain inhibitors also inhibit Wnt signalling and that this does not seem to be due to the interruption of 5-LO–mediated lipid but due to the generation of a catalytically inactive form of 5-LO which prevents nuclear translocation of β-catenin [1]. As a consequence, we found that pharmacologic inhibition of 5-LO interfered strongly with the aberrant stem cell capacity of PML/RAR□-expressing hematopoietic stem cells. Application of lipoxygenin, a direct 5-LO inhibitor, also inhibits hedgehog, TGFβ, BMP and active A signalling suggesting a more general role of 5-LO as regulator of developmental pathways [2].
Another link between cancer development and 5-LO is provided by a genome wide screen for p53 target genes. Interestingly, ChIP-seq analysis revealed that 5-LO is a direct p53 target gene and that 5-LO 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 [3]. These data suggest that 5-LO could be an interesting target for the treatment of certain types of cancer.

1. Roos, J et al. (2014) 5-Lipoxygenase is a candidate target for therapeutic management of stem cell-like cells in acute myeloid leukemia. *Cancer Res* 74, 5244-5255.
2. Brand, S et al. (2018) Combined Proteomic and In Silico Target Identification Reveal a Role for 5-Lipoxygenase in Developmental Signaling Pathways. *Cell Chem Biol*, in press.
3. Gilbert, B et al. (2015) 5-Lipoxygenase is a direct p53 target gene in humans. *Biochim Biophys Acta* 1849, 1003-1016.

Invited Speaker

PROSTAGLANDINS IN CANCER PROGRESSION

Marina Ziche, SANDRA DONNINI

Dept. Life Sciences, University of Siena, 53100 Siena, Italy

Prostaglandins are lipid compounds that mediate many patho-physiological effects, including inflammation. Prostaglandin E2 (PGE2) is the most abundant prostanoid in the human body and the synthesis of PGE2 in inflammatory milieu is driven by cyclooxygenase-2 (COX-2) and microsomal-PGE synthase type 1 (mPGES-1). Both elevated expression of COX-2/mPGES1 signaling and increased PGE2 levels have been associated with many cancers including gastrointestinal, breast, prostate and head and neck cancers. PGE2 exerts its effect by binding to the E series of prostaglandin receptors (EP) which are G-protein coupled receptors (GPCRs). Four EP receptor subtypes, EP1–4, coupled with different intracellular signalling, have been shown to play a role in many malignancies and in cancer metastasis. Further, PGE2/EPs signalling modulates pro-oncogenic and pro-angiogenic pathways such as EGFR, CSF-R1, and FGFR-1 pathways by directly transactivation of these tyrosine kinase receptors or by production of their ligands. For EGFR pathway, we unveiled a complex mechanism by which PGE2/EP3 induces EGF/EGFR signalling activation, EGFR internalization into nucleus, and transcriptional control of gene related to tumor cell proliferation. We will present our data on the role of the COX-2/mPGES-1/PGE2/EPs signalling in promoting the growth and the epithelial-mesenchymal transition (EMT) in tumor and its microenviroment and the autocrine-paracrine activity of EP receptor. We will discuss the role of PGE2 and its network of partner proteins in tumor progression and angiogenesis, and the therapeutic advances in targeting tumorigenesis and angiogenesis.

Selected Oral

STIMULATION AND MODULATION OF INFLAMMATORY MAST CELL RESPONSES BY PROSTAGLANDIN RECEPTORS

YUKIHIKO SUGIMOTO[1,2] Shuh Narumiya[3]

1) Dept. of Pharmaceut. Biochem., Kumamoto University, Japan
2) AMED-CREST, Japan
3) Grad. Sch. of Med. Kyoto University, Japan

Prostaglandins (PGs) play roles in various types of inflammatory diseases by exerting their pro-inflammatory actions. Particularly, PGE2 has been reported to work as one of the pro-inflammatory mediators during the pathological processes in various peripheral tissues. The actions of PGE2 are mediated by four PGE receptor subtypes, EP1, EP2, EP3, and EP4: EP1 receptor is coupled to intracellular Ca^{2+} mobilization, EP2 and EP4 are coupled to stimulation of adenylate cyclase, and EP3 is coupled mainly to inhibition of adenylate cyclase. Both pharmacological and genetic analyses have clarified which EP subtype are involved in each of PGE2 actions (Narumiya S, et al. Physiol. Rev. 79: 1193, 1999; Sugimoto Y et al. J. Biol. Chem. 282: 11613, 2007). For instance, EP3 receptor is involved in inflammation-associated fever generation, and EP1 is involved in thermal hyperalgesia. However, until recently, it was unknown which EP subtypes mediates PGE2-induced inflammatory response, such as enhancement of vasopermeability, edema formation and leukocyte infiltration. In order to clarify these points, we employed arachidonate-induced and PGE2-elicited dermatitis models and examined the effect of each EP gene disruption on this model. Finally, we uncovered that PGE2-EP3 signaling triggers acute inflammatory responses by mast cell activation in the skin (Morimoto K, et al. J. Immunol. 192: 1130, 2014). In addition to the roles of PGE2-EP3 signaling in mast cell activation, we would like to discuss on potential roles of PGI2 in the modulation of inflammatory mast cell responses.

Selected Oral

CONTRACTION INDUCED BY EP1 RECEPTORS ACTIVATION IN RAT DETRUSOR MUSCLE IS COUNTERACTED BY THE NO/CGMP PATHWAY

Wesam Bassiouni[1], Tahia Daabees[1], Xavier Norel[2], AMIRA SENBEL[1,2]

1) Department of Pharmacology & Toxicology, Faculty of Pharmacy, Alexandria University, Egypt
2) INSERM U1148, Laboratory for Vascular Translational Science, X.Bichat Hospital, University Paris XIII, France

Introduction: Sildenafil (PDE5-inhibitor) and alprostadil (PGE1) are used in combination clinically for the management of some cases of erectile dysfunction. Despite the roles of prostaglandins (PG) and NO pathways in contractility of bladder smooth muscle are frequently studied, the effect of sildenafil/alprostadil combination and the potential crosstalk between NO/cGMP and PG pathways on bladder activity is not documented.

Aim: To evaluate the action of some PG-receptor ligands, as well as some NO/cGMP modulators and to evaluate the effect of interaction between alprostadil and sildenafil on rat detrusor muscle contractility.

Methods: Organ bath experiments were performed using isolated rat detrusor muscle. Direct and neurogenic contractions were induced using ACh and electric stimulation (ES, 80V), respectively. A dose/frequency producing almost 50% of the maximal response was selected. The contractile responses in absence and presence of the tested drugs at different concentrations were compared. Results are expressed as mean±SEM (n=5-7).

Results: Alprostadil (0.01-10µM) and iloprost (1-10µM) concentration-dependently potentiated ACh(100µM)- and ES(4Hz)- induced contraction. Maximum potentiation of ACh- contraction in presence of alprostadil and iloprost were 40±5% and 28±7% respectively. In contrast, ONO-AE1-259 (selective EP2-agonist, 1nM-10µM) inhibited muscle contraction. As for the PG-receptor antagonists, SC51322 (EP1-blocker, 10µM) and RO1138452 (IP-blocker, 10µM) inhibited ACh response by 57.1±7% and 49.2±6%, as well as the ES-induced contraction by 53.1±6% and 38.2±5%, respectively. ONO-AE3-237 (DP1-blocker, 1µM) produced no effect.
Sildenafil potentiated ACh-induced contraction at low concentrations (0.01-1µM), but inhibited it at higher ones (10-100µM). IBMX (non-selective PDE-inhibitor, 10nM-100µM) and SNP (NO-donor, 1nM-1mM) produced the same biphasic pattern. The potentiatory phase of sildenafil was inhibited by atropine (0.1µM), L-NAME (non-selective NOS-inhibitor, 100µM), N-PLA (nNOS-inhibitor, 30µM) or MB (GC-inhibitor, 10µM).
In presence of sildenafil (0.1µM), the dose-response curve of alprostadil (0.01-10µM) on both ACh and ES-induced contraction was clearly shifted downward.

Conclusions: EP1-receptors, but not DP1, seem to play an important role in rat bladder contractility. A crosstalk between PGE1 and NO/cGMP pathways may exist. cGMP intracellularly-elevated by sildenafil, may inhibit the activity of PLC and hence the cascade of EP1, thus masking the hyperactivity of bladder caused by alprostadil, which adds to the advantages of this combination.

Selected Oral

SELECTIVE INHIBITION OF PGE2 ENHANCES THE CYTOTOXIC EFFECT OF DOXORUBICIN AND VINCRISTINE IN MULTICELLULAR TUMOR SPHEROIDS.

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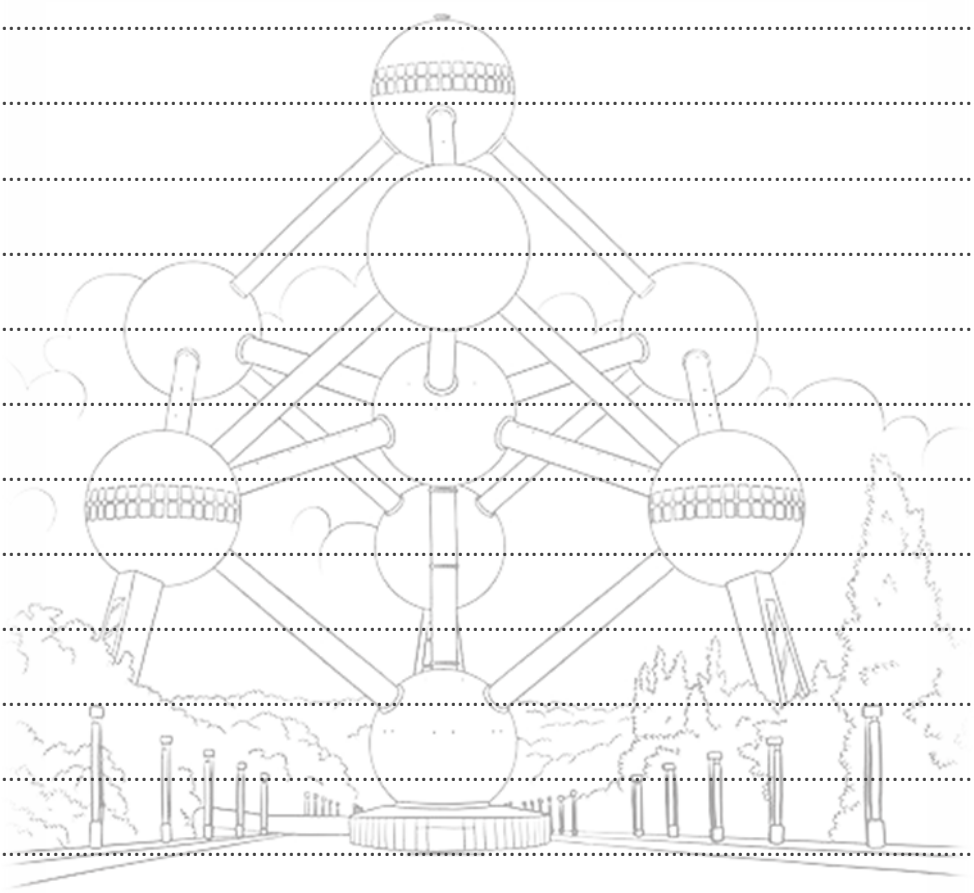
Cancer related inflammation is a complex tumor-promoting interaction between malignant cancer cells and benign stromal cells. We recently reported that the neuroblastoma microenvironment contains infiltrating cancer associated fibroblasts (CAFs) expressing microsomal prostaglandin E synthase-1 (mPGES-1), responsible for prostaglandin E2 (PGE2) synthesis. PGE2 promotes immunosuppression, angiogenesis, tumor growth and therapy resistance. Conventional therapies used in the clinic to treat cancer, i.e. chemotherapy and irradiation, induce apoptosis and cell death, processes that leads to increased inflammation and PGE2 production creating a niche for tumor cell repopulation. We propose that targeting the PGE2 production by CAFs in combination with cytotoxic drugs will abrogate tumor recovery and improve treatment outcome.

To evaluate mPGES-1 inhibition in combination with conventional cancer therapies in vitro we established a multicellular tumor spheroid (MCTS) model, simulating the neuroblastoma microenvironment by co-culturing neuroblastoma cells and fibroblasts. MCTS were treated with doxorubicin or vincristine in combination with small molecule mPGES-1 inhibitors.

The MCTS model successfully recapitulated the neuroblastoma microenvironment where fibroblasts expressed mPGES-1 and several markers for CAFs. Combination treatment of MCTS with mPGES-1 inhibitors CIII and 934 revealed an enhanced cytotoxic effect of the chemotherapeutic drugs doxorubicin and vincristine. The combination treatment also reduced post treatment MCTS recovery.

The conventional therapies used in the clinic today mainly target the cancer cells overlooking the contribution of tumor-promoting inflammation to tumor progression, and therapy resistance. In this study we developed an in vitro model mimicking the neuroblastoma microenvironment opening up for tumor- stroma investigations and screening for stroma-targeting therapies in combination with established therapies.



Invited speaker

FROM FISH TO CANNABIS – MODULATION OF INFLAMMATION BY n-3 PUFAs.

RENGER WITKAMP

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The debate on the importance of n-3 PUFAs for health and disease continues. It is clear that n-3 fatty acids are essential dietary components, and several mechanistic, epidemiological and intervention studies underline their relevance for a wide range of physiological processes and disorders. At the same time, a recent meta-analysis [1] and a Cochrane review [2] clearly questioned their clinical usefulness, at least for prevention of cardiovascular diseases. Several explanations may underlie these apparent discrepancies. For example, it is conceivable that administered amounts, intake and presence of other fatty acids, including n-6 PUFAs, and inter-individual differences, including polymorphisms, sex and age are playing a role.

Many, though not all, biological activities of n-3 PUFAs can be linked to their interaction with one or more immunological processes. However, underlying mechanisms proposed are diverse, ranging from effects on cell membranes, interactions with different receptors (PPARs, FFA 1[GPR40], FFA40[GPR120]), (other) transcription factors and enzymes. Highly intriguing, and at the same time further complicating is the role of the different metabolites of n-3 PUFAs. It is important to realise that n-3 PUFAs and their metabolites exist in dynamic equilibria with different other lipid-derived mediators, including pro-and anti-inflammatory mediators, resolvins etc.

In our group we have a special interest in 'endocannabinoid-like' derivatives of n-3 PUFAs. It has become clear that next to the 'prototypic' endocannabinoid N-arachidonoylethanolamine (anandamide), derived from the n-6 PUFA arachidonic acid, several other fatty acid-amine conjugates exist. These compounds display biological properties that go far beyond interactions with the classical cannabinoid (CB1 and CB2) receptors. By 'replacing' both the fatty acid and amine part of the conjugates, we and others synthesized a number of N-acylamine conjugates of DHA (docosahexaenoic acid, 22:6(n−3)), in particular with ethanolamine (DHEA), glycine (DHAGly), serotonin (DHA-5-HT), and dopamine (DHDA). Meanwhile, the presence in animal and human tissues of (at least) DHEA, DHAGly and DHA-5-HT has been demonstrated. Their formation depends on the relative amounts of DHA in the diet, which is a consequence from a shift in n-3/n-6 balance of membrane lipids. The same principle applies to the local availability of amines. For example, we have shown in pigs, mice and humans that DHA-5-HT is particularly present in the gut, where most of the serotonin resides. We also showed that DHA-5-HT, DHEA and DHDA possess anti-inflammatory properties in vitro. However, much remains to be discovered about their physiological role. Similar to what is known for anandamide, DHA conjugates can be metabolized via oxidative enzymes including cyclooxygenases (COXs), lipoxygenases (LOXs) and cytochrome P450 enzymes, yielding a range of prostaglandin-amides (prostamides) and hydroxy-derivatives. At least some of them appear to have biological activity. Together, these findings illustrate that we are still far away from understanding how n-3 PUFAs modulate inflammatory processes.

1. Aung T., et al., Associations of omega-3 fatty acid supplement use with cardiovascular disease risks: Meta-analysis of 10 trials involving 77 917 individuals. JAMA Cardiology, 2018. 3(3): p. 225-234.

2. Abdelhamid A.S., et al., Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. Cochrane Database of Systematic Reviews, 2018(7).

Invited speaker

ROLE OF PROSTAGLANDINS IN NEURO-IMMUNE REGULATION

JON LAMPA

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During the last decade, advances in neuroscience and immunology established a close link between nervous and immune systems. Moreover, recent data indicate a major contribution of inducible cyclooxygenase (COX-2) and its downstream prostaglandin signaling pathways in modulating neuro-inflammatory responses and neuronal function. For example, prostaglandins (PGs) have been shown to mediate pro-inflammatory cytokine effects over the blood brain barrier (BBB)1, PG receptor EP3 expression is induced in nucleus of the solitary tract (NTS) in response to peripheral inflammatory stimuli. Moreover, PG EP4 is heavily expressed by microglia and is a pivot point in a positive feedback loop, such that it can be either neuroprotective or neuro-damaging.

An important finding in the field of neuro-immune mechanisms was the discovery of the cholinergic anti-inflammatory pathway (CAP) also known as the inflammatory reflex2. The CAP comprises an important neuro-immune link whereby inflammation can be tightly controlled by the autonomic nervous system and the vagus nerve (cranial nerve X). Thus, the vagus nerve has been shown to control innate immune responses and inflammation during pathogen invasion and tissue injury. The discovery of the CAP has qualitatively expanded the understanding of how the nervous system is able to modulate immune responses.

Therapeutic implications of CAP activation have marked the beginning of a novel treatment strategy of passing electrical impulses along the left vagus nerve to alleviate acute and chronic inflammatory conditions. Extensive work has also identified key molecular and cellular involved in the CAP-associated α7 nicotinic acetylcholine receptor (nAChR) mediated control of inflammation.

Importantly, also in the CAP a role of prostaglandins has been implicated. Prostaglandin E2 (PGE2), whose concentration is increased locally following α7 nAChR activation, have been shown important as mediator of this neuro-immune crosstalk. Our group have for long time had a special interest in the role of PGs in neuro-immune communication, with focus on CAP function. We have investigated the importance of PGE2 in the cholinergic regulation of inflammation by performing both in vivo and in vitro studies with mPGES-1 (-/-) mice and human astroglial cells treated with VNS and nicotine respectively. We observed that inducible PGE2 production is crucial for VNS mediated splenic cholinergic synthesis and immunosuppressive effects during endotoxemia. These observations were applicable only to the splenic portion of the CAP, since VNS-induced norepinephrine release from the splenic nerve was not dependent on PGE2, nor was the expression of 2-adrenergic receptors on effector T lymphocytes3. Interestingly, hypothalamic mPGES-1 was found to be elevated following VNS and potentially involved in the cholinergic suppression of reactive astrogliosis and neuro-inflammatory conditions. Taken together, PGs are pivotal mediators in many CNS processes, and our finding of an important role of PGE2 in cholinergic immune regulation is in line with several other reports of PG importance in neuro-immune mechanisms, with potential therapeutic implications in chronic inflammatory diseases characterized by CNS mediated symptoms such as pain and fatigue.

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2. Tracey KJ. Nature. 2002;420(6917):853-9.
3. Revathikumar P et al, PLoS One, 2018 Feb 22;13(2):e0193210.

Selected oral

REGULATION OF INFLAMMATORY PROSTAGLANDIN SYNTHESIS BY ACYL-COA SYNTHETASE ACSL4

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Acyl coenzyme A synthetase long-chain family members (ACSLs) are a family of enzymes that convert long-chain free fatty acids into their acyl-CoAs. Among ACSL isozymes, ACSL4 prefers polyunsaturated fatty acid including arachidonic acid as a substrate and is considered to be important for removing excess free arachidonic acid liberated from membrane phospholipids following stimulation. In the present study, to clarify the role of ACSL4 in inflammatory reactions, we investigated the phenotypes of ACSL4-deficient mice. Bone marrow cells were prepared from wild-type or ACSL4-deficient mice, and then cultured with M-CSF to obtain bone marrow-derived macrophages (BMDMs). Electrospray ionization mass spectrometry of cellular phospholipids showed that the levels of phosphatidylcholine species bearing polyunsaturated fatty acid such as arachidonic acid, adrenic acid and docosahexaenoic acid were significantly lower in ACSL4-deficient BMDMs than those in wild-type BMDMs. We further found that gene deletion of ACSL4 markedly enhanced the lipopolysaccharide-induced release of arachidonic acid and its metabolites including prostaglandin (PG) E2, PGD2, and PGF2alpha from BMDMs. On the other hand, ACSL4 deficiency did not affect the cytokine release and pinocytosis of BMDMs. These results suggested that ACSL4 might function as one of regulators of inflammatory PG productions and play an important role in inflammatory reactions.

Selected oral

ALOX15-DEFICIENCY – A TWO-EDGED SWORD IN COLITIS

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Lipoxygenases (ALOX) are involved in the regulation of cellular redox homeostasis and in the biosynthesis of pro- and anti-inflammatory lipid mediators. They thus play a role in the pathogenesis of inflammatory diseases, such as the chronic inflammatory bowel diseases, Crohn's disease and Ulcerative colitis.

To explore the pathophysiological role of Alox15 (leukocyte-type 12-LOX) in mouse experimental colitis we tested the impact of systemic inactivation of the Alox15 gene on the extent of dextrane sulfate sodium (DSS) colitis. We found that in wildtype mice expression of the Alox15 gene was augmented during DSS colitis while expression of other Alox genes (Alox5, Alox15b) was hardly altered. Systemic Alox15 (leukocyte-type 12-LOX) deficiency induced less severe colitis symptoms and suppressed in vivo formation of 12-hydroxyeicosatetraenoic acid (12-HETE), the major Alox15 (leukocyte-type 12-LOX) product in mice. These alterations were paralleled by reduced expression of pro-inflammatory gene products, by sustained expression of the zonula occludens protein 1 (ZO-1) and by a less impaired intestinal epithelial barrier function. Indeed addition of 12S-HETE compromised enantioselectively transepithelial electric resistance in vitro incubations of colon epithelial cells. Consistent with these data transgenic overexpression of human ALOX15 intensified the inflammatory symptoms.

As previous studies indicate that omega-3 polyunsaturated fatty acids (PUFA) can alleviate colitis, we also employed Alox15-deficient mice to explore the role of systemic inactivation of the Alox15 gene in acute DSS- and TNBS-induced colitis in combination with the well-established fat-1 mouse as model system for a high omega-3 PUFA tissue status. While 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. Oxylipin analyses performed in colon tissues from these animals demonstrate a lack of 15-HEPE formation in fat-1 mice with Alox15 deficiency, which might explain the abrogation of the protective effect of elevated omega-3 fatty acids in these mice.

Selected oral

CEREBROSPINAL FLUID PRO-RESOLVING MEDIATORS CORRELATE WITH DISEASE SEVERITY AND OUTCOME IN ADULTS WITH TUBERCULOSIS MENINGITIS

Romain A. COLAS[3*], Guy E. Thwaites[1,2*], Nguyen Thuy Thuong Thuong[2], Lucy Ly[3], Nguyen Hoan Phu[2], Lalita Ramakrishnan[4], Nguyen Thi Hoang Mai[2] and Jesmond Dalli[3]

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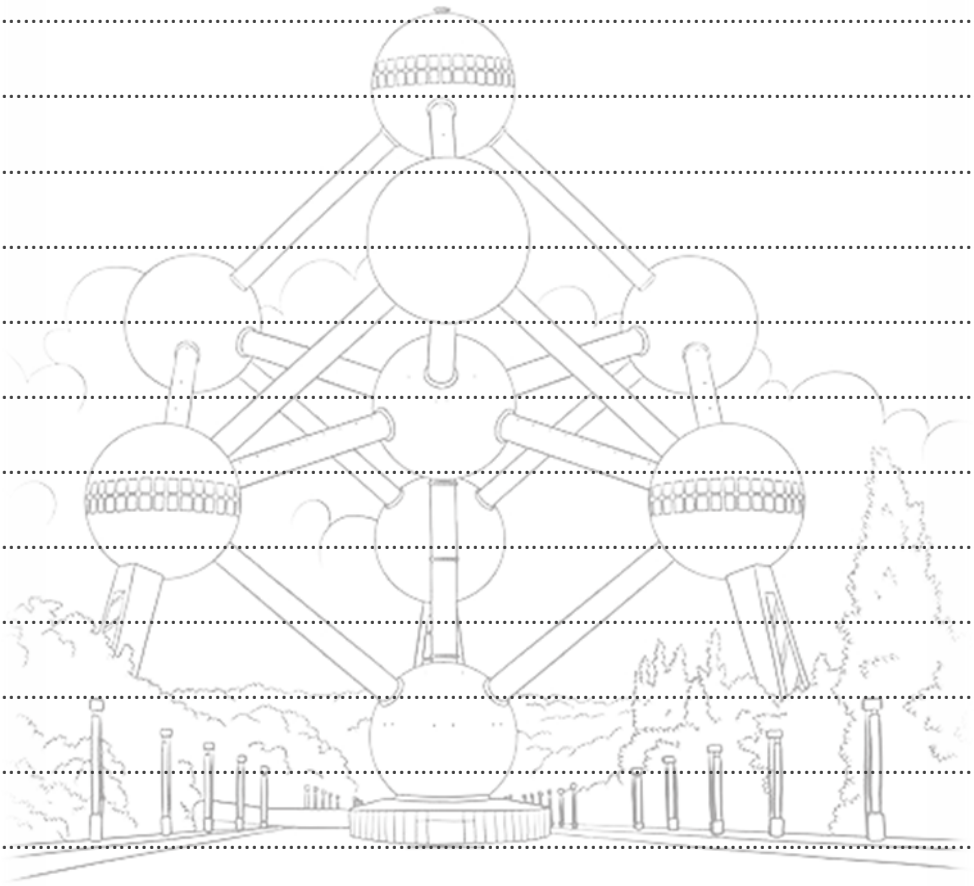
3) William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, EC1M 6BQ UK.
4) Molecular Immunity Unit, Department of Medicine, MRC Laboratory of Molecular Biology, University of Cambridge, Cambridge, United Kingdom.

Infections of the brain and the meninges by Mycobacterium tuberculosis lead to severe inflammation and are associated with poor outcomes. Tuberculosis meningitis (TBM) is the most lethal form of tuberculosis infection. It is characterized by a dysregulated immune response frequently leading to neurological injury and death despite the best available treatment. However, the mechanisms driving the inflammatory response in TBM are not well understood. To gain insights into these mechanisms, we used a targeted lipid mediator profiling approach. This allowed us to investigate the regulation of a novel group of host protective mediators, termed specialized pro-resolving mediators (SPM), in the cerebrospinal fluid (CSF) of adults with TBM enrolled into a randomised placebo-controlled trial of adjunctive aspirin. We found distinct lipid mediator profiles as the disease severity increased. These changes were linked with an upregulation of inflammatory eicosanoids in patients with severe TBM. Leukotriene A4 hydrolase (LTA4H) single nucleotide polymorphisms (SNPs) have been implicated in both the pathophysiology and outcomes of TBM. Here, this mutation regulated CSF lipid mediator production, with 15-lipoxygenase (LOX) and 12-LOX-derived SPM significantly different in CT/TT versus CC genotype patients. CSF lipid mediator profiles also correlated with outcome, where a number of SPM pathways, including the DHA-derived Maresin Conjugates in Tissue Regeneration (MCTRs), were downregulated in fatal disease compared to survivors. Furthermore, early reductions in brain infarctions and deaths observed in aspirin-treated patients were associated with increased CSF concentrations of pro-resolving DHA-derived protectins and maresins.
Together these findings indicate that the regulation of inflammatory and pro-resolving lipid mediators is important in TBM pathogenesis and outcome. Using therapeutic, such as aspirin, to manipulate SPM expression, offers novel treatment strategies to reduce the substantial morbidity and mortality caused by TBM and possibly other forms of tuberculosis.



Invited speaker

TARGETING THE PROSTACYCLIN PATHWAY: BEYOND PULMONARY ARTERIAL HYPERTENSION

JEAN-LUC CRACOWSKI

Unité de pharmacologie Clinique Inserm CIC1406 Laboratoire HP2 ; Inserm U1042 UFR de médecine-Domaine de la merci, 38700 La Tronche- France

Pioneering work demonstrated that an unstable substance isolated from rabbit and pig aortas could relax arterial smooth muscle and inhibit platelet aggregation. Since then, prostacyclin (prostaglandin I2, PGI2) and its analogs have raised much pharmacological interest. In this presentation we will detail how the PGI2 signaling pathway is much more complex than was initially anticipated, involving peroxisome proliferator-activated receptors (PPARs), prostaglandin transporters (PGTs), and PGI2–thromboxane A2 (TXA2) receptor (IP TP) heterodimerization. We will discuss the distinct affinities of PGI2 analogs for prostanoid receptors. In addition, we will introduce the new direct and indirect pharmacological approaches to targeting the PGI2 pathway within the systemic circulation, including non-prostanoid agonists of the prostacyclin receptor and transporters, as well as transcutaneous pathways using iontophoresis and nanostructured lipid carriers.

Invited speaker

COX-2 AND PROSTANOID PATHWAYS IN THE CARDIORENAL SYSTEM: MECHANISMS AND BIOMARKERS

JANE A. MITCHELL

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Cyclooxygenase (COX) is the first enzyme in the conversion of arachidonic acid to prostanoids. Prostanoids are a group of essential lipid mediators produced throughout the body. COX is present in two isoforms. COX-1 is expressed constitutively in all cells and thought to regulate homeostatic processes. COX-2 on the other hand is induced at the site of inflammation and in cancer where it drives disease and where it is the therapeutic target of nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs include common over the counter medications such as ibuprofen as well as prescription drugs such as celecoxib. However, COX-2 is also present constitutively in discrete regions of the body including the kidney where it is expressed in renal vascular cells and interstitial fibroblasts. Amongst other functions, constitutively expressed COX-2 protects the cardio-renal system. NSAIDs are effective in treating pain and inflammation and can prevent cancer but as a consequence of inhibition of constitutively expressed COX-2, they are associated with cardiovascular and renal side effects. These side effects limit NSAIDs use and have resulted in the withdrawal of celecoxib as a preventative therapy for colon cancer in Europe. The mechanisms underlying the cardio-renal side effects of NSAIDs are incompletely understood but are thought to involve the blockade of COX-2 derived prostacyclin. This lecture gives an overview of the COX-2 pathway, the constitutive and inducible nature of the enzyme and an insight into novel mechanisms and biomarkers associated with the cardio-renal protective properties of COX-2. For further details see the recent review by Mitchell and Kirkby[1].

1. Mitchell JA and Kirkby NS. Eicosanoids, prostacyclin and cyclooxygenase in the cardiovascular system. Br J Pharmacol. 2018.

Selected oral

LYSOPHOSPHATIDIC ACID INDUCES STRONG VASOCONSTRICTION IN THE CORONARIES VIA MULTIPLE SIGNALLING PATHWAYS

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4) Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee, USA

Background: Lysophosphatidic acid (LPA) is known to act on 6 documented G protein-coupled receptors (LPA1-6). It has diverse effects in the cardiovascular system including its major influence on vascular tone. Although several unsaturated LPA species are released in acute coronary syndrome, the effects of LPA on coronary vascular tone remain to be elucidated. Our aim was to describe the effects of various unsaturated LPA species (18:1, 18:2 and 18:3 LPA) on the coronary flow (CF) of isolated murine hearts and to identify the signalling pathways mediating the effect.

Results: RT-PCR analysis of LPA1-6 mRNA abundance in segments of mouse coronaries verified the expression of each LPA receptor in the vessels. Administration of 18:1, 18:2 and 18:3 LPA (10-6 M) to the perfusion line of Langendorff perfused hearts of wild type male mice (WT) caused a substantial CF reduction (up to 35%) which resulted in the drop of left ventricular developed pressure. This effect of LPA also developed in LPA1 and LPA2 deficient mice and in the presence of LPA3 antagonists (Ki16425 10^{-5} M and VPC32183 10^{-6} M). However, administration of the LPA4 antagonist BrP-LPA (10^{-8} M) abolished the effect. In mouse experimental models used for the identification of intracellular signalling pathways, the smooth muscle specific deletion of G(α)q/11 did not influence, whereas smooth muscle specific deletion of G(α)12/13 diminished by 50%, and Rho-kinase (RhoK) inhibition (Y27632 10^{-6} M) abolished the effect of LPA on CF. Moreover, deletion of endothelial NO synthase enhanced, whereas inhibition of endothelin A receptor by BQ123 diminished the response of coronaries to LPA.

Conclusion: Unsaturated 18-LPA species are strong vasoconstrictors in the coronaries. LPA4 receptor related pathways and endothelial vasoactive substances are key factors in their action. The G(α)12/13 protein - RhoK signaling pathway plays a major role in the response of the vascular smooth muscle. This process might have relevance in acute coronary events when a large amount of unsaturated LPA is released in the coronary system due to platelet activation and plaque rupture.

Funding: OTKA K125174 and K112964

Selected oral

TRANSCRIPTOMIC AND LIPIDOMIC PROFILING OF HUMAN HEART VALVES REVEAL A SPECIFIC PROSTAGLANDIN E2 SIGNATURE IN CALCIFIC AORTIC STENOSIS

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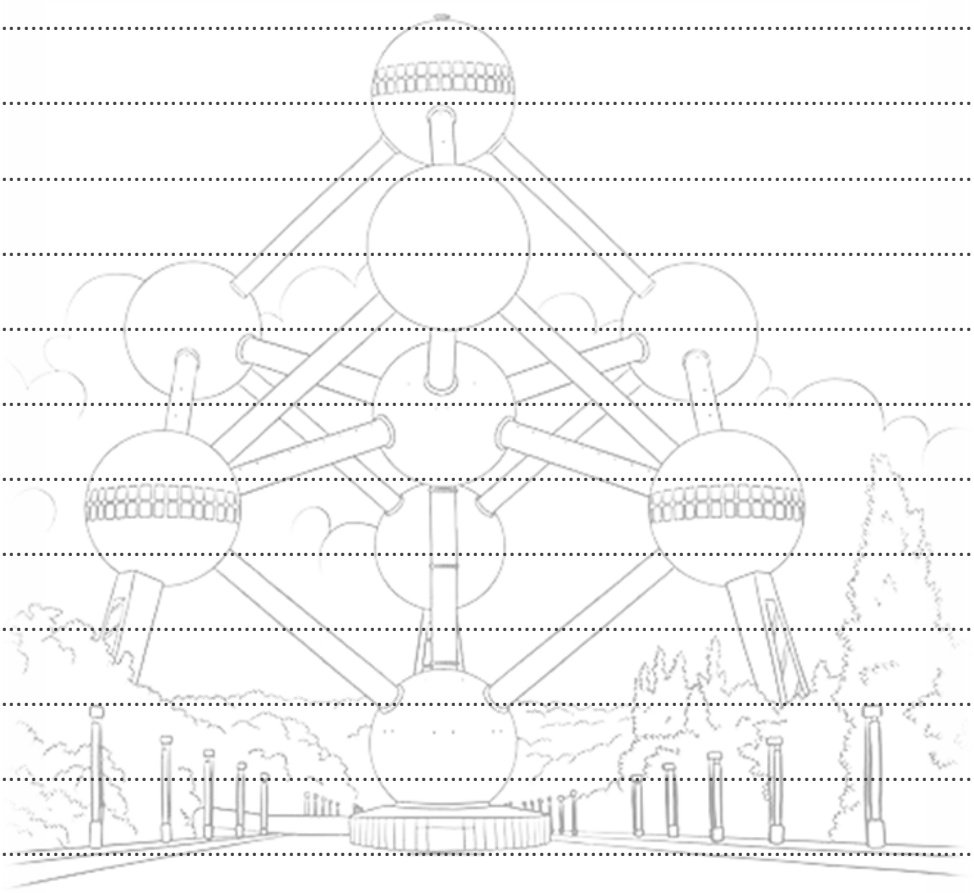
Aortic stenosis (AS) is the most common valvular heart disease in the developed world, affecting about 3% of elderly people. Inflammation and cellular calcification cause a progressive narrowing of the aortic valve, leading to heart failure and excess mortality. To date there is no treatment for AS but replacement with prosthetic valves.

We have previously shown that pro-inflammatory leukotriene mediators are associated with the severity of AS. The role of prostanoid lipid mediators in the pathogenesis of AS is, however, unclear.

Human aortic valves were collected during cardiac surgery, and tissues were macroscopically characterized as healthy, thickened, or calcified. These three types of tissues were separated and used for RNA extraction and analysis by Affymetrix HTA Array, immunohistochemistry, prostanoid analysis by liquid chromatography and tandem mass spectrometry (LC-MS/MS), and for isolation of primary cultures of valvular interstitial cells (VIC).

Expression analysis revealed that both cyclooxygenase-2 (COX-2) and microsomal prostaglandin E2 (PGE2) synthase 1 (MPGES1) mRNA levels were significantly downregulated in the calcified regions of stenotic valves compared to healthy or thickened tissue. This regulation was reflected on the metabolite level with significantly lower levels of PGE2 secreted by calcified vs. healthy or thickened valve tissue. Immunohistochemistry revealed COX-2 expression in VIC. Furthermore, we showed an induction of the PGE2 pathway in isolated VIC in response to pro-inflammatory stimuli and confirmed PGE2 production in functional in vitro experiments, which was inhibited by treatment with COX-2 or MPGES1 inhibitors.

Taken together, these results evoke the COX2-MPGE1-PGE2 pathway in aortic stenosis, being inducible in VIC but down-regulated with increasing aortic valve calcification.



PLENARY LECTURE

ISOPROSTANES, PHYTOPROSTANES AND NEUROPROSTANES: BIOMARKERS AND BIOACTIVE LIPIDS

Thierry DURAND

Institut des Biomolécules Max Mousseron UMR 5247 - CNRS, UM, ENSCM, 15 av Charles Flahault - BP 14491

Cyclic oxygenated metabolites, commonly known as isoprostanes (IsoP) are formed in vivo through non-enzymatic free radical reaction of n-6 and n-3 polyunsaturated fatty acids (PUFA) such as arachidonic acid (AA, C20:4 n-6) and eicosapentaenoic acid (EPA, C20:5 n-3). α-linolenic acid (ALA, C18:3 n-3) produced phytoprostanes (PhytoP), and docosahexaenoic acid (DHA, C22:6 n-3) led to neuroprostanes (NeuroP).[1-4] Evidences have emerged for their use as biomarkers of oxidative stress and more recently as bioactive lipids acting at molecular level as secondary messengers; the latter ones are mostly related to n-3 PUFAs.[5,6] Collectively, the existence of these metabolites are not limited to mammalian specimens, they are found as well in our food such as nuts and seeds depending on the type of PUFA.[7, 8] This lecture will focus on IsoP, PhytoP and NeuroP generated from AA, EPA, AdA, ALA, and DHA, respectively, and precisely their role in human, animals, plants, nuts, seeds, oils or macroalgae.

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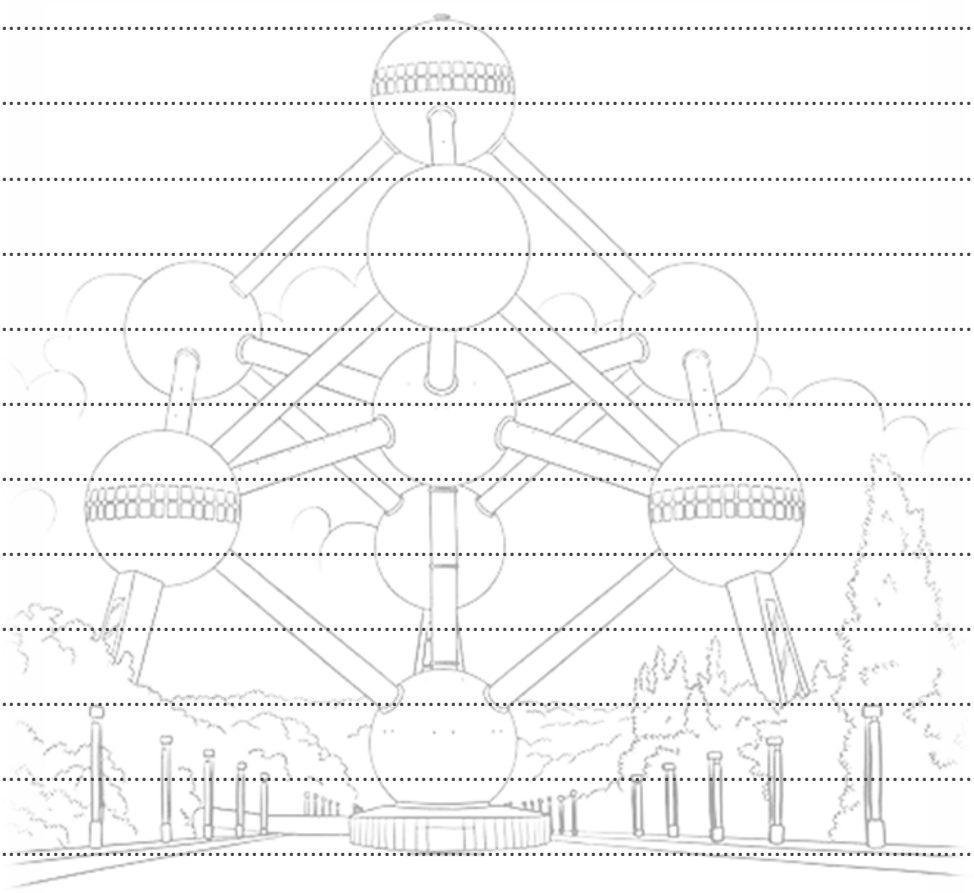

IMPAIRMENT OF DHA SYNTHESIS ALTERS THE EXPRESSION OF NEURONAL PLASTICITY MARKERS AND THE BRAIN INFLAMMATORY STATUS IN MICE

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Docosahexaenoic acid (DHA) is an ω-3 fatty acid obtained from the diet through the action of elongases (ELOVLs) and desaturases. DHA is a key central nervous system constituent and the precursor of several molecules that regulate resolution of inflammation. In the present study, we questioned whether impaired synthesis of DHA affected neural plasticity and inflammatory status and in adult murine brain. To address this question, we investigated neural and inflammatory markers from mice deficient for ELOVL2 (Elovl2$^{-/-}$), a key enzyme in DHA synthesis. From our findings, Elovl2$^{-/-}$ mice showed an altered expression of genes involved in synaptic plasticity, learning and memory formation such as Egr-1, Arc1 and BDNF noticeable in the cerebral cortex, but not in the whole brain. In parallel we also found that deficiency in DHA increased the expression of pro-inflammatory molecules (TNF-α, IL-1β, iNOS) as well as of Iba1, a marker of microglia. Interestingly we observed that neuroinflammation and neuroplasticity alterations observed in Elovl2$^{-/-}$ brains were reversed by DHA-supplementation.
Hence, impairment of systemic DHA synthesis and subsequent dietary supplementation, can modify the brain inflammatory and the neural plasticity status of mice deficient for Elovl2, supporting the view that DHA is a potent immuno-modulator and essential fatty acid in central nervous system.

THE SECRETED PRO- AND ANTI-INFLAMMATORY LIPIDOMES OF M1 AND M2 MACROPHAGES

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Introduction: Macrophages are a diverse set of innate immune cells with the important functions of immune surveillance and tissue homeostasis. M1 macrophages, which are classically activated by external stimuli like lipopolysaccharide (LPS), have a pro-inflammatory phenotype and are the first line of defense against infections. In contrast, M2 macrophages are alternatively activated by interleukin 4 (IL-4) and produce anti-inflammatory factors. While cytokine response is well-established, it is not known whether there is a lipidomic signature of M1 and M2 macrophages. Here, we profiled the entire glycerophospholipidome to investigate the link between glycerophospholipid (GPL) metabolism and inflammation. We furthermore aimed to establish whether this lipidomic signature was secreted and could be used as a marker of inflammation in animal models.

Methods: Mouse bone-marrow-derived macrophages were polarized by culturing them with either LPS or IL-4. We extracted lipids from both cells and cell media and used targeted liquid-chromatography mass-spectrometry with multiple reaction monitoring (MRM) to profile and quantify GPL species. Lipids in plasma obtained from a mouse model of acute infection were also extracted and analyzed to determine whether a circulating lipidomic signature of inflammation could be established.

Results: We found that M1 and M2 cells had strikingly different GPL profiles. For example, abundances of both lysophosphatidylcholine (LPC) and platelet activating factors (PAFs), the pro-inflammatory metabolites of alkyl-acyl-glycerophosphocholines, were significantly increased in M1 while being significantly decreased in M2 macrophages. PAFs are powerful lipid mediators of inflammation which can elicit various biological effects such as platelet activation and airway constriction. Network analysis further indicated that Lands cycle hydrolysis of glycerophosphatidylcholines is enhanced in M1 macrophages leading to elevated PAF and LPC levels in M1 cells. Principal component analysis of the glycerophospholipidome showed two distinct clusters which perfectly mirrored the M1 and M2 phenotypes. The important features identified in the PCA were lipids which were secreted.

Conclusion: We have demonstrated a distinct link between GPL metabolism in macrophages and inflammation. We are assessing whether these pro- and anti-inflammatory lipidomic signatures are present in the circulating lipidome and can be used as markers of inflammation in animal models and in patients.

INVESTIGATION OF PGE2-MMP CROSS-TALK IN HUMAN VASCULAR PREPARATIONS, THEIR ATTACHED PVAT AND PLASMA IN OBESE AND NON-OBESE PATIENTS

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Background: Perivascular adipose tissue (PVAT) which surrounds blood vessels plays an important role in the regulation of vascular functions however it becomes dysfunctional in inflammatory diseases such as obesity. In several inflammatory diseases, the release of prostaglandin E2 (PGE2) is increased via the activity of inducible microsomal PGE synthase-1 (mPGES-1). On the other hand, matrix metalloproteinase (MMP) are also involved in inflammatory processes including obesity and graft failure of coronary artery (CA) bypass grafts [internal mammary artery (IMA), saphenous vein (SV)]. Our aim was to investigate whether MMPs and their endogenous inhibitor (TIMPs) may be regulated by PGE2 under inflammatory conditions in vitro conditions by using human vessels (bypass grafts and CA) and PVAT as well as in vivo conditions by using human plasma.

Methods: MMP-1,-2 and TIMP-1,-2 densities were measured in human plasma (n=57) as well as supernatants of human vessels (IMA n=16, SV n=14, CA n=13) and their PVAT. The effects of inflammation (24-hour incubation, IL-1β and LPS) and mPGES-1 inhibitor (Compound III, 10 μM) on MMPs regulation were evaluated. The correlations between PGE2 and several parameters were calculated in plasma of obese and non-obese patients.

Results: The vascular wall and PVAT from SV exhibited the greatest MMP-1,-2 release. An increase of MMP-1,-2 and/or a decrease of TIMP-1 quantities have been detected under inflammatory condition only in vascular wall not in PVAT. These changes under inflammation were completely reversed by inhibition of mPGES-1. In obese women, C-reactive-protein (CRP), biomarker of inflammation, PGE2 and MMP-1 levels were increased. PGE2 contents were positively correlated with some anthropometric parameters including body-mass-index (BMI) and plasmatic CRP as well as MMP-1 density in women.

Conclusions: Under inflammation, the greater mPGES-1 and PGE2 levels leads to enhanced MMP activity in vitro studies by using human vascular walls. The positive association between PGE2 and MMP-1 has been also observed in vivo studies by using plasma of women with or without obesity. The greater MMP activity observed in SV may contribute to the increased prevalence of graft failure. We suggest that mPGES-1 inhibitors could prevent graft failure and obesity-related vascular remodelling mostly in women.

INHIBITION OF SPHINGOSINE-1-PHOSPATE GENERATION REDUCES BLOOD PRESSURE LEVELS BY ATTENUATING INFLAMMATION IN AN EXPERIMENTAL MODEL OF HYPERTENSION

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Background: The immune system plays a considerable role in hypertension. Particularly, T-lymphocytes are recognized as important players in its pathogenesis. Despite substantial experimental efforts the molecular mechanisms underlying the nature of T-cell activation contributing to an onset of hypertension or disease perpetuation are still elusive. Amongst other cell types, lymphocytes express distinct profiles of G-protein–coupled receptors for sphingosine-1-phosphate (S1P) – a bioactive phospholipid that is involved in many critical cell processes and most importantly, majorly regulates T-cell development, lymphocyte recirculation, tissue homing patterns, and chemotactic responses. Recent findings revealed a key role for S1P chemotaxis and T-cell mobilization for the onset of experimental hypertension, and elevated circulating S1P levels have been linked to several inflammation-associated diseases including hypertension in patients.

Objective: Our earlier work shows that blocking S1P generation attenuates the increase of blood pressure (BP) and that the inhibition of sphingosine kinase 2 (SPHK2) prevents inflammation typical of hypertension. Here, we investigate the potential therapeutic effects of pharmacological SPHK2 inhibition on BP levels, T cell activation and subtype differentiation in a murine model of hypertension.

Materials and Methods: In an Angiotensin-II murine model of hypertension, we assess the potential therapeutic effect of specific SPHK2 inhibition on (1) BP levels using tail cuff plethysmography, (2) immune cell populations using flow cytometry and (3) S1P levels using mass spectrometry.

Results: Treatment with the SPHK2 inhibitor K145 significantly lowered BP levels, which was accompanied by a decrease in the number of circulating T cells. Remarkably, our data reveal a change in T cell phenotype with reduction of both TH17 and TH1 cells after K145 treatment. This might be causative to the attenuated vascular inflammation and endothelial activation we observe in this group.

Conclusion: Our results point to a critical contribution of SPHK2-S1P signaling in immune-cell responses and vascular inflammation during AngII-induced HTN in mice and reveal potential therapeutic properties of SPHK2 inhibition. Thus, the inhibition of S1P production by antagonizing SPHK2 activity might evolve as new therapeutic strategy to efficiently controlling BP, hypertension-related inflammation and associated target organ damage.

THE NAAA INHIBITOR AM9053 ATTENUATES EXPERIMENTAL COLON CARCINOGENESIS

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Introduction: N-acylethanolamine-hydrolyzing acid amidase (NAAA) is a lysosomal enzyme highly expressed in macrophages, and peripheral tissues, including the small and large intestine, which is responsible of the hydrolysis of palmitoylethanolamide (PEA), an endogenous lipid mediator chemically-related to the endocannabinoid anandamide [Tsuboi et al. 2007, Alhouayek et al. 2015]. NAAA inhibition attenuates intestinal inflammation, a risk factor for colorectal cancer (CRC) [Alhouayek et al. 2015]. Here, we have evaluated the effect of NAAA inhibition on colon carcinogenesis.

Methods: The effect of the NAAA inhibitor AM9053 was evaluated on cell growth of human CRC and immortalized healthy colonic epithelial (HCEC) cells by using the BrdU incorporation as well as in two murine models (i.e. azoxymethane-induced and xenograft) of colon carcinogenesis. NAAA mRNA expression was evaluated by qPCR in: i) colonic samples from CRC patients; ii) different human CRC cell lines, including Caco2 and HCT116 treated or not with secretome collected from xenograft tumors.

Results: A trend toward a reduction in NAAA expression was observed in clinically-diagnosed CRC as well as in Caco-2 cells incubated with tumor secretome. AM9053 inhibited proliferation of HCT116 and Caco-2, but not of healthy colonic cells. Furthermore, AM9053 reduced the number of tumors induced by azoxymethane (AOM) and xenograft tumor growth in vivo.

Conclusions: Our results suggest that pharmacological inhibition of NAAA counteracts colon carcinogenesis.

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STRUCTURE AND REGULATION OF ARACHIDONATE 11R-LIPOXYGENASE

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Lipoxygenases (LOXs) are a diverse family of peripheral membrane proteins that catalyze the formation of various lipid mediators. While many LOXs are stimulated by Ca(2+)-induced membrane association, the arachidonate 11R-LOX from the Arctic coral Gersemia fruticosa is characterized by strict requirement for Ca(2+) and lipid membranes for any catalytic activity.

We determined the crystal structure of recombinant 11R-LOX in the absence of Ca(2+). The loops that compose the putative Ca(2+)-binding sites in the regulatory PLAT domain are not aligned as in known PLAT:Ca(2+) complexes, indicating notable conformational changes upon Ca(2+) binding. The active site in the catalytic domain is completely enclosed as the putative portal is blocked by a "broken" alpha2 helix, meaning that here, too, conformational changes are necessary. Conserved interactions were found between the PLAT domain in the vicinity of Ca(2+)-binding sites and the N-terminal side of the alpha2 region in the catalytic domain, that are crucial for proper enzymatic activity and also for structural stability.

Since 11R-LOX is a dimer in solution, we also investigated the quaternary structure of the protein by small-angle X-ray scattering, chemical cross-linking, and mutagenesis experiments. In the determined dimer assembly, the catalytic domains associate by their PDZ-like subdomains and the C-terminal sides of the alpha2 regions. In sum, the results suggest that the closed conformation of alpha2 may be imposed by stabilizing interactions from both interdomain connections on one side, and protein dimerization on the other.

Human ALOX5 is the key enzyme in the production of leukotrienes and like 11R-LOX, it is induced by Ca(2+) and features an analogous "broken" alpha2 helix. These similarities suggest that the regulatory mechanisms of the two enzymes are likely comparable. ALOX12 and ALOX15 also participate in inflammatory processes in humans. Strikingly, both can dimerize, and the interfaces of their assemblies have been narrowed down to the same region of the protein surface as in 11R-LOX. While human LOXs tend to be relatively unstable and difficult to handle, 11R-LOX is a remarkably stable enzyme facilitating extensive structural studies. As a result, 11R-LOX makes an exceptional source of information regarding the dynamic structure of LOXs.

13-Series Resolvins Mediate The Leukocyte-Platelet Actions Of Atorvastatin And Pravastatin In Inflammatory Arthritis

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Rheumatoid arthritis is characterised by excessive joint inflammation, and is associated with increased cardiovascular risk. Statins are therapeutics for patients with cardiovascular disease and exert beneficial actions in rheumatoid arthritis through unknown mechanism(s). We recently found that atorvastatin upregulates the biosynthesis of a novel family of pro-resolving mediator termed 13-series resolvins (RvT). These mediators are host-protective and promote resolution of inflammation by regulating leukocyte responses and counter-regulating pro-inflammatory mediators. In the present study, we questioned whether RvT mediated the joint protective actions of three clinically relevant statins: atorvastatin, pravastatin and simvastatin.

Male C57BL/6 mice (11 weeks old) were administered arthritogenic K/BxN serum (100 microlitres, i.p.) on days 0 and 2, then on days 3, 5 and 7 given i.v. injections of atorvastatin, pravastatin, simvastatin at 0.2mg/kg each, or vehicle ($DPBS^{-/-}$ containing 0.05% ethanol). Clinical scores were recorded daily as previously described. Blood and paws were collected on day 8. In selected experiments, mice were given 10mg/kg celecoxib 1hr prior to statin injections. Lipid mediator profiling of paws was carried out using LC-MS/MS. Flow cytometry was used to analyse blood and joint leukocytes. Bone damage was assessed by H&E staining,

Administration of atorvastatin or pravastatin to mice during inflammatory arthritis upregulated total RvT in paws by 45% and 12% respectively (vs. vehicle using column statistics), and significantly reduced joint disease ($p < 0.01$ vs. vehicle using 2-way ANOVA). Administration of simvastatin did not significantly upregulate RvT or reduce joint inflammation. Atorvastatin and pravastatin each reduced systemic leukocyte activation, including platelet-monocyte aggregates (by ~25-60%). These statins decreased neutrophil trafficking to the joint by ~45-75%, as well as joint monocyte (~60-65%) and macrophage numbers (~75-83%). Atorvastatin and pravastatin produced reductions (~30-50%) in expression of CD11b and MHC-II on both monocytes and monocyte-derived macrophages in joints. Administration of an inhibitor to cyclooxygenase-2, the initiating enzyme in the RvT pathway, reversed the protective actions of these statins on both joint and systemic inflammation. Ordinary 1-way ANOVA vs. vehicle was carried out for all presented quantitative results unless otherwise stated, where $p < 0.05$.

These results suggest a role for atorvastatin and pravastatin-driven RvT production in reducing local and vascular inflammation.

ROLE OF PALMITOYLETHANOLAMIDE IN A MOUSE MODEL OF AUTISM SPECTRUM DISORDER: COUNTERACTING CENTRAL AND PERIPHERAL INFLAMMATION

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Lipid signals play an important role in modulating behaviour, metabolism, pain and inflammation. Among endogenous bioactive lipids, palmitoylethanolamide (PEA) has been extensively studied for its anti-inflammatory effects both at central and peripheral level. Moreover, its neuroprotective effects in different neurological diseases has been recently investigated. Among the molecular mechanisms proposed to exert its effects, PEA activates the nuclear peroxisome proliferator activated receptor (PPAR)-alpha whose genetic inactivation resembles a behavioral and cognitive phenotype consistent with preclinical models of schizophrenia and autism spectrum disorder (ASD). In particular, ASD is a neurological disease with a strong genetic and environmental basis. It is recently suggested that a deficiency of anti-inflammatory cytokines and bioactive lipids ultimately results in the development of autism.

Based on this background, the aim of the study was to investigate the possible efficacy of the endogenous bioactive lipids PEA in BTBR mice and to shed light on the mechanisms underlying PEA effects.

Our results showed that PEA reverted behavioral phenotype of BTBR mice in a dose-dependent manner, and this effect was contingent to PPAR-alpha activation, since PEA failed in exerting its effect in PPAR-alpha null mice or in mice pre-treated with PPAR-alpha antagonist. Moreover, the effect of PEA was related to a modulation of the expression of neuroprotective factors and pro-inflammatory cytokines at both central and peripheral level.

In conclusion, functional and molecular findings demonstrated a therapeutic potential of PEA in limiting ASD symptoms, suggesting bioactive lipids as new candidates for the treatment of ASD, able to improve the quality of life for ASD patients.

PDN-3 DPA PATHWAY REGULATES HUMAN MONOCYTE DIFFERENTIATION AND MACROPHAGE FUNCTION

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Macrophages are central in orchestrating the clearance of apoptotic cells and cellular debris during inflammation, with the mechanism(s) regulating this process remaining of interest. Herein, we investigated the n-3 docosapentaenoic acid (DPA)-derived protectin (PDn-3 DPA) biosynthetic pathway, and the role of these mediators in regulating both human macrophage phenotype, efferocytosis and bacterial phagocytosis. Using lipid mediator profiling, human primary cells and recombinant enzymes we found that human 15-lipoxygenases (LOX) initiate the PDn-3 DPA pathway catalysing the formation of an allylic epoxide. Inhibition of human 15-LOX led to a significant reduction in several macrophage lineage markers as well as a reduction in macrophage efferocytosis. Evidence for the formation of an allyllic epoxide in the PDn-3 DPA biosynthetic pathway in human macrophages, a reaction catalysed by h15-LOX, was obtained using acid methanol trapping. The complete stereochemistry of this epoxide was determined using stereocontrolled total organic synthesis as 16S, 17S-epoxy-7Z, 10Z, 12E, 4E, 19Z-docosapentaenoic acid (16S, 17S-ePDn-3 DPA). This intermediate was enzymatically converted by epoxide hydrolases to PD1n-3 DPA and PD2n-3 DPA, with epoxide hydrolase 2 converting 16S, 17S-ePDn-3 DPA to PD2n-3 DPA in human monocytes. Incubations of human macrophages with either 16S, 17S-epoxy-PDn-3 DPA or PD1n-3 DPA restored the expression of several of the lineage markers downregulated by 15-LOX inhibition, including CD206 and CD64, and rescued their efferocytosis and phagocytosis ability. Taken together these results establish the PDn-3 DPA biosynthetic pathway in human monocytes and macrophages and its role in regulating macrophage resolution responses.

EXOTOXINS FROM S. AUREUS ARE POTENT AND SELECTIVE STIMULATORS OF 15-LIPOXYGENASE-1-DERIVED SPM IN HUMAN M2 MACROPHAGES TO RESOLVE INFLAMMATION

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Early stages of bacterial infections are characterized by excessive generation of pro-inflammatory lipid mediators (LM), followed by the biosynthesis of specialized pro-resolving mediators (SPM) such as lipoxins (LX), resolvins (Rv), protectins, and maresins that initiate resolution of inflammation and tissue repair. Pathogenicity of bacteria like S. aureus relies on several virulence factors including secreted protein toxins. Among these, exotoxins mediate multiple cellular responses including macrophage recruitment. We recently showed that pathogenic bacteria activate 5- and 15-lipoxygenase (LOX) in human macrophages to produce different LM profiles in a phenotype-dependent manner [1]. While the pro-inflammatory prostaglandins and leukotrienes (LT) are predominantly generated by M1, SPM formation is characteristic for the M2 phenotype. Here we show that exotoxins evoke LM biosynthesis in macrophages, and we identified the α-hemolysin (HLA) as potent and selective activator of the 15-LOX-1 pathway in M2. S. aureus-conditioned medium (SCM) and recombinant HLA triggered intracellular Ca^{2+} mobilization and translocation of 5- and 15-LOX from soluble to membraneous compartments in macrophages, which determines LM biosynthesis. S. aureus mutants unable to produce exotoxins failed to evoke Ca2+ mobilization, LOX translocation and LM formation. Notably, HLA specifically induced formation of 15-LOX-1-derived SPMs such as RvD5 (~2000 pg/10^6 cells) in M2. Although 5-LOX nuclear translocation was evident in macrophages upon HLA exposure, only little LTB4 was formed (<10 pg/10^6 cells), and the COX pathway was barely activated. In contrast, Ca^{2+}-ionophore failed to stimulate 15-LOX-1 product formation but strongly induced the 5-LOX and COX pathways in M1/M2. While Ca^{2+}-ionophore caused rapid mobilization of intracellular Ca^{2+}, the corresponding effects of S. aureus, SCM or HLA were striking different with delayed and gradual increases over 90 min. Together, HLA is a potent and selective activator of 15-LOX-1 in anti-inflammatory M2 which leads to marked SPM generation with little pro-inflammatory PG and LT. Our data suggest that bacterial exotoxins, besides various detrimental actions for the host, may also exert beneficial functions as stop signals for inflammation and as initiators of resolution.

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TARGETING AMPK CAN AUGMENT PROSTACYCLIN PRODUCTION FROM HUMAN LUNGS

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Introduction: Pulmonary hypertension (PH) is characterized by vasoconstriction and wall thickening of pulmonary arteries which is due to a decrease in vasodilators (prostacyclin (PGI2) and nitric oxide (NO)) levels (1). Adenosine monophosphate activated protein kinase (AMPK) is a cellular energy sensor that has ameliorates PH in animal models (2). However, no study addressed the effect of AMPK activation on eNOs activity and PGI2 production in human lung parenchyma (HLP).

Aim: First, to investigate the effect of AMPK activators (AICAR and Metformin) on the production of PGI2 and eNOS activity in HLP from control patients. Second, to compare the level of expression of eNOS and PGI2 synthase (PGIS) between control (lung cancer) and PH patients

Methods: HLP preparations obtained from control and PH patients were used for western blot analysis of the levels of eNOS and PGIS. In addition, HLP preparations or pulmonary arteries from control patients were incubated with AICAR (3mM, 30 minutes) or Metformin (3mM, 2 hours): Tissues were then used for western blot analysis of the activity of AMPK and eNOS, while supernatants were used for ELISA measurement of 6-keto-PGF1α (a stable metabolite of PGI2).

Results: Both metformin and AICAR increased the levels of pAMPK in control pulmonary arteries (n=1). Treatment of control HLP (n=4) with metformin (3mM) significantly increased the PGI2 production by 34±6 % as compared to non-treated control (P= 0.047, paired t-test). In addition, a similar result was obtained in HLP (n=3) treated with AICAR (3mM). Furthermore, Higher levels of eNOS were observed in HLP from PH patients (n=8-12) as compared to control (n=8-10). However, the PGIS levels were similar in the 2 groups.

Conclusion: This study shows that the activation of AMPK can increase the production PGI2 from HLP of control patients. Future experiments are needed to confirm the same effect in tissues derived from PH patients and to investigate the effect of AMPK stimulation on NO production, Targeting AMPK seems to represent a new therapeutic pathway for PH.

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LIPID DROPLETS INTEGRATE METABOLIC AND SIGNALLING PATHWAYS AND ARE INDISPENSABLE FOR THE CELLULAR STRESS RESPONSE IN BREAST CANCER CELLS

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For decades, lipid droplets (LDs) have been considered solely as lipid storage organelles, but recent studies suggest that they are central hubs of cellular lipid metabolism and signalling. LDs store neutral lipids, mostly triacylglycerols (TAGs) and cholesteryl esters, and coordinate the trafficking of exogenous and endogenous fatty acids (FAs) according to cellular needs. Some of the most aggressive cancer types, driven by the Ras oncogene, scavenge exogenous unsaturated FAs and form LDs in order to cope with stress. Elevated LD accumulation has been found in various tumours in vivo and, intriguingly, LD biogenesis is induced in cancer and other cells exposed to hypoxia or nutrient deprivation. Recent studies have suggested that besides providing FA for energy production, LD-derived FAs may be used as precursors for the synthesis of lipid mediators. We have found previously that a secreted phospholipase A2 (sPLA2) enzyme releases unsaturated FAs from plasma membranes of breast cancer (BC) cells and induces the formation of LDs, which enable cell survival during nutrient stress. Here we examined the role of adipose triglyceride lipase (ATGL), the main lipase that releases FAs from TAGs stored in LDs, in supplying LD-derived FAs for cell survival during stress and for the synthesis of eicosanoids in BC cells. We found that LDs induced by sPLA2-mediated phospholipid hydrolysis are enriched with polyunsaturated FAs (PUFAs). Our results further demonstrate that ATGL is involved in LD breakdown, FA transfer from LDs to mitochondria, but, surprisingly, it is not crucial for the pro-survival effects of sPLA2 or exogenous unsaturated FAs in BC cells exposed to prolonged starvation. However, we found that ATGL depletion leads to suppression of arachidonic acid- and sPLA2-induced prostaglandin E2 synthesis in BC cells. Our results therefore suggest that ATGL-mediated TAG lipolysis is involved in lipid mediator synthesis rather than cell survival in BC cells exposed to nutrient stress. Thus, LDs act as both metabolic and signalling hubs that regulate the cellular stress response and are emerging as potential targets in cancer therapy.

RESOLUTION OF INFLAMMATION THROUGH THE LIPOXIN AND ALX/FPR2 RECEPTOR PATHWAY PROTECTS AGAINST ABDOMINAL AORTIC ANEURYSMS

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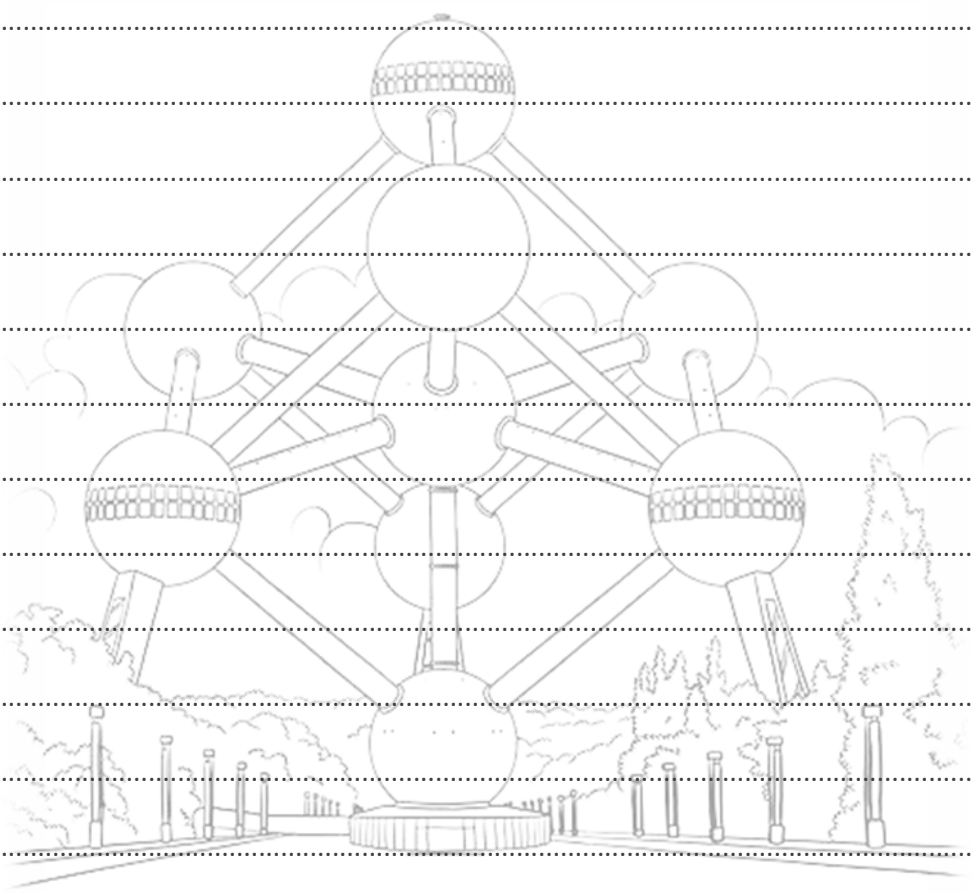
Abdominal aortic aneurysm (AAA) is a progressive aortic dilatation that may lead to a lethal rupture. Morphologically, AAA is characterized by remodeling of the vascular wall and inflammatory infiltrates, in which neutrophil recruitment and activation plays a key role. Lipoxin (LX) A4 and resolvin (Rv) D1 are products of lipoxygenase (LO) metabolism of fatty acids and mediate resolution of inflammation through engagement of the A lipoxin and formyl peptide receptor 2 (ALX/FPR2).

The aim of the present study was to investigate the role of pro-resolving signaling through the ALX/FPR2 receptor in AAA. By mapping of ALX/FPR2 mRNA expression across the vascular wall in n=89 human aortic samples we discovered a predominant adventitial ALX/FPR2 expression, which was significantly lower in samples derived from patients with AAA as compared with healthy donors. Genetic disruption of either the murine ALX/FPR2 orthologue, Fpr2, or the LX and Rv synthesizing enzyme 12/15-LO exacerbated aortic dilatation in response to angiotensin II in hyperlipidemic apolipoprotein E (apoE)-deficient mice. Aortic segments of $ApoE^{-/-}xFpr2^{-/-}$ mice exhibited less mature collagen and an increased neutrophil infiltration. The ALX/FPR2 agonist aspirin-triggered lipoxin (ATL) induced pro-resolving signaling in bone marrow derived murine neutrophils, but was devoid of activity in $Fpr2^{-/-}$ neutrophils.

These results identify a previously unknown protective role of lipoxin signaling through ALX/FPR2 to limit aortic dilatation, and indicate a key role of this pathway to promote the resolution of inflammation for the protection against AAA.



Invited speaker

THE ENDOCANNABINOID SYSTEM IN CANCER - BENEFICIAL OR DAMAGING?

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The endocannabinoid system consists of the endogenous ligands anandamide and 2-arachidonoylglycerol, their target cannabinoid (CB) receptors, and their synthetic and catabolic enzymes. There is good evidence both in vitro and in vivo in xenograft models, that CB receptor agonists have antiproliferative effects. Further, tumours frequently show an increased expression of CB receptors. This raises the prospect of using such compounds for the treatment of cancer, and a Phase II study with tetrahydrocannabinol (THC) / cannabidiol (CBD) as an add-on to temozolamide gave promising results in patients with recurrent glioblastoma multiforme according to a press-release from the company involved (https://www.gwpharm.com/about-us/news/gw-pharmaceuticals-achieves-positive-results-phase-2-proof-concept-study-glioma).

It is, however, unclear why tumours express an increased density of receptors, the activation of which will hasten their demise. Indeed, in pancreatic, prostate, oesophageal squamous cell and stage II microsatellite-stable colorectal cancer, a high expression of CB_1 receptors is indicative of a poor prognosis. In prostate cancer cells, low concentrations of THC are mitogenic secondary to activation of the Akt survival pathway, and in tumour tissue obtained at diagnosis, expression of CB_1 receptor immunoreactivity and phosphorylated Akt immunoreactivity are correlated. Given that in some tumours, circulating levels of endocannabinoids are thought to be raised, a pathway involving CB receptor-mediated Akt activation may contribute to the pathogenesis of the cancer.

Invited speaker

THE ENDOCANNABINOID SYSTEM IN THE LIVER: NOVEL PERSPECTIVES

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CB1 and CB2 receptors are G-protein-coupled receptors and components of the endocannabinoid system that also comprises enzymes that degrades the endogenous cannabinoid ligands, including MAGL and FAAH. Expression of both receptors is increased in experimental models of liver fibrosis and in liver samples obtained from patients with chronic liver disease or cirrhosis (1-3). Studies with cultured cells and in animal models have shown that an increased CB1 tone contributes to the pathogenesis of alcohol-induced liver disease and non-alcoholic fatty liver disease by enhancing hepatocyte injury (1, 4-6). In addition, CB1 receptors expressed by hepatic fibrogenic cells and macrophages promote liver fibrogenesis (3). Studies in patients with non-alcoholic fatty liver disease (NAFLD) and hepatitis C support the relevance of these experimental data to human liver diseases (7, 8). Current research efforts are directed at the development of non-brain penetrant CB1 antagonists; such compounds have shown promising results in experimental models ((6) and our unpublished observations). In contrast to CB1-dependent effects, an increase in CB2 signaling is associated with hepatoprotective effects, reduced liver inflammation, and improved liver fibrogenesis (1, 2, 10, 11). Our recent results also demonstrate that monoacylglycerol lipase is an immunometabolic target in the liver, and that inhibitors of this enzyme display anti-fibrogenic effects during chronic liver injury. The presentation will highlight the latest advances on the impact of the endocannabinoid system on the key steps of chronic liver disease progression and discuss the therapeutic potential of molecules targeting cannabinoid receptors and monoacylglycerol lipase.

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Selected oral

PROSTAGLANDIN D2-GLYCEROL ESTER, A COX-2 METABOLITE OF 2-ARACHIDONOYLGLYCEROL, DECREASES INFLAMMATORY PAIN IN MICE

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2-arachidonoylglycerol (2-AG) is known to exert anti-inflammatory effects in vivo. We previously showed that the anti-inflammatory effects of increasing 2-AG levels were in part cannabinoid receptors dependent, and in part due to the oxidative metabolism of 2-AG by cyclooxygenase-2 (COX-2) to give the anti-inflammatory mediator prostaglandin D2 glycerol ester (PGD2-G). Inflammation is characterized by the release of multiple mediators, some of which can act on nerve endings, thus triggering inflammatory pain. As PGD2-G exerts anti-inflammatory effects in vitro and in vivo, we wanted to investigate its effect in inflammatory pain. To this end, we used the model of carrageenan-induced inflammatory pain to assess the effect of PGD2-G. This model is well-described and broadly used to test new anti-inflammatory/analgesic drugs. PGD2-G (20 µg) was injected in the mouse paw 30 minutes before carrageenan injection (0.1 mg in saline). We also assessed the involvement of PGD2 and 15d-PGJ2-G, possible metabolites of PGD2-G.

We monitored over time carrageenan-induced edema and hyperalgesia, using von Frey filaments for the latter. The expression of pro-inflammatory markers was assessed in the paw tissue at different time points using RT-qPCR. Histological studies and myeloperoxidase activity (a marker for neutrophils infiltration) were used to monitor immune cell infiltration.

Mice receiving PGD2-G recovered faster from carrageenan-induced hyperalgesia while PGD2 delayed the recovery time and 15d-PGJ2-G had no effect. We also confirmed the anti-inflammatory effect of PGD2-G with a decrease of edema formation, an effect not reproduced by PGD2 while 15d-PGJ2-G worsened the edema. Moreover, PGD2-G decreased carrageenan-induced inflammatory markers expression in the mouse paw at 24h following carrageenan injection as well as immune cell infiltration.

In conclusion, while the targets mediating the effects of PGD2-G in this model are still under investigation, its anti-inflammatory and analgesic effects make it an interesting lipid mediator in inflammatory pain and support the interest to further investigate its pathophysiological role.

Selected oral

EICOSAEPOXYTRIENOIC ACID METABOLITES (EET) REGULATE KIDNEY SODIUM TRANSPORT

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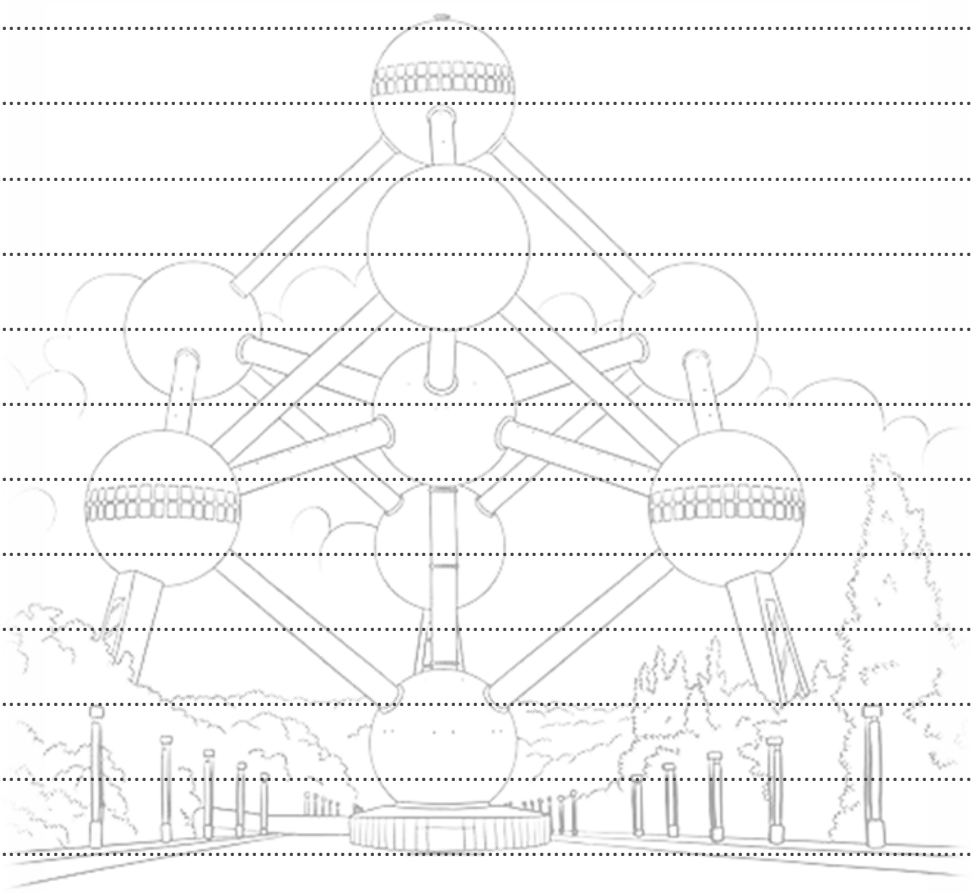
Cytochrome-p450 (Cyp) epoxygenase plays an important role in the regulation of renal Na transport. Global disruption of Cyp2c44, a major epoxygenase in mouse kidney, caused salt-sensitive hypotension. Kir4.1 (Kcnj10) is a major type of K channel expressed in the distal convoluted tubule (DCT) which is responsible for reabsorption of 5% filtered Na load. The renal phenotype of loss-of-function mutations of Kir4.1 is reminiscence of Gitelman syndrome characterized by salt wasting and hypokalemia. We have shown that the Kir4.1 activity determines the expression of thiazide-sensitive NaCl-transporter (NCC). The aim of the study is to test the role epoxygenase-dependent arachidonic acid (AA) metabolites in regulating Kir4.1 and NCC of the DCT. The patch-clamp experiments have demonstrated that AA reversibly inhibited the 40 pS K channel (Kir4.1/5.1) in the DCT in WT mice. However, the disruption of Cyp2c44 abolished the inhibitory effect of AA on the K channel. Furthermore, whole-cell recording demonstrated that the inhibition of CYP-epoxygenase with 5 µM MS-PPOH significantly increases while application of AA (5 µM) decreases the basolateral K conductance in the DCT. This suggests that AA-induced inhibition of Kir4.1 in the DCT depends on Cyp-epoxygenase activity. This notion is supported by the finding that 100 nM 14,15-EET is still able to inhibit the basolateral Kir4.1 channel in both WT and $Cyp2c44^{-/-}$ mice. Since Kir4.1 activity determines NCC activity, we next examined the expression of NCC in the kidney obtained from age- and sex-matched WT and $Cyp2c44^{-/-}$ mice. The disruption of Cyp2c44 increases the expression of NCC in comparison to those of WT mice. High Na intake inhibited Kir4.1 in the DCT and decreased NCC expression only in the WT but not in $Cyp2c44^{-/-}$ mice, suggesting the role of epoxygenase in mediating the effect of high Na intake on Kir4.1 and NCC. This notion is strongly suggested by the finding that increasing dietary Na intake caused hypertension in $Cyp2c44^{-/-}$ mice and application of thiazide almost completely restored the normal blood pressure in $Cyp2c44^{-/-}$ mice. We conclude that EET plays an important role in inhibiting NCC through inhibiting the basolateral K channel activity in the DCT.



Invited speaker

FUNCTIONAL LIPID MEDIATOR PROFILING IN ESTABLISHING DISEASE ETIOPATHOGENESIS

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Inflammation is a coordinated host response that when self-limited is protective. Many cell types upon activation produce mediators that regulate the physiological function of inflammation. Arachidonic acid derived mediators that include the leukotrienes, prostaglandins and thromboxane orchestrate the initiation of the inflammatory response. Termination or resolution of inflammation is also appreciated to be an active response coordinated by a novel genus of lipid mediators coined as specialized pro-resolving mediators (SPM). These mediators actively control leukocyte responses counter-regulating the production pro-inflammatory signals, promote leukocyte phenotype switch from inflammatory to protective and orchestrate tissue cellular trafficking. Using mass spectrometry-based structure elucidation we recently identified four new mediator families that regulate the progression of inflammation as well as fine tune the host response to clear the invading pathogens, repair and regenerate damaged tissues in tissues during ongoing infectious-inflammation. These include the thirteen series resolvins (RvT) and the protectin conjugates in tissue regeneration (PCTR). Failure to engage these protective pro-resolving pathways is implicated in the etiopathogenesis of many inflammatory diseases including infections, cardiovascular disease and neurological disease. Using a targeted mass spectrometry-based approach, measuring the flux down each of the major bioactive metabolomes we recently found that lipid mediator profiles from both experimental systems and humans provide an insight into the body's inflammation-resolution status. Using this lipid-mediator profiling approach we have recently investigated the relationships between circulating SPM concentrations and cellular responses in healthy volunteers following omega-3 fatty acid supplementation. We also assessed the relationship between the concentrations of these molecules in the cerebrospinal fluids and outcome following treatment in patients with meningeal tuberculosis. Results from these studies demonstrated a link between SPM-concentrations and outcome suggesting that functional lipid mediator profiling may represent a novel useful tool in patient stratification and to also assess treatment responsiveness. Thus, these results indicate that resolution-based personalized medicines may be useful in both preventing and treating diseases with an inflammatory component.

Invited speaker

LIPIDOMICS, OXYSTEROLS AND LIPID MEDIATORS

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Oxysterols are oxidised forms of cholesterol and of its cyclic precursors. For decades oxysterols were considered as simple intermediates in bile acid and steroid hormone biosynthetic pathways. In recent years this view has changed with the realisation that oxysterols are signalling molecules in their own right, activating both nuclear receptors and G protein-coupled receptors. Oxysterols are poorly represented in global lipidomic studies on account of their comparatively low abundance and poor ionisation characteristics. To overcome these problems many groups adopt a targeted lipidomic approach to oxysterol analysis. In this paper we will describe different lipidomic approaches to oxysterol analysis and highlight important discoveries in the areas of immunity and development. Current work in the area of oxysterol imaging of brain will be discussed.

Selected oral

PLASMA LIPIDOMIC PROFILING AND ADIPOSE TISSUE KINOMICS LINK ARACHIDONIC ACID DERIVED METABOLITES TO MAP KINASE ACTIVATION IN HUMAN OBESITY.

ESTHER TITOS[1,2,4], Cristina López-Vicario[1], Juan José Lozano[3], Mireia Casulleras[1], José Alcaraz-Quiles[1], Roger Flores-Costa[1], Marta Duran-Güell[1], Ainitze Ibarzábal[2], Juan José Espert[2], Markus Templin[5] and Joan Clària [1,3,4]

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The development of metabolic complications associated to obesity is strongly influenced by the progress of a chronic state of low-grade inflammation in the adipose tissue. In the current study we aimed at identifying a plasma lipid signature and tissue kinase nodes associated to obesity-derived complications in order to discover novel plasmatic biomarkers and tissue molecular targets. To carry out the study we performed both plasma targeted lipidomics and adipose tissue kinomics in control (CT, n=4), obese (OB, n=10) and obese diabetic (OBd, n=6) individuals. By means of LC-MS/MS targeted approach a total of 49 compounds were detected in plasma samples from our cohort, 68% were metabolites derived mainly from both arachidonic and linoleic acids and 30% were derived from omega-3 sources, mainly either from eicosapentaenoic and docosahexaenoic acids. Compared to control group, 4 arachidonic acid-derived metabolites were significantly altered in both obese groups. Data analysis revealed that monohydroxy fatty acids 8-, 11-, and 12-HETE and prostaglandin F2 alpha (PGF2a) were significantly increased in obesity. Importantly, 12-HETE was found to be up-regulated in both OB and OBd patients with positive and significant correlation with total leukocyte, neutrophil and platelet blood counts (0.54; $P< 0.02$, 0.56; $P< 0.02$ and 0.59; $P< 0.007$, respectively). Additionaly, PGF2a was found to be up-regulated during the course of the disease being significantly up-regulated in OBd patients. Phosphoproteome analysis of 80 proteins by a high-throughput western blot approach revealed a differentially regulated subset of kinases and phosphatases in visceral adipose tissue from obese patients compared to normal visceral adipose tissue. OBd patients showed a proteomic profile consisting in increased levels in the energy sensor 5'-prime-AMP-activated protein kinase (AMPK) and phosphorylated forms of kinases of MAPK family MEK1/2, ERK1/2 and tyrosine phosphatase PTPN7.

Interestingly, correlation analysis between lipidomic and kinomic data revealed a positive and significant relationship between 12-HETE and the phosphorylated form of MEK1/2 (0.66; $P<0.02$). Additionaly, 12-HETE also showed a positive correlation with the total forms of MKK4 (0.66; $P< 0.02$), MSK1 (0.62; $P<0.03$) and RSK1/2/3 (0.85; $P<0.002$). Together, these findings suggest 12-HETE as a putative biomarker related to adipose tissue-derived inflammation in obesity and identify MAPK pathway and specifically MEK1/2 as a molecular target in inflamed adipose tissue.

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Selected oral

DEVELOPMENT OF A LIQUID CHROMATOGRAPHY COUPLED MASS SPECTROMETRY ANALYSIS OF GLUTATHIONE CONJUGATES OF POLYUNSATURATED FATTY ACIDS

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Glutathione (GSH)-conjugates of polyunsaturated fatty acids comprise a group of pro- and anti-inflammatory lipid mediators formed in immunocompetent cells. The pro-inflammatory cysteinyl-leukotrienes (cys-LTs) [1] and eoxins (EXs) [2] derive from arachidonic acid (AA) via the lipoxygenase (LOX) pathways. AA is converted by 5-LOX and 15-LOX to unstable epoxides, which are subsequently conjugated with GSH by LTC4 synthase (LTC4S) yielding LTC4 and EXC4. Both are processed in the extracellular space by sequential cleavage of the tripeptide side chain to LTD4/LTE4 and EXD4/EXE4, respectively. Recently, anti-inflammatory GSH-conjugates of tissue regeneration (CTRs) have been reported [3]. They are produced from docosahexanoic acid (DHA) by various LOX enzymes (5-, 12-, 15-LOX) in conjunction with LTC4S or glutathione-S-transferases (GST). Depending on the involved LOX, maresin-, protectin- or resolvin-conjugates are formed: MCTR1, PCTR1 and RCTR1. Resembling the pro-inflammatory GSH-conjugates LTC4 and EXC4, CTR1s could be converted by sequential cleavage of the GSH-moiety to CTR2s and CTR3s.

Previously, GSH-conjugates of polyunsaturated fatty acids have been analyzed by HPLC-MS/MS with negative electrospray ionization, which was advantageous for simultaneous detection together with other eicosanoids. However, the revealed product ion scans for GSH-conjugates were devoid of significant structural information and further investigations were required to distinguish between cys-LTs, EXs and CTRs.

The main purpose of our work was to establish an UPLC-MS/MS method for parallel analysis of pro- and anti-inflammatory GSH-conjugates and to determine the influence of the instrumental parameters on the analysis of these conjugates.

Mass spectrometric analysis carried out in a negative ionization mode for GSH-conjugates (cys-LTs, EXs, MCTRs and PCTRs) was not sufficient to determine distinct conjugates due to lack of characteristic fragmentation. However, positive ionization [4] and fragmentation gave structural information for cys-LTs, EXs, MCTRs and PCTRs.

Taken together, we established a UPLC-MS/MS method carried out in a negative and a positive electrospray ionization mode to detect pro- and anti-inflammatory GSH-conjugates. Analysis in the negative ionization mode is useful for samples containing GSH-conjugates together with other eicosanoids, whereas the positive ionization mode is indispensable for structural distinction of GSH-conjugates.

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Selected oral

LEUKOCYTES FROM OBESE INDIVIDUALS EXHIBIT AN IMPAIRED SPM BIOSYNTHETIC SIGNATURE

CRISTINA LOPEZ-VICARIO, Esther Titos, Ana Isabel Martínez-Puchol, José Alcaraz-Quiles, Mireia Casulleras, Marta Durán-Güell, Roger Flores-Costa, Jesmond Dalli and Joan Clària.

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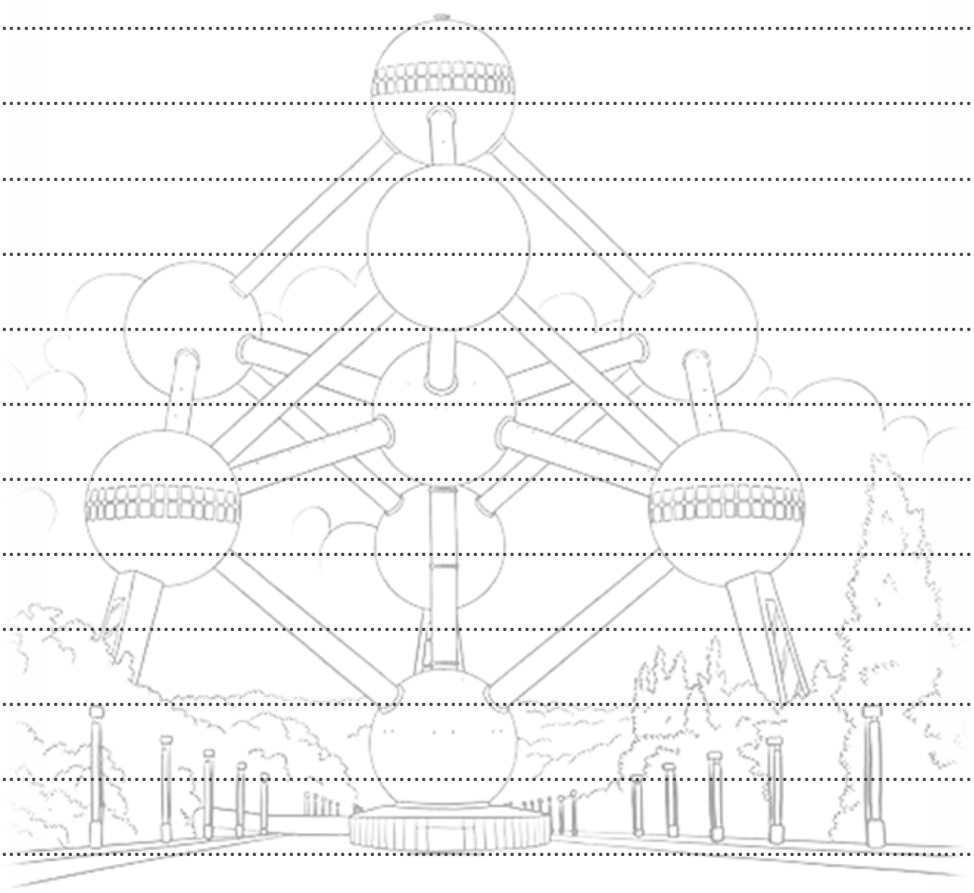
The presence of a chronic state of low-grade systemic inflammation in obesity predisposes obese individuals to the development of metabolic comorbidities. Although the mechanisms underlying systemic inflammation in obesity are not completely delineated, previous findings from our laboratory have provided evidence of unbalanced circulating levels of pro-inflammatory versus pro-resolution lipid mediators in obese individuals. The aim of the current study was to compare the profile of bioactive lipid mediators produced by peripheral leukocytes isolated from obese individuals with that of leukocytes from lean donors. LC-MS/MS lipidomic analysis revealed that peripheral leukocytes mainly produce 12-HETE and TXB2, together with monohydroxy fatty acids (i.e. 17-HDHA, 15-HETE, 14-HDHA, 18-HEPE, 5-HETE and 20-HETE), leukotrienes (i.e. LTB4), prostaglandins (PGF2alpha and PGE2) and specialized pro-resolving mediators (SPM) (i.e. RvD4, RvD5, RvE1, LXA4, RvE2, MaR2, RvD2, RvD1, MaR1, RvE3, RvD6, RvD3 and PD1) from endogenous sources. However, as compared to controls, leukocytes isolated from obese individuals produced increased levels of LTB4 and showed a remarkable deficit in the biosynthesis of omega-3-derived hydroxy fatty acids (i.e.17-HDHA and 18-HEPE), despite the levels of omega-6 and omega-3 precursors in the incubation media were similar. Moreover, leukocytes from obese individuals exhibited a 30% reduction in the endogenous production of SPM as compared to those from non-obese controls. Mechanistically, leukocytes isolated from obese individuals exhibited differential residue phosphorylation and intracellular 5-lipoxygenase localization. In addition, as compared to non-obese, obese leukocytes showed reduced protein expression of MFSD2A, a membrane transporter involved in the cellular up-take of omega-3 polyunsaturated fatty acids. Finally, exogenous addition of the omega-3-derived SPM intermediates 17-HDHA and 18-HEPE to the cell medium completely normalized the production of pro-resolving lipid mediators. Together, these data demonstrate a sub-optimal production of SPM in leukocytes from obese individuals, probably related to impaired fatty acid membrane transport and/or intracellular 5-lipoxygenase compartmentalization, alterations that can be overridden by exposing these cells to the SPM biosynthetic intermediates, 18-HEPE and 17-HDHA.



POSTER 1

IN VITRO STUDY ON PULMONARY HYPERTENSION TREATMENTS: ROLE OF PGI2 PATHWAY IN HUMAN PULMONARY VESSELS

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Introduction: Prostacyclin (PGI2) is a vasodilator and an inhibitor of cell proliferation. Low productions of PGI2 in pulmonary hypertension (PH) have been confirmed by in-vitro studies. Endothelial PGI2 synthase (PGIS) and the density of the PGI2 receptor (IP) are decreased in the lungs of PH patients (1, 2). Therefore, activation of promoters of genes encoding PGIS and IP receptor would be beneficial to PH patients (3).

Aim: To restore the endogenous production of PGI2 (by increasing the expression of PGIS) and to increase the expression of IP receptor in human pulmonary artery smooth muscle cells (hPASMC) derived from “control” and “PH” patients.

Methods: hPASMC and human pulmonary arteries (HPA) derived from control and PH patients, were used to study the basal expression of PGIS. hPASMC were incubated (4-48h) with E2 (0.01-10µM).
PGIS and IP are quantified by western blot using cells and arteries homogenates. The incubation medium is used for the measurement of 6keto-PGF1α (stable metabolite of PGI2). Organ bath experiments are conducted using HPA pre-incubated with E2. Results derived from 3-8 patients (n) are expressed as mean±SEM.

Results: The expression of PGIS is significantly decreased in untreated hPASMC and in HPA from PH patients compared with control patients (t-test, p=0.005).
The expression of PGIS in hPASMC from control patients (n=3-8), is significantly increased (37-50%) after 4, 18, 24 and 48h of treatment with 10^{-6}M of E2.
All doses of treatment (48h) of hPASMC from PH patients increased significantly PGIS expression (72-148%; n=4).
The expression of IP receptor in hPASMC from control and PH patients (n=2), is significantly increased after 48h of treatment (37%) with 10^{-5}M of E2.
The biological consequences of increased expressions (PGIS, IP) are investigated: Increased vasorelaxations and endogenous productions of PGI2 are measured after E2 incubation.

Conclusion: This study confirms the alteration of PGI2 production through the fall in the expression of its synthetic enzyme PGIS in PH patients. This decrease and the reduced IP expression could be restored by 17β-estradiol in PH arteries.

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POSTER 2

RECONSTRUCTING LIPID MEDIATORS METABOLIC NETWORKS IN OBESITY AND RELATED COMPLICATIONS

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Lipid mediators (LM) act via autocrine and paracrine signaling in different tissues and their synthesis and degradation are tightly regulated in healthy state. Dysregulation of LM signaling was associated with numerous diseases including obesity and related complications. In obesity and related metabolic disorders, alteration in LM signaling was correlated with adipocyte hypertrophy and hyperplasia, macrophage polarization and adipokine/cytokine secretion in adipose tissue. Despite the large amount of data on the role of LM in obesity associated signaling, no unified metabolic networks required for systems medicine data integration are available till now.

Based on the literature meta-study, networks of enzymatic and free-radical-driven oxidation were reconstructed for the most abundant polyunsaturated fatty acids (PUFAs) and included 570 reactions covering 480 oxidized lipid species. Reconstructed networks were further enriched in the information on enzymes involved in LMs synthesis. Using PUFA oxidation networks as a scaffold, adipocytes (preadipocytes and mature adipocyte) and adipose tissue macrophage (M1 and M2) specific regulatory networks covering four eicosanoids (prostaglandins E2, I2, F2alpha, and 15-deoxy J2), were reconstructed and enriched in the information on involved genes, enzymes, and respective LMs receptors. Quantitative data obtained in our laboratory as well as publicly available datasets were used for networks enrichment to uncover the role of LM in obesity related adipose tissue adipogenesis, lipolysis, cytokines/adipokine production and secretion. The eicosanoid metabolic networks aim to provide an unify view on regulatory function of LM in diseases development and progression with a potential to be used as diagnostic and prognostic tools by systems medicine.

POSTER 3

IMATINIB AND NILOTINIB ARE STRONG STIMULATORS OF PGE2 SYNTHESIS AND ATTENUATE INFLAMMATORY CYTOKINE RELEASE VIA EP4 RECEPTOR ACTIVATION

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Besides their effect as ABL-tyrosine kinase inhibitors, imatinib and nilotinib have been shown to produce anti-inflammatory effects through inhibition of cytokine release. We demonstrate that both drugs inhibit tumor necrosis factor (TNF)α release concentration- and time-dependently and that this effect is due to increased prostaglandin (PG)E2 release and activation of the EP4 receptor leading to inhibition of NFκB activation. Our data strongly suggest that imatinib and nilotinib inhibit thromboxane synthase, and that redirection of PGH2 towards PGE synthases accounts for the increase in PGE2 release. Most importantly, we found that patients treated with imatinib have higher plasma levels of PGE2 and reduced cytokine secretion upon stimulation of whole blood with lipopolysaccharide. The imatinib- and nilotinib-induced increase of PGE2 may thus have profound effects on cytokine regulation and immune responses and could be therefore useful for many chronic inflammatory diseases.

POSTER 4

DIFFERENT EFFECT OF MPGES-1 AND COX-2 INHIBITORS ON PROTEOMIC AND LIPIDOMIC PROFILES IN A549 CELLS

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Inhibition of mPGES-1 activity is protective in experimental models of inflammatory diseases and cancer while avoiding the adverse effects associated with COX inhibitors. Although it is established that inhibition of mPGES-1 over COX-1/2 alters the eicosanoid profile differently, little is known about how this affect the cellular proteomic or lipidomic profiles. Here we studied the effect of mPGES-1 inhibitor Compound III (CIII) and COX-2 inhibitor NS-398 in IL-1beta treated A549 cells using mass spectrometry based proteomics and lipidomics. Our proteomics analysis showed that treatment with the inhibitors altered the abundance of several proteins, many of them involved in lipid metabolism. Ingenuity pathway analysis revealed that treatment with CIII induced a pro-apoptotic and anti-proliferative state compared to treatment with NS-398. Using multiple lipidomics platforms, we found that inhibition of mPGES-1 induced an accumulation of pro-apoptotic lipids sphinganine and dihydroceramide(16:0). However, we did not observe any differences in apoptosis or cell viability as measured using IncuCyte cell imaging system. We speculate that inhibition of mPGES-1 can sensitize cancer cells to treatment with cytotoxic drugs and that this could be a valid therapeutic strategy.

POSTER 5

PROSTACYCLIN: A POTENTIAL NOVEL THERAPEUTIC TARGET TO TREAT TENDON PAIN AND INFLAMMATION?

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Inflammation in tendon disease is a significant healthcare burden whereby patients experience pain and disability. As with other chronic inflammatory diseases, there is an unmet clinical need and requirement for novel treatments that address the underlying disease biology. Herein, we studied tissues and cells isolated from biopsies of healthy and diseased human tendons to investigate the role of prostaglandins in these inflamed tissues. Immunostaining of diseased tendon tissues showed increased numbers of macrophages (CD68+ cells), increased expression of COX-1 and COX-2, prostacyclin synthase (PGIS), the prostacyclin receptor (IP receptor) and microsomal prostaglandin E synthase (mPGES)-1. PGIS co-localised with podoplanin, a marker of stromal fibroblast activation and nociceptive neuromodulator NMDAR-1. Treating cultures of isolated tendon cells with IL-1beta induced prostaglandin E2 (PGE2) in both healthy and diseased tendon cells. The same treatment potently induced the prostacyclin (PGI2) metabolite 6-keto PGF1alpha in diseased compared to healthy tendon cells. Incubation of IL-1beta treated tendon cells with the selective mPGES-1 inhibitor Compound III reduced PGE2 and increased 6-keto PGF1alpha in diseased tendon cells. Conversely, COX blockade with either naproxen or NS-398 completely inhibited both PGE2 and 6-keto PGF1alpha production from these cells. Increased expression of PGIS and IP receptor in diseased tendons and increased production of 6-keto PGF1alpha by diseased tendon cells suggests that prostacyclin may contribute to the pathogenesis of tendon inflammation and play a potential role in pain associated with disease. Targeting the prostacyclin pathway presents a novel potential therapeutic strategy to modulate inflammation and pain in tendon disease.

POSTER 6

FUNCTIONAL EICOSANOID PROFILING IN THE ISOLATED, PERFUSED MURINE HEART

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Eicosanoids generated from AA by cyclooxygenases, lipoxygenases, and cytochromes P450 pathways play an important role in myocardial physiology, bioenergetics and signalling pathways (Jenkins et al., 2009) but their role in heart failure pathophysiology is still not entirely clear.
The aim of this study was to set up the methodology to profile eicosanoids in murine heart effluents in basal conditions as well as in response to bradykinin, acetylcholine and arachidonic acid (AA).
Murine hearts were mounted in the isolated heart set-up and perfused according to Langendorff mode. Heart effluents were collected before and after addition of bradykinin, acetylcholine and AA, to the perfusion line. Eicosanoid concentrations were assessed using UFLC Nexera system (Shimadzu) combined with the triple quadrupole mass spectrometer QTrap 5500 (Sciex).
In heart effluents in basal conditions and in response to bradykinin and acetylcholine selected prostanoids, hydroxyeicosatetraenoic acids (HETEs) from lipoxygenase pathway were detected. In response to AA epoxyeicosatrienoic acids (EETs) and dihydroxyeicosatrienoic acids (DHETs) generated via cytochrome P450-dependent pathway were also detected. In heart effluents in basal conditions 12-HETE and 6-keto-PGF1alpha were released in the highest concentration. Heart stimulation by bradykinin increased the production of PGD2, PGE2, PGF2alpha more then 6-keto-PGF1alpha. On the other hand, acetylcholine prompted higher production of 6-keto-PGF1alpha than PGD2, PGE2, PGF2alpha. After AA concentrations of 6-keto-PG1alpha, PGD2, PGE2, PGF2alpha in heart effluents were higher than after bradykinin and acetylcholine. Importantly, AA stimulation caused also abundant production of 5-, 12-, 15-HETEs, 14, 15- and 11,12-DHETs.
In conclusion, the method of eicosanoids profiling in the isolated murine heart effluent is well suited for functional eicosanoids profiling. 12-HETE and 6-keto-PGF1alpha were the most abundant metabolites, and may play a major role in the coronary circulation. Acetylcholine acted on heart more via activation of prostacyclin synthase and bradykinin via activation of prostaglandin D, E, F synthases. AA acts on heart not only via cyclooxygenases and lipoxygenases pathways but also via cytP450 releasing EETs. Further study will be aimed to find differences in eicosanoid profile between healthy mice (FVB) and mice with heart failure (Tgalphaq*44).

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POSTER 7

POLYUNSATURATED FATTY ACID METABOLITES: BIOSYNTHESIS IN LEISHMANIA AND ROLE IN PARASITE/HOST INTERACTION

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Inside the human host Leishmania infection starts with phagocytosis of infective promastigotes by macrophages. To survive, Leishmania develop several strategies for manipulating macrophages functions, among them the implication of bioactive lipids has been poorly explored. We assessed the biosynthesis of polyunsaturated fatty acid metabolites by infective and non-infective stages of Leishmania and explored their role in macrophages polarization. Concentrations of docosahexaenoic acid metabolites, precursors of pro-resolving lipid mediators, were increased in the infective stage of the parasite compared to the non-infective stage. Cytochrome P450-like proteins were implicated in biosynthesis of these metabolites. Treatment of macrophages by lipids extracted from the infective stages polarized macrophages into a M2 phenotype and blocked the differentiation in M1 phenotype induced by IFNγ. In this presentation we will show that Leishmania polyunsaturated fatty acid metabolites, produced by cytochrome P450-like proteins activity, are implicated in parasite/host interactions by promoting polarization of macrophages into a pro-resolving phenotype M2.

POSTER 8

EFFECTS OF 25-HYDROXYCHOLESTEROL IN PULMONARY INFLAMMATORY DISEASE MODELS

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Respiratory diseases are one of the leading causes of death worldwide and lung inflammation plays a crucial role in most of these diseases. Several strategies have been explored over the years to tackle airway inflammation, including modulation of bioactive lipid levels.

Among these lipid mediators, the metabolic pathway of 25-hydroxycholesterol (25-OHC) was shown to be altered in lung inflammation and 25-OHC levels were increased in the sputum of COPD patients. Additionally, the levels of some oxysterols were correlated with inflammatory cell count in the bronchoalveolar lavage of asthmatic patients. Moreover, we found that the levels of 25-OHC and of its metabolite 7alpha,25-dihydroxycholesterol (7alpha,25-diOHC) were increased in a model of acute lung inflammation induced by intra tracheal instillation of lipopolysaccharides (LPS, E. coli; O55:B5, 6µg/mouse), and decreased following treatment with budesonide. This prompted us to study the effects of this oxysterol in acute lung injury.

Here, we administered 25-OHC intra-tracheally in the same model of LPS-induced lung inflammation. 25-OHC had beneficial effects on some inflammatory parameters. Indeed, it decreased leukocyte migration into the alveolar space and pro-inflammatory cytokine and chemokine expression in the lung tissue. However, 25-OHC did not reduce the increased protein concentration and production of pro-inflammatory cytokines in the bronchoalveolar lavage. Considering the interesting results we obtained with 25-OHC in acute lung injury, we further investigated its effects in a murine model of allergic asthma triggered by house dust mite extract (HDM).

Because alveolar macrophages are among the first immune cells involved in acute lung inflammation, we conducted mechanistic studies on primary alveolar macrophages to assess the role of 25-OHC on LPS-induced macrophage activation. 25-OHC decreased macrophage activation in a dose dependent manner. The same results were obtained when macrophages were activated with lipoteichoic acid, which suggests that the effects of 25-OHC are not TLR4 specific.

In conclusion, our results show beneficial effects of 25-OHC in decreasing macrophage activation and some parameters of acute lung inflammation. However, further studies are needed to decipher the molecular players involved in its effects of the 25-OHC on lung inflammation.

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POSTER 9

ALBUMIN MODULATES LIPID MEDIATOR BIOSYNTHESIS IN HUMAN PERIPHERAL LEUKOCYTES

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Background: Albumin is therapeutically used to prevent organ failure(s) and to improve survival in patients with advanced liver disease, a condition characterized by hypoalbuminemia, enhanced translocation of bacterial products from the gut to the systemic circulation and persistent systemic inflammation. Although, in addition to its well-recognized oncotic properties, albumin has emerged in recent years as an important modulator of systemic inflammation, the mechanisms underlying the protective actions of this protein are not fully understood. In the current study, we investigated the effects of albumin on peripheral leukocytes challenged with several pathogen-associated molecular pattern molecules (PAMPs), including bacterial DNA rich in unmethylated CpG motifs (CpG), lipopolysaccharide (LPS) and N-formylmethionyl-leucyl-phenylalanine (fMLP).

Material and Methods: Human leukocytes, peripheral blood mononuclear cells (PBMC) and neutrophils (PMN) isolated from healthy donors were stimulated with either CpG (2 μM) or LPS (1 μg/mL) and fMLP (1 μg/mL) in the absence or presence of human serum albumin (HSA) (15 mg/mL). Levels of bioactive lipid mediators in cell supernatants were measured by LC-MS/MS and the expression of genes coding for inflammatory cytokines was determined in cell extracts by real-time PCR. Plasma levels of bioactive lipid mediators and cytokines were also measured in patients with advanced liver disease (n=13) after receiving HSA for 6 weeks.

Results: Albumin, at therapeutic concentrations, significantly reduced the release of pro-inflammatory and vasoconstrictor eicosanoids, including leukotriene (LT) B4, prostaglandin (PG) E2, PGD2, PGF2α and thromboxane (TX)B2, in both PBMC and PMN stimulated with CpG and LPS plus fMLP. Unexpectedly, in these cells, albumin increased the formation of the monohydroxy fatty acids 15-hydroxyeicosatetraenoic acid (15-HETE), 18-hydroxyeicosapentaenoic acid (18-HEPE) and 17-hydroxydocosahexaenoic acid (17-HDHA), which are markers and precursors of the biosynthesis of potent pro-resolving lipid mediators. Consistent with these anti-inflammatory actions, albumin markedly blocked the expression of interleukin (IL) 1β, IL-6 and tumour necrosis factor (TNF) α in PBMC and PMN stimulated with CpG. These findings were translated in vivo to patients with advanced liver disease, who experienced a significant reduction in the plasma levels of TXB2, LTE4, PGD2 and 6-keto-PGF2a as well as in circulating cytokines after repeated HSA infusions. Plasma levels of 15-HETE, 17-HDHA and 18-HEPE remained unchanged.

Conclusions: Our results indicate that albumin plays an immunomodulatory role in human peripheral leukocytes, providing a mechanism for the anti-inflammatory properties of albumin infusions in patients with advanced liver disease.

POSTER 10

MUSCLE LOSS ASSOCIATED CHANGES OF OXYLIPIN SIGNATURES DURING BIOLOGICAL AGING: AN EXPLORATORY STUDY FROM THE PROOF COHORT

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Characterizations of the multiple mechanisms determining biological aging are required to better understand the etiology and identify early biomarkers of sarcopenia. Oxylipins are a large family of signaling lipids involved in the regulation of various biological processes that become dysregulated during aging.

To investigate whether comprehensive oxylipin profiling could provide an integrated and fine characterisation of the early phases of sarcopenia, we performed a quantitative targeted metabolomics of oxylipins in plasma of 81-year old subjects from the PROOF cohort with decreased (n=12), stable (n=16) or increased appendicular muscle mass (n=14).

Multivariate and univariate analyses identified significant and concordant changes of oxylipin profiles according to the muscle status. Of note, 90% of the most discriminant oxylipins were derived from EPA and DHA and were increased in the sarcopenic subjects. The oxylipins signatures of sarcopenic subjects revealed subtle activation of inflammatory resolution pathways, coagulation processes and oxidative stress and the inhibition of angiogenesis. Heat maps highlighted relationships between oxylipins and the cardiometabolic health parameters which were mainly lost in sarcopenic subjects.

This exploratory study supports that targeted metabolomics of oxylipins could provide relevant and subtle characterization of early disturbances associated with muscle-loss during aging.

POSTER 11

MASS SPECTROMETRY BASED PROFILING OF PUFA DERIVED LIPID OXIDATION IN ALZHEIMER'S DISEASE PATIENTS.

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Alzheimer's disease (AD) is a neurodegenerative disease with complex aetiology, where both gene and environmental risk factors may interact to predispose to disease. Due to the high content of polyunsaturated fatty acids (PUFA), brain is highly susceptible to the free radical oxidative damage. Previously, isoprostanes (IsoPs) and neuroprostanes (NeuroPs), non-enzymatically derived from PUFA, were proposed as a good markers of oxidative damage. We hypothesised that brain concentration of PUFA during AD is altered and these metabolites could act as markers for AD. We developed a highly sensitive and robust multiple reaction monitoring (MRM) LC-MS/MS method for the quantification of 24 different IsoPs and NeuroPs.

Lipid fraction was extracted from plasma (200µl) spiked with 1ng of 20 internal standards and 1ng of deuterated standards (d4-4(RS)-4-F4t-NeuroP, d4-10-F4t-NeuroP and d4-10-epi-10-F4t-NeuroP) by adding 1800µl of 100% methanol containing 50µg/ml BHT, followed by alternate vortexing and sonication on ice for 10min. Hydrolysis of phospholipid bound IsoPs and NeuroPs was performed in 0.5 M aqueous KOH for 30 min at 40 °C followed by addition of concentrated HCL (10µl) to quench hydrolysis. IsoPs and NeuroPs were enriched using two-step solid phase extraction column (HLB PRiME, Waters). Analyses were performed on ESI-QqLIT-MS (QTRAP 5500, AB Sciex) operated in a negative ion mode. A reverse phase LC method using a water to methanol gradient in ACQUITY UPLC HSS T3 C18-column was systematically developed for the optimal separation of IsoPs and NeuroPs. Data was processed using Analyst Software (version 1.6.2, AB Sciex). The assay was validated using quality control (QC) plasma samples.

Method validation was performed to establish linearity, sensitivity, recovery, and accuracy.

Extraction recoveries were reproducible consistently > 95% for all authentic and deuterated standards. This assay has a linear dynamic range (R^2 > 0.93) of 0.04ng/ml-20ng/ml for the 24 IsoPs and NeuroPs tested from. The method described has been established for 24 IsoPs and NeuroPs measurement in human plasma and is suitable for studies investigating the PUFA derived lipid oxidation levels in patients with Alzheimer's disease.

POSTER 12

ROLE OF PHOSPHOLIPASE D IN MIGRATION AND INVASION INDUCED BY LINOLEIC ACID IN MDA-MB-231 BREAST CANCER CELLS

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Epidemiological studies and animal models suggest an association between high levels of dietary fat intake and an increased risk of developing breast cancer. The major PUFA in most diets in linoleic acid (LA), which is an omega-6 essential fatty acid that contributes to the developing of chronic diseases. In breast cancer cells, LA induces the expression of plasminogen activator inhibitor-1, cell proliferation, migration and invasion, whereas it induces an epithelial-mesenchymal-transition (EMT)-like process in MCF10A mammary epithelial cells.

Phospholipase D (PLD) regulates various cellular processes such as proliferation, adhesion, survival, apoptosis and tumor transformation. There are mainly two mammalian PLD isoforms, PLD1 and PLD2 that catalyzes the hydrolysis of phosphatidylcholine, the major membrane phospholipid, to form phosphatidic acid (PA) and choline. PA itself is an intracellular lipid second messenger, able to regulate different cell signaling pathways in cancer. In breast cancer, PLD shows frequently elevated expression and activity that correlate with malignant progression.

In the present study, we demonstrated that stimulation of MDA-MB-231 breast cancer cells with LA induced migration, invasion and an increased in the capacity to form tumor spheroids in three-dimensional cultures. Moreover, LA induced an increase in PLD lipase activity and formation of PA, and activation of Akt2 and NFkB transcription factor. The activation of PLD, Akt2 and NFkB, as well as migration and invasion induced by LA are dependent on GPR40 and GPR120, and transactivation of epidermal growth factor receptor. In conclusion, we demonstrated that PLD participates in migration and invasion of MDA-MB-231 human breast cancer cells induced by LA.

POSTER 13

INHIBITION OF SPHK2 ACTIVITY MITIGATES EXPERIMENTAL HYPERTENSION BY REDUCING SYSTEMIC AND VASCULAR INFLAMMATION

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Background: Hypertension (HTN) is a complex condition involving functional and structural alterations of the microvasculature, and an activation of the immune-system. We recently reported a critical contribution of the bioactive phospholipid sphingosine-1-phosphate (S1P) to the pathogenesis of experimental HTN, whereby the activity of its generating enzyme SphK2 crucially affects blood pressure (BP) responses to Angiotensin II (AngII) by regulating S1P plasma levels and hence, T cell mobilization from secondary lymphoid organs (Meissner et al., 2017). Based on these findings, we presently aimed to further investigate the role of SphK2-S1P signaling in HTN-associated inflammatory responses.

Methods: In a murine model of AngII-induced HTN, S1P was quantified by mass spectrometry. Using FACS approaches, we analyzed T-cell populations and assessed T-cell phenotype. To test specific effects of SphK2 inhibition, genetic and pharmacological approaches were applied. In in vitro models of mouse or human T-cells, the effect of SphK2 inhibition on T-cell activation was tested. Standard qPCR, Western blot and immune-histochemistry techniques were applied to analyze S1P signaling components and inflammatory status of the microvasculature.

Results: In contrast to plasma, splenic S1P concentration drastically elevated with AngII treatment only when SphK2 was genetically depleted ultimately, leading to an accumulation of T-cells in secondary lymphoid tissue. Cytokine analysis of these T-cells indicated an overall Th2 phenotype in SphK2 KO mice after AngII treatment, whereas T-cells of hypertensive WT mice predominantly presented a Th17 phenotype. Thus, typical hypertension-associated accumulation of immune-cells in mesenteric arteries and concomitant vascular inflammation was devoid in SphK2 KO mice. Intriguingly, pharmacological inhibition of SphK2 lowered BP and attenuated inflammation in our mouse model. Remarkably, SphK2 inhibition mitigated T-cell activation in vitro as evident by a lower expression of early activation markers (i.e., CD40L).

Conclusion: These results point to a critical contribution of SphK2-S1P signaling in immune-cell responses and vascular inflammation during AngII-induced HTN in mice. Our findings furthermore confirm potential therapeutic properties of SphK2 inhibition in human T cell cultures. Thus, the inhibition of S1P production by antagonizing SphK2 activity might evolve as new therapeutic strategy to efficiently controlling both, BP and HTN-induced vascular inflammation.

POSTER 14

LIPOTOXICITY AND OXIDATIVE STRESS INDUCED BY PALMITATE IN HUMAN MUSCLE CELLS

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Accumulation of saturated fatty acids contributes to lipotoxicity-related skeletal muscle dysfunction. Particularly, oxidative stress has been considered as a trigger of muscle insulin resistance and atrophy. Even though it is well established that reactive oxygen species (ROS) are mainly produced by mitochondria, less is known regarding which are the main source of mitochondrial ROS. Therefore, our study investigated the effects of high level of saturated fatty acid on mitochondrial ROS production and NADPH oxidase expression using human skeletal muscle cells. To do so, myoblast and differentiated human skeletal cells were used at different stages of differentiation. The effect of palmitate (PA-300µM) on cell viability, intracellular lipid accumulation, expression of NADPH oxidase subunits and balance between oxidative stress and antioxidants were determined. PA caused a significant increase in intracellular lipid droplets along with the significant rise in mitochondrial superoxide in myoblast and differentiated myotubes. Our observations were concomitant to increased of NRF2 expression and this targets as catalase and glutathione-disulfide reductase. In summary, these results provide new information regarding PA-induced lipotoxicity in human skeletal muscle cells. Particularly, the PA induced increased superoxide level from mitochondria. Interestingly, antioxidant defenses were also increased. This highlights a protective response from human muscle cells against oxidative insult.

POSTER 15

OMEGA-3 PUFAS AND ISOPROSTANOIDS REGULATE INFLAMMATION IN MICROGLIAL CELLS

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Obesity is an energy balance disorder that increases the susceptibility of developing metabolic diseases and that is characterized by a state of chronic low-grade inflammation. Thus, obesity is as an atypical form of inflammation induced primarily by the accumulation of fatty acids in tissues (liver, adipose tissue, muscle) altering metabolic regulation. This type of inflammation is not limited to peripheral tissues as it extends to the CNS, leading to the development of neuroinflammation. Microglia, the resident immune cells of the brain, represent a novel way to target neuroinflammation in order to potentially mitigate obesity and its health consequences.

In this context, we investigated the impact of PUFAs in microglial cells on markers of inflammation and oxidation. Based on the close link between inflammation and oxidative stress, we focused specifically on non-enzymatic lipid oxidative products.

Our studies could be divided into two parts:

First, in primary microglia cultures under inflammatory conditions induced by LPS, we determined by liquid chromatography-tandem mass spectrometry (LC/MS/MS) the qualitative and quantitative profiles of isoprostanoids in cells and media, on the basis that these metabolites constitute excellent biomarkers of oxidative stress and exhibit also a wide range of bioactivities.

Then, under similar conditions, we evaluated the putative protective effects of PUFAs and some of their oxidized metabolites on LPS-induced inflammatory responses. To this end, we quantified by qPCR and ELISA the expression and secretion of pro-inflammatory cytokines (IL-1β, IL-6, TNFα, MCP-1).

To our knowledge, we demonstrate for the first time that LPS increased the production of oxidized metabolites of EPA and DHA in primary microglial cells. Our results also show that EPA, DHA and oxidized metabolites decrease the expression and secretion of pro-inflammatory cytokines.

POSTER 16

LIPID MEDIATOR FORMATION IN HUMAN MACROPHAGES – ROLE OF LONG-TERM TLR2/4 STIMULATION

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Lipoxygenases (LO) are a family of leukocytic enzymes that catalyse the oxygenation of polyunsaturated fatty acids such as arachidonic, docohexaenoic and eicosapentaenoic acid. The products of these reactions, oxidized lipids, play a central role in various immunological processes such as inflammation initiation, maintenance and resolution. 5-LO derived Leukotrienes (LTs) which are immediately released by neutrophils upon the encounter with various pathogens potently trigger the influx of immune cells into the inflamed tissue by elevation of vascular permeability and immune cell attraction. In contrast, specialized pro-resolving mediators (SPM) such as lipoxins and resolvins which are mainly formed by the sequential action of 5-LO with 12- or 15-LO coordinate the development of an adaptive immune response, inhibit leukocyte influx into the resolving tissue and orchestrate the clearance of apoptotic debris.
Macrophages are versatile cells which easily adapt to changes in the inflammatory milieu and are important regulators of the switch from innate to adaptive immunity. While the formation of pro-inflammatory lipid mediators such as LTs and cyclooxygenase derived prostaglandins is well characterized in different subsets of polarized human macrophages, little is known about the biosynthesis of pro-resolving mediators during the time course of an inflammation. It is well known that pro-inflammatory M1 macrophages mainly express 5-LO, while alternatively activated M2a macrophages, which emerge during the late phase of an inflammatory event, co-express 5- and 15-LOs and should therefore upregulate SPM biosynthesis. Therefore, we investigated LO and cyclooxygenase expression as well as lipid mediator patterns and cytokine profiles in M1 and M2 macrophages. In addition, both macrophage phenotypes where stimulated with TLR ligands. Here we found that M2 macrophages treated with TLR-2 and -4 ligands for a prolonged time develop an anti-inflammatory phenotype.

POSTER 17

ROLE OF MPGES-1-DERIVED PGE2 IN ACTIVATION OF BREAST CANCER STROMAL FIBROBLASTS

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Microsomal prostaglandin E synthase-1 (mPGES-1)-derived PGE2 is a well-known inflammation mediator and contributes to boosting progression of many epithelial tumors. Moreover, mPGES-1 inhibition has been reported to reduce pre-neoplastic lesions. We unexpectedly observed expansion of cancer-associated fibroblasts (CAF) in autochthonous polyomavirus middle T oncogene (PyMT)-induced mammary tumors of mPGES-1$^{-/-}$ mice. Although CAF are conventionally associated with promotion of tumor growth and metastasis, tumor growth in mPGES-1$^{-/-}$ PyMT mice was markedly delayed. Therefore, we asked which role mPGES-1-derived PGE2 plays in shaping stromal compartments in breast cancer, and how this would affect tumor development and metastasis.

To narrow down mPGES-1-dependent PGE2 production to the host, E0771 mammary carcinoma cells were grafted in the mammary fat pad of wildtype (WT) and mPGES-1$^{-/-}$ mice. Again, a delayed tumor progression in mPGES-1$^{-/-}$ mice and an accumulation of CAF was observed when compared to the WT. Moreover, lymphocyte infiltrates in tumors growing in mPGES-1$^{-/-}$ mice were enhanced, correlating with restricted tumor growth. Importantly, despite reduced tumor growth at the primary site, we observed enhanced metastasis in mPGES-1$^{-/-}$ mice. In vitro, PGE2 restricted the TGF-β-induced expression of CAF markers and function in cultured mammary fibroblasts. PGE2 signals predominantly through four prostanoid receptors (EP1-EP4). Mammary gland fibroblasts mainly express EP1 and EP3. mPGES-1$^{-/-}$ deficient mammary glands showed enhanced fibroblast numbers at the baseline. This baseline fibroblast expansion, together with increased collagen-1 expression, was reproduced in EP3, but not EP1, EP2 and EP4-deficient mammary glands. We therefore asked whether EP3 signaling was the main regulator of CAF expansion and activation in mammary tumors. Therefore, WT mice were co-grafted with E0771 mammary carcinoma cells and mammary fibroblasts from WT or EP3$^{-/-}$ mice. Mice that were co-transplanted with E0771 and EP3$^{-/-}$ fibroblasts largely recapitulated the phenotype observed in mPGES-1$^{-/-}$ mice that were grafted with only E0771 cells.

In conclusion, (mPGES-1)-derived PGE2 signals through EP3 in mammary fibroblasts to restrict their expansion and to promote tumor growth at the primary site, but at the same time limiting metastasis. These data need to be considered when targeting mPGES-1 or EP3 in cancer patients is envisioned.

POSTER 18

VALIDATION OF HPLC-MS/MS METHOD FOR OXYLIPINES PROFILING IN DIFFERENT CELL CULTURES

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Oxylipins, including eicosanoids, affect a broad range of biological processes, such as the initiation and resolution of inflammation. These compounds, also referred to as lipid mediators, are (non-) enzymatically generated by oxidation of polyunsaturated fatty acids such as arachidonic acid (AA) but being produced locally through specific biosynthetic pathways in response to extracellular stimuli. Meanwhile, the study of the processes of inflammation in cell cultures has a wide range of practical applications making the development of generic analytical methods for the detection and quantification of lipid mediators challenging. Herein we describe a sensitive targeted analysis platform on the basis of high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) operated in multiple reaction monitoring (MRM) mode for detection and quantification of oxylipins in cell cultures. Besides AA metabolites, the approach covers oxylipins derived from li noleic, dihomo-γ-linolenic, α-linolenic, eicosapentaenoic and docosahexaenoic acids. The work was carried out using a Shimadzu 8040 triple quadrupole mass spectrometer equipped with a Nexera ultra-HPLC separation system (Phenomenex C8 chromatographic column (2.1×150 mm×2.6 µm) was used. The flow rate was 0.4 ml/min. The temperature of the auto sampler and column was 5°C and 40°C, respectively. The volume of injection was 20 µl. Mobile phases, 0.1% of formic acid and acetonitrile-MS grade were used). Detection of oxylipins was carried out using both positive and negative modes in combination with MRM. The use of the HPLC-MS/MS preset system parameters included in the Shimadzu lipid mediator method package Ver.2, simplifies the process allowing to obtain optimal chromatographic and mass-spectrometric characteristics of the compounds to be determined, which ensure their acceptable separation and high detection sensitivity in complex biological matrices as cell cultures. For quantitation of metabolites, isotope dilution method was used. The resulting data of preliminary experiments were useful in the selection of optimal extraction conditions (LL, SPE) of the studied metabolites from complex matrices of cell cultures. Validation was carried out and important parameters were established for precisioness, correctness, linearity, limit of detection, etc.). This work has been prepared with the support of the «RUDN University Program 5-100».

POSTER 19

STIMULATORY EFFECTS OF C. ALBICANS ON 5-LOX ACTIVITY IN HUMAN IMMUNE CELLS

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Candida albicans is one of the most life-threatening opportunistic fungal pathogens. As a benign colonizer in healthy individuals it causes superficial mycoses of skin and mucous membranes. However, in immunosuppressed patients, C. albicans can induce nosocomial blood-stream infections that are associated with a mortality rate of ~ 40%. Unfortunately, only a small number of effective anti-Candida drugs is currently licensed and fungal pathogenicity mechanisms as well as evading strategies in humans are still only partially understood. Inflammatory processes are substantial defence mechanisms mediated by immune cells to eradicate the pathogen and to restore homeostasis. Within the inflammatory reponse, 5-lipoxygenase (5-LOX) converts arachidonic acid (AA) to potent pro-inflammatory leukotrienes (LT), which play a key role in the host immune defence strategy. We therefore raised the question whether C. albicans is able to activate 5-LOX along with LT biosynthesis and thus to stimulate the host immune defence. C. albicans grows in distinct morphological forms, as yeast and hyphae. The latter is associated with an increased virulence. Here we have investigated the effects of the different morphological forms and their potentially released virulence factors on 5-LOX activation and LT formation in human immune cells. In order to generate LTs, cytosolic 5-LOX needs to form a biosynthetic complex with the 5-LOX-activating protein (FLAP) at the nuclear membrane. We found that C. albicans germ tubes, which display the morphological state of pseudohyphae, induced 5-LOX redistribution at the nuclear membrane in neutrophils, while conditioned medium and C. albicans yeast cells were insufficient to cause this effect. These findings are in accordance with an increase of intracellular Ca^{2+} levels in neutrophils challenged by germ tubes, whereas yeast cells or conditioned medium failed to cause Ca^{2+} mobilization. In a cell integrity assay, LDH-release of neutrophils treated with germ tubes was comparable to the LDH released in unstimulated cells, which excludes C. albicans-induced cell lysis as 5-LOX activation mechanism. Conclusively, we demonstrate that the virulent morphological state (hyphae) of C. albicans induces an immune response in neutrophils by activation of a Ca^{2+} mediated 5-LOX activation and LT formation.

POSTER 20

SELECTIVE DETERMINATION OF LIPID MEDIATORS AS MARKERS FOR PRO- AND ANTI-INFLAMMATORY PATHWAYS AND RESOLUTION OF INFLAMMATION USING CHIRAL CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Inflammatory processes are regulated by potent signaling molecules derived from polyunsaturated fatty acids such as arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid via discrete enzymatic pathways. In order to better describe the inflammatory response in human plasma a selective and sensitive quantification method for the determination of lipid mediators involved in inflammation has been developed using solid phase extraction for sample cleanup and chiral chromatography coupled with tandem mass spectrometry for detection. The developed method is able to overcome potential common pitfalls resulting in false positive or false negative results such as first and foremost stereo- and constitutional isomerism, but also instability related to temperature, light and pH, as well as matrix effects. Method performance and quality is ensured by full validation according to guidelines by the U.S. Food and Drug Administration. In summary, a single run of 25 min is able to distinguish and quantify 34 lipid mediators in human plasma including inflammatory pathway markers like prostaglandins, thromboxane, lipoxins, resolvins, maresins as well as several precursors of specialized pro-resolving lipid mediators with LLOQ values ranging from 0.1 to 0.2 ng/mL. The dramatically improved separation of isomers outweighs the slightly higher detection limits caused by the chiral chromatography, overall reducing the risk of wrong assignment of analytical identities. The developed and fully validated methodology can be applied to current research questions dealing with resolution of inflammation and chronification of inflammatory diseases.

POSTER 21

A SPECIFIC COMBINED LONG-CHAIN POLYUNSATURATED FATTY ACID SUPPLEMENTATION RESCUED ASTHMA-DYSREGULATED MIRNA-146A-5P

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Background: Specialized pro-resolving mediators (SPM) are well shown to exhibit pro-resolving effects on asthma and are proposed to be involved in the regulation of miRNA expression. However, little is known about the modulative capacity of omega-(n)-3 and n-6 long-chain polyunsaturated fatty acids (LCPUFA) as precursors of endogenously biosynthesized lipid mediator derivates and their effects on miRNAs.

Objective: Therefore, we investigated whether a specific combined (sc)-LCPUFA supplementation strategy, containing eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), γ –linolenic and stearidonic acid, can influence miRNA profiles in murine house dust mite (HDM)- induced asthma. Thus, we investigated how miRNA profiles are altered in allergic asthma and whether the sc-LCPUFA supplementation is able to rescue dysregulated miRNA expression.

Methods: miRNA sequencing from lung tissues of asthmatic and control mice with normal diet, as well as of sc-LCPUFA supplemented mice was performed using the Illumina MiSeq platform. Moreover, LCPUFA and lipid mediators were determined by capillary gas chromatography (C-GC) or mass spectrometry (LCMS), respectively.

Results: A total of 62 miRNAs were significantly dysregulated in murine HDM-induced allergic asthma. Sc-LCPUFA supplementation normalized 21 of these dysregulated miRNAs surviving correction for multiple testing. Specifically, mmu-miR146a-5p, a counter regulator of NF-κB, COX-2 and pro-inflammatory cytokines was significantly rescued by sc-LCPUFA supplementation in asthmatic mice. Furthermore, sc-LCPUFA supplementation induced the expression of miRNAs, such as mmu-miR-141-3p and mmu-miR-22-3p, described to be potential therapeutic miRNAs in inflammation. The C-GC and LCMS analysis i) confirmed the incorporation of EPA, n-3 docosapentaenoic acid (DPA) and DHA, ii) the increased biosynthesis of distinct SPMs as well as iii) a significant reduction of pro-inflammatory mediators in lung tissue.

Conclusion: These results demonstrate the modulative capacity of LCPUFA on miRNA expression and specifically the rescuing potential of the sc-LCPUFA supplementation on mmu-miR-146a-5p in the HDM mouse model for asthma.

POSTER 22

EVALUATION OF THE BRAIN DISTRIBUTION OF OLEOYLETHANOLAMIDE AND ITS ANALOGUES AFTER ACUTE SYSTEMIC ADMINISTRATION IN RATS

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The gut-derived satiety signal oleoylethanolamide (OEA), which belongs to the family of N-acylethanolamides is a potent activator of the alpha type peroxisome proliferator-activated receptor (PPAR-alpha) and is able to inhibit food intake and control lipid homeostasis both in rodents and humans. The hypophagic action of OEA has been associated to the activation of selected brain areas, although how the OEA signal reaches the brain remained to be fully elucidated. Recent findings demonstrated a necessary role of the area postrema (AP), a circumventricular organ with a weak blood brain barrier (BBB) and a high density of PPAR-alpha receptors, for i.p. injected OEA to reduce eating, suggesting that this brain area could represent a receptive region for circulating OEA.
To test this hypothesis, male rats were sacrificed at different time points (2.5, 5, 15, 30, 60 minutes) after acute administration of OEA (10 mg kg^{-1}, i.p.); plasma and different brain areas (AP, median eminence, nucleus of the solitary tract, ventral and dorsal hippocampus) were microdissected and N-acylethanolamines, including OEA, were extracted and measured by HPLC-MS. Vehicle-treated animals were used as controls.
Our results revealed that OEA was able to permeate all the brain areas analysed as early as 5 minutes after its systemic administration. OEA levels resulted considerably higher in OEA than in vehicle-treated animals at 15 minutes in all the brain areas studied and particularly in the AP, were it reached the highest increase. In accordance, plasma OEA levels increased as early as 5 minutes after i.p. administration with a maximum increase registered at 15-30 minutes. OEA administration did not significantly affect the levels of other N-acylethanolamines in the brain, whereas in the plasma we observed an increase of linoleoylethanolamide, palmitoylethanolamide and stearoylethanolamide.
Overall these findings suggested that OEA is able to reach the BBB as an intact molecule within few minutes after i.p administration. Furthermore our data indicated that OEA has a very extensive brain penetration capability, and its effect on feeding behavior following systemic administration may be mediated by either the brain regions in close proximity to the circumventricular organs or sites outside of the BBB, including AP.

POSTER 23

STAPHYLOCOCCUS AUREUS-DERIVED PHENOLE SOLUBLE MODULINS INDUCE LIPID MEDIATOR FORMATION IN NEUTROPHILS

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Staphylococcus aureus is a human pathogen that can cause severe invasive diseases accompanied by vast inflammatory processes including neutrophil activation and leukotriene (LT) formation. LTs are generated from arachidonic acid (AA) by the 5-lipoxygenase (5-LOX) pathway. Beside Ca^{2+} mobilization, LT formation requires 5-LOX translocation from the cytosol to the nuclear membrane where it forms a LT-biosynthetic complex with the 5-LOX-activating protein (FLAP). Recently, we showed that pathogenic bacteria induce lipid mediator formation (including LTs) in human macrophage phenotypes with distinct pattern towards pro- and anti-inflammatory properties [1]. Here we present that not only intact pathogenic bacteria, but also secreted soluble molecules from S. aureus induce 5-LOX activation in human neutrophils and in HEK293 cells stably co-expressing 5-LOX and FLAP. Cell-free conditioned medium of S. aureus mutant strains deficient in virulent exotoxin production failed to activate 5-LOX. In HEK cells expressing 5-LOX and FLAP cells, two types of exotoxins released from S. aureus activated 5-LOX: (i) the pore-forming toxin α-hemolysin and (ii) the amphipathic α-helical phenol-soluble modulin (PSM) peptides. Surprisingly, in neutrophils, only PSMs were able to activate 5-LOX along with LT formation, which strongly correlated with elevated intracellular Ca^{2+} levels. These results support the proposed roles of LTs in the combat of immune cells with pathogenic bacteria and highlight PSMs of S. aureus as bacteria-derived elicitors of LT biosynthesis neutrophils.

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POSTER 24

CHARACTERIZATION OF ESTERIFIED LIPID MEDIATORS, A BIOSYNTHETIC APPROACH

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Eicosanoids are an important class of lipid mediators including prostaglandins, leukotrienes and hydroperoxyl fatty acids that are formed in the arachidonic acid cascade. These mediators play a crucial role in a variety of physiological and pathophysiological processes like allergic reactions, inflammation and tumor diseases1-3. They were commonly believed to mediate their signalling actions as free acids while newer studies indicate that they also occur attached to phospholipids or glycerol. Although the signalling actions of phospholipids are well studied today, only few information is available on their esterified counterparts. First studies demonstrate that they seem to be involved in the regulation of immune reactions and coagulation4, which might be a promising starting point for the development of new drugs.

Thus, the need to investigate this new class of mediators, where a major part, concerning the effects of several esterified lipids together with their mode of action, is unknown4, arises. Unfortunately, when it comes to study these lipid mediators as well as their signalling actions, one has to overcome the fact that these compounds are either quite expensive or that they are not commercially available at all.

Therefore, we work on establishing a biosynthetic approach including recombinant versions of the human enzymes long-chain acyl CoA synthetase 4 (ACSL4) and lysophosphatidylcholine acyltransferase 2 (LPCAT2). With this approach, polyunsaturated fatty acids can be activated by ACSL4 via conversion into CoA esters and then transferred by LPCAT2 to lyso-phospholipids in vitro. This allows a highly flexible synthesis of a large variety of lipids in order to facilitate the investigation of esterified lipid mediators in a more cost-efficient manner.

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POSTER 25

EFFECT OF THREE-DIMENSIONAL (3D) CELL CULTURE ON 5-LIPOXYGENASE EXPRESSION AND ACTIVITY IN COLON CANCER CELLS

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5-lipoxygenase (5-LO) is mainly expressed in leukocytes and catalyzes the first two steps in leukotriene (LT) biosynthesis. Its enzymatic activity is embedded in a complicated network regulating LT synthesis by various factors dependent on the cell type and nature of the stimulus in intact cells. 5-LO overexpression is also well documented in colon cancer where it is correlated with poor prognosis regarding patient survival. In addition, 5-LO products such as LTs were shown to promote the proliferation of tumour cells. Yet, the exact role of 5-LO in cancer development and tumour cell survival remains somewhat elusive.

A systematic approach dealing with 5-LO expression and LT biosynthesis under 3D cell culture has never been investigated. But, cancer development and progression are strongly dependent on the tumour microenvironment. In two-dimensional (2D) culture of cancer cell lines, a system where cells are grown as monolayers, cell-matrix and cell-cell interactions present in native tumours are lacking. Here, cells develop artificial polarity due to cytoskeletal rearrangements as a cause of layer growth. In addition, absence of factors such as hypoxia, nutrient deprivation and accumulation of metabolic waste can change gene expression substantially. In contrast, 3D culture systems mimic the in vivo situation much closer.

In the present study we aimed at investigating the expression of proteins involved in LT biosynthesis as well as LT formation in spheroids of the 5-LO overexpressing colon cancer cell lines HT-29 and HCT-116. In addition, 5-LO phosphorylation and cytokine release was measured. Furthermore, the influence of hypoxia, nutrient deprivation and metabolic waste accumulation was investigated in 2D cell culture of the cells.

POSTER 26

CROHN'S DISEASE AND ULCERATIVE COLITIS ALTER OXYSTEROL LEVELS AND METABOLISM IN THE COLON

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Inflammatory bowel diseases (IBD) represent a challenging health issue with a complex etiology implicating, among others, genetic and environmental factors. In this context, bioactive lipids, more specifically oxysterols, represent an interesting avenue to investigate. Indeed, oxysterols or their receptors are involved in inflammation and immune regulation. Moreover, several oxysterol receptors such as LXR and ROR, have been implicated in IBD. However, less is known about the effects of oxysterols in IBD or the impact of colon inflammation on oxysterol levels.

We have already shown, using several mouse models, that colitis profoundly affects oxysterol levels, not only in the colon, but also in the liver and plasma. We identified some oxysterols and metabolic pathways that were consistently altered and we further found correlations between oxysterol levels and inflammatory markers expression.

Here, we set out to study the oxysterome in IBD patients. We used colon biopsies from patients with Crohn's disease (CD) or ulcerative colitis (UC) as well as control biopsies. Oxysterol levels were quantified by HPLC-MS and mRNA expression of the enzymes implicated in oxysterol metabolism was measured by RT-qPCR.

We found that the oxysterome is altered by IBD in human biopsies. Interestingly, two of the oxysterols quantified, 4beta-hydroxycholesterol and 25-hydroxycholesterol that were consistently altered in several mouse models of colitis, were also profoundly affected by IBD in human colon. Indeed, 4beta-hydroxycholesterol levels were decreased in the colon of CD and UC patients compared to control subjects and this was also accompanied by a decrease in the expression of CYP3A4 and CYP3A5, the enzymes responsible for its synthesis. Conversely, 25-hydroxycholesterol levels were increased in the colon of CD and UC patients.

Furthermore, some alterations in colon oxysterol metabolism display specific variations according to the type of IBD considered. For instance, we found that the expression of cholesterol-25-hydroxylase and CYP7A1 was only altered in UC biopsies.

These data further support the interest of studying the metabolism of oxysterols in colon inflammation as well as their implication in the pathophysiology of IBD.

POSTER 27

THE SENSITIVITY OF CHOLINE INCLUSIVE LIPIDS OF RAT BRAIN AND KIDNEY CHROMATIN TO CISPLATIN IN VIVO ACTION

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Taking into consideration the regulatory role of chromatin phospholipids, their implication in various processes such as DNA replication, transcription, chromatin assembly, acetylation and methylation of histones and in other distinct nuclear processes as well as the concentration depending disposition of lipid caused regulatory effects, the special interest are rendered to the interdependent alterations of two choline inclusive chromatin lipids after the cisplatin action.

The results of our investigations have revealed the significant alterations in absolute quantity of phospholipid fractions of chromatin preparations from rat brain and kidney cells after the antitumor drug cisplatin in vivo action. The ratio of sphingomyelin and phosphatidylcholine absolute contents for chromatin preparations of rat brain and kidney cells in baseline and after the cisplatin 24h. in vivo action have been calculated as well. These results confirm that cisplatin action caused decrease of two choline inclusive lipids absolute quantity in chromatin preparation from rat brain and kidney cells. Therefore, the decreasing effect of cisplatin was more significant in case of chromatin from rat kidney cells phosphatidylcholine. That is why the ratio of sphingomyelin:phosphatidylcholine was increased up to 15% in chromatin preparation from rat kidney cells, while this ratio remains invariable after the cisplatin treatment in case of chromatin from rat brain cells. Thus, the results confirm that cisplatin action shift the crosstalk between choline inclusive lipids in sphingomyelin direction, that in turn may lead to apoptose.

These alterations in content of choline inclusive lipids in chromatin preparations caused by cisplatin action likely are connected with the antitumor effects of this drug.

POSTER 28

LC-MS/MS BASED ANALYSIS OF CYCLOOXYGENASE 2 EXPRESSION AND ACTIVITY

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Eicosanoids and other oxylipins are formed in the arachidonic acid (ARA) cascade via three main enzymatic pathways, cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) enzymes. These important lipid mediators play a major role in the regulation of many physiological functions, e.g. intracellular signaling, inflammation and pain.

The investigation of oxylipin formation is dominantly carried out by LC-MS/MS, directly quantifying lipid mediator concentrations. This approach allows analyzing changes in the oxylipin pattern but only little information regarding the responsible mechanisms is obtained. As e.g. reduced oxylipin levels may result from direct enzyme inhibition or decreased expression, quantification of enzyme expression levels is additionally required to fully understand molecular mechanisms.

Here, an LC-MS/MS method was developed for the targeted analysis of COX-2 enzyme expression, accompanying an established targeted oxylipin method and thus allowing comprehensive monitoring of the COX-2 branch of the ARA cascade.

Expression levels of COX-2 are measured via unique peptides (“proteotypic peptides”, PTP), arising from tryptic digestion of the target enzyme. For selection of PTPs, in silico digestion was performed beforehand and different databases and prediction platforms were considered to avoid peptides containing sites of natural variants, e.g. nonsynonymous single nucleotide polymorphisms (nsSNPs), and post-translational modifications. Also, peptides predicted with reduced cleavage probability were excluded, as well as peptides containing chemically unstable residues such as tryptophan or asparagine, as far as possible.

For sample preparation, the protein fraction is extracted from cells using a denaturing lysis buffer and sonication. Before performing tryptic digestion, unfolding of the proteins is mediated by a chaotropic reagent, intramolecular disulfide bridges are reduced and subsequently alkylated with iodacetamide (IAA). After desalting the samples, the peptides are chromatographically separated on an RP18 column and measured on a QTRAP instrument following positive ionization in scheduled multiple reaction monitoring (sMRM) as well as MS^3 mode, allowing sensitive detection in the low ng/mL range. Three peptides were carefully optimized and used for quantitation.

The method was applied on the investigation of COX-2 expression in different human cell lines incubated with and without inhibitors, and the enzyme concentration is correlated with the COX-2 activity determined based on oxylipin formation.

POSTER 29

COMPREHENSIVE TRACKING OF LIPID MEDIATOR REPROGRAMMING IN TYPE 2 IMMUNE SETTINGS REVEALS MACROPHAGE EICOSANOID PLASTICITY DURING ALLERGEN EXPOSURE

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Background: Eicosanoid lipid mediators play key roles in allergy and asthma. Macrophages represent major source cells of these mediators, but the complex and dynamic eicosanoid output of these cells during type 2 immune responses is not understood.
Objective: We aimed to comprehensively track lipid mediator production in type 2 immune responses.
Methods: We established an LC-MS/MS workflow for the quantification of 52 oxylipins to track lipid mediator reprogramming in human monocyte derived macrophages (MDM) during exposure to house dust mite (HDM) or during nematode infection in vivo. Expression of eicosanoid enzymes was studied by qPCR and western blot and cytokine production was assessed by multiplex cytokine assays.
Results: Differentiation of macrophages with GM-CSF and TGFβ1 resulted in a phenotype ("aMDM") with characteristic features of airway macrophages such as high expression of 5-lipoxygenase (5-LOX), which resisted IL-4-mediated transcriptional repression. Exposure of aMDM to HDM resulted in the suppression of 5-LOX expression and product formation. In contrast, HDM triggered an increased prostanoid production with thromboxane and prostaglandins D2 and E2 as major metabolites. HDM also induced pro-inflammatory cytokines and chemokines, resulting in an overall M1-like mediator profile. Finally, distinct changes in lipid mediator profiles occurred during the type 2 immune response to nematodes in vivo.
Conclusion: Our findings show that type 2 immune responses are characterized by a fundamental reprogramming of the lipid mediator metabolism with macrophages representing particularly plastic responder cells. Targeting mediator reprogramming in airway macrophages may represent a viable approach to regulate pathogenic lipid mediator profiles in allergy or asthma.

POSTER 30

THE ROLE OF PEDF AND ITS RECEPTOR IN DHA-DEPLETED (ELOVL2 KO) MICE.

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Pigment epitelium-derived factor (PEDF) is a secreted 50-kDa polypeptide with established neuroprotective, and neurotrophic activities. Neural retinal and CNS cells have demonstrated binding sites for PEDF on their extracellular surfaces, therefore, a ligand-cell-surface receptor mechanism for PEDF activity has been proposed and demonstrated. The polypeptide product of the pnpla2 gene, Adipose Trygliceride Lipase (ATGL), had been identified as a binding partner for PEDF and suggested as the main PEDF's neurotrophic/neuroprotective receptor (PEDF-R). The PEDF-R has phospholipase activity, which can potentially hydrolyze biologically-active fatty acids (e.g. n-3 and n-6 PUFAs, particularly enriched in retina and CNS from the plasma membrane phospholipids. We are using Elovl2 knock-out mice, which have decreased DHA synthesis and incorporation in membranes, compared to wild type mice, as a model organism demonstrate the role of DHA (and other n-3 and n-6 fatty acids in the same pathway) in the molecular signaling mechanism deriving by PEDF binding to its receptor. Our results show that PEDF-R is upregulated, both at the mRNA and protein levels, in aged DHA-depleted (Elovl2 KO) mice. Further, lipid analysis in retinal and brain tissues, confirmed that DHA was significantly decreased in these mice when compared to WT.
These data suggest a functional correlation between the expression and activity of Elovl2 and the action of PEDF/PEDF-R, and provide a strong evidence that they are part of the same pathway.

POSTER 31

PHYLOGENETIC INSIGHTS INTO THE REACTION SPECIFICITY OF 15-LIPOXYGENASE ORTHOLOGS (ALOX15) OF DIFFERENT MAMMALIAN SPECIES

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ALOX15 orthologs have been implicated in maturational degradation of intracellular organelles and in the biosynthesis of anti-inflammatory and pro-resolving eicosanoids. We previously hypothesized, that lower mammals (mice, rats, pigs, cattle) express 12-lipoxygenating ALOX15 orthologs. In contrast, 15-lipoxygenating isoforms are found in highly developed primates (orangutans, chimpanzee, men) and these data suggested an evolution of ALOX15 specificity. To put this hypothesis on a broader experimental basis, we first extracted the ALOX15 sequences of different mammals from the NCBI and ENSEMBL protein databases and identified the triad determinants by amino acid sequence alignment with human ALOX15. Care was taken to include representatives of evolutionary more ancient Prototheria (monothremes) but also representatives of highly developed Metatheria (marsupials) and Eutheria (scandentia, primates, rodents, dermoptera, lagomorphs). Our database searches indicated that for mo notremes no complete ALOX15 sequence was available and thus, no functional data for ALOX15 specificity could be obtained. In contrast, complete ALOX15 sequences could be extracted for Metatheria (opossum) and Eutheria (marmoset, philippine tarsier, bushbaby, sunda flying lemur, Ord's kangaroo rat) and the sequence alignments suggested 12-lipoxygenating ALOX15 orthologs for most of them. Next we expressed selected ALOX15 orthologs as recombinant proteins and confirmed their reaction specificity. The ALOX15 orthologs of highly developed primates (men, H. neanderthalensis, H. denisovan, chimpanzee, orangutan, gorilla) express 15-lipoxygenating ALOX15 orthologs and these data are consistent with the Evolutionary Hypothesis of ALOX15 specificity. Gibbons express an ALOX15 ortholog, which exhibits a pronounced dual reaction specificity. Thus in ALOX15 evolution this protein represents a transition enzyme. However, among the more than 100 mammalian ALOX15 orthologs we analyzed, there were just two exceptions. The ALOX15 orthologs of rabbit and kangaroo rats express 15-lipoxygenating ALOX15 orthologs, although these mammals are ranked below gibbons in mammalian evolution. The lipoxin synthase activities of 15- compared to 12-lipoxygenating ALOX15 variants were more than 5-fold higher ($p < 0.01$), suggesting an evolution of ALOX15 specificity, which is aimed at optimizing the biosynthetic capacity for lipoxins.

POSTER 32

FOOD POLYPHENOLS INHIBIT THE CYP-DEPENDENT FORMATION OF EPOXY AND HYDROXY FATTY ACIDS IN VITRO

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Polyphenols (a large group of secondary plant metabolites) can lead to food-drug-interactions, causing side effects of pharmaceuticals by modulation of cytochrome-P450-enzyme (CYP) activity. One of the best known examples is the flavonoid naringenin: by an inhibition of CYP3A4, resulting in a prolonged half-life of several drugs, this polyphenol contained in grapefruit can lead to toxicity of drugs predominantly metabolized by this CYP isoform. However, the effects of food ingredients on CYP families involved in the metabolism of endogenous substrates like fatty acids (FA) have been sparsely investigated so far.

In the third branch of the arachidonic acid cascade, CYP catalyze the formation of epoxy and hydroxy fatty acids from arachidonic acid (ARA) and other polyunsaturated fatty acids. The resulting oxylipins have important functions in human physiology, for example in the regulation of inflammatory processes, renal functions and angiogenesis. Regarding the vascular tone, different metabolites show ambivalent effects: while epoxy-FA, primarily formed by CYP2J and CYP2C, show vasodilatory effects, the omega-hydroxylation-product of ARA (20-HETE), formed by CYP4A or CYP4F, acts vasoconstrictive.

We investigated the effects of food polyphenols on the third branch of the ARA cascade utilizing microsomes from different tissues as well as individual CYP isoforms. The metabolites were extracted by liquid-liquid-extraction and the formation of CYP products was quantitatively determined by means of RP LC ESI(-)-MS/MS. The method covered all ARA derived epoxy-FA and their hydrolysis products, dihydroxy-FA, as well as terminal, subterminal and mid-chain hydroxylation products.

Based on the obtained product patterns of several PUFA-oxidizing CYP and human liver microsomes, inhibitory effects of a library of selected polyphenols, covering different flavonoids, stilbenoids and their oligomers, were examined. A comparison of the effects to those of well described specific (17-ODYA, MS-PPOH) or non-specific (imidazole derivatives) CYP inhibitors allowed an evaluation of the potency of the secondary plant metabolites. We could show that several polyphenols inhibit the CYP dependent oxidation of ARA with same potency compared to the known inhibitors. Furthermore, selectivity regarding CYP isoforms was observed: the flavone apigenin inhibits the formation of epoxy- and hydroxy-FA, including 20-HETE. The latter occurs by inhibition of CYP4F2, while no effect on CYP4A11 activity was observed. The structurally related isoflavone genistein inhibits the formation of 20-HETE as well, however has little effect on the formation of other oxylipins.

In the presentation the inhibitory effects of the test compounds are characterized by the modulation of the overall CYP product pattern and the results are discussed with respect to potential effects on human physiology and health.

POSTER 33

EFFECT OF PARENTERAL OMEGA-3 FATTY ACID SUPPLEMENTATION ON ENDOGENOUS FATTY ACID AND OXYLIPIN PROFILES IN PATIENTS WITH CHRONIC INTESTINAL FAILURE

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Long-term parenteral nutrition (PN) of chronic intestinal failure (CIF) patients bears an increased risk of intestinal failure associated liver disease (IFALD). Long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFA) attracted attention for preventing IFALD. However, the relative impact and mechanisms of this approach have not been fully established yet, as required for recommending general use of n-3 LC-PUFA containing PN solutions in CIF patients.

CIF patients permanently (1 - 174 months) received either PN without (n = 13) or with (n = 33) fish oil n-3 LC-PUFAs (cross-sectional study). The fatty acid (FA) profile of red blood cells (RBC) yielded the HS-Omega-3 Index® (OM3I = (EPA + DHA) / total FA in %). Free and total plasma oxylipins were analyzed by LC-MS/MS.

The OM3I ranged from 3.3 to 14.9 % in the cross-sectional study, with means of 5.6 ± 1.4 % and 11.0 ± 2.7 % in the subgroups without and with n-3 LC-PUFA supplementation. The OM3I positively correlated with fish oil–dosage ($p < 0.0001$, $r = 0.74$; dose range: 0 – 1.6 g/kg BW/week). Liver transaminases did not correlate with the OM3I, but albumin and pseudocholinesterase ($r > 0.46$, $p < 0.005$) and leucocyte count ($r = -0.29$, $p = 0.047$) did. Overall, prominent oxylipins that increased upon fish oil supplementation included the CYP epoxygenase products of EPA (17.18-EEQ) and DHA (19.20-EDP) as well as 18-HEPE and 17-HDHA, whose plasma levels linearly correlated with the availability of their precursor FA. In contrast, increased formation of EPA-derived COX metabolites (PGE3 or TXB3) was only detectable in a few patients. All patients showed relatively high plasma levels of cis-EODA (13 ± 4 ng/ml), a monoepoxide derived from oleic acid. The formation of cis-EODA did not correlate with the relative content of its precursor FA (13 – 20 % of total RBC FAs), interestingly, however, with a functional test (LiMAx), reflecting liver CYP1A2 activity ($p = 0.014$, $r = 0.38$).

Fish oil supplementation in PN formulations dose-dependently and highly efficiently increases endogenous EPA / DHA levels and antiinflammatory oxylipins, which stabilize liver function tests and thus may protect against the development of IFALD.

POSTER 34

EXTRACTION OF LIPIDS AND OXYLIPINS FROM PLASMA FOR QUANTIFICATION OF TOTAL OXYLIPINS – CHALLENGES AND STRATEGIES

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Several eicosanoids and other oxylipins are potent lipid mediators which are involved in the regulation of 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, e.g. in polar lipids.

Only little information is available on the biological activity of these esterified oxylipins. An important step towards a better understanding of their biological role is a comprehensive comparison of the changes in the pattern of free vs. esterified mediators induced by pharmacological intervention or dietary supplementation as well as during onset and progression of diseases.

While several LC-MS based methods for the detection of free oxylipins have been developed, esterified oxylipins are commonly quantified as a sum of free and esterified oxylipins following base hydrolysis. However, different approaches have been described for preparation of samples before hydrolysis and for the solid phase extraction. Here, we present a three-step strategy for the quantification of total oxylipins including extraction of total lipids, saponification to liberate esterified oxylipins and solid phase extraction of free oxylipins. We thoroughly investigated different liquid-liquid extraction procedures and protein precipitation in terms of extraction efficiency for various lipid classes from plasma. Moreover, to optimize sample throughout we compared extraction of free oxylipins via solid phase extraction on cartridges with 96-well plates.

In conclusion, our results emphasis the challenges related to the extraction procedures and provide different strategies for reliable oxylipin quantification with a focus on efficient and reproducible extraction.

POSTER 35

ENDOGENOUS METABOLITES OF VITAMIN E LIMIT INFLAMMATION BY TARGETING 5-LIPOXYGENASE

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Vitamin E is essential for immune functions and protects against diseases with an inflammatory component. While the fat-soluble vitamin has long been viewed as a mere antioxidant, non-redox mechanisms have recently moved into research focus, and oxygenated metabolites have been proposed as signalling molecules. However, the mode of action and physiological targets of these metabolites are unknown. Hence, we constructed a library of long-chain vitamin E derivatives that encompasses 20 metabolites with potential physiological relevance in humans as well as 55 semi-synthetic analogues. From this in-house library, we identified omega-hydroxyls and omega-carboxylates as high-affinity inhibitors of 5-lipoxygenase (5-LO) - a key enzyme in the biosynthesis of chemoattractant and vasoactive lipid mediators. The lead compound delta-trans-tocotrienolic acid (so-called garcinoic acid) potently and selectively inhibited 5-LO (IC50 = 35 nM) in a reversible and substrate concentration-ind ependent manner by allosterically targeting a so far unexploited cavity of the enzyme, as proposed by molecular docking studies and confirmed by pull-down assays and site-directed mutagenesis [1]. Another hit compound from our screening, 13-((2R)-6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)-2,6,10-trimethyltridecanoic acid (alpha-T-13'-COOH), was biosynthesized from alpha-tocopherol in a biochip-based human liver organoid and was detected in human and mouse plasma at concentrations (9 to 49 nM) that inhibited 5-LO in human leukocytes. This physiological metabolite of vitamin E accumulates at sites of inflammation and essentially contributes to the anti-inflammatory properties of vitamin E in mouse models of acute peritonitis and asthma through selective inhibition of 5-LO. Further structural optimization of vitamin E metabolites yielded orally active derivatives that inhibit leukotriene formation in human leukocytes with an IC50 ≥ 19 nM [2] and show favorable efficacy and selectivity in vitro and in vivo. To sum up, our data suggest that the well-recognized immune functions and protection against inflammation by α-tocopherol critically depend on its metabolic conversion to the endogenous metabolite α-T-13'-COOH that inhibits 5-LO as high-affinity target.

[1] Pein et al., Nat. Commun. 2018, in revision [2] Richomme et al., 2017, WO A1 2017032881

POSTER 36

OPTIMIZED QUANTIFICATION OF SPECIALIZED PRO-RESOLVING MEDIATORS IN BIOLOGICAL SAMPLES BY MEANS OF LC-MS

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The intake of long chain omega-3 polyunsaturated fatty acids (n3 PUFA) is associated with positive effects on human health, which are believed to be – at least in part – mediated by the oxygenated metabolites (oxylipins) of these essential fatty acids. In the last two decades interest in a novel group of autacoids termed specialized pro-resolving mediators (SPMs) increased. SPMs are di- or tri-hydroxylated fatty acids, including resolvins, maresins and protectins derived from n3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as lipoxins derived from EPA and arachidonic acid (ARA). This class of substances is actively involved in the resolution of inflammation and might contribute to the anti-inflammatory effects of n3 PUFA. However, as levels of these potent mediators in blood and tissue of healthy subjects are low, quantitative analysis is challenging.

We developed an LC-MS method for a comprehensive set of 18 SPMs derived from ARA, EPA and DHA, which was integrated in our targeted metabolomics platform for the simultaneous quantification of enzymatically and autoxidatively formed oxylipins (doi.org/10.1016/j.aca.2017.11.002). Quantification is carried out based on external calibration utilizing five deuterated internal standards in combination with a second internal standard for quality assessment of each sample. The tandem mass spectrometric parameters were carefully optimized in order to ensure sensitive and specific detection and the influence of source parameters of the used AB Sciex 6500 QTRAP instrument, such as probe position, temperature and source gas as well as electronic parameters and the selection of transitions are discussed. The method was validated/characterized oriented at the EMA guideline on bioanalytical method validation and method performance is demonstrated regarding extraction efficacy of SPMs in spiked plasma (0.1 nM to 3 nM) and ion suppression. The method was applied on plasma and serum samples from healthy human subjects as well as on clinically relevant human samples from patients with and without septic shock or peritonitis.

POSTER 37

CHALLENGES IN ABSOLUTE QUANTIFICATION OF OXYLIPINS - A STRATEGY FOR THE PREPARATION OF STANDARD SERIES AND VERIFICATION OF CONCENTRATIONS

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Oxygenated metabolites of polyunsaturated fatty acids (PUFA), i.e. oxylipins, are formed in the arachidonic acid (ARA) cascade via three enzymatic pathways by cyclooxygenases (COX), lipoxygenases (LOX) or cytochrome P450 monooxygenases (CYP) or by autoxidation. Oxylipins can function as potent lipid mediators that are involved in maintaining the physiological homeostasis in the human body. Moreover, oxylipins derived from different precursor PUFA as well as different pathways can have similar or opposing effects, e.g. the conversion of ARA by COX among others leads to PGE2, which is involved in the regulation of inflammation, fever and pain. The epoxidation of PUFA is catalyzed by CYP leading to e.g. 17(18)-EpETE derived from EPA and 19(20)-EpDPE derived from DHA that act anti-arrhythmic. Furthermore, multiple hydroxylation of EPA and DHA can lead to potent inflammation resolving lipid mediators, such as resolvins, maresins or protectins. In order to investigate the biological effects of oxylipins it is important to analyze and quantify a wide oxylipin pattern, since their effects do not result from the modulation of one metabolite but rather crosstalk between the pathways.

A major challenge in absolute quantification is to characterize quality and concentration of standards.

On the poster we present a tiered approach for the evaluation and verification of the concentration of multianalyte standards utilizing few well-characterized MaxSpec Standards, UV spectroscopy and LC-MS. A standard series was prepared comprising 160 analytes as well as 25 internal standards. Standards used for calibration and MaxSpec Standards (Cayman) were measured by means of LC-ESI(-)-MS in selected ion monitoring (SIM) mode. Resulting areas were compared utilizing the MaxSpec Standard of the corresponding analyte group as reference to determine nominal concentrations of the analytes in the standards. In case the molecules contain at least one conjugated electron system, the UV absorption was determined by UV spectroscopy using extinction coefficients from literature and assuming similar coefficients for molecules bearing the same absorbing structural moiety. Based on the results of these approaches, factors were determined to correct the stated concentration of non-certified standards.

Our results reveal in part significant differences between determined nominal concentrations and the stated concentrations of certified standards and thus emphasize the need of routine quality assurance analysis of standards in each lab. Such a strategy is presented and discussed in this presentation.

POSTER 39

DYSREGULATED CIRCADIAN PRO-RESOLVING MEDIATOR BIOSYNTHESIS AND PERIPHERAL BLOOD T-CELL SUBSET IN MURINE CARDIOVASCULAR DISEASE

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Mounting evidence demonstrates that dysregulation in diurnal mechanisms is central to the development and exacerbation of many diseases that afflict western societies. T-cell subsets are implicated in the onset and progression of cardiovascular disease with mechanisms leading to their dysregulated responses remaining of interest. In the present studies, we found that western diet led to the dysregulation of the diurnal levels of peripheral blood regulatory T-cells and effector cells circadian in both wild-type (WT) and mice deficient in Apolipoprotein E ($ApoE^{-/-}$). Western diet also led to the development of early lesions in the aortic branch of $ApoE^{-/-}$ and increased peripheral blood platelet-leukocyte aggregates. Using lipid mediator profiling, we also found a dysregulation in the diurnal regulation of both plasma and aortic tissue pro-resolving lipid mediator concentrations. In WT mice, western diet led to a down-regulation of a number of SPM including n-3 docosapentaenoic acid resolvins RvDn-3 DPA as well as upregulation of inflammation initiated by eicosanoids prostaglandin E2 and Thromboxane B2, the further metabolite of the pro-thrombotic mediator TxA2. This dysregulation in peripheral blood mediators was further exacerbated in $ApoE^{-/-}$ mice. Administration of RvD5n-3 DPA, one of the mediators found to be dysregulated in $ApoE^{-/-}$ mice peripheral blood, reduced both the expression IL-17A and IFNγ in CD4+ T-cells as well as the number of these cells in circulation. RvD5n-3 DPA also downregulated the expression of RORγt on circulating CD4+ cells, numbers of circulating neutrophil-platelet aggregates and decreased the occurrence of early lesions in $ApoE^{-/-}$ mice fed western diet. Together these findings suggest that impaired diurnal regulation of peripheral blood lipid mediators leads to the dysregulation of T-cell responses contributing to cardiovascular disease development.

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POSTER 40

SIGNAL TRANSDUCTION PATHWAYS OF PROSTANOIDS AND ISOPROSTANES IN URINARY BLADDER SMOOTH MUSCLE

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The overactive bladder (OAB) is a clinical condition characterized by symptoms of frequency, urgency with/without incontinence. It affects 50-100 million people worldwide. The current treatment mainly consists of anticholinergic drugs with several side effects. According to prior experimental results the arachidonic acid (AA) derivate prostanoids and isoprotanes, the latter produced non-enzymatically during oxidative stress (e.g. cystitis), may have a role in the pathogenesis of the OAB.
Our aim was to examine the effects and the signal transduction pathways of prostanoids and isoprotanes in the urinary bladder smooth muscle, and potentially provide theoretical basis for the development of more specific medication of OAB with less adverse effects.
Detrusor muscle strips were prepared from wild type (C57BL/6) and knockout mice, deficient for the thromboxane receptor (TP) or the alpha-subunits of heterotrimeric G proteins (Galphaq/11-KO, Galpha12/13-KO) without urothelium under dissection microscope. Contraction force was measured by myograph under isometric conditions and normalized to the reference contractions evoked by 124 mM K^+.
The prostaglandin E2 (PGE2) and prostaglandin F2alpha (PGF2alpha), as well as the isoprostane 8-epi-PGE2 and 8-iso-PGF2alpha evoked contraction in the bladder strips. The effect of the prostanoids was decreased, and the effect of the isoprostanes was abolished in the strips of TP KO mice, suggesting that the effect of the prostanoids is mediated partially, whereas that of the isoprostanes mainly by the TP. The contracion responses were decreased in the strips of the Galpha12/13-KO mice. Correspondingly, the responses evoked by the prostanoids and isoprostanes were reduced by the Rho-kinase (ROCK) inhibitor Y-27632. In the strips of the Galphaq/11-KO mice, the responses were also decreased and in the presence of Y-27632 abolished completely.
In conclusion, the contractile effects of the examined prostanoids and isoprostanes are mediated mainly by the TP receptor and linked to the Galphaq/11 and to the Galpha12/13-Rho-ROCK intracellular signaling pathways in the murine urinary bladder. The Galpha12/13-Rho-ROCK signaling pathway may provide a novel, more specific pharmacological target with less adverse effects in the treatment of OAB.

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POSTER 41

COMMUNICATION BETWEEN HUMAN MACROPHAGE PHENOTYPES AND CANCER CELLS ORCHESTRATES LIPID MEDIATOR BIOSYNTHESIS

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Unresolved inflammation in the tumor milieu and tumor-associated macrophages are hallmarks of cancer. Depending on their polarization state and functional phenotype (designated as M1 or M2), M2 support tumor development or can phenotypically switch into M1 with tumor-suppressive properties. Lipid mediators (LM) comprise hydroxylated PUFAs produced by LOXs and COXs isoenzymes in macrophages and related leukocytes, as well as in cancer cells, with pivotal bioactivities in inflammation and cancer. We recently showed that human macrophages express COX and LOX enzymes in a phenotype-dependent manner with consequent phenotype-specific LM profiles upon activation by pathogenic bacteria. Here, we investigated how the communication of M1 and M2 phenotypes with cancer cells affects LM biosynthesis versus monocultures of either subtype. Co-cultures of human macrophages during polarization for 48 hrs with human A549 epithelial lung carcinoma cells substantially increased formation of 5-LOX products (i.e. leukotriene B4 and 5-hydroxyeicosatetraenoic acid) in M1 and M2 upon subsequent activation. In M2, also 15-LOX-derived LM including lipoxin A4, resolvin D2 and D5, and protectin D1 were elevated due to the presence of A549 cells. In parallel, co-culture of A549 cells during polarization towards the M2 phenotype selectively enhanced the expression of 15-LOX-1 while other LM biosynthetic enzymes (e.g. COX, 5-LOX) were not markedly affected. Notably, co-incubation of M1 and M2 with non-transformed human umbilical vein endothelial cells (HUVEC) did not affect LM production as compared to A549 cells. Vice versa, co-culture with M1 or M2 prompted COX product formation in A549 cells upon subsequent stimulation, accompanied by tremendous increase in COX-2 protein expression. Conclusively, our data reveal that the communication between macrophage phenotypes and cancer cells can strikingly modulate the biosynthetic capacities to produce bioactive LM with potential relevance for tumor biology.

POSTER 42

LUNG INFLAMMATION IN MICE ALTERS LUNG OXYSTEROL LEVELS

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Respiratory diseases remain one of the main causes of death worldwide. In most of these diseases, lung inflammation plays a crucial role and can be of multiple origins and diverse types. For instance, acute inflammation is observed in diseases such as pneumonia and acute lung injury (ALI) whereas more chronic forms of inflammation are present in asthma or COPD.

Oxysterols are oxygenated derivatives of cholesterol that are increasingly studied as bioactive lipids in their own right. However, apart from a recent paper showing increased levels of 25-hydroxycholesterol (25-OHC) in the sputum of COPD patients, not much is known on the effect of lung inflammation on oxysterol levels. As oxysterol levels can be altered in inflammatory settings, we sought to investigate the effect of lung inflammation on oxysterol levels.

We used two murine models of lung inflammation, an acute form of ALI induced by intra-tracheal instillation of lipopolysaccharides (LPS) to swiss mice, and a mouse model of asthma induced by intranasal instillation of house dust mite (HDM) leading to a more chronic lung inflammation. To better characterize the acute inflammation induced by LPS, lungs were recovered after 6h and 24h. For the asthma model that presents chronic inflammation, we recovered tissues 14 days after the first HDM challenge. In both models, mice were treated with budesonide in order to assess the effect of inflammation and treatment on oxysterol levels. Oxysterols were quantified using our previously validated HPLC-MS/MS method.

The levels of some oxysterols, such as 25-OHC, 7alpha-OHCnone and 27-OHC were altered in the acute lung inflammation induced by LPS at 6 hours. At the 24 hours time point, only the levels of 25-OHC remained increased and were normalized following budesonide administration. We also measured mRNA expression of the oxysterol metabolizing enzymes in order to see how this acute inflammation affected oxysterol metabolism. mRNA expression of several of these enzymes was also altered by inflammation.

In conclusion, we show here that oxysterol levels are altered by lung inflammation and normalized following treatment. This further supports our interest to study the implication of oxysterols in lung inflammation.

POSTER 43

IDENTIFICATION OF KEY LIPIDS CRITICAL FOR PLATELET ACTIVATION BY COMPREHENSIVE ANALYSIS OF THE PLATELET LIPIDOME

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Platelet integrity and function critically depend on lipid composition. However, the lipid inventory in platelets was hitherto not quantified. Here, we examined the lipidome of murine platelets using lipid-category tailored protocols on a quantitative lipidomics platform. We could show that the platelet lipidome comprises almost 400 lipid species and covers a concentration range of seven orders of magnitude. A systematic comparison of the lipidomics network in resting and activated murine platelets, validated in human platelets, revealed that less than 20% of the platelet lipidome is changed upon activation, involving mainly lipids containing arachidonic acid. Sphingomyelin phosphodiesterase-1 (Smpd1) deficiency resulted in a very specific modulation of the platelet lipidome with an order of magnitude up-regulation of lyso-sphingomyelin (SPC), and subsequent modification of platelet activation and thrombus formation. In conclusion, this first comprehensive quantitative lipidomic analysis of platelets sheds light on novel mechanisms important for platelet function, and has therefore the potential to open novel diagnostic and therapeutic opportunities.

POSTER 44

PROSTAGLANDIN E2 STIMULATES ADAPTIVE IL-22 PRODUCTION AND PROMOTES ALLERGIC CONTACT DERMATITIS

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BACKGROUND: Atopic dermatitis (AD) and allergic contact dermatitis (ACD) are both forms of eczema and are common inflammatory skin diseases with a central role of T cell-derived IL-22 in their pathogenesis. Although prostaglandin E2 (PGE2) is known to promote inflammation, little is known about its role in processes related to AD and ACD development, including IL-22 upregulation.

OBJECTIVES: We set out to investigate whether PGE2 has a role in T cell-derived IL-22 induction and development of ACD, which has augmented prevalence in patients with AD.

METHODS: T cell cultures and in vivo sensitization of mice with powerful haptens (oxazolone and dinitrofluorobenzene) were used to assess the role of PGE2 in IL-22 production. The involvement of PGE2 receptors and their downstream signals was also examined. The specific effects of PGE2 during ACD pathogenesis were evaluated by using the oxazolone-induced ACD mouse model. Gene expression of PGE2 and IL-22 signaling pathways was also investigated by using genomic profiling in human lesional AD skin biopsies.

RESULTS: PGE2 promotes IL-22 production from T cells through its receptors, E prostanoid receptor 2 (EP2) and EP4. This is mediated by its downstream cAMP-PKA signaling and probably involves the transcription factor aryl hydrocarbon receptor (AHR). Selective deletion of EP4 in T cells prevents hapten-induced adaptive IL-22 production in vivo. Importantly, blockade of endogenous PGE2 production by a COX inhibitor indomethacin or deletion of EP4 in T cells limit atopic-like skin inflammation in the oxazolone-induced mouse ACD model. Moreover, both PGE2 and IL-22 pathway genes were coequally upregulated in human AD lesional skin but were down-regulated after treatment with betamethasone or ultraviolet B (UVB) radiation, both common therapies for AD.

CONCLUSIONS: Our results thus define a crucial role for PGE2 in promoting ACD by facilitating T cell-derived IL-22 production.

POSTER 45

IN VIVO ASSESSMENT OF THE SOLUBLE EPOXIDE HYDROLASE INHIBITORY EFFECT OF SORAFENIB IN PATIENTS WITH HEPATOCELLULAR CARCINOMA: IS THERE SCOPE FOR A SYNERGISTIC THERAPEUTIC EFFECT OF OMEGA-3 FATTY ACIDS?

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Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related mortality globally and mostly diagnosed at advanced stages. Advanced HCC has a poor prognosis and treatment options are limited. Sorafenib, a multikinase inhibitor, is currently the only systemic agent approved for use in advanced HCC. Moreover, Sorafenib is an inhibitor of the soluble epoxide hydrolase (sEH). sEH catalyses the conversion of epoxides derived from long-chain polyunsaturated fatty acids (PUFAs) 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 treatment with 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 HCC patients focusing on EDP and EET.
By using tandem liquid chromatography mass spectrometric methods (LC-MS/MS) we were able to simultaneously analyze broad oxylipin profiles of all PUFA, particularly epoxides and their corresponding diols, in plasma samples from eight HCC patients pre- and post-therapy. The analyzed datasets confirm a sEH-inhibitory role of sorafenib, showing a significant increase of epoxy metabolites derived from omega-6 PUFA (EET´s) and from omega-3 PUFAs (EEQs and EDPs) after sorafenib treatment in comparison to baseline measurement.
The demonstrated stabilization of epoxides due to the sEH-inhibitory effect of sorafenib of both, tumor promoting EET and tumor suppressing EDP, could lead to a rationale for a supplementary therapy with the omega-3 PUFA DHA in HCC patients treated with sorafenib to promote the increase of 19,20-EDP.

POSTER 46

ANTI-INFLAMMATORY NITRO-FATTY ACIDS SUPPRESS TUMOR GROWTH BY TRIGGERING MITOCHONDRIAL DYSFUNCTION AND ACTIVATION OF THE INTRINSIC APOPTOTIC PATHWAY IN COLORECTAL CANCER CELLS

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Nitro-fatty acids (NFAs) are endogenously occurring lipid mediators exerting strong anti-inflammatory effects and acting as anti-oxidants in a number of animal models of inflammation. These NFA effects are mediated by targeting important regulatory proteins involved in inflammatory processes, such as 5-lipoxygenase, soluble epoxide hydrolase, or NF-κB. In the present study, we investigated the anti-tumorigenic effects of NFAs on colorectal cancer (CRC) cells in cell culture-based experiments and in a murine xenograft model of human CRC. We could show that 9-NOA suppresses the viability of CRC cells (HCT-116 and HT-29) by inducing a caspase-dependent apoptosis via the intrinsic apoptotic pathway as determined by the assessment of phosphatidylserine exposure, activation of caspase 3 and 9, alterations in the mitochondrial membrane potential, and the release of cytochrome c. Co-treatment with the pan-caspase inhibitor Q-VD-OPH were able to counteract the NFA-mediated apoptosis in both cell lines. Furthermore, NFAs affected the cell cycle transition by triggering an arrest at the G2/M phase. Using oxygraphic analysis, we could show that 9-NOA reduces the oxygen consumption rate of human CRC cells by approximately 30% after treatment of the cells for 1 h. On the contrary to their well-known anti-oxidative properties, NFAs mediated the generation of mitochondrial oxidative stress in human CRC cells. Additionally, similar to the cytostatic drug mitomycin, the nitro-fatty acid 9-NOA used at 16 mg/kg/day significantly reduced tumor growth in a murine xenograft model of human colorectal cancer. In contrast to the cytostatic drug, 9-NOA treatment was not associated with a prominent loss of body weight. In fact, mice tolerated the treatment with 9-NOA well and neither conspicuous behavior nor physical abnormalities could be observed in the NFA-treated group. This study delivers a novel mechanistic approach for nitro-fatty acid-induced inhibition of CRC cell growth by targeting mitochondrial functions such as the mitochondrial membrane potential and mitochondrial respiration. We suggest these naturally occurring lipid mediators as a new class of well tolerated chemotherapeutic drug candidates for treatment of CRC or potentially other inflammation-driven cancer types.

POSTER 47

CORRELATION OF THE EPOXY-FATTY ACID PATTERN WITH ISOPROSTANE FORMATION DURING OXIDATIVE STRESS

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Oxidative metabolites from polyunsaturated fatty acids (PUFA), i.e. eicosanoids and other oxylipins, are formed in the arachidonic acid (ARA) cascade by three enzymatic pathways and autoxidation.
Enzymatic conversion of the naturally occurring all-cis PUFA by cytochrome P450 monooxygenases (CYP) leads to cis-epoxy-PUFA. Also non-enzymatic chemical peroxidation gives rise to epoxy-PUFA. However during lipid peroxidation formation of both cis- and trans-epoxy-PUFA is observed [1,2]. We detected high concentrations of trans-epoxy-PUFA in different biological samples. Though, information about the formation of epoxy-PUFA following oxidative stress is scarce.
In order to evaluate trans-epoxy-PUFA and the trans/cis-epoxy-PUFA ratio as potential new biomarker for lipid peroxidation we investigated the formation of epoxy-PUFA and analyzed its correlation with the formation of isoprostanes (IsoP), a commonly used marker for autoxidation.
A comprehensive set of IsoP and epoxy-PUFA was quantified by LC-ESI(-)-MS/MS monitoring 27 IsoP and 8 isofurans derived from 6 different PUFA (ALA, ARA, EPA, AdA, n6-DPA, DHA) as well as 17 cis-epoxy-PUFA and 17 tentatively identified trans-isomers. These S,S- and R,R-enantiomers elute 0.15-0.35 min after their corresponding CYP derived cis-isomers (R,S- and S,R-enantiomers).
On the poster the time and dose dependent formation of epoxy-PUFA in different cell lines (HCT-116, Caki-2 and HepG2 cells) following incubation with the oxidative stressor tert-butyl hydroperoxide (t-BOOH) is shown and correlated with the formation of isoprostanes. Moreover, the formation of these autoxidatively formed oxylipins in the model organism Caenorhabditis elegans is presented.

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POSTER 48

OMEGA-3 PUFA MODERATELY ATTENUATE ACUTE KIDNEY INJURY IN A MURINE MODEL OF RENAL ISCHEMIA REPERFUSION INJURY

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Background: Renal ischemia reperfusion injury (IRI) is an important comorbidity in the context of solid organ transplantation and causes acute kidney injury (AKI). Here we present a dietary omega-3 polyunsaturated fatty acid (PUFA) supplementation study to investigate whether pre-treatment with omega-3 PUFA can reduce renal IRI.

Methods: Male 12-14 week old C57BL/6J mice received omega-3 PUFA supplementation (2 % in the chow containing 10 % fat) and a control group had chow with low omega-3 PUFA for 2 weeks prior to IRI. Bilateral 30 min IRI was done and mice were sacrificed at 24 h. Serum-creatinine and blood urea nitrogen (BUN) elevation were measured. Kidney damage was analyzed by histology and immunohistochemistry. Proinflammatory cytokines (IL-6, MCP-1) as well as FA and oxylipin patterns were determined in blood and kidneys.

Results: The feeding massively increased the levels of omega-3 PUFA. Consistently eicosanoids and other oxylipins derived from omega-3 PUFA were elevated while omega-6 PUFA derived mediators such as proinflammatory prostaglandins were decreased. Omega-3 PUFA feeding attenuated s-creatinine increase significantly. Similar effects were seen for BUN. PAS stain revealed similar degrees of AKI and tublar NGAL elevation. However, the tubular transport marker A1M was significantly higher expressed in omega-3 PUFA compared to vehicle treated mice indicating better integrity of proximal tubular epithelial cells. IL-6 and MCP-1 elevation due to IRI in renal tissue were not affected by omega-3 PUFA treatment.

Discussion: There are various reports on treatment strategies with omega-3 PUFA in the context of renal diseases. Here, we show that omega-3 PUFA pre-treatment attenuated worsening of renal function after IRI and that tubular transport was protected as well. However, inflammation markers were similar in the vehicle treated and the omega-3 PUFA treated groups.

In conclusion dietary omega-3 PUFA supplementation resulted in beneficial effects on renal function impairment in experimental renal IRI in mice but did not attenuate tissue inflammation.

POSTER 49

A METABOLIC PATTERN OF INFLUENZA A VIRUS INFECTED SUS SCROFA: ESTABLISHMENT OF THE PIG AS NEW NFECTION MODEL FOR BACTO-VIRAL INFECTIONS

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Introduction: Virus infections of the upper respiratory tract in combination with secondary bacterial infections can lead to severe lung infections. The aim of the current project KoInfekt is to elucidate the host-pathogen interactions establishing the pig as an animal infection model due to high genetic and physiological similarities to human beings.

Material and Methods: Animal experiments were done on the Federal Research Institute for Animal Health (Isle of Riems, Germany). A group of 25 pigs were infected with Influenza A virus (H1N1, Germany) and samples were collected over 31 days. For metabolic analysis tissues samples (lung, spleen), biofluids (blood plasma, BAL) and feces were collected and analyzed by a combination of 1H-NMR, GC-MS and LC-MS/MS.

Results: Extraction protocols for pig fecal material was established for analysis of host and gut microbiota digestion processes analyzed by 1H-NMR and GC-MS. For eicosanoid detection of tissue and biofluid samples the extraction steps were optimized for a LC-MS/MS method working on dynamic MRM.

Discussion: Perturbations in the eicosanoid profile of Influenza A virus infected pigs were detected. The analysis of the fecal metabolites enables an overview about the gut microbiota, which is linked to the host immune response and the interplay of the host and the bacteria community. This is the first step for the metabolic analysis of bacto-viral co-infections, which play an important role in human and animal health.

POSTER 50

CANCER-INDUCED INFLAMMATION AND INFLAMMATION-INDUCED CANCER IN COLON: A ROLE FOR S1P LYASE

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Objective: A role of sphingolipids for IBD and cancer is evident. However, the relative and separate contribution of sphingolipid deterioration in inflammation and carcinogenesis in the pathophysiology of colitis associated colon cancer (CAC) is unknown and was aimed to be determined in this study.

Methods: We performed isogenic bone marrow transplantation of inducible sphingosine-1-sphosphate (S1P) lyase knockdown mice to specifically modulate sphingolipids and associated genes and proteins in a compartment-specific way in a DSS/AOM mediated CAC model. 3-D organoid cultures were used in vitro.

Results: S1P lyase (SGPL1) knockdown in either immune cells or tissue, caused local sphingolipid accumulation leading to a dichotomic development of CAC: Immune cell SGPL1 knockdown (I-SGPL$^{-/-}$) augmented immune cell infiltration initiating colitis with lesions and calprotectin increase. Subsequent pathological crypt remodeling plus extracellular S1P-signaling facilitated tumor formation characterized by S1P receptor 1 (S1P1) and signal transducer and activator of transcription 1 (STAT1) bot not STAT3 regulation as well as programmed cell death 1 ligand 1 (PD-L1) expression. In contrast, tissue SGPL1 knockdown (T-SGPL$^{-/-}$) provoked immediate occurrence of epithelial-driven tumors with sphingosine kinase 1 (SphK1), S1P2 and epidermal growth factor receptor (EGFR) expression upregulation. Progressing carcinogenesis was accompanied by an IL-12/IL-23 shift with a consecutive development of a Th2-driven tumor-favoring microenvironment. Moreover, the knockdown models showed distinct lymphopenia and neutrophilia, different from the full SGPL knockout.

Conclusions: This study shows that depending on the initiating cellular S1P source, the pathophysiology of inflammation-induced cancer versus cancer-induced inflammation can be defined by sphingolipid modulation.

POSTER 51

A NOVEL FUNCTION FOR 15-LIPOXYGENASES IN CHOLESTEROL HOMEOSTASIS AND CCL17 PRODUCTION IN HUMAN MACROPHAGES

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Arachidonate 15-Lipoxygenase (ALOX15) and arachidonate 15-Lipoxygenase, Type B (ALOX15B) catalyze the dioxygenation of polyunsaturated fatty acids and are upregulated in human alternatively activated macrophages (AAMs) induced by Th2 cytokine interleukin-4 and/or interleukin-13. Known primarily for roles in bioactive lipid mediator synthesis, 15-lipoxygenases (15-LOXs) have been implicated in various macrophage functions including efferocytosis and ferroptosis. Using a combination of inhibitors and siRNAs to suppress 15-LOX isoforms, we studied the role of 15-LOXs in cellular cholesterol homeostasis and immune function in naïve and AAMs. Silencing or inhibiting the 15-LOX isoforms impaired SREBP-2 signaling by inhibiting SREBP-2 processing into mature transcription factor and reduced SREBP-2 binding to sterol regulatory elements (SREs) and subsequent target gene expression. Silencing ALOX15B reduced cellular cholesterol content in untreated and IL-4-stimulated macrophages.

Additionally, attenuating both 15-LOX isoforms did not generally affect IL-4 gene expression but rather uniquely impacted IL-4-induced CCL17 production in a SREBP-2-dependent manner resulting in reduced T cell migration to macrophage conditioned media. In conclusion, we identified a novel role for ALOX15B, and to a lesser extent ALOX15, in cholesterol homeostasis and CCL17 production in human macrophages.

POSTER 52

IMPAIRED PRODUCTION AND DIURNAL REGULATION OF VASCULAR RVDN-3 DPA INCREASE SYSTEMIC INFLAMMATION AND CARDIOVASCULAR DISEASE

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Circadian regulation of many physiological functions, such as leukocytes and platelets, play an important role in host responses. Recently, a new family of mediators, termed as specialized proresolving mediators, were discovered to play an important role in the inflammatory process. These mediators are better known for their capacity to terminate inflation without interfering with the immune response. Thereby, the diurnal regulation of specialized proresolving mediators and their role in controlling peripheral blood leukocyte and platelet activation is of interest. Using lipid mediator profiling and healthy volunteers, we found that plasma concentrations of n-3 docosapentaenoic acid-derived D-series resolvins (RvDn-3 DPA) were regulated in a diurnal manner. The production and regulation of these mediators was markedly altered in patients at risk of myocardial infarct. These changes were associated with decreased 5-lipoxygenase expression and activity, as well as increased systemic adenosine concentrations. We also found a significant negative correlation between plasma RvDn-3 DPA and markers of platelet, monocyte, and neutrophil activation, including CD63 and CD11b. Incubation of RvDn-3 DPA with peripheral blood from healthy volunteers and patients with cardiovascular disease significantly and dose-dependently decreased platelet and leukocyte activation. In addition, administration of RvD5n-3 DPA to $ApoE^{-/-}$ (apolipoprotein E deficient) mice significantly reduced platelet–leukocyte aggregates, vascular thromboxane B2 concentrations, and aortic lesions. In conclusion, these findings show a novel protective pathway played by the diurnal regulation of vascular n-3 DPA-derived resolvins that is lost in patients with CVD.

POSTER 53

CHARACTERIZATION OF THE BIOSYNTHESIS OF 15-DEOXY Δ12,14 PROSTAGLANDIN J2 (15DPGJ2) IN MACROPHAGES UPON MPGES-1 INHIBITION

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15-deoxy delta12,14 prostaglandin J2 (15dPGJ2) is a naturally occurring degradation product of PGD2. It consists of a highly reactive polyunsaturated carbonyl moiety that can form adducts with thiol-containing biomolecules like Glutathione (GSH) or cysteine residues on proteins such as Keap1 or NFkB via Michael addition and thereby exert anti-inflammatory effects in vivo. The depletion of microsomal prostaglandin E synthase 1 (mPGES-1), a novel key target of anti-inflammatory drugs might lead besides the reduction of pro-inflammatory PGE2 levels, to the activation of anti-inflammatory pathways, directing excessive PGH2 into the PGD2/15dPGJ2 pathway or generating anti-inflammatory mediators from altered fatty acid metabolism.

However the production and anti-inflammatory mechanisms of 15dPGJ2 are not clearly understood, due to the lack of appropriate model systems and detectability with current methods. Here we aim to develop a suitable method for the detection of 15dPGJ2 and novel 15dPGJ2-GSH-metabolites. Moreover we aim to characterize 15dPGJ2 biosynthesis and investigate the impact of selective mPGES-1 inhibitors on anti-inflammatory mediators in macrophages.

In order to identify endogenous 15dPGJ2 conjugated to GSH we have developed an UPLC-MS/MS multiple reaction monitoring method to analyze 15dPGJ2-GSH and 15dPGJ2-L-Cysteine metabolites. Our results show endogenous PGD2, 15dPGJ2, 15dPGJ2-GSH and 15dPGJ2-LCys formation in RAW264.7 cells and bone marrow derived macrophages upon LPS stimulation. Exogenously applied 15dPGJ2 gets metabolized in the presence of both murine macrophage types via GSH adduction.

Our results indicate an enzymatic dependency for the formation of 15dPGJ2-GSH and 15dPGJ2-L-Cysteine conjugates, however further studies are warranted to elucidate the underlying mechanisms of 15dPGJ2 biosynthesis in macrophages and upon mPGES-1 inhibition.

POSTER 54

APOPTOTIC DEPLETION OF STEAROYL-COA DESATURASE-1-DERIVED PHOSPHATIDYLINOSITOLS ACTIVATES P38 MAPK

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Apoptosis is characterized by a loss of membrane asymmetry, rendering phospholipids as hallmark of the initial phase of apoptosis [1]. Here, we describe comprehensive changes in phospholipid-bound monounsaturated fatty acids (MUFAs) under apoptotic conditions. Mechanistically diverse cytotoxic agents decrease the cellular proportion of MUFA-containing phospholipids in a time-dependent manner. The central enzyme in the biosynthesis of MUFAs is stearoyl-CoA desaturase-1 (SCD-1) which introduces a cis-Δ9 double bond into saturated fatty acids (SFAs) [2]. Plenty of studies report SCD-1 as molecular target for the treatment of cancer, metabolic diseases, and skin disorders [2]. During apoptosis, we observed a time-dependent decrease of SCD-1 expression along the depletion of MUFAs. In fact, specific inhibition of SCD-1 with the selective inhibitor CAY10566 reduced the cellular proportion of MUFA-bound phospholipids. The most pronounced effects on the time-dependent loss of phospholipid-bound MUFAs were observed in the minor phospholipid subclass of phosphatidylinositols (PIs). Furthermore, we have recently shown that changes in the cellular ratio of SCD-1-derived MUFAs can be ascribed to a negative regulation of p38 MAPK [3], a stress-activated protein kinase with crucial functions in apoptosis. MUFA-PI ratios and p38 MAPK activation were counter-regulated across cell lines and primary cells. To investigate whether MUFA-PIs guide the activation of p38 MAPK activation during fibroblast apoptosis and to identify the involved signaling molecules, we inhibited SCD-1 while providing MUFA-containing phospholipids. These exogenous phospholipids were supplied as liposomes, and their uptake was confirmed by UPLC-MS/MS. Of all tested phospholipid species, only PI(18:1/18:1) substantially prevented the phosphorylation of p38 MAPK, reduced ER stress, impaired apoptosis induction and restored fibroblast morphology and cell proliferation. Additionally, the activation of p38 MAPK, caused by cytotoxic agents was diminished by supplementation of PI(18:1/18:1)-containing liposomes, while cellular levels of free MUFAs were not altered, which points towards the phospholipid rather than released fatty acids as signaling molecule. In conclusion, we here identified PI(18:1/18:1) as novel bioactive lipid that regulates p38 MAPK with apparent relevance for stress-signaling in apoptosis.

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POSTER 55

SEX BIAS IN COLITIS: PROTECTIVE ROLE OF SPECIALIZED PRO-RESOLVING MEDIATORS IN FEMALE MICE

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Specialized pro-resolving mediators (SPM) are bioactive molecules with critical roles in the resolution of inflammation, tissue repair and organ protection. Non-resolved inflammation is a hallmark of several chronic pathological conditions including inflammatory bowel disease (IBD) that is characterized by intestinal mucosal damage, with different incidence and progression between men and women. Here, we provide evidences for sex differences in experimentally induced colitis in mice related to sex-biased levels of SPMs in the colon. Oral dextran sodium sulfate (DSS) administration to mice over five days resulted in more pronounced colon inflammation and wasting disease in male versus female animals. Accordingly, myeloperoxidase (MPO) activity and interleukin (IL)-1β levels in colon tissue as well as circulating levels of chemoattractant proteins in plasma were significantly higher in male mice during the acute inflammatory phase. Targeted liquid chromatography-tandem mass spectrometry-based metabololipidomics (LC-MS/MS) of colonic tissues revealed superior amounts of SPMs (i.e. resolvins RvD2 and RvD5, protectins PD1 and PDX and maresin-1 as well as SPM precursors 14-HDHA and 17-HDHA) in female versus male mice prior to colitis induction. Along these lines, analysis of the enzymes involved in SPM biosynthesis showed higher expression of 5-lipoxygenase (5-LO) and 12-lipoxygenase (12-LO) in colons from female mice before colitis, and no sex differences in cyclooxygenases-1 and -2 (COX-1/2) expression. To investigate if SPMs may play a protective role in connection to the lower susceptibility of female animals to develop colitis, male mice were treated with a mixture of RvD2, RvD5 and maresin-1, 30 minutes prior to dinitrobenzene sulfonic acid (DNBS) intrarectal injection. This SPM treatment prevented the DNBS-induced colon inflammation, the wasting disease and the chemokine production in male mice, and it almost completely abolished the sex bias in experimental colitis. Together, our findings reveal a sex dimorphism in the SPM biosynthetic pathway and identify SPMs in the colon as key molecules mediating the sex-biased susceptibility to IBD.

POSTER 56

LYSOPHOSPHATIDIC ACID (LPA) MEDIATED VASOCONSTRICTION DEPENDS ON FATTY ACID CHAIN UNSATURATION AND CHANGES WITH AGE

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Our research group has recently reported that LPA1 receptor activation in the endothelium induces vasorelaxation, whereas in the vascular smooth muscle (VSM) it evokes cyclooxygenase 1 (COX1) activation with consequent thromboxane A2 (TXA2) release, which elicits vasoconstriction.
In this study, we aimed to compare the vasoactive effects of saturated and unsaturated LPA species on the vascular tone and to identify their signal transduction. Since vascular diseases are more common in the elderly, we also aimed to observe the age-related changes of the 18:2 LPA induced vasoconstriction.
In aortic segments isolated from young and middle-aged (8 and 32 weeks old, respectively) wild-type and knock-out (KO) mice the vasoactive effects of different LPA species were determined with wire myography. To compare the LPA induced signal transduction in the two age groups general contractility and TXA2 receptor sensitivity were tested by examining phenylephrine (PE) and U-46619 dose-response relationship, respectively. Expression profile of LPA1-3 receptors were demonstrated by qPCR in the VSM cells.
Saturated 14:0, 16:0 and 18:0 LPAs had no vasoconstrictor activity, and their vasorelaxant effect increased with the reduction of the fatty acid chain length. Highest vasorelaxant effect was mediated by the mono-unsaturated oleoil-LPA, whereas increase in the number of double bonds results in decreased vasorelaxing and increased vasoconstricting activity. Both relaxation and constriction disappeared in the LPA1-KO vessels. 18:2 LPA mediated vasoconstriction was increased in the aortae of the 32-week old mice. There was no difference between the two age groups neither in the expression of LPA1 receptor nor in the PE and U-46619 dose-response curves. 18:2 LPA mediated constriction was diminished in vessels of both the middle-aged and young COX1-KO animals.
In conclusion, polyunsaturated LPAs appear to have strong, age-dependent vasoconstrictor activity. This is particularly interesting in the case of endothelial injury and platelet activation where polyunsaturated LPAs can contribute to the progression of vasospasm and they may have an enhanced role in the cardiovascular diseases in the elderly.

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POSTER 57

INFLUENCE OF CRISPR-CAS MEDIATED KNOCK-OUT OF 5-LIPOXYGENASE ON TUMOUR CELLS

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5-Lipoxygenase (5-LO) catalyses the first two steps in the formation of pro-inflammatory leukotrienes (LT) and is predominantly expressed in immune cells like granulocytes, macrophages and mast cells. 5-LO derived LTA4 can be further converted into LTB4, a potent chemotactic agent, or cysteinyl LTs which play an important role in smooth muscle cell contraction. Through this, 5-LO products contribute to the development and progression of various pathophysiological conditions such as allergic reactions or chronic inflammation.

It is well known that tumours and cell lines of different origin such as prostate, breast, pancreas and colon, frequently overexpress 5-LO even though these tissues do not express the enzyme under physiological conditions. These tumours show a poor prognosis regarding the survival expectancy of the patients as well as bad responsiveness to cytostatic treatments. In addition, 5-LO products were shown to promote proliferation of tumour cells. Until now, little is known about the functional consequences of 5-LO overexpression in these cells and its importance for cell survival, proliferation, invasiveness and metastasis.

Therefore, we have established a 5-LO knockout in different overexpressing tumour cell lines via CRISPR-Cas technology. Subsequently, we performed various in-vitro assays dealing with cell proliferation, survival and invasiveness of these knock-out cell lines and compared them to their wild-type counter parts.

POSTER 58

OXYLIPIN FORMATION IN HUMAN COLON TISSUE

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Eicosanoids, including prostaglandins and thromboxanes, have been implicated to increase colon tumorigenesis possibly through chronic inflammatory mechanisms. Epidemiological and experimental data suggest that acetylsalicylic acid (ASA) helps prevent colorectal cancer (CRC), possibly through cyclooxygenase (COX)-mediated suppression of eicosanoid - particularly prostaglandin E2 (PGE2) - formation. Recent studies suggest that statins also help to prevent CRC and improve survival after CRC diagnosis.

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, Furthermore, we identified subsets of patients on ASA and/or statin treatment undergoing colonoscopy and measured oxylipin levels in macroscopically healthy colonic mucosa with targeted metabolomics technology (LC-MS/MS). We were able to confirm in vivo that ASA-treated individuals had significantly lower tissue eicosanoid levels of most COX-derived metabolites as compared to untreated individuals. In contrast, COX-derived lipid metabolites tended to be higher in patients with statin treatment as compared to those not receiving statins. This effect was not discernible in subjects treated with ASA and statins, as individuals treated with both drugs showed a pronounced suppression of COX-derived eicosanoids in colon tissue even when compared to subjects treated with ASA alone. Our data from a routine clinical setting thus support the hypothesis that ASA and statins could inhibit CRC development via lipid mediator modification.

POSTER 59

EFFECT OF DDHD1 PHOSPHOLIPASE A1 ON GROWTH AND SURVIVAL OF PANCREATIC CANCER CELLS.

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Phospholipase A1 (PLA1) catalyzes the hydrolysis of ester bond at sn-1 position of glycerophospholipids. The intracellular phosholipase A1, DDHD domain containing 1 (DDHD1), was previously identified as phosphatidic acid (PA)-preferring PLA1 (PA-PLA1). We previously proposed that DDHD1/ PA-PLA1 might be involved in the biosynthesis of lysophosphatidylinositol (LPI), a lysophospholipid mediator, that is an agonist for GPR55, a novel cannabinoid receptor. We further identified mutations in DDHD1/ PA-PLA1 gene in individuals with autosomal-recessive forms of hereditary spastic paraplegia (HSP). The mitochondrial respiration and ATP synthesis were reduced in the lymphoblasts derived from the patient. In the present study, we investigated the effects of DDHD1/ PA-PLA1 on the proliferation and survival of PANC-1, the pancreatic cancer cells. DDHD1/ PA-PLA1 and its non-catalytic mutant (S537A) were expressed in PANC-1 cells by the recombinant retrovirus system. The expression of DDHD1/ PA-PLA1 and S537A mutant significantly reduced the proliferation of PANC-1 cells with normal growth medium. The cell cycle analysis revealed that the expression of DDHD1/ PA-PLA1 and S537A mutant significantly decreased the G0/G1 cells and increased G2/M cells, suggesting that the expression caused the G2/M cell cycle arrest. The expression of DDHD1/ PA-PLA1 and S537A mutant significantly increased the mitochondrial respiration and membrane potential. Our results suggest that DDHD1/ PA-PLA1 is involved in the regulation of G2/M cell cycle transition and mitochondrial function and the dysregulation of DDHD1/ PA-PLA may be lead to the reduced cell proliferation of pancreatic cancer cells.

POSTER 60

EFFECT OF OMEGA-3 FATTY ACID SUPPLEMENTATION ON BLOOD FATTY ACIDS AND LIPID MEDIATORS IN A PATIENT COHORT UNDERGOING REGULAR APHERESIS TREATMENTS

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An elevated intake of long-chain polyunsaturated omega-3 fatty acids (n-3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is associated with reduced cardiovascular risk and attenuation of inflammatory processes. In contrast, omega-6 polyunsaturated fatty acids (n-6 PUFA) such as arachidonic acid (AA) have been shown to enhance predominantly pro-inflammatory effects. PUFAs can be oxygenated by different pathways, resulting in a wide spectrum of bioactive lipid mediators. It is of particular interest to assess the impact of n-3 PUFA supplementation on plasma oxylipin patterns in the context of concomitant treatment with other cardioprotective drugs. Therefore we examined the plasma lipidome of 35 patients with severe atherosclerosis and hyperlipidemia undergoing long-term weekly lipoprotein apheresis. All patients had standard lipid lowering drugs and aspirin. 11 patients received steady-state treatment with 840 mg n-3 PUFA (460 mg EPA and 380 mg DHA as ethyl esters) and 8 patients received 1680 mg n-3 PUFA in addition to their standard medical treatment. 16 patients served as controls.

First of all, supplementation of n-3 PUFA had no impact on any of the investigated AA-derived metabolites. In contrast, n-3 PUFA supplementation had a significant influence on several DHA- and EPA-derived metabolites. Most importantly, supplementation increased the level of 18-hydroxyeicosapentaenoic acid (18-HEPE), an EPA-derived metabolite and precursor of the E-resolvin family. Furthermore, an increase in DHA- (19,20-epoxydocosapentaenoic acid; 19,20-EDP) and EPA-derived epoxy metabolite (14,15-epoxyeicosatetraenoic acid; 14,15-EEQ) could be observed. N-3 PUFA supplementation also enhanced the level of 14,15-dihydroxyeicosatetraenoic acid (14,15-DiHETE) which is the corresponding diol of 14,15-EEQ.

In summary, plasma levels of AA-derived metabolites were not significantly modified by treatment with n-3 PUFA whereas several DHA- and EPA-derived metabolites which are known for their anti-inflammatory properties, in particular 18-HEPE, were significantly elevated. Interestingly, levels of 17-hydroxydocosahexaenoic acid (17-HDHA, precursor of D-series resolvins) and 14-HDHA (precursor of maresins) were not enhanced by n-3 PUFA supplementation, diverging from results obtained from healthy individuals. Our hypothesis is that this could be in part attributed to concomitant medication with statins which are known to have inhibiting effects on LOX- and COX-enzymes under certain conditions.

POSTER 61

VALIDATION OF HPLC-MS/MS METHOD FOR OXYLIPINES PROFILING IN BIOFLUIDS AND TISSUES

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The modern scientific approach to understanding the occurrence of diseases and its clinical diagnosis, as well as optimization of drug treatment should be associated with the study of the body response to pathophysiological effects by evaluating the levels of low molecular weight metabolites in biological fluids and tissues, as well as the study of its dynamics that leads to a better understanding of the role they play in the early detection of diseases. As an example Archadionic Acid metabolites (Prostaglandins and Related Compounds) have a crucial role in the pathobiology of hypertension and diabetes mellitus, therefore the detection and quantification of such compounds in different biofluids is important in the better comprehension of these biological processes. Herein we describe a sensitive targeted analysis platform on the basis of high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) operated in multiple reaction monitoring (MRM) mode for detection and quantification of oxylipins in biofluids. Besides AA metabolites, the approach covers oxylipins derived from linoleic, dihomo-γ-linolenic, α-linolenic, eicosapentaenoic and docosahexaenoic acids.

The work was carried out using a Shimadzu 8040 triple quadrupole mass spectrometer equipped with a Nexera ultra-HPLC separation system (Phenomenex C8 chromatographic column (2.1×150 mm×2.6 μm) was used. The flow rate was 0.4 ml/min. Mobile phases, 0.1% of formic acid and acetonitrile-MS grade were used). Detection of oxylipins was carried out using both positive and negative modes in combination with MRM. The use of the HPLC-MS/MS preset system parameters included in the Shimadzu lipid mediator method package Ver.2, simplifies the process allowing to obtain optimal chromatographic and mass-spectrometric characteristics of the compounds to be determined, which ensure their acceptable separation and high detection sensitivity in complex biological matrices as biofluids. For quantitation of metabolites, isotope dilution method was used. The resulting data of preliminary experiments were useful in the selection of optimal extraction conditions (LL, SPE) of the studied metabolites from complex matrices of biofluids. Validation was carried out and important parameters were established for precisioness, correctness, linearity, limit of detection, etc.). The publication has been prepared with the support of the «RUDN University Program 5-100».

POSTER 62

RAPID DETECTION OF UNKNOWN COX-2 DERIVED METABOLITES OF N-3 POLYUNSATURATED ENDOCANNABINOIDS IN LPS STIMULATED MACROPHAGES

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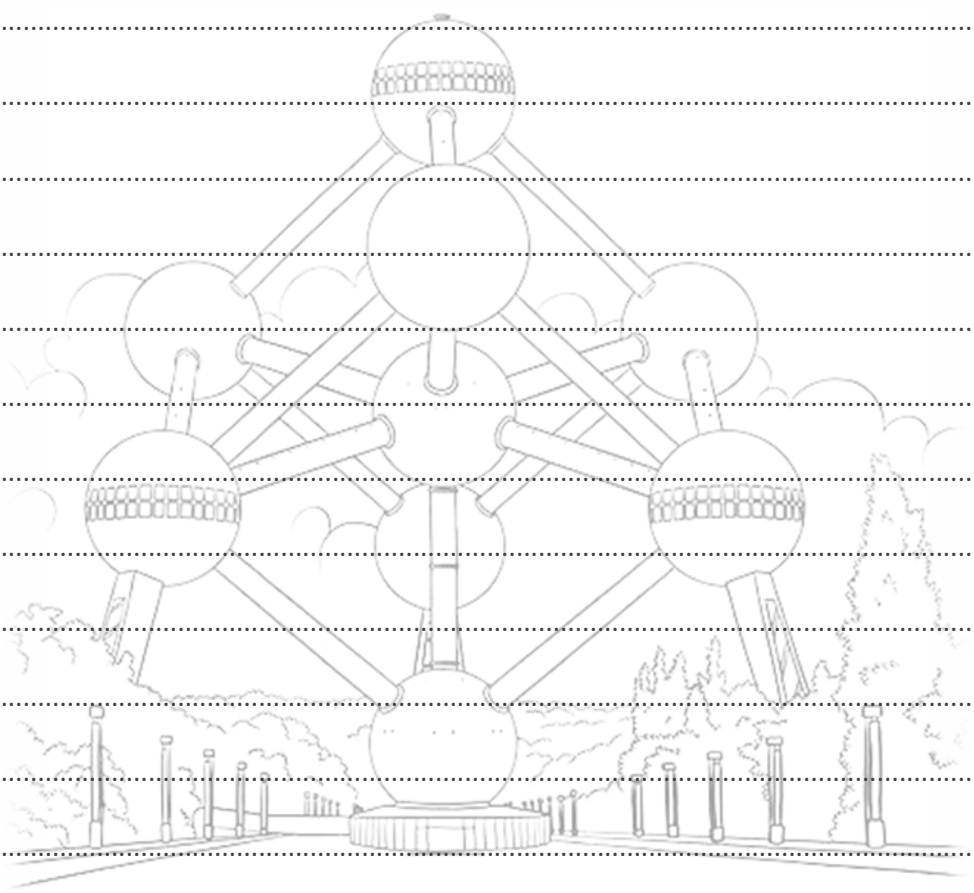
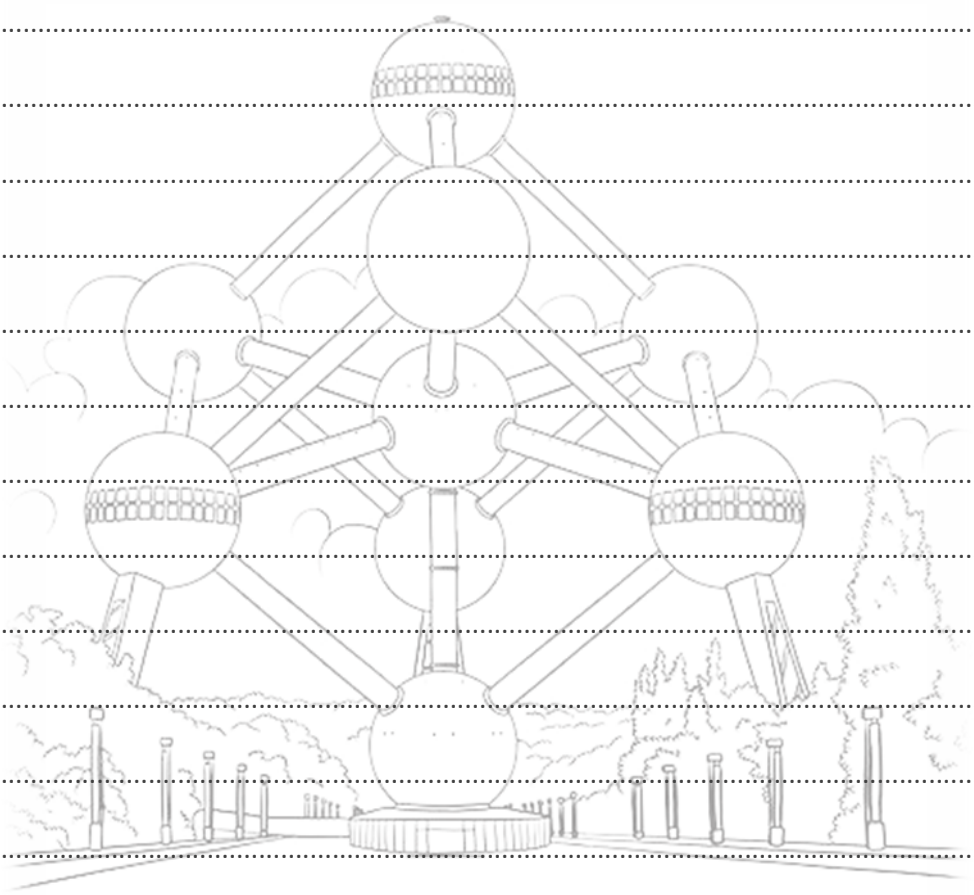
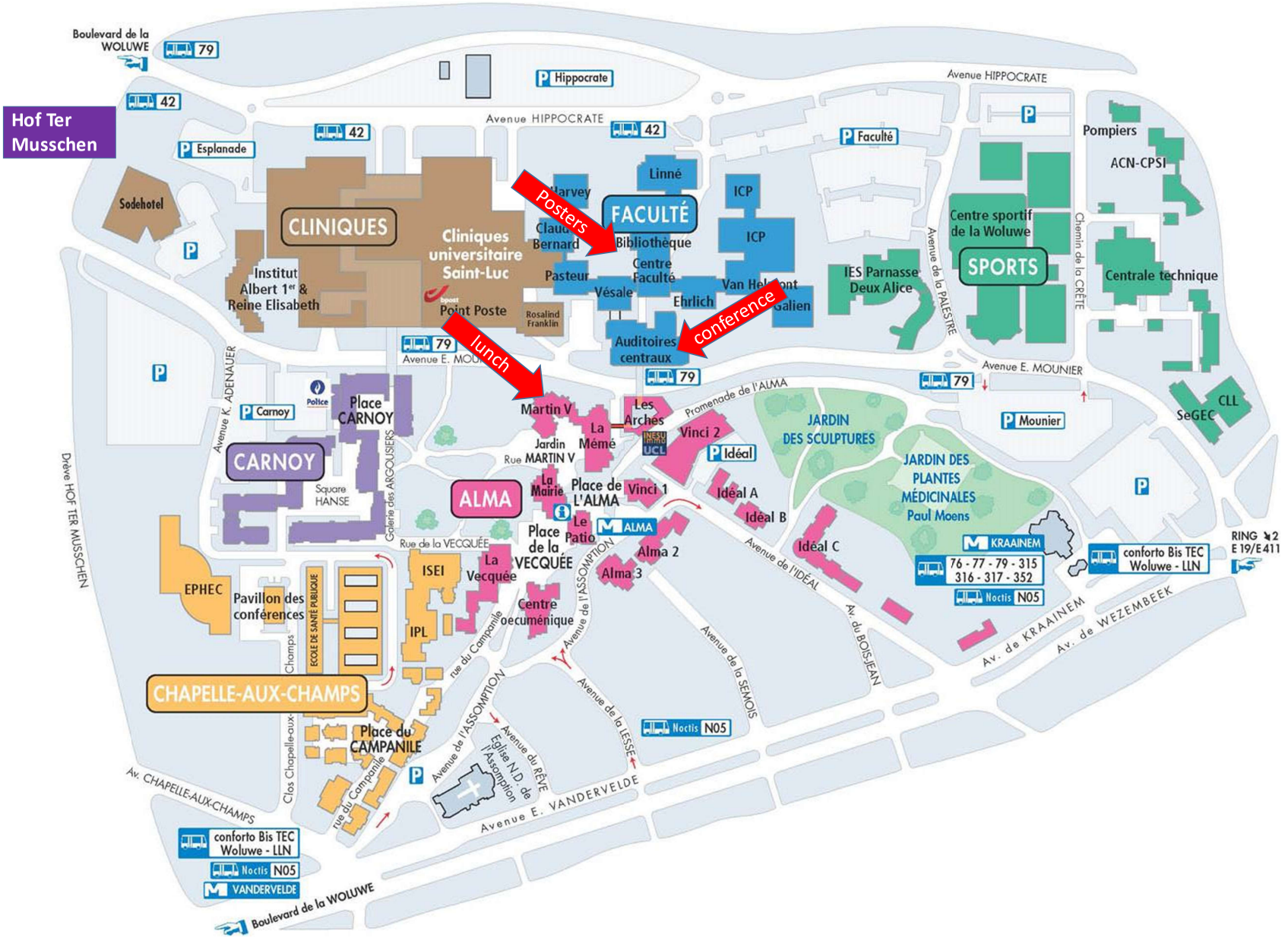
Cyclooxygenase-2 (COX-2) is a non-constitutional enzyme that is specifically expressed during inflammation. The enzyme oxygenates poly-unsaturated fatty acids (PUFAs) such as arachidonic acid (AA) as well as neutral lipids like the endocannabinoid arachidonoyl ethanolamide (AEA), leading to various metabolites that have regulatory roles in inflammation. Previously published data on the interaction between the ω-3 derived endocannabinoid docosahexaenoylethanolamide (DHEA) and COX-2 suggested that DHEA may also be a substrate for COX-2. To better understand the interactions between endocannabinoids and COX-2, we designed a new cell free screening assay consisting of hCOX-2 incubations followed by LC-HRMS analysis to qualitatively identify metabolites. To show the validity of this rapid screening assay, we first successfully demonstrated the enzymatic synthesis of known prostaglandins and monohydroxylated PUFA's from AA, and ω-3 PUFAs, respectively. Next, the formation of several novel oxygenated metabolites of DHEA and related congeners was demonstrated, of which 13-HDHEA and 16-HDHEA were shown to be the hCOX-2 products of DHEA. Finally, we showed that 13-HDHEA and 16-HDHEA were also produced in LPS-stimulated murine RAW264.7 macrophages. These results underline the potential of our new LC-HRMS screening assay to rapidly detect in vitro formed hCOX-2 products. In addition, this assay demonstrates that hCOX-2 can metabolize DHEA into two previously unknown metabolites, 13-HDHEA and 16-HDHEA.



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