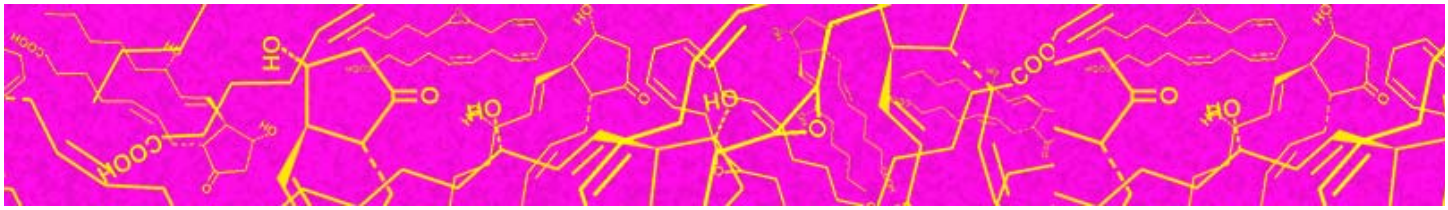
Book of Abstracts



Thursday, September 27th

SESSION 1: LIPIDOMICS

SESSION 2: OMEGA-3

SESSION 3: RESOLUTION OF INFLAMMATION

Friday, September 28th

YOUNG SESSION

SESSION 4: INNATE IMMUNITY

SESSION 5: PROSTANOID RECEPTOR PHARMACOLOGY

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LOCAL ORGANIZING COMMITTEE

The 4th European Workshop on Lipid Mediators has been organized under the auspices of

Group for Research and Studies on Mediators of Inflammation

(French Inflammation Society)

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Véronique Witko-Sarsat, INSERM U845, Necker Hospital, Paris.

Program
Thursday, September 27th, 2012

9:10 Welcome by the Organizers (Xavier Norel)
Jesús Balsinde introduces 1st Lecture: Ed Dennis (University of California, San Diego, USA)
9.15-10:00 Eicosanoid lipidomics and omega-3 role in the innate immune and inflammatory response

SESSION 1 LIPIDOMICS (Chairs: Jesús Balsinde & Gerard Bannenberg)

10:00-10:30 Lipid mediator profiling as part of a systems-based approach to investigating inflammatory disease
Craig Wheelock (Karolinska Institutet, Stockholm, Sweden)
10:30-11:00 Identification and characterisation of esterified eicosanoids generated by immune cells using lipidomics approaches
Valerie O'Donnell (School of Medicine, Cardiff University, Cardiff, UK)

11:00-11:30 Coffee Break

11:30-12:00 Novel analytical approaches for the quantitative and qualitative analysis of lipids in biofluids
Rob Vreeken (Netherlands Metabolomics Centre, Leiden University, Leiden, The Netherlands)
***12:00-12:20 Eicosanoids and docosanoids in plasma and aorta of healthy and atherosclerotic rabbits: Establishment of a multiplex HPLC ESI MS-MS method**
Ralph Rühl (University of Debrecen, Hungary)
***12:20-12:40 Generation of lysophosphatidylinositol, an endogenous agonist for novel cannabinoid receptor GPR55, by intracellular phospholipase A1**
Atsushi Yamashita (Teikyo University, Japan)

12:40-14:10 Lunch

SESSION 2 OMEGA-3 (Chairs: Joan Clària & Francis Berenbaum)

14:10-14:40 Transfer of essential fatty acids from aquatic to terrestrial ecosystems
Michail Gladyshev (Siberian branch of Russian Academy of Sciences, Krasnoyarsk, Russia)
14:40-15:10 Omega-3-derived lipid mediators counteract obesity-induced adipose tissue inflammation
Esther Titos (Hospital Clinic-CIBERehd, Barcelona, Spain)
15:10-15:40 Omega 3 and the aging brain: from epidemiological data to intervention studies in humans
Pascale Barberger-Gateau (Inserm, Université de Bordeaux, Bordeaux, France)
***15:40-16:00 Quantitative Profiling of Omega-6 and Omega-3 Eicosanoids by LCMSMS in Health and Disease**
Cecil Pace-Asciak (The Hospital for Sick Children Research Institute, Toronto, Canada)
***16:00-16:20 Isoprostanes and Neuroprostanes, Metabolites of omega-6 and omega-3**

Program
Thursday, September 27th, 2012

PUFAs: Not only Biomarkers of Lipid Peroxidation
Thierry Durand (Institut des Biomolécules Max Mousseron, Montpellier, France)

16:20-16:50 Coffee Break

SESSION 3 RESOLUTION OF INFLAMMATION (Chairs: Joan Clària & Gerard Bannenberg)

16:50 -17:20 Pro-resolution lipids: gamekeepers turn poachers
Derek Gilroy (Centre for Clinical Pharmacology and Therapeutics, University College London, UK)
17:20-17:50 Macrophage remodeling during the resolution of inflammation
Amiram Ariel (University of Haifa, Haifa, Israel)
***17:50-18:10 Emerging roles of eosinophils and eosinophil-derived lipid mediators in acute inflammation and resolution**
Makoto Arita (University of Tokyo, Japan)
***18:10-18:30 Method of identification and quantification of resolvin D5 in biological samples: application to an in vivo animal model for evaluation of inflammation**
Marc Dubordeau (Ambiotis, Toulouse, France)

18:30-19:45 POSTER SESSION + Wine and Cheese

Program
Friday, September 28th, 2012

Francis Berenbaum introduces 1st Lecture: Per-Johan Jakobsson (Karolinska Institutet, Stockholm, Sweden)
8:30-9:15 Microsomal prostaglandin E synthase, a multifaceted drug target

<u>YOUNG SESSION</u> (Chairs: Lhousseine Touqui & Xavier Norel)

9:15-11:15: (10+2 min each speaker)

***The endocannabinoid 2-arachidonoylglycerol controls macrophage activation through its oxidative metabolite prostaglandin 2-glycerol (PGD2-G)**
Mireille Alhouayek (Université catholique de Louvain, Bruxelles, Belgium)
***Endogenous epoxygenases regulate endothelial cell TNFalpha secretion**
Ara Askari (William Harvey Research Institute, University of London, UK)
*** Comparison of the relaxations induced by PGI2 analogues (used clinically), in isolated human pulmonary vessels: role of the DP receptor**
Chabha Benyahia (INSERM U698, Université Paris Nord, France)
***Simultaneous Activation of p38 and JNK by Arachidonic Acid Stimulates the Cytosolic Phospholipase A2-dependent Synthesis of Lipid Droplets in Human Monocytes**
Carlos Guijas (Instituto de Biología y Genética Molecular, Valladolid, Spain)
***Lysophosphatidic acid acyltransferase 3 – the missing link between polyunsaturated fatty acids and fertility?**
Andreas Koeberle (Friedrich-Schiller-University, Jena, Germany)
***A Regulatory Loop Between Desaturases and Omega-3 Fatty Acids Plays a Major Role in Non-Alcoholic Steatohepatitis**
Cristina López-Vicario (University of Barcelona, Spain)
*** Mechanisms of *P. aeruginosa*-induced expression of secretory phospholipase A2 type IIA in cystic fibrosis lung epithelial cells**
Erwan Pernet (INSERM U874, Institut Pasteur, Paris, France)
*** Resolvin D1 activates Specific GPCRs and regulates miRNAs in novel resolution circuits**
Antonio Recchiuti (Harvard Institutes of Medicine, Boston, USA)
***Adipocytes modulate the phenotype of macrophages through secreted lipids**
Inge Klein-Wieringa (Leiden University Medical Center, The Netherlands)
***Investigating the Role of Cyclooxygenase in Toll-Like Receptor Responses**
William Wright (Imperial College, London, UK)

11:15-11:45 Coffee Break

Program
Friday, September 28th, 2012

SESSION 4 INNATE IMMUNITY (Chairs: Lhousseine Touqui & Francis Berenbaum)

11:45-12:15 Lipids, apoptosis, and immunity to tuberculosis
Samuel M Behar (Harvard Medical School, Boston, USA)
12:15-12:45 Role of lipid mediators in airway inflammation
Massimo Triggiani (University of Naples, Naples, Italy)
***12:45-13:05 Adipocytes modulate T cell function through release of lipids**
Andreea Ioan-Facsinay (Leiden University Medical Center, The Netherlands)
*** 13:05-13:25 Elevated prostaglandin E2 mediates immune suppression in liver cirrhosis**
Alastair O'Brien (University College London, United Kingdom)

13:25-15:25 Lunch + POSTER SESSION

SESSION 5 PROSTANOID RECEPTOR PHARMACOLOGY (Chairs: Xavier Norel & Robert Jones)

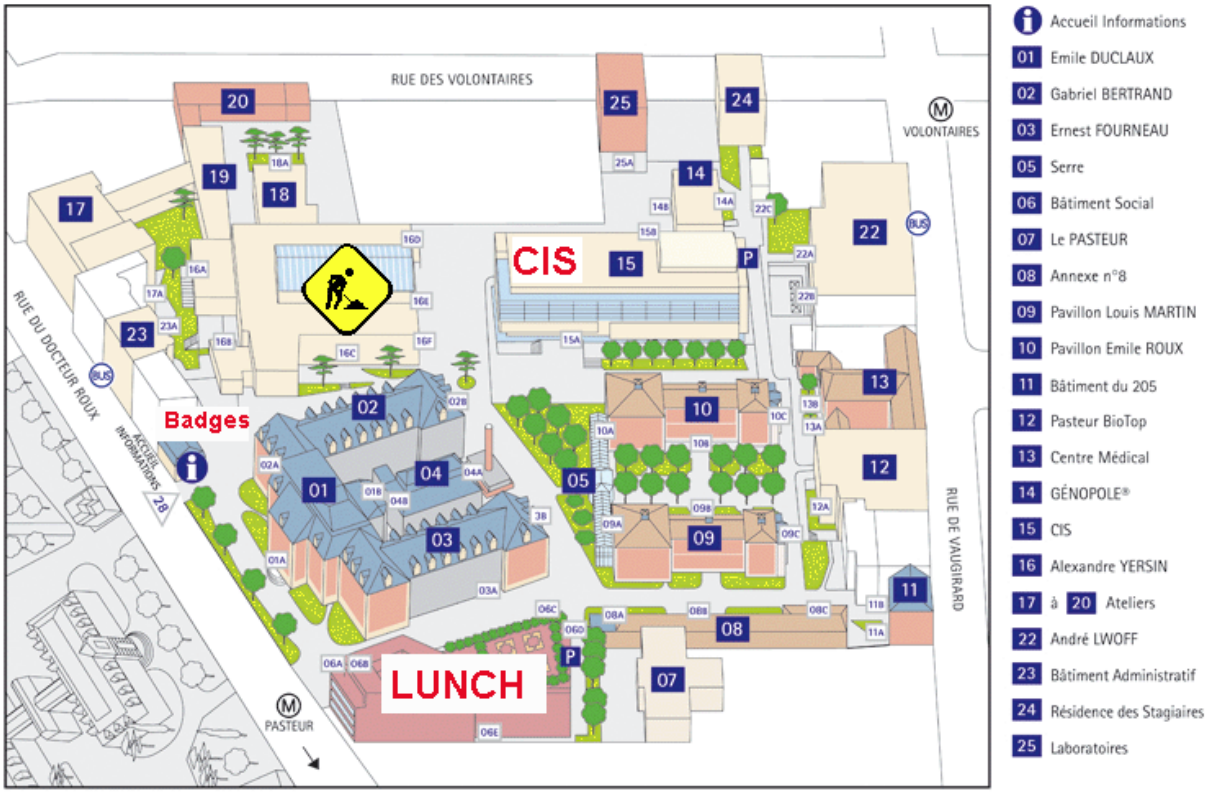
15:25-15:55 Prostacyclin analogs in pulmonary hypertension
Lucie Clapp (Metabolism and Experimental Therapeutics, University College London, London, UK)
15:55-16:25 The pharmacology and therapeutic application of COX-2 metabolites of mammalian endocannabinoids
David Woodward (Allergan, Department of Biological Sciences, Irvine, CA, USA)
16:25-16:55 Prostanoid receptors in chronic inflammation
Shuh Narumiya (Department of Pharmacology, Kyoto University, Kyoto, Japan)
***16:55-17:15 Investigation of the slow kinetics of a non-prostanoid EP2 receptor agonist**
Robert Leslie Jones (University of Strathclyde, UK)
*** 17:15-17:35 The arachidonic acid epoxygenase: functional roles and relevance to the pathophysiology of hypertension and tumor angiogenesis**
Jorge Capdevila (Vanderbilt University Medical School, Nashville, USA)

17:35 Adjourn (Closing Remarks Joan Clària & Gerard Bannenberg)
last Coffee Break

***indicates a communication selected from the submited abstracts**

Pasteur Institute, Map of campus 28 rue du Dr Roux
(right side, coming from Pasteur Métro)
Conference center (CIS)

Oral communications, poster sessions, stands, registration on site and wellcome reception (wine an cheese on September 27th evening) will be held at the CIS.



ORAL COMMUNICATIONS

Thursday, September 27th, 2012

EICOSANOID LIPIDOMICS AND OMEGA-3 ROLE IN THE INNATE IMMUNE AND INFLAMMATORY RESPONSE

Author: Edward A. DENNIS

Institute address: Distinguished Professor of Chemistry, Biochemistry, and Pharmacology, University of California at San Diego, La Jolla, California, USA

Abstract: The omics evolution began at the end of the 20th century with the cloning of the human genome. The 21st century has already seen the development of comprehensive proteomics analyses, but the emerging evolution is to metabolomics, the definition of which is the identification and quantification of all of the molecular constituents of the cell including its nucleic acids, amino acids, sugars, and fats. But by far, the largest number of distinct molecular species in cellular metabolism lies in the fats (or lipids) where tens of thousands of distinct molecular species exist in cells and tissues [Dennis, Proc.Natl.Acad.Sci.*U.S.A.*,*106,* 2089-2090 (2009)]. We have now applied novel liquid chromatographic-mass spectrometric based lipidomics techniques termed "CLASS" [Harkewicz & Dennis, Annual Reviews of Biochemistry, **80**, 301-25 (2011)] generally in the context of an overall omics analysis of immunologically-activated macrophages integrating transcriptomics, proteomics, and metabolomics of lipid metabolites [Dennis et al, J Biol Chem, 285, 39976-85 (2010)]. As part of the LIPID MAPS Consortium [www.lipidmaps.org], our laboratory has developed a robust and comprehensive approach to the lipidomics analysis of hundreds of fatty acids, acylethanolamines and inflammatory eicosanoids, including their numerous metabolites arising from an array of cyclooxygenases, lipoxygenases, cytochrome P450s and non-enzymatic oxidation producing isoprostanes, as well as combinations thereof [*J. Lipid Res. 50,* 1015-1038 (2009)]. We will discuss the application of lipidomic analysis to characterize cellular lipid signaling of Toll-like (TLR) and purinergic receptors and their "synergy" in endotoxin stimulated macrophages as models for inflammation and infection [*J. Biol. Chem., 282*, 22834-22847 (2007)]. New results comparing various primary macrophages [*J Leukocyte Biology*, 90, 563-74 (2011)] and analysis of the fluxes of metabolites as well as "directed proteomics" of the system will be presented. Also lipidomic analysis of cells supplemented with small amounts of the omega-3 fatty acids eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) provides information on the overall effects of EPA and DHA on the inflammatory eicosadome [*Proc Natl Acad Sci U S A*, **109**, 8517-22 (2012)]. Human plasma has also been profiled to quantify almost six hundred distinct lipid molecular species present across all mammalian lipid categories and the implications for the future of clinical medicine and the understanding of the mechanisms of disease has been discussed [Quhenberger & Dennis, New Eng J Med, 365, 1812 (2011)].

[Supported by LIPID MAPS Glue Grant U54 GM069338, R01 GM020501, and R01 GM064611]

LIPID MEDIATOR PROFILING AS PART OF A SYSTEMS-BASED APPROACH TO INVESTIGATING INFLAMMATORY DISEASE

Authors: Fabio Luiz D'Alexandri[a], Dmitry Grapov[b], Peddinti Gopalacharyulu[c], Diego Diez[d], Susumu Goto[d], Theresa Pedersen[b], Anton Razuvaev[e], Sivonne Arvidson[e], Ulf Hedin[e], Kent Lund[f], Tomas Gustavsson[f], Kenneth Caidahl[f], Jesper Z. Haeggström[a], Reijo Laaksonen[g], Minna Jänis[g], Tuulia Hyötyläinen[c], Matej Orešič[c], John Newman[b], Craig E. WHEELOCK[a].

Institute address: [a]Department of Medical Biochemistry and Biophysics, Division of Physiological Chemistry II, Karolinska Institutet, SE-17177, Stockholm, Sweden. [b]USDA ARS Western Human Nutrition Research Center, Davis, CA, USA. [c]VTT Technical Research Centre of Finland Tietotie 2, Espoo, FIN-02044, Finland. [d]Bioinformatics Center, Kyoto University, Uji, Japan. [e]Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden. [f]Clinical Physiology, Karolinska Institutet, Stockholm, Sweden. [g]Zora Biosciences Oy, Espoo, Finland.

Abstract: Cardiovascular disease is the major cause of premature death in Europe, resulting in >4 million deaths annually. Cardiovascular disease is often regarded as a "men's" disease; however, it is the leading cause of mortality among women. In particular, since 1984, more US women than men have died annually from cardiovascular disease. Studies have suggested a key role for oxidized fatty acids (oxylipins) in inflammatory reactions of atherosclerosis; however, it is still unclear to what extent pro- and anti-inflammatory factors determine whether an atherosclerotic lesion develops into a stable plaque or ruptures, leading to stroke or myocardial infarction. To further probe the etiology of plaque development and subsequent rupture, we performed a combination of lipidomics and metabolomics as well as targeted oxylipin, free fatty acid and endocannabinoid lipid mediator profiling in human carotid atherosclerotic plaques and matching circulating plasma. These data were combined with sonographic gray-scale median plaque imaging and Affymetrix GeneChip® data as well as patient clinical parameters to develop a multivariate model of plaque gender-specificity. PCA analysis showed that plaque and plasma have unique composition (R^2=0.54, Q^2=0.41), which was mainly driven by oxylipin levels, despite the fact that endocannabinoids represent >90% of the lipids measured. Orthogonal projections to latent structures (OPLS) analysis based on gender resulted in a robust oxylipin-driven model with high predictive power, which was specific for plaque (R^2=0.87, Q2^=0.43). No model could be generated using data from circulating plasma. The most important variables driving the gender separation were primarily products of the 12/15-lipoxygenase pathway (12/15-LOX), suggesting gender-specific differences in this key pathway. These trends were supported by gene set enrichment analyses (GSEA) showing enrichment in linoleic- and eicosanoid-specific pathways in plaque, but not plasma. Accordingly, the observed changes in lipid and gene data agreed in the vector and magnitude of the alterations, suggesting that gender-specific pathways do exist in carotid plaques. Imaging data suggested that plaques in women had distinct morphological differences, with women having overall more calcified plaques. Collectively, results point to gender-specific shifts in inflammatory lipid species as well as morphological differences that could potentially explain the higher incidence of cardiovascular disease in women.

IDENTIFICATION OF NOVEL FAMILIES OF BIOACTIVE OXIDIZED PHOSPHOLIPIDS GENERATED BY IMMUNE CELLS

Author: Valerie O'DONNELL

Institute address: School of Medicine, Cardiff University, UK

Abstract: Phospholipids are centrally important in cell biology, as they provide a permeability barrier, and act as substrates for generation of bioactive lipid mediators. Recently, we used mass spectrometry approaches to identify several new families of bioactive lipids that form by the enzymatic oxidation of membrane phospholipids in circulating innate immune cells and platelets. These comprise eicosanoids attached to either phosphatidylethanolamine (PE) and phosphatidylcholine (PC) and they are generated within 2 - 5 min of cell activation by pathophysiological agonists, via the coordinated action of receptors and enzymes. These include thrombin, collagen and bacterial products. In contrast to traditional eicosanoids, they are not secreted and remain cell-associated for up to 3 hr following their generation. Enzymes that generate these lipids include 5-, 12- and 15-lipoxygenases (LOX) and cyclooxygenase-1 (COX-1). This presentation will summarize what is currently known regarding the structures, mechanisms of formation, cell biology, and signaling actions of enzymatically-generated esterified eicosanoids. Phospholipid oxidation by acutely activated immune cells is shown to be a controlled event, proposed to play a central role in regulating membrane biology and innate immune function during health and disease. The mass spectrometry methods used for identification of these lipids will also be reviewed, and how these approaches can be used for discovery of new lipid mediators in complex biological samples described.

NOVEL ANALYTICAL APPROACHES FOR THE QUANTITATIVE AND QUALITATIVE ANALYSIS OF LIPIDS IN BIOFLUIDS

Author: Rob J. VREEKEN

Institute address: Netherlands Metabolomics Centre & Division of Analytical Biosciences/LACDR, Leiden University, Leiden, Netherlands

Abstract: Lipids and more specific, Oxylipins (Including eicosanoids, like prostaglandins, leukotriens and thromboxanes, but also hydroperoxides, alcohols, epoxides, and diols) are important biological molecules involved in numerous processes, like, e.g., cell signalling, inflammation, trafficking of lipid particles (HDL, VLDL and LDL), and associated to various pecific phenotypes. In order to understand their specific biochemical role better, a detailed quantitative and qualitative analysis in a variety of bio-samples from various compartments, as well as between species, is required. Especially in view of translational studies related the latter is the more important. The quantitative analysis of oxylipin compounds was accomplished using U/HPLC coupled to triple quadrupole mass spectrometry using dynamic MRM. Eicosanoids produced from the omega-6 polyunsaturated fatty acid arachidonic acid, linoleic acid and dihomo-γ-linolenic acid as well as from the omega-3 poly unsaturated fatty acids α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid are inluced in our target library. Both pro-and anti-inflammatory oxylipns are included. Our approach allows detection and quantification of more than 100 oxylipin compounds down to nanomolar level. A variety of studies involving different biofluids from human (plasma, serum, etc) and rodents like mouse, rat etc has been excecuted. Large variations were observed both in concentration as well as in the in the general profile, especially in between different species. Moreover, in different human (plasma) samples compositional- as well as quantitative differences are observed due to biological variation, circadean rhytms, differences in sampling time and storage conditions as well as compartment of sampling. Next, applying the developed method to samples from patients undergoing cardiac surgery and experiencing myocardial ischemia/reperfusion injury and having harsh inflammation, a clear upregulation of some LOX derived oxylipins confirmed their clinical diagnosed inflammed state. Levels though were upregulated to a lower level than expected. Does this point to moe local effects as opposed to the expected more systemic presence? Distribution of our targets compounds between lipid particles, like HDL, VLDL and LDL showed an intriguing distribution of these compounds, leading to new hypothesis on trafficking of inflammation. Especially, the distribution of epoxides in relation to the hydroxylated compounds was strickingly different in the various compartments.

EICOSANOIDS AND DOCOSANOIDS IN PLASMA AND AORTA OF HEALTHY AND ATHEROSCLEROTIC RABBITS: ESTABLISHMENT OF A MULTIPLEX HPLC ESI MS-MS METHOD

Authors: Monika Szklenar, Janine Kalkowski, Verena Stangl, Mario Lorenz, Ralph RÜHL

Institute address: Laboratory of Nutritional Bioactivation and Bioanalysis, Department of Biochemistry and Molecular Biology, Medical and Health Science Center, University of Debrecen, Hungary

Abstract: Various eicosanoids and docosanoids have been described to be positively and negatively involved in atherosclerosis. These docosanoids and eicosanoids are metabolites from poly-unsaturated fatty acids (PUFAs) which were important nutrients and then metabolized by lipoxygenases and cycloxygenases to various mono-hydroxy-metabolites of PUFAs which can be further metabolized by specific enzymes to eicosanoids and docosanoids. In this study rabbits were fed with a control or a high-cholesterol diet to induce atherosclerotic lesions and to determine pro- or anti-inflammatory lipid mediators in atherosclerotic vessels. For the determination we especially developed an HPLC MS-MS method for the determination of various PUFAs, mono-hydroxylated PUFA-metabolites and furthermore bioactive derivatives like leukotrienes, prostaglandins, resolvins and other eicosanoid classes. This HPLC ESI MS-MS method was established for 53 PUFA-metabolites and applied for plasma and tissu e analysis. In aortic samples from atherosclerotic rabbits we determined for the first time various eicosanoids / docosanoids and observed an increased concentration of 12-lipoxygenase metabolites. Especially, increased levels of 12-hydroxy-eicosatetraenoic acid (12-HETE) in high-cholesterol versus control animals as well as increased ratios of 12-HETE / arachidonic acid (AA) ratios indicates that 12-lipoxygenase metabolites may have importance in atherosclerosis. In addition, decreased concentrations of the 5-lipoxygenase metabolite leukotriene B4 (LTB4), LTB4/AA ratio and hepoxilin levels were detected in high-cholesterol animals. A positive correlation of total plaque area with plasma levels of 12-HETE and a negative correlation with aortic levels of endogenous PPARγ-ligand 13-oxo-octadecadienoic acid (13-KODE) were found. This study let us conclude that the cholesterol content in the diet might influence atherosclerosis via increased 12-lipoxygenase and cycloxygenas e mediated pathways and reduced 5-lipoxygenase pathways.

GENERATION OF LYSOPHOSPHATIDYLINOSITOL, AN ENDOGENOUS AGONIST FOR NOVEL CANNABINOID RECEPTOR GPR55, BY INTRACELLULAR PHOSPHOLIPASE A1

Authors: Atsushi YAMASHITA, Tsukasa Kumazawa, Hiroki Koga, Saori Oka, Yoko Nemoto-Sasaki, Yasuhiro Hayashi, Takashi Tanikawa and Takayuki Sugiura

Institute address: Faculty of Pharmaceutical Sciences, Teikyo University Kaga 2-11-1, Itabashi-Ku, Tokyo 173-8605, Japan

Abstract: GPR55 is a seven-transmembrane G protein-coupled receptor. Recently, several groups reported that GPR55 is a possible novel type of cannabinoid receptor, yet the details remain obscure. Very recently, we explored a possible endogenous ligand for GPR55 using HEK293 cells expressing GPR55 and found that lysophosphatidylinositol (LPI) induces rapid phosphorylation of the extracellular signal-regulated kinase (ERK) and a rapid transient increase in the intracellular free Ca2+ concentration ([Ca2+]i) in these cells 1). Structure-activity relationship of various molecular species of LPI using HEK293 cells expressing GPR55 revealed that the biological activity of the arachidonic acid-containing species is markedly higher than those of others. These results strongly suggest that the 2-arachidonoyl species of LPI is the natural ligand for GPR552). However, the biosynthetic pathway of LPI, in particular, the arachidonic acid-containing LPI have not fully understood. Since 1-stearoyl-2- arachidonoyl species is most abundant in PI from mammalian tissues, phospholipase A1 may be involved in the formation of 2-arachidonoyl-LPI. We examined whether intracellular phosholipase A1, DDHD domain containing 1 (DDHD1), previously identified as phosphatidic acid (PA)-preferring PLA1 (PA-PLA1)3), involved in the formation of 2-arachidonoyl-LPI4). We found that purified FLAG-DDHD1 have PLA activity toward [3H]PI to form [3H]LPI. The purified enzyme showed apparent Km of 10 mol% for PI and a Vmax of 190 μmol/min/mg in the presence of 1% of Triton X-100. This enzyme has PA-hydolytic activity and showed apparent Km of 4 mol% and a Vmax of 200 μmol/min/mg. [3H]arachidonic acid-containing LPI formed when HEK293 cells expressing DDHD1 were prelabeled with [3H]arachidonic acid and activated by ionomycin. We also found that rat brain contains a substantial amount of the arachidonic acid-containing LPI (22.1%) as well as the stearic acid-containing species (50.5%). These results suggest that DDHD1 may be involved in the synthesis of arachidonic acid-containing LPI since DDHD1 is expressed in brain.

(1) Oka et al, Biochem. Biophys. Res. Commun. (2007) 362, 928-934, (2) Oka et al, J. Biochem. (2009) 145, 13-20, (3) Higgs et al, J. Biol. Chem. (1998) 273, 5468-5477, (4) Yamashita et al, Biochim Biophys Acta. (2010) 1801, 711-720

TRANSFER OF ESSENTIAL FATTY ACIDS FROM AQUATIC TO TERRESTRIAL ECOSYSTEMS

Author: Michail I. GLADYSHEV

Institute address: Institute of Biophysics of Siberian Branch of Russian Academy of Sciences, Akademgorodok, Krasnoyarsk, 660036, Russia Siberian Federal University, Svobodny av. 79, Krasnoyarsk, 660041, Russia

Abstract: Omnivorous animals, including humans, must get the physiologically important polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid, (EPA) and docosahexaenoic acid (DHA) from food. Only some taxa of microalgae, rather than higher plants can synthesize de novo high amounts of EPA and DHA. Once synthesized by microalgae, PUFA are transferred through trophic chain to organisms of higher levels. Thus, aquatic ecosystems play the unique role in the Biosphere as the principal source of EPA and DHA for most omnivorous animals, including inhabitants of terrestrial ecosystems. PUFA are transferred from aquatic to terrestrial ecosystems through riparian predators, global flux ~ $2\ 10^6$ kg y^{-1}, through emergence of amphibiotic insects ($240\ 10^6$ kg y^{-1}), and through water birds ($432\ 10^6$ kg y^{-1}). The essential PUFA are transferred through trophic chains with about twice higher efficiency than bulk carbon. Thereby, PUFA are accumulated, rather than diluted in biomass of organisms of higher trophic levels, e.g., in fish.

Man withdraws from aquatic ecosystems through fishery ~$180\ 10^6$ kg y^{-1} of EPA+DHA. However, global average personal daily consumption of EPA+DHA is only about 0.1 g. Thus, mankind is faced with a severe deficiency of the physiologically important long-chain PUFA in diet. Aquatic ecosystems should be protected from anthropogenic impacts, such as eutrophication, pollution and warming, which reduce PUFA production. For instance, in European lakes climate warming in conjunction with eutrophication stimulates a shift from Salmoniformes fish, which contain 2.1-4.4 g kg^{-1} of EPA+DHA, to Cypriniformes fish, which contain only 0.8-1.6 g kg^{-1} of EPA+DHA.

Besides the essential nutrients, PUFA, toxic materials, such as heavy metals and pesticides can be transferred through aquatic food chains and accumulated in organisms of higher trophic levels, namely in fish. Various fish species from diverse locations have different contents of PUFA, as well as different concentrations of toxic compounds. Thus, a quantitative estimation of risks versus benefits of fish intake for human health is very desirable. A formula for calculation of the risk-benefit ratio is suggested. Data are given on quantity of various fish products to be consumed for obtaining the recommended appropriate intake of EPA+DHA for humans.

OMEGA-3-DERIVED MEDIATORS COUNTERACT OBESITY-INDUCED ADIPOSE TISSUE INFLAMMATION

Author: Esther TITOS

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Abstract: Obesity has become an epidemic in developed countries and is considered the main driver for the development of metabolic syndrome and its complications namely insulin resistance, type 2 diabetes, cardiovascular disease and non-alcoholic fatty liver disease. Unresolved and chronic low-grade inflammation in visceral adipose tissue has been recognized as a key step in the development of obesity-associated complications. During weight gain, the accumulation of infiltrating macrophages and to a greater extent, their phenotypic switching to M1-type macrophages in adipose tissue, exacerbates the production of proinflammatory adipokines and establishes the link between obesity and insulin resistance. Resolvins and Protectins are potent anti-inflammatory and pro-resolving mediators endogenously generated from omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These lipid mediators act as "stop-signals" of the acute inflammatory response counteracting the proinflammatory effects of arachidonic acid lipid mediators and thus promoting the resolution of inflammation. Recently, it has been shown that obese adipose tissue has a deficit of these endogenous anti-inflammatory signals and that their partial restoration using enriched diets improve the inflammatory status of adipose tissue and ameliorate metabolic dysfunction. Mechanisms for resolvins and protectins and their precursors in the amelioration of insulin resistance and prevention of hepatic steatosis in obese mice, include up-regulation of adiponectin, IRS-1 and GLUT-4 expression and induction of AMPK phosphorylation in adipose tissue. Moreover, these changes are accompanied by down-regulation of proinflammatory adipokines MCP-1, IL-6, TNF-alpha, up-regulation of anti-inflammatory adipokine IL-10 and the more relevant, the switch in the polarization of adipose tissue macrophages toward an anti-inflammatory M2-like phenotype. Reinforcing these results, recent data demonstrated that transgenic restoration of omega-3 fatty acids in obesity improves resolution capacity and alleviates adipose tissue inflammation, insulin resistance and protects obese mice from fatty liver disease. Together, our results and other recently published data, support a strong rationale for using omega-3 derived mediators in the resolution of adipose tissue inflammation and prevention of obesity-derived complications.

OMEGA3 AND THE AGING BRAIN: FROM EPIDEMIOLOGICAL DATA TO INTERVENTION STUDIES IN HUMANS

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Abstract: The two main causes of late-life cognitive decline and dementia are Alzheimer's disease (AD) and vascular dementia, including many mixed forms. AD is characterized by the accumulation of beta-amyloid and hyperphosphorylated tau proteins in the brain for decades. Thus, late-life dementia results from a complex interaction between aging, genetics (apopolipoprotein E4 allele, ApoE4), and lifelong environmental factors including dietary omega3 fatty acids.

Long-chain omega3 (n-3) polyunsaturated fatty acids (PUFA), especially docosahexaenoic acid (DHA), have a fundamental role in the brain. Indeed, DHA is a major component of neuronal membranes under the form of phospholipids, contributing to membrane fluidity and synaptic plasticity. In addition, long-chain n-3 PUFA exert protective effects on the vascular system including lowering triglyceride levels, anti-arrhythmic effects, vasodilatation, lower blood pressure, decreased inflammatory response and decreased platelet aggregation. Moreover, fatty acids can modulate the expression of gene receptors since they are potent ligands for nuclear receptors. Eicosapentaenoic acid (EPA), DHA and some derived eicosanoids are activators of peroxysome proliferator activated receptors (PPAR). PPARs inhibit NFkappaB, a transcription factor that plays an important role in various inflammatory processes.

Longitudinal epidemiological studies have reported that people with high dietary intake of fish or long-chain n-3 PUFA or higher blood levels of n-3 PUFA have lower risk of dementia or cognitive decline, although some contradictory results exist as well (Cunnane Prog Lipid Res 2009). This protective effect seems to be more pronounced in individuals who are not ApoE4 carriers.

However, most randomized controlled trials of n-3 PUFA for the prevention or treatment of dementia or cognitive decline have yielded disappointing results as shown by a recent meta-analysis (Mazereeuw Neurobiol Aging 2012). Thus, there is presently no evidence supporting the use of omega3 supplements for the prevention of dementia or cognitive decline in older adults (Dangour Br J Nutr 2012). More research is needed to understand the gene-by-diet interactions, determine the best quantities and proportions of EPA and DHA that should be administered, and identify groups of subjects who could most effectively benefit from supplementation with n-3 PUFA.

QUANTITATIVE PROFILING OF OMEGA-6 AND OMEGA-3 EICOSANOIDS BY LCMSMS IN HEALTH AND DISEASE

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Abstract: Over the past twenty years we have been involved in the quantitative profiling of eicosanoids and their metabolites using deuterium labeled internal standards and separation of the analytes on a capillary GC and detection by MS in the NICI mode. Due to the cumbersome extraction and purification methods required for such analyses and the low throughput of the analysis, and the newer emerging MS technology over the last decade, we shifted our analytical strategy to LCMSMS for faster and improved analysis with better sample throughput. Hence over the past decade we have set up and used quantitative profiling techniques employing LCMSMS (Sciex 4000 and the more sensitive Sciex 5500) to measure eicosanoids derived from arachidonic acid, i.e. eight prostanoids including 8-isoprostaneF2 and 11-dehydro thromboxane B2, LOX products including six HETEs, LTB4, two Hepoxilins, four EETs and AA (22 in total + internal standards) using deuterium labeled internal standards of each analyte. Recently, we expanded this technique to the omega-3 eicosanoids to provide quantitative profiling of 40 compounds in a given sample including EPA and DHA plus deuterated internal standards. The limit of sensitivity of the assay is in the low picogram level depending on the analyte investigated. Additionally, we have employed ms3 technology to 'clean-up' the signal obtained, useful when investigating a specific analyte, e.g. 8-isoprostaneF2, in difficult-to-purify samples as urine. We have employed these techniques to monitor eicosanoid changes in tumours from mice xenograft models and the effect of certain drugs on tumour growth and eicosanoid patterns. Data will be presented on an experimental drug related to the hepoxilins that caused inhibition of tumour growth in a leukemic xenograft model. Other practical applications in animal models and in man will be presented. (Supported by private funding)

ISOPROSTANES AND NEUROPROSTANES, METABOLITES OF OMEGA-6 AND OMEGA-3 PUFAS : NOT ONLY BIOMARKERS OF LIPID PEROXIDATION

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Abstract: Since the discovery by Roberts et al. in 1990 that isoprostanes (IsoPs) were formed by a free radical mediated, non-enzymatic mechanism from arachidonic acid (AA, C20:4 omega n-6) in vivo,1 an important field of research has been developed.2 Docosahexaenoic acid (DHA, C22:6 n-3) located mainly in the grey matter and, more recently, adrenic acid (AdA, C22:4 n-6) located in the white matter, undergoe such a lipid peroxidation to produce, neuroprostanes (NeuroPs)3 and dihomo-isoprostanes (Dihomo-IsoPs),4 respectively. In order to fully assess the physiological importance of each of the enantiomerically pure IsoPs, NeuroPs and dihomo-IsoPs, we have developed different chemical strategies.5 The total synthesis of these new metabolites, preliminary data on physiological activities and the use of such lipids as biomarkers of oxidative stress in human, will be presented.

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PRO-RESOLUTION LIPIDS: GAMEKEEPERS TURN POACHERS

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Abstract: Inflammation is a primordial response to infection and injury that seeks to neutralise and eliminate foreign organisms and/or material. Thus, inflammation is no trivial event. Life depends upon it. In general, the innate inflammatory response initiates within minutes and resolves within hours. Chronic inflammation, on the other hand, persists for weeks, months or even years and, unlike the acute response, is the side of host immunity we need to avoid. Notwithstanding, dispersed among this black and white view of inflammation are shades of grey. We are constantly reminded that defining inflammation is not so easy and that while acute inflammation can resolve, it can also be recurrent and that, over time, chronic inflammation can also resolve or persist with devastating consequences to the host. In this presentation, one of these many aspects of the inflammatory response – how acute inflammation resolves and the role of macrophages in this process will be discussed. I will present new data on so-called resolution-phase macrophages, describe their phenotype in the context of conventional nomenclature and speculate on their function in resolution and homeostasis.

MACROPHAGE REMODELING DURING THE RESOLUTION OF INFLAMMATION

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Abstract: Macrophages are highly plastic leukocytes that adopt different molecular and functional phenotypes during the onset, progression, and termination of immune responses. The engulfment of apoptotic leukocytes (efferocytosis) by macrophages during the resolution of inflammation is essential for homeostasis and results in macrophage reprogramming/immune-silencing. We found CD11b-high macrophages convert to CD11b-low ones and stop efferocytosing apoptotic PMN after reaching an engulfment threshold in vivo. In addition, CD11b-low macrophages are distinct from either M1 or M2 in their expression of iNOS, arginase-1, COX-2, 12/15-lipoxygenase, and matrix metalloproteinase-9 and express pro-resolving properties, such as diminished responses to different TLR ligands ex vivo and propensity to emigrate from resolving inflammation sites to lymphoid organs. Of interest, we found the pro-resolving lipid mediators resolvin (Rv) E1 and RvD1, as well as the glucocorticoid dexamethasone (Dex) to enhance satiated-efferocytosis, whereas genetic deficiency in the chemokine scavenging receptor D6 resulted in delayed satiation and reduced immune-silencing of macrophages, and inhibits their departure from resolving inflammation sites. In sum, we suggest satiated-efferocytosis is a novel phagocyte property displayed by CD11blow macrophages and regulated by pro-resolving mediators. Moreover, satiated-efferocytosis is required for CD11blow macrophage emigration from resolving inflammation sites and the return of tissue homeostasis. Thus, satiated-efferocytosis is essential for the completion of timely- and spatially-coordinated resolution of acute inflammation.

EMERGING ROLES OF EOSINOPHILS AND EOSINOPHIL-DERIVED LIPID MEDIATORS IN ACUTE INFLAMMATION AND RESOLUTION

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Abstract: Acute inflammation in healthy individuals is self-limiting and has an active termination program. The mechanisms by which acute inflammation is resolved are of interest. In murine zymosan-induced peritonitis, we found that eosinophils are recruited to the inflamed loci during the resolution phase of acute inflammation. In vivo depletion of eosinophils caused a resolution deficit, namely impaired lymphatic drainage with reduced appearance of phagocytes carrying engulfed zymosan in the draining lymph node, and sustained numbers of polymorphonuclear leukocytes in inflamed tissues. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based lipidomics of the resolving exudates revealed that locally activated eosinophils in the resolution phase produced 12/15-lipoxygenase-derived mediators including protectin D1 (PD1) from docosahexaenoic acid. The resolution deficit caused by eosinophil depletion was rescued by eosinophil restoration or the administration of PD1. Eosinophils deficient in 12/15-lipoxygenase were unable to rescue the resolution phenotype. The present results indicate that mouse eosinophils and eosinophilderived lipid mediators have a role in promoting the resolution of acute inflammation, expanding the roles of eosinophils in host defense and resolution.

METHOD OF IDENTIFICATION AND QUANTIFICATION OF RESOLVIN D5 IN BIOLOGICAL SAMPLES: APPLICATION TO AN IN VIVO ANIMAL MODEL FOR EVALUATION OF INFLAMMATION

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Abstract: Inflammation is a normal process of defence in Humans and Animals as well. It is a phenomenon that appears, increases and resolves. It has been described that mediators issued from the arachidonic, the docosahexaenoic and the eicosapentaenoic acids are actively involved in the resolution and good ending of the inflammatory process. Those bioactive mediators are called specialized pro-resolving mediators (SPMs). Recently, CN Serhan's team has described that Resolvins (Rv) D5 and D1 and protectin D1 (PD1) each directly enhanced phagocytosis of E. coli, opening then a new role for these bioactive mediators on inflammation, which occurs during infection. In conjunction with these results, we have developed a new and sensitive method for concomitant detection and quantification of RvD1, RvD5 and PD1 by using liquid chromatography-tandem mass spectrometry. As no commercial source of RvD5 was available, we have developed a new method to generate and detect this compound. Using an elegant in vitro approach with its known precursor DHA, RvD5 was produced in amount sufficient to allow its complete characterization. Identification was done according to mass spectrometry and literature description. Then, quantification was achieved using chemically related molecule on a triple quadrupole mass spectrometer. The linearity and the accuracy of the method, the limit of detection and the limit of quantification were considered on a mixture of pure standards for RvD1 and PD1. All compounds were separated and quantified on a C18 column and analyzed on a triple quadrupole detector. Mass Hunter quantitative (MH Quant) analysis software was used to generate calibration lines. The limit of detection (LOD) and the limit of quantification (LOQ) were determined for the 3 compounds. Armed with this information, we have applied this methodology of dosage to quantify RvD1, RvD5 and PD1 in samples issued from a zymosan-induced peritonitis to follow production of those mediators during sterile inflammation.

MICROSOMAL PROSTAGLANDIN E SYNTHASE-1 – A MULTI FACETTED DRUG TARGET

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Abstract: Microsomal prostaglandin E synthase-1 (mPGES-1) constitutes a drug target for inflammation, fever and pain. mPGES-1 catalyzes the biosynthesis of prostaglandin (PG) E_2 from cyclooxygenase (Cox) -derived PGH_2, which in turn is derived from arachidonic acid. mPGES-1 is mainly associated with inflammation and it is known to be up regulated by various pro-inflammatory cytokines like IL-1 beta and TNF-alpha. Mice devoid of mPGES-1 activity display resistance to development of experimental arthritis, fever, pain, symptoms following a stroke, atherosclerosis and breathing anomalies induced by hypoxia. Alternatively, the enzyme seems to have a protective role in wound healing as well as after myocardial infarction. Inhibitors of mPGES-1 have been developed by several groups. However, their characterization in animal models of inflammation, or other models previously used to study mPGES-1 knock-out mice, remains limited. One reason is the fact that potent inhibitors of human mPGES-1 in many cases are significantly less potent or even completely inactive towards rodent mPGES-1. In addition, the impact of mPGES-1 inhibitors on the prostaglandin profile should be further investigated in various cells and *in vivo* systems, as their effects but also side-effects will largely depend on the particular prostaglandin profile they elicit. In fact, one may argue that an mPGES-1 inhibitor, in terms of mechanism of action, will have little in common with upstream Cox inhibitors. In this presentation, an update will be provided on 1) mPGES-1 biochemistry; 2) its persistent expression in arthritic joints/muscles from patients with rheumatoid arthritis/myositis despite ongoing anti-inflammatory treatments; and 3) our current work on the characterization of mPGES-1 inhibitors.

YOUNG SESSION

Friday, September 28th, 2012

THE ENDOCANNABINOID 2-ARACHIDONOYLGLYCEROL CONTROLS MACROPHAGE ACTIVATION THROUGH ITS OXIDATIVE METABOLITE PROSTAGLANDIN 2-GLYCEROL (PGD2-G)

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Abstract: Macrophages are key players in innate and adaptive immune responses. Their role during an inflammatory process is to eliminate the threat and protect the body from noxious agents. However, under persistence of the pro-inflammatory phase or when macrophages trigger an altered response, inflammation becomes chronic and thus deleterious. Therefore pro-inflammatory macrophages responses must be controlled in chronic inflammatory settings. The endocannabinoid 2-arachidonoylglycerol (2-AG) exerts anti-inflammatory actions in various settings, classically through activation of the cannabinoid receptors CB1 and CB2. Here, using two macrophage cell lines in culture, we show that 2-AG controls macrophage activation (LPS, 100ng/mL). Indeed, 2-AG counteracts the LPS-induced production of pro-inflammatory cytokines, such as interleukin (IL)-1beta (IC50 = 6 microM), IL-6 and tumor necrosis factor-alpha, as well as nitric oxide production. Interestingly, in both cell lines analyzed, we show, using receptorselective antagonists (SLV319 and SR144528), that the effects of 2-AG are cannabinoid receptors independent. Moreover, this response seemed to be 2-AG specific, since other fatty acid glycerols (e.g. 2-oleoylglycerol) had no effect on the LPS-induced response. Thus, considering that LPS induces cyclooxygenase (COX)-2 expression and that 2-AG could also be oxidized by COX enzymes, similarly to arachidonic acid, to give protanglandin-glycerols (PG-G), we sought to investigate the biological effects of these PG-G. In the same setting, PGD2-G reduced the LPS-induced expression of pro-inflammatory cytokines and nitric oxide production, whereas PGE2-G and PGF2alpha-G strongly exacerbated the LPS-induced macrophage response. The effect of PGD2-G is not due to its hydrolysis into PGD2, since the latter had no effect in the same setting, or to the activation of the PGD2 receptors DP1 and DP2. Additionally, we show through RT-qPCR analysis, that in the cell lines used, prostaglandin D synthase is more expressed than prostaglandin E synthase. This is corroborated by the fact that these cells produce significantly more PGD2 than PGE2 as assessed by HPLC-MS. Thus 2-AG could be oxidized by COX-2 into PGH2-G and then preferentially give rise to PGD2-G in this setting. In conclusion, we describe here a novel pathway through which the endocannabinoid 2-AG can control inflammation.

ENDOGENOUS EPOXYGENASES REGULATE ENDOTHELIAL CELL TNF-ALPHA SECRETION

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Abstract: Members of the cytochrome p450 family known as epoxygenase enzymes are responsible for the conversion of arachidonic acid into epoxyeicosatrienoic acids (EETs) and are rapidly metabolised by soluble epoxide hydrolase (sEH) into dihydroxyeicosatrienoic acids (DHETs) which are biologically inactive. Whilst the role of EETs as a vasodilator and mediator of cell proliferation is relatively well established, its anti-inflammatory effects are still poorly understood. This study aimed to examine the anti-inflammatory role of epoxygenase enzymes in the human vascular endothelial cells. EAhy.926 and human blood derived primary endothelial cells (hBlEC) were used in culture. Epoxygenase expression was determined by RT-PCR or real-time RT-PCR. Cells were treated with the epoxygenase inhibitor N-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MS-PPOH) or with sEH inhibitor 12-tricyclodecylamino-carbonylamino-dodecanoic acid (AUDA) in the presence and absence of bacterial lipopolysaccharide (LPS). TNFalpha release was quantified using ELISA. BlEC and EAhy.926 cells expressed the CYP2J2 epoxygenase which was further induced (3.3±1.1 and 2.1±0.4 fold respectively; n=3-4, $p<0.05$) by LPS (1ug/ml; 4h). EAhy926 also constitutively expressed CYP2C8, but not CYP2C9 mRNA, under basal conditions. In Eahy926 inhibition of epoxygenases using MS-PPOH (10uM) increased TNFalpha release on its own, but had no significant effect on LPS induced TNFalpha release (Control, 1; MS-PPOH 2.6±0.8*; LPS 6.9±2.0*; MS-PPOH+LPS 7.5±1.9* fold; n=5 from 3 separate experiments; *$p<0.05$ compared to control). In contrast, elevating endogenous epoxygenase products using AUDA decreased TNFalpha release in the presence and absence of LPS (Control 1; AUDA 0.7±0.04*; LPS 1.7±0.11; AUDA+LPS 0.4±0.05* fold compared to control; n=8 from 4 separate experiments; *$p<0.05$ AUDA compared to respective control or LPS stimulated cells). These findings support the concept that epoxygenases have a vasoprotective and anti-inflammatory role in human endothelial cells and that elevating epoxygenase products by using sEH inhibitors may provide therapeutic benefit to vascular and inflammatory disorders.

This work was supported by a grant from the British Heart Foundation (PG PG/11/39/28890)

COMPARISON OF THE RELAXATIONS INDUCED BY PGI2 ANALOGUES (USED CLINICALLY), IN ISOLATED HUMAN PULMONARY VESSELS: ROLE OF THE DP RECEPTOR

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Abstract: Introduction: Pulmonary hypertension (PH) involves pulmonary vasoconstriction. Among the treatments of PH, prostacyclin (PGI2) and its analogues (iloprost, treprostinil, beraprost and MRE-269) are potent vasodilatators thought to act predominantly by activating the PGI2 receptor (IP). A recent study has shown that iloprost and treprostinil also have high affinity for some other prostanoid receptors including EP1 and DP, respectively (ref1). Additionally, in human pulmonary artery (HPA) and vein (HPV), the IP receptor and the IP/EP4/DP receptors are the prostanoid receptors responsible for vasorelaxation, respectively (ref2-3). The aims of this study are to compare the relaxant effects (in vitro) of PGI2 analogues used clinically and to determine which relaxant receptors (IP/EP4/DP) are involved in HPA and HPV. Methods: HPV (n=8) and HPA (n=12) preparations obtained from lung cancer patients were placed in an organ bath system for vascular tone measurements. After precontraction with norepinepherine (NE, 10μM), dose-response curves with PGI2 analogues were generated. In addition, some preparations were incubated (30 min) with one of the following antagonists (1, 10μM) of various prostanoid receptors: [Cay10441 (IP), GW627368X (EP4/TP), L-877499 or BWA868C (DP)]. Results: Iloprost and treprostinil were the more relaxant agonists in both HPA and HPV when compared with the other analogs. Maximal relaxation (% of NE-precontraction) and pEC50 values were in HPA: [iloprost (-86±08%, 7.68±0.15); treprostinil (-86±05%, 6.84±0.08)] and in HPV: [iloprost (73±06%, 8.13±0.19); treprostinil (-74±12%, 7.63±0.17)]. No significant antagonism with the EP4-antagonist was observed. In contrast, the DP-antagonists inhibited the relaxations induced by treprostinil (not iloprost) only in HPV. Conclusions: Iloprost is a more potent vasorelaxant agonist than treprostinil in HPA. This difference could be explained by their affinities for the IP receptor (Ki= 4nM and 32nM, respectively; ref1). In contrast, in HPV both agonists are equipotent, and this could be explained through both DP- and IP- receptor activation by treprostinil. In conclusion, these data suggest iloprost and treprostinil should be the more potent agonists to facilitate pulmonary blood circulation in PH patients.

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Ref2: Foudi et al., (2008), Br J Pharmacol, 154:1631-39.
Ref3: Walch et al., (1999), Br J Pharmacol, 126:859-66.

We acknowledge United Therapeutics for financial support.

SIMULTANEOUS ACTIVATION OF p38 AND JNK BY ARACHIDONIC ACID STIMULATES THE CYTOSOLIC PHOSPHOLIPASE A2-DEPENDENT SYNTHESIS OF LIPID DROPLETS IN HUMAN MONOCYTES

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Abstract: Exposure of human peripheral blood monocytes to free arachidonic acid (AA) results in the rapid induction of lipid droplet (LD) formation by these cells. This effect appears specific for AA in that it is not mimicked by other fatty acids, whether saturated or unsaturated. LD are formed by two different routes, namely (i) the direct entry of AA into triacylglycerol and (ii) activation of intracellular signaling leading to increased triacylglycerol and cholesteryl ester formation utilizing fatty acids coming from the de novo biosynthetic route. Both routes can be dissociated by the arachidonyl-CoA synthetase inhibitor triacsin C, which prevents the former but not the latter. LD formation by AA-induced signaling predominates, accounting for by 60-70% of total LD formation, and can be completely inhibited by selective inhibition of the group IVA cytosolic phospholipase A2alpha (cPLA2alpha), pointing out to this enzyme as a key regulator of AA-induced signaling. LD formation in AA-treated monocytes can also be blocked by the combined inhibition of the mitogen-activated protein kinase family members p38 and JNK, which correlates with inhibition of cPLA2alpha activation by phosphorylation. Collectively, these results suggest that concomitant activation of both p38 and JNK by AA cooperate to activate cPLA2alpha, which is in turn required for LD formation possibly by facilitating biogenesis of this organelle, not by regulating neutral lipid synthesis

LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE 3 – THE MISSING LINK BETWEEN POLYUNSATURATED FATTY ACIDS AND FERTILITY?

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Abstract: Long-chain polyunsaturated fatty acids (PUFAs) accumulate in mammalian testis during puberty and are essential for fertility. Testes contain three major cell types – germ cells, Sertoli cells and Leydig cells. We recently reported that PUFAs are synthesized by Sertoli cells (the nursing cells of germ cells), stored in a membrane lipid pool and then supplied to germ cells [1]. The enzymatic basis for the accumulation of PUFAs in germ cells and the consequences for fertility were still unknown. We speculated that lysophospholipid acyltransferases – a group of isoenzymes that incorporate fatty acids into phospholipids - might mediate the fertile effect of PUFAs. Thus, we compared the mRNA expression, in vitro activity and specificity of lysophospholipid acyltransferases with changes of the lipidome (= entirety of cellular lipids) during mouse testis maturation. Lysophosphatidic acid acyltransferase (LPAAT)3 was identified as key acyltransferase for PUFA incorporation and testis function [2]. The accumulation of PUFAs in testicular phosphatidylcholine correlated with increased LPAAT3 expression and activity. LPAAT3 was induced during germ cell maturation as shown for mouse testis sections and differentiating GC-2spd(ts) spermatocytes. Accordingly, germ cell differentiation elevated the proportion of polyunsaturated phospholipids and shifted the specificity towards an incorporation of PUFAs into phosphatidylcholine. Stable knockdown of LPAAT3 in GC-2spd(ts) cells decreased LPAAT3 activity, reduced levels of polyunsaturated phospholipids and inhibited cell proliferation during geneticin selection. We conclude that LPAAT3 is induced during germ cell development, incorporates PUFAs into testicular phospholipids and possibly promotes sperm cell production.

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A REGULATORY LOOP BETWEEN DESATURASES AND OMEGA-3 FATTY ACIDS PLAYS A MAJOR ROLE IN NON-ALCOHOLIC STEATOHEPATITIS

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Abstract: The mechanisms underlying non-alcoholic steatohepatitis (NASH) are not completely elucidated. In this study we integrated gene expression profiling of liver biopsies from NASH patients with translational studies in mouse models of steatohepatitis and pharmacological interventions in isolated hepatocytes to identify a novel mechanism implicated in the pathogenesis of NASH. By using high-density oligonucleotide microarray analysis we identified a significant enrichment of known genes involved in the multi-step catalysis of long-chain polyunsaturated fatty acids, namely delta-5 and delta-6 desaturases. A combined inhibitor of delta-5/delta-6 desaturases significantly reduced intracellular lipid accumulation and inflammatory gene expression in hepatocytes subjected to experimental conditions to induce fat accumulation and inflammation. Gas chromatography analysis revealed impaired delta-5 desaturase activity toward the omega-3 pathway in livers from high-fat diet (HFD)-induced obese mice. Consistently, restoration of hepatic omega-3 content in transgenic fat-1 mice expressing an omega-3 desaturase, which allows the endogenous conversion of omega-6 into omega-3 fatty acids, produced a significant reduction in hepatic insulin resistance, steatosis, macrophage infiltration and necroinflammatory injury, accompanied by attenuated expression of genes involved in hepatic inflammation, fatty acid uptake and lipogenesis. These results were comparable to those obtained in a group of HFD-induced obese mice receiving EPA/DHA supplementation in the diet. Of interest, omega-3 enrichment in obese steatotic livers not only repressed delta-5 and delta-6 desaturase expression but diverted their activities toward the omega-3 pathway. Finally, hepatocytes from fat-1 mice or supplemented with EPA exhibited synergistic anti-steatotic and anti-inflammatory actions with the delta- 5/delta-6 desaturase inhibitor. Conclusion: These findings indicate that modulation of desaturase activities and restoration of the hepatic balance between omega-6 and omega-3 fatty acids exert a positive loop in the prevention of NASH.

MECHANISMS OF P. AERUGINOSA-INDUCED EXPRESSION OF SECRETORY PHOSPHOLIPASE A2 TYPE IIA IN CYSTIC FIBROSIS LUNG EPITHELIAL CELLS

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Abstract: Cystic fibrosis (CF) is an inherited lethal disease due to mutations of cftr gene and chronic bacterial infection by opportunistic pathogens such as Pseudomonas aeruginosa is a hallmark of this disease. Our preliminary data showed that in human CF epithelial cells P. aeruginosa stimulated expression of secretory phospholipase A2 type IIA (sPLA2-IIA). The latter has been shown to exhibit bactericidal property against Gram-positive bacteria like Staphylococcus aureus. However, the virulence factors of P. aeruginosa and signalling pathways involved in this expression are still unknown. Human bronchial CF epithelial cell line IB3-1 was used in the present study. Cells were infected by P. aeruginosa and 24 h later sPLA2-IIA expression was analyzed by western blot and qPCR. In certain experiment, cells were pre-incubated with pharmacological inhibitors 1h before infection. Cells were also infected by bacteria for 30min and then protein phosphorylations were examined by western blot. Various strains of P. aeruginosa including PAK, CHA, PAO1 and mucoid strain induced sPLA2-IIA expression in IB3-1 cells at almost similar levels. Two mutants of P. aeruginosa, deltafliC (flagellin deficient) and L88 strain (bearing flagellin mutant not recognized by TLR5) induced sPLA2-IIA expression at similar levels compared to parent strain. Purified flagellin of P. aeruginosa failed to induce sPLA2-IIA expression. Incubation of cells with other P. aeruginosa components including LPS, CpG and quorum sensing (HSL) did not induce sPLA2-IIA expression. Interestingly, sPLA2-IIA expression decreased when cells were treated with pili-deficient compared to corresponding wild type strains. Furthermore, purified pili induced sPLA2-IIA expression. Application of receptor ligands, neutralizing antibodies or inhibitor indicated that TLR2, 4, 5 and intracellular receptors did not participate in P. aeruginosa-induced sPLA2-IIA expression. MAPK Erk and AP-1, but not p38 and NF-kappaB, were proven to be involved in this process. These results indicate that P. aeruginosa induces sPLA2-IIA expression in human bronchial CF epithelial IB3-1 cells via a Erk MAPK and AP-1 dependent process and that the virulence factor pili plays a major role in this induction.

RESOLVIN D1 ACTIVATES SPECIFIC GPCRs AND REGULATES miRNAs IN NOVEL RESOLUTION CIRCUITS

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Abstract: Resolution of acute inflammation is an active process regulated by specialized pro-resolving mediators. Among them, resolvin (Rv) D1 acts on two separate G protein coupled receptors (GPCRs), ALX/FPR2 and RvD1 Receptor (DRV1)/GPR32, and regulates cellular pathways to curtail excessive inflammation and promote resolution. Here, we report the ligand selectivity of RvD1 activation for both ALX/FPR2 and DRV1/GPR32. In addition to RvD1, its aspirintriggered epimer (AT-RvD1) and synthetic RvD1 mimetics, each dose dependently activated both human GPCRs in recombinant cell systems with EC50 (10^-12 to 10^-11 M range) the reported levels of RvD1 present in healthy human plasmas. In murine peritonitis stimulated by zymosan, RvD1 significantly up-regulated miR-21 (70%), 146b (30%), and 219 (50%) as well as down-regulated miR-208a (40%). In transgenic mice expressing human RvD1 receptor ALX/FPR2, miR-208a and miR-219 were significantly up-regulated by RvD1 compared to wild typ e littermates, further corroborating that these regulations occur via activation of RvD1 receptors. The RvD1-GPCR-regulated miRNAs targeted cytokines and proteins involved in the immune response. For instance, miR-146b targeted NF-κB, IKK, TRAF6, TLR1 and 10; miR-208a controlled PDCD4, a negative regulator of IL-10 production; miR-219 significantly reduced 5-lipoxygenase expression and leukotriene B4 biosynthesis. Together, these findings establish the ligand selectivity for pro-resolving agonists of both hALX/FPR2 and hGPR32. Moreover, they indicate that RvD1 regulates specific “Resolution miRs” and their target genes in novel GPCR-resolution circuits for controlling the magnitude and duration of the acute inflammatory response and stimulating its timely resolution.

ADIPOCYTES MODULATE THE PHENOTYPE OF MACROPHAGES THROUGH SECRETED LIPIDS

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Abstract: Adipose tissue secretes a wide range of soluble factors that can influence whole body metabolism. Previous studies have shown an accumulation of macrophages and an enhanced pro-inflammatory profile of these cells in adipose tissue of obese mice. Modulation of macrophages by soluble mediators released by adipocytes has been proposed as a possible mechanism underlying these changes. In humans, an increased number of macrophages in adipose tissue of obese individuals has been observed, although no clear change in macrophages phenotype could be established. Moreover, no information exists about the interaction between macrophages and adipocytes in humans. In the present study, we explored the possibility that adipocytes modulate the phenotype of macrophages and studied the possible molecular pathways involved in this modulation. Treatment of macrophages with adipocyte-conditioned medium (ACM) resulted in a strong reduction in IL12p40 secretion upon LPS stimulation, whereas TNFα and other cytokines remained largely unaffected. This effect was independent of the source of ACM. Interestingly, the inhibition increased with increase in Body Mass Index (BMI) of the adipocyte donor. Therefore, it was hypothesized that the effect is mediated by a soluble factor whose release is correlated to the BMI of the adipocyte donor. To this end, we measured several cytokines, adipokines and lipids present in ACM. Among these, the release of several free fatty acids (FA) and PGE2 correlated with the BMI of the adipocyte donor. Further tests indicated that oleic and linoleic acid, as well as PGE2, were able to inhibit IL12p40 secretion whereas palmitic acid could not. Upon separation of ACM protein and lipid fractions, we confirmed that inhibition of IL12p40 resides mainly in the ACM lipid fraction. These results provide first evidence that obesity-related changes in macrophage phenotype could be mediated by adipocytes in humans. These effects are mainly mediated through lipids released by adipocytes. Intriguingly, modulation appears different than in murine obesity, indicating that the immunomodulatory effects of obesity could be different in humans and mice.

INVESTIGATING THE ROLE OF CYCLOOXYGENASE IN TOLL-LIKE RECEPTOR RESPONSES

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Abstract: Cyclooxygenase (COX) is the first enzyme required for the conversion of arachidonic acid to the prostanoids. There are two isoforms of COX; COX-1 is constitutively expressed in most tissues and plays a homeostatic role, whereas COX-2 is induced by growth factors and at sites of inflammation. Toll-Like Receptors (TLRs) recognise specific, conserved pathogen-associated molecular patterns (PAMPs). They are differentially expressed throughout the body and are essential mediators of the innate immune response. Research has shown that COX products such as prostaglandin E2 suppress TLR4-induced cytokine production [1]. Here, the role of COX in TLR responses in vivo in mice and in vitro, in lung fibroblasts, was investigated. For in vitro studies, lung fibroblasts isolated from human patients or wild-type (WT; C57Bl/6), COX-1-/- and COX-2-/- mice were stimulated with agonists to TLRs 2/1, 2/6, 3, 4, 5, 7 and 8. After 24hrs, media was removed, and IL-8/KC and IP-10 measured by ELISA. Human cells were pre-treated for 30mins with vehicle (0.1% DMSO) or the non-selective COX-1/2 inhibitor diclofenac (10 μM). For in vivo studies, WT, COX-1-/- and COX-2-/- mice were treated with LPS (10mg/kg) or poly (I:C) (8mg/kg). After 4hrs, blood was collected and plasma cytokines measured by ELISA and multi-cytokine array. Human lung fibroblasts released more IL-8 and IP-10 in response to the TLR3 agonist poly (I:C) than to other TLR agonists. Inhibition of COX in human lung fibroblasts resulted in significantly enhanced TLR3-induced IL-8 and IP-10 release. In mouse lung fibroblasts, deletion of COX-1 or COX-2 also resulted in augmented IP-10 and KC release in response to TLR3 ligands, as well as to TLR4 and TLR2/6 ligands. In vivo, COX-2 but not COX-1 deletion significantly increased all measured interferon-related genes (interferon-alpha, -beta, -gamma and -lambda, IP-10). These data indicate that TLR3, which recognises viral-type PAMPs, is the dominant TLR pathway in lung fibroblasts, and that cytokine release stimulated by TLR3 activation is limited by COX activity in vitro and in vivo. We speculate that COX-2 inhibition by non-steroidal anti-inflammatory drugs may augment the innate immune response to viral infection.

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ROLE OF EICOSANOIDS IN TUBERCULOSIS INFECTION

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Abstract: Lipids, apoptosis, and immunity to tuberculosis.
Two different forms of death are commonly observed when *Mycobacterium tuberculosis* infected macrophages die: 1) necrosis, a death modality defined by cell lysis; and 2) apoptosis, a form of death that maintains an intact plasma membrane. Necrosis is a mechanism used by the bacteria to exit the macrophage, evade host defenses, and spread. In contrast, apoptosis of infected macrophages is associated with diminished pathogen viability. Apoptotic vesicles derived from infected macrophages are also an important source of bacterial antigens that can be acquired by dendritic cells to prime antigen-specific T cells. Cell death during mycobacterial infection is regulated by the eicosanoids PGE_2 (pro-apoptotic) and lipoxin LXA_4 (pro-necrotic). Following apoptosis, *M. tuberculosis*-infected macrophages are rapidly engulfed by uninfected macrophages through the process of efferocytosis. Engulfment of *M. tuberculosis* sequestered within an apoptotic macrophage further compartmentalizes the bacterium and delivers it along with the apoptotic cell debris to the lysosomal compartment. Thus, virulent *M. tuberculosis* subvert eicosanoid regulation to promote necrotic death to foil innate and adaptive defense mechanisms.

LIPID MEDIATORS IN AIRWAY INFLAMMATION

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Abstract: Airway inflammation plays a key role in the pathogenesis of chronic lung diseases including bronchial asthma and chronic obstructive pulmonary disease (COPD).Many inflammatory cells, either resident in the lung, e.g., macrophages and mast cells, or recruited from bloodstream, e.g., T cells, neutrophils and eosinophils, are involved in development and perpetuation of airway inflammation. When activated within human airways, these inflammatory cells produce a wide range of lipid mediators including eicosanoids, platelet-activating factor (PAF) and endocannabinoids. Lipid mediators are primarily responsible for several features of asthma and COPD such as bronchoconstriction, amplification of the inflammatory response and tissue damage. Both in vitro and in vivo studies have recently emphasized the role of phospholipases A_2 (PLA_2s) as crucial molecules to regulate production of lipid mediators in the human lung and as potential pharmacological targets to modulate airway inflammation. PLA_2s are currently classified as cytosolic and secreted molecules. Cytosolic PLA2s are the main enzymes involved in mobilization of arachidonic acid within human inflammatory cells and their inhibition almost completely suppress generation of eicosanoids and PAF. Secretory PLA_2s ($sPLA_2$s) are released in inflamed airways by a variety of cells such as mast cells, neutrophils and eosinophils. $sPLA_2$s are not directly involved in arachidonate mobilization but they can potently enhance cPLA2-mediated generation of eicosanoids. We and others have recently shown that $sPLA_2$s, particularly group IA, IB and X, are released in the airways of patients with bronchial asthma. These $sPLA_2$s activates exocytosis and production of cytokines and chemokines from human macrophages, eosinophils and monocytes by a mechanism unrelated to their enzymatic activity. These effects are, in fact, reproduced by $sPLA_2$ mutants in which catalytic activity has been suppressed by chemical inactivation or site-directed mutagenesis. $sPLA_2$-induced cytokine production from macrophages is suppressed by inhibitors of the M-type receptor, a putative receptor for $sPLA_2$s expressed on macrophages. Thus, generation of lipid mediators in human airways is mostly dependent on $cPLA_2$s but it can be strongly potentiated by $sPLA_2$s that can both enhance arachidonate mobilization at cellular level and promote inflammatory cell recruitments in the airways of patients with asthma or COPD:

ADIPOCYTES MODULATE T CELL FUNCTION THROUGH RELEASE OF LIPIDS

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Abstract: Obesity is characterized by the presence of inflammation in adipose tissue. Accumulation of several immune cell types, including CD4+ T cells, has been previously reported in the increasing adipose tissue. This accumulation is also paralleled by changes in cytokine profiles and phenotype of the infiltrating cells. One of the possible mechanisms involved in these changes is the modulation of T cell function by tissue-resident adipocytes. Therefore, we investigated whether adipocytes derived from various adipose tissues can modulate CD4+ T cell cytokine production and proliferation and studied the mechanisms involved in this process. CD4+ T cells produced increased levels of IFN-gamma when activated in the presence of adipocytes. This effect is mediated by soluble mediators, as shown in transwell and adipocyte-conditioned medium (ACM) transfer experiments. Additionally, ACM induced increased proliferation of CD4+ T cells upon activation. Furthermore, adipose tissue contained more IFN-gamma-producing CD4+ T cells than peripheral blood of the same individuals, in 3 out of 3 cases tested, which indicates a possible in vivo relevance of our results. To investigate the possible molecular mechanisms involved in this effect, we separated the protein and lipid fraction of ACM. Surprisingly, despite previous data indicating that several adipocyte-derived proteins can modulate T cell function, we have found that the increased proliferation of T cells is mainly due to the lipids isolated from ACM. Further separation of these lipids based on polarity revealed that the modulatory effect is mainly confined to fractions containing free fatty acids. All identified fatty acids were able to individually enhance T cell proliferation. These data indicate that adipocytes can modulate CD4+T cell function through release of soluble mediators. Remarkably, within the soluble mediators identified, lipids and especially free fatty acids are the most prominent modulators of T cell proliferation.

ELEVATED PROSTAGLANDIN E2 MEDIATES IMMUNE SUPPRESSION IN LIVER CIRRHOSIS

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Abstract: Liver cirrhosis is a leading cause of death worldwide. Cirrhotic patients have a seven-fold increased risk of infection which, if associated with organ dysfunction, carries an 80-90% mortality rate. This susceptibility to infection was first demonstrated 30 years ago, however, the underlying mechanism is unknown. We found reduced leukocyte trafficking coupled with impaired leukocyte function in rodent models of liver injury (Bile Duct Ligation (BDL) and Carbon Tetrachloride (CCL4)). Nitric oxide (NO) was elevated in the plasma of these animals (91(12) vs 50.5(9.6) micromoles, $p<0.05$) preventing extravascular polymorphonuclear leukocyte accumulation (sham vs BDL, $p<0.01$) in a zymosan (0.1mg/kg) model of peritonitis which was fully reversed by L-NAME (50mg/Kg). We found immune-dysfunction in the liver injury rodents, as defined by impaired leukocyte cytokine synthesis (reduced TNFalpha and IL6 and increased IL10, $p<0.001$ c.f. sham) and bacterial killing, was caused by elevated plasma prostaglandin (PG)E2 (BDL 1.66(0.4) vs Sham 0.36(0.1) nanograms/ml, $p<0.05$, ESI/LC mass spectroscopy). A trend towards an increase was seen in 5, 8 and 15-HETrE and 10-, 13-, 14- and 17-HDOHA in liver injury animals. Importantly, cyclooxygenase (COX) inhibition with Indomethacin (3mg/kg) fully reversed immune-suppression in rodents as assessed by Group B Streptococcus bacterial killing in vivo, independent of leukocyte trafficking. PGE2 was also significantly elevated in humans with decompensated cirrhosis (0.091(0.01)vs 0.0015(0.003) nanograms/ml, $p<0.001$) and correlated with immunesuppressive properties of cirrhotic plasma on human macrophage cytokine (reduced TNFalpha and increased IL10, $r2=0.72$, $p<0.01$) and bactericidal (E.coli) function ($p<0.05$). Plasma taken from cirrhotic patients' for up to 6 days after admission was found to be immune suppressive – despite these patients receiving antibiotics. Other lipids were significantly elevated e.g. 5/12/15 HETE and PGF2alpha (but not PGD2) but these had no immune modulatory properties at the concentrations detected in plasma. We also discovered that PGE2 bioavailability is controlled by the plasma protein albumin, which is substantially lowered in cirrhotic patients leading to greater PGE2-mediated immune-dysregulation. Thus, we propose that PGE2 underpins susceptibility to infection in cirrhosis; that its inhibition will be clinically beneficial in this setting while concomitantly easing antibiotic over-usage and that PGE2/albumin represents a robust bio-index for infection vulnerability.

PROSTACYCLIN ANALOGUES IN PULMONARY HYPERTENSION

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Abstract: Prostacyclin and its stable analogues are used to treat pulmonary arterial hypertension (PAH) and therapeutic benefit may be derived from their potent actions on the prostacyclin (IP) receptor. However, increasing evidence suggest these drugs have differential actions at other prostaglandin receptors which might serve to enhance or dampen their therapeutic action. Iloprost has been known for over a decade to be more or less equipotent at activating both human EP_1 and IP receptors (Abramovitz *et al.*, 2000). This contrasts with treprostinil which has a high affinity for the DP_1 and EP_2 receptor while having a 200 fold lower affinity for the EP_1 receptor (Whittle *et al.*, 2012). The consequence of EP_1 receptor activation is to increase intracellular calcium and provoke vasoconstriction, and this may offset the IP-receptor mediated vasodilator and antiproliferative effects of iloprost. On the other hand activation of IP, DP_1 and EP_2 receptors, all of which are linked to cyclic AMP generation through coupling to Gs could act in concert to produce vasodilatation, platelet disaggregation and inhibition of cell growth. Other potential targets for prostacyclin analogues include EP_3 receptors (Abramovitz *et al.*, 2000), which may negatively modulate vasorelaxation induced by prostacyclin analogues' and may thus limit their effects particularly under conditions where IP receptors are down regulated as occurs in PAH (Falcetti *et al.*, 2010). EP_4 receptors appear only to be significantly activated by iloprost and treprostinil at concentrations well outside the therapeutic dose range (Whittle *et al.*, 2012), suggesting such receptors are not a clinically relevant target. Lastly one should consider selexipag (NS-304), an oral, IP receptor agonist currently undergoing clinical trials for the treatment of PAH (Simonneau *et al.*, 2012). MRE-269, the active metabolite of selexipag, potently binds to the human IP receptor but has little binding affinity for other prostanoid receptors. Thus MRE-269 should prove useful in distinguishing between IP and other prostanoid receptor effects. In summary, IP receptor agonists represent a heterogeneous class of agents, probably giving rise to variable therapeutic and side-effect profiles. Further studies are required to understand the nature of prostanoid receptor expression in the lung and the impact of disease on the overall function of these receptors.

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THE PHARMACOLOGY AND THERAPEUTIC APPLICATION OF COX-2 METABOLITES OF MAMMALIAN ENDOCANNABINOIDS

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Prostamide (prostaglandin ethanolamide) research evolved from two separate avenues of research (a) the discovery that anandamide is a substrate for cyclo-oxygenase-2 (COX-2), (b) the unique, pharmacology of bimatoprost. During the past decade the biosynthetic pathways for prostaglandin (PG) ethanolamides have been elucidated in detail, including the discovery of prostamide / PGF synthase. This was of some pharmacological significance, since prostamide $F_{2\alpha}$ and its synthetic analogue bimatoprost are the most extensively studied neutral prostanoid to date.

The first evidence for pharmacological differentiation of prostamide $F_{2\alpha}$ and its analogs from other PGs, including $PGF_{2\alpha}$ was obtained from isolated tissue preparations. These divided into two categories: preparations that responded to bimatoprost, prostamide $F_{2\alpha}$, and $PGF_{2\alpha}$ and those that responded only to $PGF_{2\alpha}$. Subsequent cell pharmacology studies presented a similar pattern, those cells critically involved in the ocular hypotensive and hypertrichotic activities showing potent and marked responsiveness to bimatoprost. The pharmacological characterization was significantly advanced by the discovery of "prostamide" receptor antagonists, which selectively blocked the effects of bimatoprost and prostamide $F_{2\alpha}$ but not those of $PGF_{2\alpha}$ and other PGs. These antagonists were also critical for the cell modeling of the "prostamide" receptor. This model involved transfects for the wild type and alternative mRNA splicing variants for human FP receptor. Co-transfection of the wild-type and an alternative mRNA splicing variant (alt FP_4) reproduced the pharmacology of the "prostamide" receptor in all respects for Ca^{2+}, Cyr 61 expression, and MLC-phosphorylation.

Bimatoprost is widely used for treating glaucoma and, at the recently formulated 0.01%, is virtually devoid of meaningful side effects, such as ocular surface redness. Bimatoprost is therapeutically used for stimulating eyelash growth and increases human scalp hair growth in vitro. Both effects appear to result from extending the anagen phase .The most recent therapeutically targeted studies have involved adipocytes and fat reduction. Bimatoprost inhibited adipogenesis in 3T3-L1 cells and primary human subcutaneous pre-adipocytes, according to analysis of mature adipocyte genetic markers and triglyceride accumulation. In common with human dermal papilla cells, pre-adipocytes and mature adipocytes expressed the wild-type FP and the alt4FP variant receptors, a requisite for prostamide/bimatoprost activity.

PROSTANOID RECEPTORS IN CHRONIC INFLAMMATION

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Abstract: Inflammation is in principle a body defense mechanism that is evoked by noxious stimuli and terminated on exclusion of the initial stimuli by local vascular and cellular responses. However, inflammation often becomes chronic by repeated exposure to stimuli, enhanced immune responses, and tissue remodeling such as anginogenesis and fibrosis, where various inflammatory mediators are induced through gene expression and interact each other. Prostaglandins (PGs) such as PGD2, PGE2, PGF2α, PGI2 and thromboxane (TX) A2 are produced and released in response to various stimuli and exert their action by acting on their cognate receptors such as PGD receptor (DP), EP1 to EP4 subtypes of PGE receptor, PGF receptor (FP), PGI receptor (IP) and TXA receptor (TP). Since aspirin-like drugs elicit their actions by inhibiting PG biosynthesis, PGs are believed to mediate various symptoms of acute inflammation such as swelling, pain and fever. We cloned the above family of PG receptors, generated mice deficient in each receptor, and examined the role of PGs in inflammation. Our study has revealed that PGs not only mediate acute inflammatory responses described above but also are involved in induction and maintenance of chronic inflammation by collaborating with cytokines, chemokines and growth factors through regulation of gene expression. For example, in collagen-induced arthritis, PGI2-IP signaling and IL-1β collaborate to amplify expression of IL-6 and RANKL in synoviocytes, and, in bleomycin-induced lung fibrosis, PGF2αFP signaling and TGFβ independently induce a variety of genes including those of collagen. Furthermore, PGE2-EP2/EP4 signaling can facilitate Th1 differentiation and Th17 amplification in experimental allergic encephalomyelitis. These findings are changing our concept on the role of PGs in inflammation.

INVESTIGATION OF THE SLOW KINETICS OF A NON-PROSTANOID EP2 RECEPTOR AGONIST

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Abstract: Several prostanoid receptors are activated by non-prostanoid ligands – we have recently investigated (o-(o-benzyloxy)-cinnamyl)-cinnamic acid (coded (L)-9), originally reported to be a EP2 agonist by Belley et al. (2005). While (L)-9 appeared to be selective for the EP2 receptor over DP1, EP4 and IP receptors, its relaxation of guinea-pig trachea was characterized by slow onset and marked persistence following washout. Two possibilities were considered: 1. (L)-9 has unusually small association (k1) and dissociation (k2) rate constants for the EP2 receptor; 2. Access of (L)-9 to the centre of the muscle mass is retarded by sequestration process(es). Hypothesis 1 appeared unlikely for the following reason. A potent and selective EP2 antagonist (member of a series described by Forselles et al., 2011) rapidly reversed established relaxation induced by (L)-9. The reversal half-time was similar to those of other EP2 agonists, e.g. ONO-AE1-259, treprostinil and CP-533536 (another non-prostanoid). Hypothesis 2 seemed more likely. Firstly, the onset time for 70% relaxation by L-(9) on rabbit vena cava was 2.4 min compared to 17 and 42 min on mouse trachea and guinea-pig trachea respectively. Corresponding T70 values for ONO-AE1-259 were 1.1, 3.9 and 5.4 min. Light microscopy showed a thin smooth muscle layer in vena cava (2 cells thick), while the tracheas showed thicker muscle layers and considerable subendothelial tissue. Secondly, in the presence of extensive EP2 receptor blockade, L-9 inhibited contraction induced by the TP agonist U-46619 with onset rate order: rabbit vena cava > mouse trachea > guinea-pig trachea. CP-533536 did not exhibit significant TP antagonism. We suggest that the high lipophilicity of (L)-9 (logP = 6.69) results in uptake into lipophilic cellular domains, thereby retarding its diffusion into the ecf of the muscle mass; the greater the thickness of the muscle mass, the slower the diffusion. Our data will be compared to previous rate studies on EP3 and TP ligands of varying potency and lipophilicity (Jones et al., 2011).

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THE ARACHIDONIC ACID EPOXYGENASE: FUNCTIONAL ROLES AND RELEVANCE TO THE PATHOPHYSIOLOGY OF HYPERTENSION AND TUMOR ANGIOGENESIS

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Abstract: In addition to roles in xenobiotic metabolism, cytochrome P450 (P450) bio-activates arachidonic acid (AA) to 5,6-, 8,9-,11,12-, and/or 14,15- epoxyeicosatrienoic acid (EET)(AA epoxygenase); with human CYP2C8 and CYP2C9, mouse Cyp2c44, and rat CYP2C23 identified as predominant epoxygenases in the liver, kidney, and endothelium. The characterization of the AA epoxygenase as a formal metabolic pathway, suggested functional roles for its EET metabolites, and led to the identification of their roles in cell proliferation, vascular reactivity, and Ca++, K+, and Na+ ion channel regulation. Experimentally induced or genetically determined changes in the expression and/or the activity of the Cyp2c44 epoxygenase alters renal and vascular function with important physiological and/or pathophysiological consequences. Thus, disruption of the Cyp2c44 epoxygenase gene leads to alterations in: a) sodium transport in the distal nephron, and dietary salt sensitive hypertension, and b) tumor angiogenesis and growth. On the other hand, PPAR(alpha) ligands down-regulate endothelial Cyp2c44 expression, and reduce tumor angiogenesis and growth. The demonstration of a tumor selective expression of human CYP2C9, a functional homologue of murine Cyp2c44, and of its regulatory control by PPARα ligands suggest a role for this enzyme in human cancer, and for human PPARα as a target for the development of novel and better tolerated anti-tumor strategies. These and other studies introduce a paradigm shift that could influence our views of the roles played by the P450 enzyme system in disease and toxicology, from that of vehicles for drug disposition and/or activation, to that of active participants in the pathophysiology of hypertension and cancer. It is expected that they will stimulate efforts to: a) develop novel, CYP2C based, inhibitors of tumor angiogenesis, and b) to a consideration during drug evaluation of the physiological and/or pathophysiological consequences of interfering with the activity and/or expression of P450s involved in endogenous metabolic pathways.

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POSTER SESSION

POSTER 1

THE ANTINEOPLASTIC EFFECT OF MOLECULAR IODINE IS MEDIATED BY 6-IODOLACTONE (6-IL) AND THE PROLIFERATIVE PEROXISOME ACTIVATED RECEPTOR GAMMA (PPARg) IN SEVERAL CANCER CELL LINES THAT TAKE UP IODINE.

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Abstract: Iodine is crucial for thyroid hormone synthesis; however, considerable evidence indicates that iodine per se could also be implicated in the physiopathology of several organs that take up iodine. Previous reports have documented the antineoplastic properties of molecular iodine (I2) and the iodinated arachidonic acid (AA) derivative 6-IL in several cancer cells. Our group has characterized the cellular pathways activated by these molecules and their effects on proliferation, apoptosis, and differentiation in mammary, prostate, and neuroblatoma cancer cells. The results show that low to moderate concentrations of I2 (100-200 μM) cause G1 and G2/M phase arrest in normal cells, and caspase-dependent apoptosis in cancer cells. In contrast, when normal or cancer cells are treated with 5-10 μM 6-IL, both types of cells trigger apoptosis pathways. In mammary MCF-7 cells these apoptotic events are accompanied by the significant induction of PPARg expression (mRNA and protein), and using a ligand assay (EMSA) we find that 6-IL binds PPAR proteins with high affinity (6-fold greater than AA). Our data indicate that both I2 and 6-IL trigger the same intracellular pathways and suggest that the antineoplastic effect of I2 in cancer cells capable of iodine uptake involves the intracellular formation of 6-IL. Cancer cells are known to contain high concentrations of AA, which might explain why low concentrations of I2 exert apoptotic effects only in tumour cells.

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POSTER 2

POLYUNSATURATED FATTY ACID METABOLISM IN MONOCYTE DIFFERENTIATION

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Abstract: Polyunsaturated fatty acids (PUFA) regulate of cell function by determining membrane fluidity and by acting as substrates for the synthesis of secondary lipid mediators. Previous studies have shown that macrophage activation increases PUFA synthesis which has been associated with the production of eicosanoids [1], but the role synthesis plays is unknown. The effect of monocyte to macrophage differentiation on the expression of key genes in PUFA synthesis, delta-6 and delta-5 desaturases, and on the fatty acid composition of cell membranes is a matter for debate [2]. In this study, we have used THP-1 cells as an in vitro model of monocyte to macrophage differentiation to determine whether the cellular transformation has an effect on membrane PUFA content and on the mRNA expression of delta-6 and delta-5 desaturases.

Human THP-1 monocytes were differentiated to macrophages using 100nM PMA for 72 hours. Differentiation was confirmed by measurement of CD11c and CD36 expression by flow cytometry. Delta - 6 and delta - 5 desaturase mRNA expression was measured by real-time RT-PCR [3].

Membrane fatty acid composition was measured by gas chromatography. Incubation with 100nM PMA was sufficient to differentiate the monocytes to macrophages, which was confirmed by a 6-fold increase of CD36 and CD11c expression ($P \leq 0.0001$). Macrophages had 20% lower delta 6- and delta 5- desaturase mRNA expression ($P < 0.05$) than monocytes. Arachidonic acid (AA), eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA) concentrations were 10 % lower in macrophages than monocytes ($P \leq 0.0001$).

These findings suggest that unstimulated macrophages have lower capacity for PUFA synthesis than monocytes. This is consistent with the lower proportions of AA, EPA and DHA in macrophage cell membranes compared to monocytes. While the precise function of PUFA synthesis in macrophage function remains unclear, these findings imply specific regulation of this pathway during differentiation and thus may provide novel insights into the function of these cells.

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POSTER 3

HOMOCYSTEIN AND TRACE ELEMENTS LEVELS IN PATIENT WITH ISCHEMIC HEART DISEASE

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Abstract: This paper includes the determination homocysteine level and trace elements magnesium (Mg), zinc (Zn) and iron (Fe) in ppm (part per million); lead (Pb), cadmium (Cd), selenium (Se), chromium (Cr) and germanium (Ge) in ppb (part per billion)) in random serum of patients with pure ischemia, ischemia with hypertension and ischemia with diabetes. Homocysteine level was significantly increased ($P< 0.01$) in pure ischemic patients, ischemia with hypertension and ischemia with diabetes in comparison with control group. A comparison had also been done between male & female groups in patients and control groups and no significant changes ($P >0.05$) were observed. The result of this study showed that concentration of the trace elements (Pb & Cd) were significantly increased ($P< 0.01$) in patients groups in comparison with control group and the concentration of (Mg, Zn, Se, Cr and Ge) were significantly decreased ($P< 0.01$) among patients groups in comp arison with control group.

Keywords: Homocysteine, High performance liquid chromrtography, Trace elements, Atomic absorption spectrography, Ischemic heart disease.

POSTER 4

IMMUNOMODULATION OF n-3 POLYUNSATURATED FATTY ACIDS

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Abstract: There is interest in the enrichment of poultry meat with n-3 polyunsaturated fatty acids (PUFA) so as to increase consumption of these fatty acids by humans. However, there is concern that high levels of n-3 PUFA may have detrimental effects on immune function. The purpose of this study was to investigate the effects of various dietary sources of n-3 PUFA on natural killer (NK) cell activity and cell proliferation in broiler chickens. One day old male Ross 308 broilers (n=20) were fed on one of four sources of n-3 PUFA: linseed oil-, echium oil-, fish oil (FO)- or algal biomass-enriched diets until slaughter. At slaughter, samples of blood, thymus and spleen were collected from each bird. The source of n-3 PUFA had a strong influence on fatty acid composition across the tissues. Algal biomass was as efficient as FO in enriching chicken meat with DHA. NK activity was highest in splenocytes and PBMCs from broilers fed linseed oil, followed by those fed algal biomass or echium oil, and lowest for those from broilers fed FO. There was a significant positive relationship between NK activity/cell proliferation and splenocyte/PBMC linoleic acid, AA and total n-6 PUFA, and a negative relationship between NK activity and EPA/total n-3 PUFA. However, there was no relationship between NK activity/cell proliferation and DHA content. These results suggest that the immunosuppressive effects of FO are primarily dependent on the EPA content, and that a DHA-rich algal product may enrich chicken meat with n-3 PUFA without significant detrimental effects on chicken immunity.

POSTER 5

THROMBIN-STIMULATED PLATELETS ACUTELY GENERATE PHOSPHOLIPID-ESTERIFIED PROSTAGLANDINS

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Abstract: Generation of free acid eicosanoids by human platelets is well known. Here, we reveal a new family of 16 phospholipid-esterified prostaglandins (PGs) acutely generated by agonist-activated human platelets. Precursor scanning LC/MS/MS demonstrated multiple lipids that fragment on collisioninduced-dissociation to generate a daughter ion of m/z 351 (common m/z [MH]- of several PGs), in thrombin-stimulated platelet lipid extracts (m/z 770, 796, 798 and 814). These are consistent with PGs esterified to phosphatidylethanolamine (PE), with sn1 fatty acids, 16:0p, 18:1p, 18:0p, and 18:0a. Four different PGs were attached to each PE, making a total of 16 distinct PG-PE molecular species. Hydrolysis using PLA2 released PGE2 and PGD2 as well as other unknown PGs, but isoprostanes for PGE2 were absent. The lipids were generated via activation of PAR-1/4 receptors and exploiting several intracellular signalling intermediates including phospholipase C (PLC) cytosolic Ca2+, cytosolic phospholipase A2α (cPLA2 α), p38 mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinase (ERK) and src-family tyrosine kinases. Interestingly, in vitro and in vivo aspirin treatment showed complete inhibition of both free and esterified PGs, indicating requirement for cyclooxygenase-1 (COX). Inhibition by thimerosal and in vitro experiments using ovine COX-1 enzyme suggested that esterified prostaglandin are first synthesised as free prostaglandins and then esterified into phospholipids. This is the first demonstration of acute generation of phospholipid-esterified PGs in platelets from endogenous substrate. The data indicates that membrane-bound PGs are generated under physiological conditions. Their potential role in regulation of haemostasis is now being elucidated.

POSTER 6

A NOVEL BACTERIAL RESISTANCE MECHANISM AGAINST HUMAN TYPE-IIA SECRETED PHOSPHOLIPASE A2: ROLE OF STREPTOCOCCUS PYOGENES SORTASE A

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Abstract: Human type-IIA secreted phospholipase A2 (sPLA2-IIA) is a bactericidal molecule important for the innate immune defence against Gram -positive bacteria. Here, we analyse the role of sPLA2-IIA in the host defence against Streptococcus pyogenes, a major human pathogen, and demonstrate that these bacteria have evolved a previously unidentified mechanism to resist killing by sPLA2-IIA. Analysis of a set of clinical isolates demonstrated that a ~500-fold higher concentration of sPLA2-IIA was required to kill S. pyogenes compared to strains of the group B streptococcus, which previously has been shown to be sensitive to sPLA2- IIA, indicating that S. pyogenes has developed a specific mechanism to resist sPLA2-IIA. We found that an S. pyogenes mutant lacking Sortase A (SrtA), a transpeptidase responsible for anchoring LPXTG-proteins to the cell wall in Gram-positive bacteria, was significantly more sensitive to sPLA2-IIA compared to the parental strain, indicating that one or more LPXTG surface proteins protect S. pyogenes against sPLA2-IIA. This resistance mechanism is specific, as SrtA does not confer resistance against the human cathelicidin LL-37. Importantly, using transgenic mice expressing human sPLA2-IIA, we showed that the SrtA-mediated sPLA2-IIA resistance mechanism in S. pyogenes also occurs in vivo. Moreover, we demonstrate in this mouse model that human sPLA2-IIA is important for the defence against lethal S. pyogenes infection. Thus, we have demonstrated a novel mechanism by which pathogenic bacterium can evade the bactericidal action of sPLA2-IIA and we have shown that sPLA2-IIA contributes to the host defence against S. pyogenes infection.

POSTER 7

HUMAN GROUP IIA SECRETED PHOSPHOLIPASE A2 IS ESSENTIAL FOR INNATE IMMUNITY AGAINST THE GROUP B STREPTOCOCCUS

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Abstract: The group B streptococcus (GBS) is the leading cause of lifethreatening bacterial infections in newborn infants and is a growing cause of serious invasive disease in adults. Despite its clinical importance, little is known about innate immunity against GBS in humans. Here, we analyze the role of human group IIA secreted phospholipase A2 (sPLA2-IIA), a bactericidal enzyme induced during acute inflammation, in innate immunity against GBS. We show that clinical GBS isolates are highly sensitive to killing by pure sPLA2-IIA, but not human antimicrobial peptides (AMPs), and that the bactericidal activity of sPLA2-IIA against GBS is dependent on its catalytic activity. Employing transgenic mice expressing human sPLA2-IIA, we show that this enzyme is crucial for host protection against experimental systemic infection and lung colonization by GBS. Moreover, we show that acute sera from adults diagnosed with invasive GBS disease contain high levels of sPLA2-IIA compared to normal serum from healthy individuals, and we demonstrate that sPLA2-IIA in the acute sera rapidly kills clinically relevant GBS strains. Thus, we demonstrate a direct link between sPLA2-IIA induced in patients with invasive GBS disease and the ability of this sPLA2-IIA to kill clinically important GBS strains. Altogether, we present both experimental and clinical evidence that sPLA2-IIA may be of major importance for the innate immune defence against GBS in humans.

POSTER 8

ALTERED ARACHIDONATE DISTRIBUTION IN MACROPHAGES FROM CAVEOLIN-1 NULL MICE LEADING TO REDUCED EICOSANOID SYNTHESIS

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Abstract: In this work we have studied the effect of caveolin-1 deficiency on the mechanisms that regulate free Arachidonic acid (AA) availability. The results presented here demonstrate that macrophages from caveolin-1- deficient mice exhibit elevated fatty acid incorporation and remodeling and a constitutively increased CoA-independent transacylase activity. Mass spectrometrybased lipidomic analyses reveal stable alterations in the profile of AA distribution among phospholipids, manifested by reduced levels of AA in choline glycerophospholipids but elevated levels in ethanolamine glycerophospholipids and phosphatidylinositol. Furthermore, macrophages from caveolin-1 null mice show decreased AA mobilization and prostaglandin E2 and LTB4 production upon cell stimulation. Collectively, these results provide insight into the role of caveolin-1 in AA homeostasis and suggest an important role for this protein in the eicosanoid biosynthetic response.

POSTER 9

OXYLIPINS FROM MICROALGAE ARE INVOLVED IN THE RESOLUTION OF INFLAMMATION IN EXPERIMENTAL INTESTINAL COLITIS

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Abstract: BACKGROUND. The inflammatory bowel disease is a chronic, recurrent, and uncontrolled inflammation of the intestinal mucus which can affect any part of the gastrointestinal tract. Although its initiation is well-established, its pathogenesis is not fully understood. Recent findings that the resolution of inflammation is an active process have provided new insights for understanding and treating these conditions.
Currently we are interested in the characterization of anti-inflammatory molecules from natural origin; specifically we are studying lipid-fractions from microalgae which are particularly rich in fatty acids and derived metabolites as oxlylipins (OXL), a structurally diverse class of signaling molecules derivative from fatty acids oxidation. In this line, we have obtained several OXL which are being studied in our laboratory for inflammation and colon cancer treatments.

OBJECTIVE. To explore the anti-inflammatory properties of two OXL isolated from microalgae (13-HOTE and 13-HODE) and to test a lyophilized from microalgae in both animal models of acute/chronic colitis by trinitrobenzenesulfonic acid (TNBS).

MATERIAL AND METHODS. The anti-inflammatory activity of OXL was evaluated on LPS-stimulated THP-1 cells through quantification of TNF-alpha (ELISA) and COX-2 (western-blot); Adherent macrophages were treated with OXL followed by LPS-stimulation. We tested the effect of these OXL on PPAR-gamma localization in HT-29 colon cells by immunofluorescence.
We also examined the effects of a p.o. lyophilized-microalgae (rich in OXL), following an acute (5days) and a chronic (14 days) experimental approaches of colitis by TNBS in rats; behavior data, stool consistency and adhesions were determined as well as inflammation response by histological and myeloperoxidase activity analyses.

RESULTS. OXL significantly reduced TNF-alpha production (13-HOTE, 77%; 13-HODE, 53%; $P<0.01$) and COX-2 expression on THP-1. Preliminary data in HT-29 cells showed an important presence of PPAR-gamma into the nucleus after treatment.
In the TNBS models, lyophilized ameliorated: diarrhea, colon damage score and inflammation. These responses were more evident in the acute study.

CONCLUSION. OXL from microalgae are potential biomolecules involved in the resolution and recovery of intestinal homeostasis in experimental IBD. Complementary researches to analyze the mechanisms are currently being completed in our lab.

This research has been made possible by a grant from MICIIN-FEDER(Spanish Government) and BTM.

POSTER 10

HYPOTHALAMIC PROSTAGLANDIN E2 SYNTHESISED BY A CYCLOOXYGENASE-1 GENE-DERIVED PROTEIN IS INVOLVED IN THE MAINTENANCE OF NORMOTHERMIA

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Abstract: Prostaglandin E2 (PGE2) synthesised through inducible cyclooxygenase-2 (COX-2), expressed in endothelial cells of the pre-optic area of the hypothalamus, is involved in induction of pyrexia. On the other hand, there is no evidence to support a role for PGE2 in the maintenance of normal body temperature (normothermia). In the current study, administration of paracetamol to mice resulted in the induction of hypothermia, which correlated with a reduction of brain PGE2 levels in a time and concentration-dependent manner. These effects were attenuated in COX-1 knockout mice, but not in COX-2 knockout mice, suggesting that the paracetamol-induced hypothermia and reduction in brain PGE2 levels are mediated through inhibition of a COX-1 gene-derived protein. Inhibition of COX-1 in the induction of hypothermia is ruled out as SC560, COX-1 selective inhibitor, failed to induce hypothermia at a concentration were significant reduction of PGE2 levels was observed.
Futhermore, paracetamol reduced elevated body temperature to a hypothermic level in pyrexic wild-type mice, but not in pyrexic COX-1 knockout mice, suggesting that, unlike non-steroidal anti-inflammatory drugs (NSAIDs) that induce anti-pyresis by inhibition of inducible COX-2, the antipyretic action of paracetamol is mediated through inhibition of a COX-1 genederived protein that is involved in the synthesis of PGE2 that plays an important role in the maintenance of normothermia.

POSTER 11

DECIPHERING THE INTERACTION OF 5-LIPOXYGENASE AND FLAP

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Abstract: Leukotrienes are pro-inflammatory lipid mediators involved in chronic inflammatory diseases like asthma and atherosclerosis. An array of proteins are involved in the leukotriene (LT) biosynthesis pathway that stems from oxygenation of arachidonic acid by 5-lipoxygenase (5LO). 5-lipoxygenase activating protein (FLAP) is an integral membrane protein, belonging to the MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism) family and is localized in the nuclear membrane. The hypothesis is that 5LO localizes near the FLAP on increase in intracellular calcium concentration and then FLAP presents the arachidonic acid to 5LO which then converts it to LTA4. The main aim of our project is to decipher the ambiguous interaction between FLAP and 5LO. We employ soluble phospholipid bilayers called Nanodiscs which mimic a membrane environment to characterize the interaction both structurally and functionally.

POSTER 12

RELATIONSHIP BETWEEN PLASMA C-REACTIVE PROTEIN (CRP) AND PARAOXONASE-I (PON-I) ACTIVITY IN HUMAN OBESITY

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Abstract: High density lipoproteins (HDL) exert several physiological roles. They have a role in reverse cholesterol transport, behave as antioxidant, antithrombotic and anti-inflammatory particles. Moreover HDL inhibit also the expression of endothelial cell adhesion proteins induced by C-reactive protein (CRP), an acute-phase protein synthesized by liver in response to inflammation (Barter et al., 2004).

The multifunctional enzyme paraoxonase-1 (PON1), associated to HDL surface, is able to modulate HDL function. PON1 is able to prevent the accumulation of oxidized lipids from lipoproteins (HDL and LDL) and membranes preventing the atherogenic and inflammatory response induced by lipid peroxidation products. However, systemic inflammation and oxidative stress convert HDL to a dysfunctional form that loses anti-inflammatory and anti-atherogenic effects.

Recent studies have suggested that the PON1/CRP ratio could be a useful indicator for disturbances between intensity of inflammation processes and anti-inflammatory and antioxidant effects of HDL. Higher plasma levels of CRP are associated with low PON1 activity and a decrease of the PON1/CRP ratio has been observed in diabetic and in end-stage renal disease patients with respect to healthy subjects. To confirm that PON1 activity and CRP levels are related in diseases associated with inflammation, we studied the PON1/CRP ratio in 25 controls and 22 obese subjects (BMI range 30.5-71.2 kg/m2; mean value 47.0 (9.5) kg/m2). Our results demonstrated that the PON1/CRP ratio is significantly lower in obese subjects with respect to controls ($p<0.001$). A relationship between BMI and PON/CRP has been demonstrated; a lower ratio was observed in obese patients with higher BMI.

These results confirm the relationship between PON1 and CRP levels. CRP is a commonly used marker of systemic inflammation and elevated CRP levels may predict cardiovascular disease. These findings raise the possibility that low paraoxonase-1 activity in obesity could contribute to increased cardiovascular risk via an effect on enhanced systemic low grade inflammation.

POSTER 13

LIPIN-2 REDUCES PROINFLAMMATORY SIGNALING INDUCED BY SATURATED FATTY ACIDS IN MACROPHAGES

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Abstract: Lipin-2 is a member of the lipin family of enzymes, which are key effectors in the biosynthesis of lipids. Mutations in the human lipin- 2 gene are associated with inflammatory-based disorders, however the role of lipin-2 in cells of the immune system remains obscure. In the present work we have investigated the role of lipin-2 in the proinflammatory action of saturated fatty acids in murine and human macrophages. Depletion of lipin-2 promotes the increased expression of the proinflammatory genes Il6, Ccl2 and Tnfα, which depends on the overstimulation of the JNK1/c-Jun pathway by saturated fatty acids. On the contrary, overexpression of lipin-2 reduces the release of proinflamatory factors. Metabolically, the absence of lipin-2 reduces the cellular content of triacylglycerol in saturated fatty acid-overloaded macrophages. Collectively, these studies demonstrate a protective role for lipin-2 in proinflammatory signaling mediated by saturated fatty acids that occurs concomitant with an enhanced cellular capacity for triacylglycerol synthesis. The data provide new insights into the role of lipin-2 in human and murine macrophage biology, and may open new avenues for controlling the fatty acid-related low grade inflammation that constitutes the sine qua non of obesity and associated metabolic disorders.

POSTER 14

POLYUNSATURATED FATTY ACIDS REMODEL MEMBRANE RAFTS ORGANIZATION AND PLD1 TRAFFICKING OF MAST CELLS

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Abstract: Phospholipase D (PLD), a receptor-regulated signalling enzyme, is involved in numerous cellular processes including exocytosis and phagocytosis. In mast cells, the two isoforms PLD1 and PLD2 appear to be essential for the stimulated secretion of granules. Using the canine mastocytoma cell line C2, a model for atopic dermatitis in dogs, the aim of the present work was to investigate the modulatory effect of polyunsaturated fatty acids (PUFA) on intracellular PLD trafficking. Supplementation of the cell culture medium with α-linolenic acid (LNA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA) or arachidonic acid (AA) resulted in an enrichment of mast cell membranes with the fatty acid added, at this modulating the lipid composition of both raft and non-raft membrane domains. To investigate the effect of the membrane remodeling on PLD trafficking, the C2 cells were transfected with EGP plasmids encoding PLD1 or PLD2, stimulated with mastoparan and imaged using confocal microscopy. Before stimulation PLD1 is situated within intracellular vesicular structures; PLD2 is located at the plasma membrane. After stimulation, PLD1 migrates to the plasma membrane, whereas PLD2 maintains at its peripheral location. Enrichment of the mast cells with PUFA affected the stimulation-induced trafficking of PLD1. All PUFA examined, except AA, prevented the peripheral migration thus retaining PLD1 intracellular. For PLD2 no PUFA effects could be observed. In conclusion, membrane remodeling due to PUFA supplementation affects intracellular PLD1 trafficking which in turn impact mast cell function.

POSTER 15

CELL SIGNALING PATHWAYS INVOLVED IN CACO-2 CELL GROWTH INDUCED BY EICOSANOIDS

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Abstract: Increasingly evidence indicates that enzymes, receptors and metabolites of the arachidonic acid (AA) cascade play a role in intestinal epithelial cell proliferation and colorectal tumorigenesis. However, the information available is does not provide a complete picture and contains a number of discrepancies. For this reason it might be appropriate a holistic study into the impacts of the AA cascade on intestinal epithelial cell growth. Our data show that PGE2, LTB4 and 5-, 12- and 15-HETE at concentrations reached in 10% FBS Caco-2 cultures (1-10 nM) were able to induce Caco-2 cell growth and DNA synthesis. To explore the cell signaling mechanisms involved in these events, we studied the capacity of these eicosanoids to phosphorylate pivotal elements in the cell signaling pathways implicate in the regulation of cell growth such as AKT1, AKT2, ERK1/2, p38α, CREB, and GSK beta, as well as the dephosphorylation of Beta-catenin. Interestingly, we provide evidence that PGE2 (1 nM) stimulates ERK, P38 alpha, CREB and GSK beta/Beta-catenin involved in the regulation of Caco-2 growth. Moreover, LTB4 (10 nM) and HETEs (12-S-HETE) (100 nM) only increase the phosphorylation of p38 alpha and ERK in a lesser extent than PGE2. Furthermore, our results show that ERK and AMPc-PKA inhibitors such as PD98059 and KT5720, respectively, significantly reduced CREB phosphorylation induced by PGE2. On the basis of our results we can conclude that several eicosanoids are involved in the regulation of cell signaling involved in Caco-2 cell.

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POSTER 16

ACTIVATION OF LIPOXIN A4 RECEPTOR REDUCES INFARCT SIZE AND NEUROINFLAMMATION IN A RAT MODEL OF ISCHEMIC STROKE

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Abstract: Lipoxin A4 (LXA4) is an anti-inflammatory mediator derived enzymatically from arachidonic acid by the concerted action of lipooxygenases. Binding of LXA4 to its receptor (FPR2/ALX) has pro-resolution effects on inflammation. Virtually nothing is known of the role of LXA4 on neuroinflammation following cerebral ischemia. BML-111 is a FPR2/ALX receptor agonist and has been recognize to play a similar role as LXA4 in anti-inflammatory responses. We hypothesized that treatment with BML-111 would reduce post-ischemic inflammation and provide neuroprotection in a rat model of ischemic stroke. Rats were subjected to 90 minutes of transient middle cerebral artery occlusion and sacrificed after 48 hours of reperfusion. Animals received BML-111 or the vehicle. The first dose of BML-111 (1 mg/kg) was given intravenously 10 minutes after the onset of reperfusion and another dose 24 hours after reperfusion. Pro-inflammatory markers such as myeloperoxidase (MPO), a neutrophil marker, and matrix metalloproteinases-2 and -9 (MMP-2 and MMP-9) were evaluated at 48 hours after reperfusion. Post-ischemic treatment with BML-111 significantly reduced infarct size and blood-brain barrier (BBB) damage as seen with 2,3,5-triphenyltetrazolium chloride (TTC) staining and brain IgG extravasation, respectively. BML-111-treated animals had significantly reduced neutrophil infiltration as seen by decreased MPO expression in the ipsilateral cerebral cortex compared to vehicle-treated animals as determined by immunoblotting. Rats given BML-111 also had significantly decreased levels of MMP-9 in the ipsilateral cortex and striatum compared to the vehicle group. These data indicate that activation of the FPR2/ALX receptor with BML-111 results in reduced infarct size possibly through the attenuation of the inflammatory response following ischemic stroke.

POSTER 17

QUANTIFICATION OF BIOACTIVE LIPIDS IN TISSUES FROM IRRITABLE BOWEL SYNDROME (IBS) PATIENTS

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Abstract: The activation of different transient receptor potential receptors (TRPV1, TRPV4 and TRPA1) induces visceral hypersensitivity in mice. However, the presence of potential endogenous activators of TRP in the digestive tract in the context of IBS has never been investigated. TRP channels can be activated or inhibited in vitro by polyunsaturated fatty acid (PUFA) metabolites. In the present study we have quantified PUFA metabolites that are able to regulate TRP channel in human biopsies and in mouse tissues in a model of visceral hypersensitivity. Methods: Biopsies from control and IBS patients were immersed in oxygenated Hank\'s solution. Those supernatant were administered intracolonically (IC) to mice, thereby inducing hypersensitivity symptoms with supernatants from IBS patients) or not (supernatants from control individuals). Lipids from biopsy, biopsy supernatant and mouse colons were extracted and agonists of TRPV1 (12-HpETE, 15-HpETE, 5-HpETE, LtB4), TRPV4 (5, 6-EET; 8, 9-EET) and TRPA1 (PGA1, 8-iso-PGA2, 15-d-PGJ2) as well as PGE2 and resolvins, were quantified by tandem mass spectrometry after HPLC (LCMS/ MS). Pharmacological properties of the lipid extracted from mouse colon were assessed in vitro by calcium flux experiments in sensory neurons and in vivo by recording viscero-motor responses to colorectal distention. Results: By LC-MS/MS, in IBS biopsies we detected an increase in PGE2, 5, 6-EET and in 12-HETE, TRPA1 agonist and others metabolites quantified were unchanged compared to control. The quantity of PUFA metabolites in supernatants was below the limit of detection (5pg). In mouse colons, 5, 6 -EET and 15-HETE were increased 3 hours after IC administration of IBS biopsy supernatants. In sensory neurons, calcium flux induced by lipids extracted from mouse colon treated with IBS biopsy supernatant was decreased by a pretreatment with TRPV4 antagonist. In mice, hypersensitivity induced by IC administration of IBS patient biopsy supernatants was decreased by intrathecal administration of TRPV4 SiRNA. Conclusions: Our study shows that TRPV4 and TRPV1 endogenous agonists are increased in human biopsies of IBS patients. Moreover, mediators released from IBS patient tissues are able to increase the production of several PUFA metabolites by mouse colonic tissues, in particular TRPV4 agonist. Thus, TRPV4 and its endogenous agonists seem to play a pivotal role in IBS related visceral pain.

POSTER 18

ALTERING PROSTAGLANDIN E AND F ISOMER PRODUCTION IN OVINE ENDOMETRIAL CELLS BY n-3 AND n-6 POLYUNSATURATED FATTY ACID SUPPLEMENTATION

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Abstract: N-3 polyunsaturated fatty acid (PUFAs) are the precursors of 3- series prostaglandins (PGs), while n-6 PUFAs produce 1- and 2-series PGs. Both PUFA families utilise the same enzymes for their metabolism and PG production, so supplementation of one PUFA family may influence the metabolism and PG production of the other family. Many reproductive processes involve inflammation, for which regulation of uterine 2-series PG production is essential. PGE are predominantly produced by endometrial stromal cells and PGF by epithelial cells. Most previous studies did not distinguish different PG isomers due to high cross-reactivity of the antisera used in the assays. The aims of this study were to investigate the effect of n-3 and n-6 PUFA supplementation on PGE and PGF isomer production by ovine uterine endometrium.

Studies were performed on confluent (a) epithelial cells alone and (b) mixed epithelial and stromal endometrial cells isolated from cyclic ewes. Cultures were supplemented with 0 (CONT)-100 microM of n-3 PUFAs [alphalinolenic acid (ALA), stereodonic acid (SDA) or eicosapentaenoic acid (EPA)] or n-6 PUFAs [linoleic Acid (LA), gamma-linolenic acid (GLA) and arachidonic acid (AA)] in serum free medium for 24 h. PG isomers in spent medium were isolated using HPLC and quantified by radioimmunoassay. In the mixed endometrial cells EPA and SDA significantly suppressed PGE1 production by up to 4-fold ($P<0.05$-0.01). The n-3 PUFAs altered PGE2 moderately (only SDA increased PGE2 production, $P<0.01$); 100μM EPA increased PGE3 by 2-fold ($P<0.01$), whereas other tested PUFAs did not change PGE3 production significantly. In the epithelial cells, LA did not alter PGF isomer production. GLA increased both absolute and proportional PGF1alpha production by up to 7-fold ($P<0.01$) and enhanced PGF2alpha generation by up to 3-fold ($P<0.05$). AA increased PGF2alpha generation by up to 9-fold and raised its isometric proportion ($P<0.01$). It also increased PGF1alpha output by up to 3-fold ($P<0.05$). PGF3alpha production was not altered by n-6 PUFA supplementation ($P>0.05$).

These results suggest that consumption of n-3 and n-6 PUFAs are likely to alter uterine PG isomer production differentially. This may have implications for the control of a variety of reproductive processes.

POSTER 19

OMEGA-6 DOCOSAPENTAENOIC ACID DERIVED RESOLVINS ALLEVIATE EXPERIMENTAL COLITIS IN MICE

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Abstract: Enzymatic oxygenated lipid metabolites derived from omega-3 and omega-6 polyunsaturated fatty acids (n-3 PUFA and n-6 PUFA) play an important role in inflammation dampening. In the data presented here we demonstrate significantly alleviated dextran sodium sulphate (DSS) induced colitis in mice treated with n-6 docosapentaenoic acid derived lipid products 17-hydroxy-docosapentaenoic acid (17-HDPAn-6) or 10,17-dihydroxydocosapentaenoic acid (10,17-HDPAn-6).

Our results show that treatment with 17-HDPAn-6 and 10,17-HDPAn-6 protect mice against DSS-induced colitis and significantly improved disease activity index, body weight loss, colon epithelial damage and macrophage infiltration. Furthermore, these compounds were able to promote a resolution phenotype in macrophages in vitro.

To our knowledge, our findings show for the first time the antiinflammatory effects of treatment with DPAn-6 derived resolvins in preventing experimental colitis.

These results suggest that DPAn-6 derived 17-HDPAn-6 and 10,17-HDPAn-6 have inflammation-dampening and resolution-promoting effects that could be used to treat inflammatory conditions such as inflammatory bowel disease.

POSTER 20

EXPRESSION AND ACTIVITY OF HUMAN TYPE IIA SECRETORY PHOSPHOLIPASE A2 IN SERUM PATIENTS WITH IDIOPATHIC PARKINSON'S DISEASE

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Abstract: It has been postulated that sPLA2-IIA and cytokines plays an important role in several responses at inflammatory sites and contributed to neurodegeneration in neurodegenerative diseases but studies relating sPLA2 expression and activity with PD have been lacking. We investigated serum levels of sPLA2, oxidative stress and GSH concentration in 31 patients with idiopathic PD (iPD) patients, 17 idiopathic PD with atherosclerosis (iPD-ALTS) patients and 20 age-matched controls (healthy, non-parkinsonian patients). We observed the increased sPLA2 level and activity (2 fold) in serum iPD-ALTS and AP patients compared to control group. sPLA2 concentration did not change in serum iPD patients compared to the control. The enhancement of oxidative stress assessed by TBARS, cGMP concentration and reduction of GSH level were observed in serum of all parkinsonian groups compared to control ($p<0.05$). Moreover, the serum levels of lipid peroxidation products and cGMP level were enhanced in PD. These results argue in favor of the involvement of immunological events in the process of neurodegeneration in AP and iPD.

Key words: secretory PLA2, idiopathic Parkinson Disease, oxidative stress, cGMP, glutathione level, atherosclerosis.

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POSTER 21

RESOLVIN D1 AND RESOLVIN D2 GOVERN LOCAL INFLAMMATORY TONE IN OBESE FAT

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Abstract: The unprecedented rise in the prevalence of obesity and obesityrelated disorders is causally linked to a chronic state of low-grade inflammation in adipose tissue. Timely resolution of inflammation and return of this tissue to homeostasis are key to reducing obesity-induced metabolic dysfunctions. Here, with inflamed adipose, we investigated the biosynthesis, conversion and actions of Resolvin (Rv) D1 and RvD2, potent anti-inflammatory and pro-resolving lipid mediators, and their ability to regulate monocyte interactions with adipocytes. LC-MS/MS-based metabololipidomics identified RvD1 and RvD2 from endogenous sources in human and mouse adipose tissues. We also identified pro-resolving receptors (i.e. ALX/FPR2, ChemR23 and GPR32) in these tissues. Compared to lean tissue, obese adipose showed a deficit of these endogenous antiinflammatory signals. With inflamed obese adipose tissue, RvD1 and RvD2 each rescued impaired expression and secretion of adiponectin in a time- and concentration-dependent manner while decreasing pro-inflammatory adipokine production including leptin, TNFalpha, IL-6 and IL-1beta. RvD1 and RvD2 each reduced MCP-1 and leukotriene B4-stimulated monocyte adhesion to adipocytes and their transadipose migration. Adipose tissue rapidly converted both resolvins to novel oxo-resolvins. RvD2 was enzymatically converted to 7-oxo-RvD2 as its major metabolic route that retained adipose-directed RvD2 actions. These results indicate, in adipose, D-series resolvins (RvD1 and RvD2) are potent pro-resolving mediators that counteract both local adipokine production and monocyte accumulation in obesity-induced adipose inflammation.

POSTER 22

FATTY ACID CHLOROHYDRINS GENERATED DURING ACUTE PANCREATITIS INCREASES THE INFLAMMATORY RESPONSE

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Abstract: Background: Acute pancreatitis is an inflammatory process of the pancreatic gland that in the severe forms triggers the inflammation in remote organs. An additional characteristic of pancreatitis is the necrosis of peripancreatic adipose tissue due to the release of lipolytic enzymes. These areas of adipose tissue release in turn inflammatory mediators. We have evaluated the generation of halogenated free fatty acids by necrotic adipose tissue in a model of acute pancreatitis. Methods: Pancreatitis was induced in rats by intraductal administration of 3.5 % sodium taurocholate. We obtained samples of adipose tissue and ascitic fluid 3h, 6h and 18h after induction, and the levels of free fatty acids as well as fatty acid chlorohydrins were evaluated by GC-MS. In additional experiments we administered fatty acid chlorohydrins, generated by chlorination of adipose tissue lipid extracts, on the peritoneum of control animals. Three hours later we obtained peritoneal macrophages and the expression of TNFα and IL1B on these cells was evaluated by RT-PCR. Results: During pancreatitis, necrotic areas of adipose tissue generate and release free fatty acids as well as its chlorohydrins. We identified oleic acid chlorohydrin and mono- and bis- chlorohydrin of linoleic acid in both adipose tissue and ascitic fluid. Administration of chlorinated lipids in the peritoneal cavity results in an increased expression of TNFα and IL1B by peritoneal macrophages. Conclusion: During severe acute pancreatitis, the necrotic areas of the peripancreatic adipose tissue generate chlorinated fatty acids. These halogenated lipids could activate macrophages and plays a role in the progression of the systemic inflammation.

POSTER 23

PLA2 AND AQP4 MECHANISMS IN CHRONIC ALCOHOL-INDUCED BRAIN DAMAGE: NEUROPROTECTION BY OMEGA-3 DHA SUPPLEMENTATION

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Abstract: Repetitive binge ethanol intoxication, which causes oxidative stress and neurodegeneration in the hippocampus and entorhinal cortex of adult rats, can be modeled in organotypic brain slice cultures. Studies with these cultures demonstrate the neuroinflammatory importance in the ethanol-dependent brain damage of PLA2 activation and increased arachidonic acid (AA) mobilization, possibly linked to aquaporin-4 (AQP4) upregulation and glial edema. Supplementation with docosahexaenoic acid (DHA) suppresses AQP4 increases and AA mobilization, while preventing neurodegeneration. Further studies in vivo in binged rats show selective elevations in the above-mentioned proteins in the two brain regions. These studies lend support to the overall idea that neuroinflammatory lipid based mechanisms underlie brain damage in chronic alcoholism.

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POSTER 24

PGE2 INHIBITS MACROPHAGE-DEPENDENT IMMUNITY TO INFLUENZA A H1N1

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Abstract: The Spanish Influenza A virus (Flu) pandemic, which claimed up to 50 million lives at the beginning of the 20th century, was a major factor that led to increased popularity of the first generation of nonsteroidal anti-inflammatory drugs (NSAIDs). Today, these drugs are used on a regular basis, including for the management of Flu symptoms, despite our limited knowledge of their effects on anti-viral immunity. NSAIDs mainly act by inhibiting cyclooxygenase-1 (COX-1) and/or COX-2 enzymes involved in the synthesis of prostanoid lipid mediators. Studies investigating the effects of COX deficiency or inhibition during Flu infection have yielded controversial findings potentially due to the fact that prostaglandin H2 (PGH2), the product of COX enzymatic activity, is the precursor of all prostanoids including four principal PGs (PGE2, PGI2, PGD2 and PGF2alpha) and thromboxane A2 (TXA2). PGE2, a key inflammatory mediator with emerging function in immune regulation, is specifically generated from PGH2 via the microsomal prostaglandin E-synthase 1 (mPGES-1) enzyme and is critically implicated in inflammatory responses. Using mice deficient in mPges-1, we demonstrated that PGE2 inhibits macrophages type I interferon production and apoptosis via EP2 and EP4 receptors. This inhibitory role of PGE2 was not only limited to innate immunity since both antigen (Ag) presentation and T cell mediated immunity were also suppressed during Flu infection. Furthermore, the loss of mPGES- 1 function in human macrophages was protective against Flu infection. Thus, our study identifies a previously unknown mechanism implicating PGE2 in immune evasion during Flu infection and provide a specific target for anti-Influenza treatments.

POSTER 25

MAMMALIAN EPOXIDE HYDROLASES IN HEPOXILIN METABOLISM

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Abstract: Hepoxilins are lipid signalling molecules derived from arachidonic acid through the 12-lipoxygenase pathway formed in various organs like liver, brain and skin. These trans-epoxy hydroxy eicosanoids play a role in a variety of physiological processes, including inflammation, neurotransmission, insulin signalling and formation of skin barrier function. Mammalian soluble epoxide hydrolase (sEH, EC 3.1.3.76; EC 3.3.2.10) is involved in diverse physiological processes, which is mainly due to the turnover of arachidonic acid derived lipid epoxides with signalling function such as epoxyeicosatrienoic acids (EETs). Mammalian hepoxilin hydrolase - partly purified from rat liver - has earlier been reported to degrade hepoxilins to trioxilins. Here, we report that hepoxilin hydrolysis in liver is mainly catalysed by mammalian soluble epoxide hydrolase (sEH, EC 3.1.3.76; EC 3.3.2.10). We used LC-MS/MS analysis followed by kinetic evaluation to analyse the metabolism of hepoxilins. (i) Purified mammalian sEH hydrolyses hepoxilin A3 and B3 with a Vmax of 0.4 – 2.5 mmol/mg/min, (ii) the highly selective sEH inhibitor Nadamantyl- N'-cyclohexyl urea (ACU) greatly reduced hepoxilin hydrolysis in mouse liver preparations (iii) hepoxilin hydrolase activity correlates with the sEH presence after separation of mouse liver cytosol by gel permeation chromatography, (iv) hepoxilin hydrolase activity was abolished in liver preparations from sEH-/- mice and (v) liver homogenates of sEH-/- mice show elevated basal level of hepoxilins but lowered levels of trioxilins compared to WT animals. We conclude from our results that sEH is identical to previously reported hepoxilin hydrolase. Furthermore, in skin protein extracts isolated from sEH-/- mice HxA3 and HxB3 turnover is largely reduced as compared to the WT mice and sEHIs inhibit hepoxilin turnover in a human keratinocyte cell line. However, we cannot exclude the partly contribution of an AUDA sensitive EH to hepoxilin metabolism in the skin. A likely candidate is EH3, a new epoxide hydrolase which was recently cloned and functionally expressed in our group. EH3 turnes over hepoxilins in vitro, is like sEH AUDA sensitive and predicted to be localised in the skin. This new epoxide hydrolase might contribute to hepoxilin metabolism under certain conditions.

POSTER 26

SECRETORY PHOSPHOLIPASE A2 SUBTYPE V (PLA2G5) POLYMORPHISM AND ACUTE RESPIRATORY DISTRESS IN INFANTS

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Abstract: Background: Secretory Phospholipase A2 (PLA2) has been linked with acute respiratory distress syndrome (ARDS) and its clinical severity and mortality. The enzyme subtype -V(PLA2G5) is known to be expressed in the lung tissue. We aimed at sequencingitsgene (HGNC:9038) in infants with ARDS. This study is a part of a multicentre project whose protocol has been published elsewhere.[1]

Methods: 24 ARDS infantsand 24 age-matched babies with no lung disease were enrolled. 50 healthy adult volunteers, who never had neither ARDS nor chronic pulmonary diseases, served as another control group.Genomic DNA was extracted from leukocytes, amplifiedbyPCR andsequenced, analyzing the coding regions by SeqScape. Basic clinical data were recorded.

Results: A polymorphism (p.G3G= c.9C>T) was detected in the gene PLA2G5 (exon 1). This variation was present in heterozygosis in 42% of controls and in 17% of patients, while homozygosis was detected in 21% of patients and in no controls (p=0.022). Heterozygosis and homozygosis were present in 54% and 10% of adult controls, respectively. Homozygosis for such polymorphism led to an increased risk of ARDS (OR: 6.7;95% C.I.: [1.3- 34.2]). Patients carrying this polymorphism had lower PaO2/FiO2 ratio (104±29vs147±53;p=0.039) and higher lung injury score at the diagnosis (3.7±0.2vs3.2±0.4;p=0.031).

Discussion: These are the first findings aboutgenetic association between PLA2 and ARDS. Variation in the PLA2G5 gene mightbe associated to an increased risk for ARDSas it may represent a marker of variations in other genes nearby PLA2G5, that may be involved in inflammation pathway.

[1] De Luca D,CapoluongoE,Rigo V & Study group on Secretory Phospholipase in Paediatrics.BMC Pediatr 2011;11:101.

POSTER 27

POLY UNSATURATED FATTY ACIDS (PUFAs) MODULATE IL-6 SIGNALING PATHWAY IN MICROGLIA CELLS

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Abstract: Sickness behavior depends on brain cytokine production by microglia. Interleukin-6 (IL-6) plays a central role in the development of these behavioral changes. IL- 6 binds to its receptor GP80 which forms a complex with GP130, inducing phosphorylation of STAT3, a transcription factor activating inflammatory genes expression. This process needs to be tightly controlled to prevent adverse effects of inflammatory factors on neurons.

N-3 or n-6 Polyunsaturated Fatty Acids (PUFAs) are essential nutriments with distinct physiological and immunoregulatory properties. The n-6/n-3 ratio in the diet is of particular importance. A dietary deficiency of n-3 reduces Docosahexanoic Acid (DHA) content of the brain and increases n-6 PUFAs, particularly Docosapentaenoic Acid (DPA) and in a lesser extends Arachidonic Acid (AA). In response to an immune challenge, mice fed with n-3 deficient diet exhibit IL-6 peripherical and central overproduction but a weak IL-6 induced STAT3 activation in the brain, associated with an impaired sickness behavior (1). Alternatively, n-3 PUFAs supplemented diets display anti-inflammatory effects. Incorporation of DHA in phospholipids modifies physical properties of membranes (fluidity and rafts domain integrity) and cell surface expression of lipopolysaccharid (LPS) receptors (TLR4 and CD14) in microglial cells (2). Consequently, DHA limits LPS-induced cytokine production. The role of PUFA on IL-6 signaling has not been studied.

We aimed at compare the impact of n-6 and n-3 PUFAs on IL-6-signalling pathway. For this purpose, BV2 cells (murine microglial cell line) were incubated with AA, DPA or DHA and then treated or not with IL-6. Receptor expression at cell surface (Flow cytometry) and STAT3 activation (westernblot) were evaluated. Both AA and DHA treatments impaired surface presentation of IL-6 receptors. AA weakly reduced IL-6-induced STAT3 activation while DHA totally abolished IL-6 action. On the opposite, DPA n-6 treatment had no effect on IL-6 signaling.

All together, these results indicate that long chain PUFA differently act on IL-6 signaling pathway by targeting microglial cells. In vivo studies are in progress to investigate the impact of dietary PUFAs on membrane composition and its consequences on cytokine signaling pathways.

1Mingam, et al. (2008) European J.of Neuroscience 28:1877-1886.
2De Smedt-Peyrusse et al., (2008) J Neurochem. 105(2):296-307

POSTER 28

SEX DEPENDENT EICOSANOID BIOSYNTHESIS IN AN IN VIVO MODEL OF ACUTE INFLAMMATION

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Abstract: The pleurisy induced by carrageenan in rats is a well-establish animal model to study acute inflammatory responses and to evaluate the in vivo effects of anti-inflammatory compounds. The intrapleural injection of carrageenan induces exudate formation and infiltration of leukocytes, which are regulated by the production of lipid mediators (e.g., eicosanoids). Significant differences in the exudate volume and in the amount of infiltrated inflammatory cells have been previously observed between male and female rats. In order to evaluate the responsible factors for the observed sex-differences, we have here performed a detailed analysis of the pleural levels of the formed eicosanoids. Leukotriene (LT) B4, 6-keto prostaglandin (PG)F1alpha, PGE2 and thromboxane (TX)B2 peaked 2 h after intrapleural carrageenan injection in both male and female rats. Interestingly, a significant sex-related difference was observed in the amounts of LTB4, being 2 fold higher in the exudate from female rats as compared to male rats. On the contrary, 6-keto PGF1alpha as well as PGE2 were significantly higher in males (6-keto PGF1alpha about 2 fold, PGE2 at 2 and 4 h about 1.5 fold and at 8 h 7 fold), while no significant difference was observed for TXB2. This early phase release of pro-inflammatory mediators was followed by significant sex-differences in exudate formation and infiltration of inflammatory cells, which were two fold higher in male than in female rats, 8 h after carrageenan injection. Taking together, our data disclose novel sex-related differences in the production of lipid mediators in the acute phase of inflammation in carrageenan-treated rats, and emphasize the need for an accurate analysis of sex-related differences in the inflammatory response.

POSTER 29

FATTY ACID COMPOSITION IN BLOOD SERUM OF PATIENTS WITH POST-TRAUMATIC STRESS DISORDER

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Abstract: Objective: Fatty acids play a significant role in the structure and function of cell membranes and cells in general. Cells from central nervous system are extremely rich in lipids and therefore highly susceptible to damages caused by disturbances in fatty acids pattern. Certain psychiatric disorders, including depression, bipolar disorder, schizophrenia and others, are thought to be associated with essential fatty acids deficiency. Post-traumatic stress disorder (PTSD) is an anxiety disorder that can develop after exposure to a psychologically traumatic event. Diagnostic symptoms include re-experiencing the original trauma(s) usually accompanied by anger, hostility, and depression. Bearing in mind the fact that PTSD and other psychiatric disorders have many symptoms in common, this study was aimed to analyze the fatty acid composition in blood serum of patients with PTSD compared to healthy individuals, and to check for possible essential fatty acids deficiency.

Methods: The study included 50 male war veterans diagnosed with PTSD and 30 control subjects who also served in the war, but did not develop PTSD. The participants (aged 34-58 years) were interviewed about their nutritional and life style habits and anthropometric measures were taken. Vein blood samples were taken from cubital vein after overnight fasting; from serum total lipids were extracted, and fatty acids trans-esterified to methyl esters. Fatty acid composition was analyzed by gas chromatography.

Results: In PTSD group a decreased ratio of linoleic (C18:2n-6) and arachidonic acid (C20:4n-6) was found, followed by an increase in saturated fatty acids ratio.

Conclusion: Changes in fatty acid composition might be involved in the onset and the development of post-traumatic stress disorder.

POSTER 30

EVALUATION OF THE 5-LIPOXYGENASE INHIBITORY ACTIVITY OF COUMARINS

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Abstract: Nine natural product of the coumarin family with diverse substitution patterns were tested for their capacity to inhibit leukotriene biosynthesis via the inhibition of human 5-lipoxygenase (5-lox) enzyme. The coumarins tested showed good activity against 5-lox extracted from human polymorphonuclear leukocytes. Docking and the structureactivity relationships were evaluated with respect to their ability to inhibit the human 5-lox enzyme. The results can be exploited for the design of potentially valuable anti-inflammatory agents for treating diseases in which leukotriene generation are involved.

POSTER 31

HEIGHTENED RISK OF CORONARY ATHEROMA CONFERRED BY A DECREASE IN THE PLASMA CONCENTRATIONS OF LITHOCHOLIC ACID

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Abstract: Context: Bile acids are cholesterol derivated molecules, synthetized by the liver. The bile acids receptors Farsenoid X (nuclear) and TGR5 (membranar) protect against the formation of atheroma in mice, though no evidence have linked coronary atheroma and bile acid in human.
Objective: To test the hypothesis that changes in concentrations of circulating bile acid species influence the risk of developing coronary atheromas in humans.
Design: Pilot, prospective, observational study conducted between June and September 2010. The serum concentrations of the four main circulating bile acids species (cholic, chenodeoxycholic, deoxycholic, and lithocholic acids) were measured by high-pressure liquid chromatography coupled with tandem mass spectrometry.
Study participants: Consecutive hospitalized or ambulatory patients undergoing emergency or elective coronary angiograms were eligible. Postcardiac arrest, non-fasting states, hepatic disease, and treatment with antimicrobials, corticosteroids, statins or fibrates were exclusion criteria. Of 393 screened patients, 44 met the study criteria, and were divided between 27 patients with (Group A) and 17 without (Group B) angiographically visible coronary atheromas.
Main outcomes measures: Blood was collected after 8 h of fast before elective angiography, or after 8 h of fast after angiography in patients who presented with acute coronary syndrome. The pool of circulating bile acids was analyzed to measure the plasmatic concentrations of 28 different bile acid species. The variables associated with the presence of angiographically visible coronary atheromas were examined by single and multiple variable logistic regression analysis.
Results: The serum lithocholic acid concentration was significantly lower in group A. (median = 0.03 μmol/L; interquartile range 0.02 to 0.05) than in group B (0.08 μmol/L; interquartile range 0.05 to 0.11; P=0.015). By multiple variable analysis, lithocholic acid was the only predictor of coronary atheroma independently of patient gender (odds ratio 2.41 per 0.05 decrease; 95% confidence interval 1.11 to 5.25, P=0.027)
Conclusion: A low serum concentration of lithocholic acid was an independent predictor of coronary atheroma in humans. Both TGR5 and FXR receptor protects against atheroma in animal models and are activated by LCA. Further human studies will be necessary to better understand how far LCA is implicated in coronary atheroma plaque development.

POSTER 32

PLASMA 4-HYDROXYNONENAL PROTEIN ADDUCTS AS A NEW MARKER IN MULTIPLE SCLEROSIS

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Abstract: Background: Multiple sclerosis (MS), with a prevalence ranging between 2 and 150 per 100,000, is an inflammatory central nervous system disease of social relevance, leading to demyelination of neurons. MS is more common among females and no known cure exists to date. The pathogenesis of MS is still incompletely understood, although several genetic and environmental factors have been associated with the risk of developing the disease. The role of oxidative stress in MS has not been explored in-depth. Levels of 4-hydroxynonenal protein adducts (4-HNE PAs) in MS have not been explored.

Aim: To investigate plasma 4-HNE PAs as a possible marker of lipid peroxidation-induced protein damage in MS.

Methods: The study involved a total of 20 patients with diagnosed MS (age at examination: 42.2 ± 8.2 yrs; age at onset: 33 ± 8.8 yrs; M: 6; F: 14) and healthy controls (n=11). MS subtypes included clinically isolated syndrome (CIS), relapsing-remitting MS (RRMS) and secondary progressive MS (SPMS). Neurological disability in the MS patients were evaluated by the Expanded Disability Status Scale (EDSS). Plasma 4-HNE PAs were evaluated by Western blot and expressed as Arbitrary Units (A.U.) based on optical densities.

Results: Plasma 4-HNE PAs was found to be significantly increased in MS patients compared to controls (p=0.0001). A cut-off value of > 14 819 962 A.U discriminated MS from controls with 100% sensitivity and specificity (95% CI: 71.5-100%). No significant differences were detectable among MS clinical types (ANOVA, P=0.81), and no significant correlations were observed for plasma 4-HNE PAs with disease severity or duration.

Conclusions: Our findings indicate that lipid peroxidation is a common feature in MS and that plasma 4-HNE PAs may become an interesting new marker for the disease.

POSTER 33

LIPID PEROXIDATION PATTERNS IN ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT

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Abstract: Introduction: Lipid peroxidation is implicated in the pathogenesis of Alzheimer Disease (AD) and mild cognitive impairment (MCI). However, it is unclear whether it is a primary or a secondary contributor to the MCI/AD phenotype, and whether oxidative stress markers in peripheral samples could represent reliable indicators for the presence of neurodegenerative disease.

Methods: Patients with AD and MCI were included, as well as healthy controls comparable for age and gender. Plasma was used for determination of free F2-Isoprostanes (F2-IsoPs), F2-dihomo-isoprostanes (F2-dihomo- IsoPs), F3-Isoprostanes (F3-IsoPs), F4-neuroprostanes (F4-NeuroPs) and non -protein-bound iron (p-NPBI) and erythrocytes were used for determination of intraerythrocyte NPBI (IE-NPBI). F2-IsoPs, F2-dihomo-IsoPs, and F4-NeuroPs were measured by gas chromatography/negative ion chemical ionization tandem mass spectrometry method (GC/NICI-MS/MS). IE-NPBI and p-NPBI were assessed by HPLC.

Results: Levels of IE-NPBI and p-NPBI in MCI and AD patients were significantly higher as compared to controls ($p=0.001$ and <0.001, respectively) and p-NPBI was higher in MCI than in AD. Plasma F2-IsoPs and plasma F2-dihomo-IsoPs concentrations were significantly higher in MCI and AD as compared to controls ($p<0.001$), with F2-IsoPs concentration being higher in AD as compared to MCI patients. F3-IsoPs were significantly reduced in both MCI and AD patients. No significant differences were observed for F4-NeuroPs ($p=0.216$).

Conclusions: Increased lipid peroxidation end-products from arachidonic and adrenic acids are detectable in the peripheral blood from MCI and AD patients with distinct patterns, while lower levels of eicosapentaenoic acid oxidation end-products are observed. The data indicate that an early screening for AD is feasible and suggest a primary contributing role of lipid peroxidation in the pathogenesis of this neurodegenerative disease.

POSTER 34

CYTOCHROME P450 PRODUCTS AND THE REGULATION OF VASCULAR FUNCTION

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Abstract: Cytochrome P450 (CYP) enzymes produce a number of bioactive epoxy-fatty acids from polyunsaturated fatty acids, e.g., from arachidonic acid and docosahexaenoic acid, that are further metabolized by the soluble epoxide hydrolase (sEH) to their corresponding diols. Given that our previous work data attributed arachidonic acid epoxides a role in angiogenesis we assessed angiogenesis in sEH-/- mice. Retinal vascularisation was delayed in sEH-/- mice at postnatal days (P) 2 and 5, and was associated with reduced tip cell numbers and filopoida extensions at the angiogenic front as well as induction of Notch-dependent transcription factors Hes1 and Hey1. The sEH was mainly expressed in Müller glia cells and Müller cell specific sEH knockout mice also displayed a delayed retinal angiogenesis phenotype. Lipid profile analysis of wild-type and sEH-/- Müller cells reveled marked differences in 19,20-dihydroxydocosapentaenoic acid (DiHDPA) levels. Moreover, 19,20-DiHDPA was able to significantly decrease Notch signaling and intravitreal injection of 19,20-DiHDPA resulted in increased primary network density, as well as tip cell and filopodia numbers in the angiogenic front in sEH-/- mice (P5). These data indicate that the sEH is required for retinal vascularization. This is the first indication that Müller glia cells play an important role in the development of the retinal vasculature and that a sEH metabolite is essential for normal vascularization.

POSTER 35

PROSTACYCLIN IS RESPONSIBLE FOR VASODILATATION IN HUMAN MAMMARY ARTERY DURING INFLAMMATORY CONDITIONS

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Abstract: Background: Cyclooxygenase-2 (COX-2) has been reported to be induced in human internal mammary arteries (IMA) during inflammation and to cause a decrease of the vasoconstriction induced by norepinephrine. This effect was accompanied by an increase of COX metabolites releases such as Prostaglandin E2 (PGE2) and Prostacyclin (PGI2).

Aims: The present study was designed to determine the role of PGE2 and PGI2 and their receptors in the control of human vascular tone during inflammation.

Methods: IMA from 14 patients undergoing bypass surgery were cultured in normal or inflammatory conditions (interleukin-1β; and lipopolysaccharide) for 24h in an incubator with 5% CO2/air at 37°C. Subsequent to this exposure, different protocols were performed: prostanoid receptors, COX, PGI- and PGE- synthases isoforms (mRNA and proteins) were detected and quantified by real time PCR and immunohistochemistry. In addition, a pharmacological study was also performed on isolated vessel placed in an organ bath system: concentration -effect curves induced by norepinephrine or prostanoid-receptor agonists were performed after 30min incubation with or without selective prostanoid -receptor antagonists.

Results: A significant increase (187±76% and 20±4%) of COX-2 and IP receptor mRNA expressions, respectively, was observed under inflammatory conditions. Using immunohistochemistry, the IP, EP1-4, receptors, PGIS were similarly detected between both conditions. The IP receptor antagonist (CAY10441, 1μM) increased significantly norepinephrine contraction under inflammatory conditions. This effect was not observed with selective EP2/4 blockades using (AH6809+GW627368x). PGE2, TP agonist (U46619) and selective EP agonists induced potent vasoconstriction in IMA. These effects were similar between both conditions. No relaxation was observed with PGE2 or the EP2/4 agonists in pre-contracted IMA under both conditions. In contrast, the vasodilatation induced by iloprost, a PGI2 stable analogue, was significantly reduced under inflammatory conditions.

Conclusion: The reduced IMA relaxation induced by iloprost is probably due to an impairment of the IP receptor signaling pathway during inflammation. However, the increased release of PGI2 could surmount this impairment. Our study demonstrates that PGI2 but not PGE2 is responsible for the decreased reactivity to norepinephrine in human IMA under inflammatory conditions. This could be an explanation of the detrimental vascular events related to the use of COX-2 inhibitors.

POSTER 36

FATTY ACID COMPOSITION OF THREE MEDICINAL PLANTS FROM BAHRAIN: NEW POTENTIAL SOURCES OF γ-LINOLENIC ACID AND DIHOMO-γ- LINOLENIC

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Abstract: The fatty acids of three medicinal plant species from Bahrain, Asphodelus tenuifolius, Aizoon canariense, and Emex spinosus were identified. The fatty acid composition varied among species. Lauric acid (12:0) was the major saturated fatty acid in all species studied, while α-linolenic (18:3n3) and eicosatrienoic (20:3n3) acids were the major polyunsaturated fatty acids. The polyunsaturated omega 3 fatty acids detected in all plants studied are well known for their role in the prevention of Coronary Heart Disease. The fatty acid γ-linolenic acid (18:3n6, GLA) was only detected in Aizoon canariense in noticeably high quantity in plant leaves, whereas tearidonic acid (18:4n3) was detected in all species but it was significantly ($p<0.05$) higher in Aizoon canariense compared to Asphodelus tenuifolius and Emex spinosus. Therefore, Aizoon canariense can be considered as a promising new source of GLA that could be useful in treating many diseases and/or cosmetic applications. The lowest omega 6 to omega 3 ratio was found in Emex spinosus, while the highest ratio was found in Aizoon canariense.

POSTER 37

REGULATION OF EXCITATORY NEUROTRANSMITTER RELEASE BY METABOLITES OF LONG CHAIN POLYUNSATURATED FATTY ACIDS IN BOVINE RETINA, IN VITRO

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Abstract: Neuroprostanes (NeuroPs) and F3-isoprostanes (IsoPs) are a series of F2-IsoP-like compounds that are spontaneously generated via free-radical catalyzed peroxidation of the long chain polyunsaturated fatty acids (LCPUFA), docosahexaenoic acid (DHA) and eicosapentanoic acid (EPA), respectively (Musiek et al., 2005; VanRollins et al., 2008). Although endogenous level of LCPUFA-metabolites is elevated in neurodegenerative conditions (Musiek et al., 2005), their pharmacological effect on retinal neurons has not been fully elucidated. In this study, we investigated the regulatory effect of DHA-derived NeuroPs, 4-F4t-NeuroP (CO5-738) and 4(RS)-4-F4t-NeuroP (CO3-475) and EPA-derived F3-IsoP epimer pair, 5-F3t-IsoP (CO5-667) and 5-epi-5-F3t-IsoP(CO5-668) on K+-induced glutamate release (using [3H]D-aspartate as a marker) in isolated bovine retina.

POSTER 38

DEVELOPMENT OF A CELLULAR MODEL FOR STUDYING THE FUNCTIONAL INTERACTION BETWEEN 5-LIPOXYGENASE AND ITS HELPER PROTEIN FLAP

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Abstract: The enzyme 5-lipoxygenase (5-LOX) metabolizes arachidonic acid in a two-step catalysis via 5-hydoperoxyeicosateraenoic acid (5-HpETE) to the unstable leukotriene A4 (LTA4). The latter is converted enzymatically and non-enzymatically to LTB4, whereas the peroxide can also be reduced to the corresponding alcohol 5-HETE. Upon stimulation of intact cells, 5-LOX translocates to the nuclear membrane and associates with 5-LOX activating protein (FLAP), an essential step in LT-biosynthesis during inflammation and allergic rhinitis. Therefore, FLAP serves as a promising pharmacological target.

In order to study 5-LOX/FLAP interaction and putative FLAP inhibitors, HEK -cell systems with stably transfected 5-LOX plus/minus FLAP were developed. Exogenous arachidonic acid was metabolized in both systems to 5-HETE and LTB4. However, expression of both 5-LOX and FLAP promotes conversion to LTB4 with a LTB4/5HETE ratio of 1:1.5, whereas 5-LOX alone forms LTB4/5-HETE in a 1:7 ratio. This finding suggests that 5-LOX together with FLAP facilitates the epoxide formation of LTA4, the second step of catalysis.

Immunofluorescence data show 5LOX/FLAP co-localization upon ca-ionophore stimulation at the nuclear membrane and demonstrate the well described translocation of 5-LOX as shown in neutrophils and monocytes. Furthermore, MK886 did not inhibit the 5-LOX activity in 5-LOX expressing HEK cells, but reduced the formation of the 5-LOX metabolites when FLAP is co-expressed with 5-LOX. This observation is consistent with the fact that MK886 is a FLAP inhibitor.

In conclusion, we present an appropriate cell system with stably transfected 5-LOX with or without FLAP that offers investigation of the 5- LOX/FLAP interaction by immunofluorescence and studies of putative FLAP inhibitors. The initial results suggest FLAP as a key element in both the retention of 5-LOX at the nuclear membrane and promotion of the second step of catalysis, the formation of LTA4.

POSTER 39

TARGETED PROFILING OF INFLAMMATION RELATED LIPID MEDIATORS IN BIOFLUIDS BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY – TRIPLE QUADRUPOLE MASS SPECTROMETRY

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Abstract: It is increasingly recognized that dysregulated inflammatory response contributes to the pathogenesis of a diverse range of clinical disorders including obesity, atherosclerosis, type 2 diabetes and Parkinson's disease. The existing molecular assays for profiling inflammatory cascade have thus far largely relied on measurement of cytokine or chemokine levels.

The recent findings indicate the important regulatory role of lipid mediators during inflammatory processes. For example, fatty acids derived metabolites such as eicosanoids and endocannabinoids have been shown to exhibit potent pro-inflammatory and pro-resolving properties.

Metabonomics is now a well established systems biology approach that brought insights – without a priori - on the phenotype of a population at a molecular level mainly using untargeted profiling of metabolites based on nuclear magnetic resonance spectroscopy (NMR) or high resolution mass spectrometry (HRMS). If this approach is supposed to be as comprehensive as possible, due to sensitivity and identification issues, trace level bioactive mediators are difficult to monitor without a targeted method, including dedicated sample preparation and analytical procedures. Here, we present an analytical strategy for improved profiling of a wide range of inflammation related metabolic mediators using Ultra Performance Liquid Chromatography – Tandem Mass Spectrometry (UPLC-MS/MS) analysis of biofluids. Analyte concentration and sample clean-up is performed by solid phase extraction using mixed mode anion exchanger to achieve selective enrichment of the analytes. Qualitative and quantitative data are obtained by UPLC-MS/MS with a last generation triple quadrupole mass spectrometer.

The proposed method has been validated for accuracy, precision, linearity, limits of detection and quantitation in order to be applied to large-scale metabolic profiling studies and in vivo experiments on animal models.

POSTER 40

PRO-RESOLVING LIPID MEDIATORS ARE PRESENT IN THE JOINTS OF ARTHRITIS PATIENTS

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Abstract: Background: Specialized pro-resolving lipid mediators (SPM), such as resolvins, protectins and maresins, are potent molecules that play a critical role in limiting inflammation and promoting tissue regeneration. Generally they appear in the late phases of self-resolving inflammation, as shown in several experimental models. Chronic inflammation may thus result from failure of resolution. However, the mechanisms underlying incomplete resolution in human tissues are not yet fully understood. To investigate whether resolution pathways are activated during chronic inflammation, we studied the presence and abundance of SPM and their lipid precursors in two chronic inflammatory diseases: Osteoarthritis (OA) and Rheumatoid Arthritis (RA). Lipid profiling and targeted lipid mediator metabolomics measurements were carried out with synovial fluids (SF) from OA and RA patients.

Methods: SF samples from OA (n = 8) and RA (n = 8) patients visiting the outpatient clinic of the department of Rheumatology were collected. Untargeted LC-(MS/MS) lipidomics profiling, and targeted LC-(MS/MS) lipid mediator metabolomics were applied.

Results: Over 70 different lipid species were determined in both OA and RA SF, including poly unsaturated fatty acids (PUFA) such as arachidonic acid (AA) and docosahexanoic acid (DHA) as well as their hydroxy-containing products. Targeted lipid mediator analyses revealed the presence of SPM in 4 of the OA and 5 of the RA patient samples. Among these, Resolvin D5 and Maresin 1 were readily detectable and were accompanied by the presence of anti-inflammatory lipid mediators such as lipoxin A4 and B4. They were accompanied by pro-inflammatory lipid mediators such as 5S,12S-diHETE an isomer of LTB4 and several prostaglandins. Hydroxy derivatives of polyunsaturated fatty acids (HETE, HEPE and HDHA) which are precursors and biomarkers for lipid mediators were additionally detected in both patient groups. Of interest, 5-HETE levels were higher in OA than in RA, while 12- and 15-HETE were more abundant in RA patient material, suggesting a possibly differential involvement of LOX isoforms in these diseases.

Conclusions: This study represents first evidence for the activation of resolution pathways during chronic inflammation in human disease, indicating that tissue repair mechanisms are activated, albeit inefficient in chronic inflammatory diseases such as OA and RA.

POSTER 41

ANTI-ATHEROGENIC ACTION OF DHA IN LDLR-/- MICE: ARE DHA OXYGENATED METABOLITES PLAYERS IN THE RESOLUTION OF INFLAMMATION?

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Abstract: The omega 3 PUFA docosahexaenoic acid (DHA) exerts potent antiinflammatory action with benefit in numerous inflammatory diseases including atherosclerosis. The molecular mechanisms of its antiinflammatory action are complex and not still fully understood, especially with regard to an array of potentially bioactive lipid species generated via enzymatic and non-enzymatic oxidation. We aimed to determine the impact of DHA supplementation on 1) the extent of atherosclerosis and inflammation in aorta, 2) the aortic overall gene expression and 3) the profiles of fatty acid and their oxygenated metabolites. LDLR-/- mice (n=30/group) were fed for 20 weeks a diet enriched with lard (10%, w/w) and cholesterol (0.045%, w/w) in parallel with daily oral gavages (5 days/week) with either oleic acid rich oil (Control) or a mixture of oils providing 2% of energy as DHA (DHA). DHA reduced the systolic blood pressure (-16 mmHg, $p<0.01$), plasma cholesterol (-28%, $p<0.001$) and triacy lglycerol (-37%, $p<0.01$) concentrations, and the extent of atherosclerosis (-35%, $p<0.001$). The macrophage infiltration in the lesion (Mac3 positive) was not modified by DHA supplementation. However, macrophages were preferentially oriented towards a reparative M2 phenotype (Arginase I positive: + 111%, $p=0.01$). The transcriptomic analysis of aorta reveals that DHA supplementation was associated with anti-inflammatory and immunomodulatory effects, namely downregulation of adhesion molecules (e.g. ICAM-2, fold change (FC)=-1.34, $p<0.01$), pro-inflammatory cytokines (e.g. CCL5, FC=-1.49, $p<0.01$) or of genes of the major histocompatibility complex (e.g. HLA-DRB1, FC=-1.68, $p<0.01$). Transcription factors analysis reveals the inhibition of the NFkappaB pathway and the activation of PPARgamma. Lipidomic analysis was conducted to determine profiles of PUFA and their enzymatic and non-enzymatic oxygenated metabolites (oxylipins, hydroxyalkenals and F4-neuroprostanes) in plasma and liver. Dietary treatment induced an accumulation of DHA in liver (x8, $p<0.001$) and plasma (x2, $p<0.001$) in parallel with increased levels of liver 4-hydroxyhexenal-protein adducts (+59%, $p<0.05$), F4-neuroprostanes (x11, $p<0.01$) and plasma DHA oxylipins (x3, $p<0.001$). All together, these results show that DHA exerts anti-inflammatory and atheroprotective effects, which occur in the presence of an enhanced production of oxygenated metabolites. Further research is now needed to determine specifically the biological properties of these new potentially bioactive lipid metabolites.

POSTER 42

NOVEL BRAIN ANTIOXIDANT ACTIVITY

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Abstract: Antioxidant activity in the brain protects against a variety of neurodegenerative disorders in which oxidative stress play a role such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis. One antioxidant activity is related to the ability to hydrolyze oxidized polyunsaturated fatty acids (PUFA) from esters, thus defining the role of esterases as antioxidants. We have demonstrated thet oxygen exchange is indicative of esterase activity. Therefore, we looked at the exchange of oxygen on prostaglandin E2 (PGE2), an arachidonic acid oxidation product that is formed in situ on PUFA esters via nonenzymatic oxidative pathways. We determined the esterase activity in different rat brain compartments both in vivo and in vitro by measuring 18O exchange on the carboxyl group of deuterium labeled PGE2 using LC-MS. While there was oxygen exchange in all of the compartments, the choroid plexus showed the highest exchange rate of 18O. We also treated a primary neuronal culture with increasing concentrations of H2O2 up to 500 μM in the presence and absence of an esterase inhibitor, and measured LDH release as an indicator of cytotoxicity. The inhibitor caused a dramatic, up to 5 fold, increase in H2O2 cytotoxicity with increased H2O2 concentration. The inhibitor alone did not display cytotoxic effect. Using pharmacological inhibition, we demonstrated that this esterase activity is distinct from known esterases that have antioxidant properties such as paraoxonase or platlet activating factor acetyl hydrolase. These results indicate that there is a high esterase activity in the brain and this activity may be neuroprotective against oxidative damage.

POSTER 43

INVOLVEMENT OF PGE2 AND MMP IN VASCULAR WALL REMODELLING OF HUMAN SAPHENOUS VARICOSE VEINS

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Abstract: Varicose saphenous veins are characterized by venous backflow, blood stagnation and vascular wall remodelling. This pathology affects approximately one-third of the adult population. However, the pathogenesis is unclear. PGE2 is synthetized from arachidonic acid metabolism by cyclooxygenases and by PGE synthase (PGES) isoenzymes (mPGES-1, -2 and cPGES). The 15dPGDH is the enzyme responsible for its degradation. Studies have shown that PGE2 is involved in vascular wall remodelling by activating the matrix metalloproteases (MMP, Cipollone et al., Circulation 2001, Lee et al., Mol. Cell. Endocrinol 2010) and is also able to induce saphenous vein dilatation. These points could be involved in varicose vein formation.

Aim: The aim of the present work was to study the PGE2 metabolism and MMP activities during varicose veins development.

Methods: In order to study the expression of different PGESs and 15dPGDH by western blot, proteins were extracted from human saphenous veins (large (LDv) and small (SDv) diameter varicosities from the same patients (n=5) or healthy donors (n=5). Measurements for PGE2, MMP and TIMP production in varicose veins (n = 5) and healthy veins (n=5) supernatant were realised by ELISA. Collagen content was measured by histomorphometry on formalinfixed venous preparations (n=5).

Results: mPGES-1/-2 and 15dPGDH proteins are expressed in varicose and healthy veins. However, mPGES1 expression decreases in LDv. In contrast, we observed a significant increased expression of 15dPGDH in varicose veins. We also observed a significant decrease of PGE2 concentration in supernatant of varicose veins. Futhermore, the MMP-1/-2 activities are lower in LDv than in the other preparations. Furthermore, TIMP-2 is expressed in all the preparation, and TIMP-1 is significantly higher in LDv. Finally, we observed a significant increase in collagen content in LDv.

Conclusion: PGE2 is involved in varicose veins vascular wall remodelling by activating MMP activities. Our results suggest that the lower PGE2 concentrations measured in LDv in comparison with SDv, could be associated to a reduced MMP- and an increased TIMP-activity. These effects could explain the increased collagen quantity observed in LDv. These results show that PGE2 metabolism plays a role in varicosity formation.

POSTER 44

VLDLR IS A DETERMINANT FACTOR IN ADIPOSE TISSUE INFLAMMATION AND ADIPOCYTE-MACROPHAGE INTERACTIONS.

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Abstract: OBJECTIVE: Obesity is associated with adipose tissue remodeling characterized by macrophage accumulation and local inflammation. In this scheme, secretion of pro-inflammatory mediators and expression of cell membrane receptors are involved in the crosstalk macrophage-adipocyte. Very low density lipoprotein receptor (VLDLR) is highly expressed in adipocytes and macrophages but its role in adipocyte-macrophage interaction is not known. The objective of this study was to investigate the role of VLDLR in adipose tissue inflammation and adipocyte-macrophage interplay.

EXPERIMENTAL APPROACH: Adipose tissue inflammation and macrophage infiltration was compared in VLDLR deficient (VLDLR-KO) and wild type (WT) mice. The inflammatory response to lipopolysaccharide (LPS) was studied in primary adipocytes and macrophages isolated from VLDLR-KO and WT mice. In addition, adipose tissue macrophages (ATMs) were characterized with flow cytometry and confocal microscopy according to the expression of selective surface antigens of the classical (M1) and alternative (M2) phenotypes. Furthermore, the role of VLDLR in adipocyte-macrophage interactions is examined in a co-culture system of adipocytes-macrophages.

RESULTS: Compared to WT mice, VLDLR-KO mice exhibited reduced adiposity and adipose tissue inflammation, with decreased pro-inflammatory cytokine production (-40%), and macrophage and T-cell accumulation (-52%). In primary cell culture, the absence of VLDLR expression in ATMs decreased pro-inflammatory cytokine, and endoplasmic reticulum (ER) stress gene expression in response to LPS. Likewise, VLDLR deficiency in primary adipocytes reduced pro-inflammatory cytokine and chemokine secretion (30- 57% reduction), and increased adiponectin production (+46%). Primary macrophage and adipocyte co-culture experiments showed that these cell types act synergistically in their inflammatory response to LPS and that VLDLR modulates such synergistic effects. Flow cytometry analysis showed that VLDLR deficiency reduced the content of the pro-inflammatory M1 (CD11b+ and CD11c+) and increased the proportion of the anti-inflammatory M2 macrophage (CD206+) in adipose tissue. Similarly, confocal microscopic analysis of adipose tissue showed low staining with CD11c and high staining with M2 marker arginase-1.

CONCLUSIONS: Collectively, these results show that VLDLR enhances adipose tissue inflammation through its expression in both macrophages and adipocytes.

POSTER 45

CHARACTERIZATION OF A NEW FAMILY OF ELECTROPHILIC PHOSPHOLIPIDS GENERATED BY MONOCYTES AND MACROPHAGES AND IN VIVO IN HUMAN LUNG DISEASE.

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Abstract: We previously reported that activated human monocytes acutely generate four phosphatidylethanolamine-esterified 15-hydroxyeicosatetraenoic acids (HETE-PEs) via the enzymatic action of 15- Lipoxygenase1 (15-LOX). Herein, we show formation of related lipids, including 15-HpETE-PEs and 15-KETE-PEs. The latter comprises four molecular species, specifically 16:0p/15-KETE-PE, 18:1p/15-KETE-PE, 18:0p/15-KETE-PE and 18:0a/15-KETE-PE. 15-KETE-PE generation is transiently elevated on ionophore activation of monocytes, with average levels reaching a maximum of 800 pg/106 cells. The lipids were also detected in bronchoalveolar lavage fluid from cystic fibrosis patients (up to 3.6ng/ml lavage), with levels elevated significantly from controls. Using a selective inhibitor, CAY10397, the formation of 15-KETE-PEs was shown to require 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which catalyses their oxidation from 15-HETE-PE. Murine peritoneal macrophages also generated four 12-KETE-PEs, analogous to those detected in human monocytes, appearing within 15 minutes of activation and disappearing after 3 hours. Little is currently known about the pharmacology of these ketone containing phospholipids, however due to the electrophilic nature of the α,β- unsaturated ketone group, 18:0a/15-KETE-PE readily adducts to glutathione or cysteine in vitro, demonstrating its potential to form protein or peptide adducts, and activate electrophile sensitive transcriptional regulatory events. Both 15-HETE- and 15-KETE-PEs displayed low affinity PPARγ stimulating activities, but did not activate Nrf2. Electron microscopy showed that murine peritoneal 12/15-LOX-/- macrophages contain increased numbers of double membrane intracellular vesicles, and defective mitochondria, suggesting defective autophagy. Furthermore, the autophagic vesicle membrane protein LC3II is deficient in 12/15-LOX-/- macrophages. Future studies aim to determine how the presence of these oxidized phospholipid families alters the structure or behaviour of the lipid bilayer during inflammation.

POSTER 46

PPARbeta/delta AGONISTS GW0742 AND GW501516 INDUCE VASODILATATION OF HUMAN PULMONARY ARTERY

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Abstract: Pulmonary arterial hypertension is defined as a mean pulmonary artery pressure of greater than 25mmHg at rest. Current therapies modulate or mimic endogenous hormones released by endothelial cells that regulate vascular tone. These include prostacyclin (IP) receptor agonists (e.g. treprostinil sodium), phosphodiesterate type 5 inhibitors (e.g. sildenafil), and endothelin receptor antagonists (e.g. bosentan). However, none of these drugs cure the condition and new therapeutic approaches are currently under investigation. We and others have recently shown that the PPARbeta/delta agonist GW0742 induces vasodilatation of mouse and rat pulmonary arteries (Harrington et al 2010; Li et al 2012). However, the effect of PPARbeta/delta agonists on human pulmonary vessels has not been tested. Here we investigated the effects of two PPARbeta/delta agonists on human pulmonary artery tone in vitro using resting pressures in line with those seen in patients with pulmonary hypertension.

Human pulmonary arteries dissected from human lung samples were loaded on to Mulvany myographs and normalised to an effective pressure of 4kPa (equivalent to 30mmHg). Following equilibration, arteries were exposed to 3x10-8M U46619 followed by increasing concentrations of GW0742 (10-6 to 3x10-5M), GW501516 (10-6 to 3x10-5M), or the mixed IP/PPARbeta/delta agonist treprostinil sodium (10-9 to 10-6M). The drug vehicle, DMSO plus bovine serum albumin (BSA; maximum bath concentration of 0.033% for both) was added to control tissues.

All agonists tested induced reductions in tone induced by U46619 with a range of potencies: GW0742, EC50 1.4x10-5M; max response, change in induced tone, -71±9%: GW501516, EC50 9.6x10-6M; max response -76±7%: treprostinil sodium, EC50 1.74x10-8; max response -67±9%: n=3 for all. Addition of vehicle had minimal effect on vascular tone (max change in induced tone, -9±7%).

Two chemically distinct PPARbeta/delta agonists induced vasodilatation in human pulmonary artery with similar efficacies to that of treprostinil sodium. These results support the idea that PPARbeta/delta agonists could have therapeutic utility in the treatment of pulmonary arterial hypertension.

Harrington LS et al PLoS ONE (2010) vol5, 3 e9526; Li Y et al Am J Respir Cell Mol Biol (2012) vol 46, 372-379.

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POSTER 47

NOVEL INSIGHTS INTO CYCLOOXYGENASES, LIPOXYGENASES, AND 8R - DIOXYGENASES FROM OXIDATION OF CIS-TRANS ANALOGUES AND DEUTERIUM KINETIC ISOTOPE EFFECTS

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Abstract: Cyclooxygenases (COX) and lipoxygenases (LOX) transform arachidonic acid into prostaglandins and leukotrienes which have numerous functions in physiological and pathophysiological processes as pain, inflammation, homeostasis, and reproduction.

The prototype of LOX is soybean LOX-1 which contains non-heme iron and catalyzes bis-allylic hydrogen abstraction and antarafacial oxygen insertion. This occurs with an enigmatically large deuterium kinetic isotope effect (D-KIE) of ~80. COX and the 8R-dioxygenase (DOX) domains of linoleate-diol-synthases are heme-dependent DOX which generate a tyrosyl radical that abstracts hydrogen prior to antarafacial oxygen insertion. LOX are limited to hydrogen abstraction from bis-allylic carbons, whereas COX also perfom the more energy demanding abstraction of allylic hydrogens. The D-KIE during bis-allylic hydrogen abstraction of COX-1 is 2-3.

The first goal was to address the large difference in D-KIE of LOX and COX. We investigated the reaction mechanisms of COX-1, COX-2, 8R-DOX, sLOX -1 and 13R-MnLOX with selectively deuterated fatty acids. The second goal was to compare the D-KIE of sLOX-1 and 13R-MnLOX, representing LOX with Fe and Mn as catalytic metals. The third goal was to study cis-trans isomers of 18:2n-6 by COX-1, 8R-DOX, and sLOX-1 to get more insight into the relation of hydrogen abstraction and oxygen insertion.

Oxygenation of bis-allylic carbons by COX-1 and COX-2 occurred with a DKIE of 3-5, whereas oxygenation of the allylic carbon C-13 of 20:1n-6 by COX-1 and C-8 of 18:1n-9 by 8R-DOX were accompanied by large D-KIE (>20).

sLOX-1 and 13R-MnLOX oxidized [11S-2H]18:2n-6 with similar D-KIE (~53), which implies that the different redox potentials of the catalytic metals did not alter the D-KIE.

COX-1 oxidized (9Z,12E)18:2 with bond rotation at C-13, whereas 8R-DOX shifted hydrogen abstraction from the allylic C-8 to the bis-allylic C-11 yielding 9R-linoleic acid as main metabolite.

We conclude that COX oxidize allylic and bis-allylic positions with different D-KIE.

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POSTER 48

EVALUATION OF UP-STREAM AND DOWN-STREAM CHANGES IN mPGES-1 KNOCK-OUT MICE

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Abstract: Background: Microsomal prostaglandin E synthase-1 (mPGES-1) is the terminal enzyme in the induced PGE2 production at the sites of inflammation and well-recognized target for the development of novel antiinflammatory drugs. Genetic deletion of mPGES-1 in arthritic mice reduces inflammation and protects them from pain and joint destruction. However, molecular mechanisms involved in anti-inflammatory effects of mPGES-1 inhibition/deletion at sites of inflammation have not been explored.

Objective: To investigate the effect of mPGES-1 deletion on the eicosanoid profile (down-stream cascade) and total fatty acid composition (up-stream cascade).

Methods: Peritoneal macrophages (PM) from WT and KO mice were induced with LPS for 16 h and supernatants were harvested for eicosanoid analysis. Eicosanoid profiling of approximately 30 compounds was performed using LCMS/ MS. The total fatty acid composition in spleen and brain homogenates of WT and KO mice was determined using GC-FID.

Results: Compared to WT, mPGES-1 deficient PM displayed a markedly attenuated increase in PGE2 production upon LPS stimulation, and exhibited significantly increased levels of 15-deoxy-PGD2 and PGF2a ($p<0.01$) indicating shunting towards the PGD2 pathway. There were significant differences in the total fatty acid composition in spleen (e.g., palmitoleic acid, $p<0.05$) and brain (e.g., alpha linolenic acid, $p<0.01$) of KO and WT mice, suggesting that feed-back mechanisms are alternated by the deletion of mPGES-1.

Conclusion: Data reveals that mPGES-1 deficient PMs displayed shunting towards production of PGD2 metabolites upon LPS stimulation compared to WT. In addition, mPGES-1 depletion alters the fatty acid composition. These effects of inducible PGE2 have important implications regarding potential consequences of pharmacologic mPGES-1 inhibition. It is of importance to apply a lipidomics approach to obtain a more holistic picture of the studied system.

POSTER 49

ENDOCANNABINOID, GHRELIN, AND THEIR POTENTIAL CROSS-TALK IN THE BRAIN

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Abstract: Endocannabinoid (eCB) has been well characterized for its regulatory role in the immune system and peripheral metabolism. Recent evidence, however, suggests that eCBs also exert neuroprotective roles in the brain and stimulate appetite through its central receptor, CB1R. Indeed, a brain-gut peptide, ghrelin, and eCBs are two representative molecules that stimulate appetite. Although both molecules are critically involved in the induction of orexigenic activities in the hypothalamus, they also play an important role in hedonic eating governed in the hippocampus. We previously reported ghrelin activated CREB (cAMP responseelement binding protein) and phosphorylated the NMDA receptor subunits via the ghrelin receptor (also known as GHSR1a). Ghrelin phosphorylated NR1 by activating cAMP/PKA signaling pathways. On the other hand, for NR2B, ghrelin triggered more complex signaling pathways including phophatidylinositol-3-kinase and AMP kinase. Interestingly, the effect of ghrelin was counteracted by exogenous application of eCBs. Twenty nM of R(+)-methanandamide (a non-degrading form of anandamide, a typical eCB in the brain) negated the effect of ghrelin on the phosphorylation of NRl. However, this inhibitory effect was not blocked by either AM251, capsazepine, or iodoresiniferatoxin suggesting that R(+)- methanandamide exerted its effect independent of the cannabinoid receptor and the vanilloid receptor. When 2-arachidonoyl-glycerole (2-AG, another typical eCB in the brain) was co-applied with ghrelin, it negated the effect of ghrelin on the subunit phosphorylation. However, the inhibitory effect of 2-AG was blocked by the CB1R antagonist, AM251, suggesting that the effect of 2-AG was CB1R-dependent. Similarly to 2-AG, WIN 55, 212 (a CB1R agonist) also blocked the stimulating effect of ghrelin on the NR1 phosphorylation. NMDA receptors are critical for learning and memory, and the ghrelin's stimulatory effect on the NMDA rec eptor indicated the likely-involvement of the NMDA receptor in appetiterelated learning. eCBs, on the other hand, are central in healthy and maladaptive reward learning. Our present finding for the presence of interaction, a possible crosstalk, between the ghrelin and eCB system in the brain may pave a future direction towards the understanding and management of maladaptive appetite and related metabolic diseases in the brain.

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POSTER 50

EFFECT OF INTERLEUKIN-1BETA ON THE FATTY ACID COMPOSITION OF CULTURED NASAL FIBROBLASTS DERIVED FROM NASAL MUCOSA AND NASAL POLYPS

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Abstract: Background. Interleukin 1 beta (IL-1β) is a proinflammatory cytokine that plays an important role in a number of chronic and acute inflammatory diseases including asthma. It is produced by a variety of cells in the body among them the fibroblasts. Fibroblasts from asthmatics, especially those with aspirin sensitivity, produce little prostaglandin E2 and have an abnormal regulation of cyclooxygenase 2. The objective of this study is to determine and compare the fatty acid composition before and after stimulation with interleukin-1beta in culture airway fibroblasts from non-asthmatics and from aspirin-tolerant and aspirin-intolerant asthmatics.

Methods. Fibroblasts obtained from nasal surgery from healthy subjects and asthma patients with and without aspirin intolerance were stimulated with interleukin-1beta. Fatty acids composition was analyzed by gas chromatography-flame ionization detector.

Results. There was a significant difference before and after stimulation with IL-1β in aspirin-tolerant and aspirin-intolerant asthmatics fatty acid composition. In non-asthmatics, the fatty acid contents increased significantly in palmitic acid (C16:0) ($p<0.05$), and alphalinolenic acid (C18:3n3) ($p<0.01$), while decreased significantly in dihomo -gamma-linolenic acid (C20:3n6) ($p<0.05$), arachidonic acid (C20:4n6) ($p<0.05$) and eicosatrienoic acid (C20:3n3) ($p<0.05$). In aspirin-tolerant asthmatics, there was a significant increase in fatty acid contents of eicosadienoic acid (C20:2n6) ($p<0.05$), while there was a decrease in fatty acid contents of eicosatrienoic acid (C20:3n3) ($p<0.036$). In aspirinintolerant asthmatics, there was a significant increase in the fatty acid contents of palmitic acid (C16:0) ($p<0.002$).

Conclusion. Asthmatic and non-asthmatic fibroblasts show different changes in fatty acid composition after stimulation with interleukin-1beta especially in polyunsaturated omega-3 and omega-6 fatty acids. Omega-3 and omega-6 fatty acids have an adverse effect in inflammation in which the former reduces inflammation while some of the later promote inflammation. These data reinforce the concept that interleukin-1beta might be important in the inflammatory diseases.

POSTER 51

OXIDIZED LDL INDUCES CO-STIMULATION AND PHYSICAL INTERACTION OF CD36 AND PAF-RECEPTOR IN MACROPHAGES

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Abstract: Introduction: The uptake of oxidized LDL (oxLDL) by macrophages is a key initial event in atherogenesis. CD36 is a scavenger receptor expressed by macrophages responsible for oxLDL uptake and foam cell formation. In previous study we showed that oxLDL activates the receptor for Platelet-activating factor (PAFR) and increases CD36 expression, enhancing oxLDL uptake by mouse macrophages. Here we investigated the involvement of PAFR in oxLDL uptake by human macrophages, the signaling pathways involved and whether co-localization of PAFR and CD36 is required. Methods: LDL was obtained from human plasma and oxidized by CuSO4. Human monocytes were differentiated to macrophages by culture in RPMI/10% FCS for 72h. PAFR antagonists, WEB2170 or CV3988 and the PI3- kinase inhibitor LY-294002 were added 30 min before oxLDL. Bone marrowderived macrophages were obtained by culture in L929-conditioned medium. FITC-oxLDL uptake and CD36 expression were determined by FACS. Co-local ization and co-immunoprecipitation of PAFR and CD36 were evaluated by confocal microscopy and western blot, respectively. HEK293T cells were cotransfected with plasmids encoding PAFR and/or CD36.

Results: In human macrophages, addition of oxLDL increased CD36 expression, PI3K/AKT pathway activation and IL-8 and MCP1 production. Pretreatment with PAFR antagonists or inhibitors of the PI3K/AKT pathway significantly reduced CD36 expression, IL-8 production and oxLDL uptake. The uptake was significantly lower in PAFR KO compared to WT macrophages. By confocal microscopy, we observed that oxLDL induces the co-localization of CD36 and PAFR on the plasma membrane and in the intracellular compartment. This was confirmed by co-immunoprecipitation. HEK293T cells transfected with either PAFR or CD36 were able to uptake FITC-oxLDL but only HEK293T cells transfected with both receptors produced IL-8 following activation with oxLDL.

Conclusion: It is shown that PAFR is directly involved in oxLDL uptake by activating PI3K/AKT pathway; that oxLDL induces co-stimulation and physical interaction of PAFR and CD36 and that this dual interaction is required for optimal oxLDL uptake and transcription of cytokine genes.

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POSTER 52

ROLE OF ANTI-INFLAMMATORY LIPID MEDIATORS IN THE RESOLUTION OF INFLAMMATION IN MICROGLIAL CELLS

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Abstract: Microglial cells are the cellular components of the brain immune system. In response to systemic bacterial infection they become activated and are strongly involved in the local inflammatory response. They produce pro- and anti-inflammatory cytokines, in particular IL-1b, IL-6 and TNFalpha, and IL-10, respectively. Dietary polyunsaturated fatty acids (PUFA) are able to regulate neuroinflammation. N-6 and n-3 PUFA are precursors of anti-inflammatory mediators such as lipoxin A4 (LXA4) synthesized from arachidonic acid and resolvin E1 (RvE1) and D1 (RvD1) produced from eicosapentaenoic acid and docosahexaenoic acid, respectively. These mediators are synthesized by 5- and 15-lipoxygenases and exert their anti-inflammatory actions by acting on specific G-protein coupled receptors in particular on ALX and ChemR23. The aim of this study was to determine the effects of lipoxin and resolvins on the resolution of inflammation in microglial cells stimulated with lipopolysaccharide (LPS). We used BV-2 cells, a murine microglial cell line. In a first experiment aimed at determining the time course of the induction of the expression of the lipoxygenases and receptors, they were incubated with LPS. In a second experiment aimed at determining the effects of the resolvins and lipoxin on the production of IL-10, IL-1b, IL -6 and TNFalpha, they were firstly incubated with LXA4, RvD1 and RvE1 and then incubated with LPS. Our results indicated that LPS only enhanced the expression of ALX, the receptor for RvE1 and LXA4 (x2) ($p<0.05$). The expression of the 15-lipoxygenase was also affected by LPS ($p<0.01$) and varied during the time course, reaching a maximum after 6h of incubation ($p<0.05$). The production of the proinflammatory cytokines decreased after 18h of incubation: IL-1b with RvD1 (100 nM, $p<0.001$) and RvE1 (10 nM, $p<0.01$); IL-6 with RvE1 (1 and 10 nM, $p<0.05$ and $p<0.001$); TNFalpha with RvE1 (1 and 10 nM, $p<0.001$ and $p<0.01$). The production of the anti-inflammatory cytokine IL-10 increased only when cells were incubated with LXA4 for 6h (1 and 10 nM, $p<0.01$). These results suggested a potential role of PUFA-derivates in the resolution of inflammation.

POSTER 53

VASODILATORY EFFECTS OF PGI2 AND ITS ANALOGUES ON HUMAN UMBILICAL VESSELS: ROLE OF THE CAMP PATHWAY

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Abstract: Background: For pregnant women, the effects of the treatment against pulmonary hypertension on umbilical blood vessels tone are unknown. Prostacyclin (PGI2) and its analogues (like iloprost) are used in this case, in combination with phosphodiesterase inhibitors and/or calcium channel antagonists. Agonists of IP receptor induce vasodilatory response on many systemic blood vessels through cyclic AMP (cAMP) production. Nevertheless, there are few studies about the role of IP receptor in umbilical vessels.

Aim: The relaxations induced by PGI2 and its analogues on human umbilical and pulmonary vessels are compared. The role of cAMP pathway is investigated.

Methods: The effects induced by PGI2, analogues and pharmacological treatments on human umbilical and pulmonary vessels are measured with an organ bath system. IP receptors are quantified by Western Blot. Amount of cAMP in umbilical vessels is measured by ELISA.

Results: PGI2 and analogues induce weaker vasodilatory responses on human umbilical vessels as compared to human pulmonary vessels. Western Blot analysis reveals no difference on IP receptor density between umbilical and pulmonary vessels. In umbilical arteries (HUA), the non-specific phosphodiesterase inhibitor (IBMX) enhances the relaxations induced by PGI2 analogues. The opposite effect is observed on umbilical veins. The L-type calcium channel antagonist, nifedipine, enhances relaxations induced by forskolin (adenylyl cyclase activator) on HUA.

Conclusions: The functionality of IP receptors in umbilical vessels is shown. Moreover, the weak vasodilatory responses induced by IP receptor agonists in umbilical vessels could be due to an impaired production/effect of cAMP. During pregnancy or birth, the use of IP receptor agonists in combination with calcium channel inhibitors in case of pulmonary hypertension is safe for the umbilical circulation.

POSTER 54

P2Y(12) RECEPTOR ACTIVATION LIMITS THE ANTI-PLATELET EFFECTS OF NITRIC OXIDE AND PROSTACYCLIN BY DISTINCT MECHANISMS

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Abstract: ADP, released from activated platelets, acts on P2Y(12) receptors, the target of widely used anti-platelet drugs, to inhibit adenylyl cyclase activity. This provides a mechanism by which platelet activation can proceed in the presence of inhibitory substances that elevate platelet cAMP content such as prostacyclin. The role of P2Y(12) activation in regulating the platelet response to nitric oxide (NO) is less clear, however, as NO acts via a distinct, but related cGMP-dependent signaling cascade. Here we have explored how blockade of the P2Y(12) receptor influences the sensitivity of platelets to inhibition by NO and prostacyclin. In human washed platelets and whole blood, blockade of the P2Y(12) receptor with prasugrel-active metabolite enhanced the inhibition of thrombin- and collagen-induced platelet aggregation produced by prostacyclin (by up 20-fold) and NO (by up to 100,000-fold). In both cases, this was accompanied by similarly enhanced inhibition of platelet ATP secretion, and Rap1b activation. For prostacyclin, this was accompanied by increased platelet levels of cAMP and VASP phosphorylation, suggesting the enhanced activity of prostacyclin was related to loss of P2Y(12)-mediated adenylyl cyclase inhibition. In agreement, inhibition of platelet aggregation to exogenous cAMP was not altered by P2Y(12) blockade. In contrast, the enhanced inhibitory activity of NO produced by P2Y(12) blockade was not accompanied by changes in cAMP, cGMP or phospho- VASP levels. Further, the inhibition produced by exogenous cGMP was also enhanced when P2Y(12) receptors were blocked suggesting a mechanism downstream of cGMP formation, which may involve PY2(12)-dependent activation of phosphoinositide-3 kinase. Taken together, these data clearly demonstrate that activation of P2Y(12) by secreted ADP is a crucial means by which platelets sustain aggregation responses, even in the presence of these endothelium-derived inhibitors. This may provide an alternative pathway by which P2Y(12) inhibitors can exert their powerful anti-thrombotic effect in vivo, one that is intrinsically influenced by the health of the vascular endothelium.

POSTER 55

MODIFIED PHOSPHOLIPIDS FORMED IN INFLAMMATION ARE INVOLVED IN SECRETORY PHOSPHOLIPASE A2 GROUP IIA REGULATION

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Abstract: Secretory phospholipase A2 group IIA (sPLA2-IIA) is an active participant of inflammation. The enzyme destroys bacterial cell membranes and induces generation of bioactive lipid mediators, including lysophospholipids, prostaglandins and leukotriens. SPLA2-IIA may also contribute to various pathologic processes. It has been shown that elevated level and activity of sPLA2-IIA in blood serum are associated with the development of adverse cardiovascular events. The study of sPLA2- IIA regulation is of great physiological and clinical importance and can extend the knowledge of inflammation development.

Levels of sPLA2-IIA are normally extremely low in blood serum, but increase sharply during various inflammatory processes and in diseases that involve systemic inflammation. This suggests the generation of various sPLA2-IIA stimulators in inflammation. We have shown that oxidized lipoproteins activated sPLA2-IIA in human blood serum. Phosphatidylcholine is the major and easily modified phospholipid of lipoproteins. As known, oxidative stress leads to production both oxidized and halogenated phosphotidylcholine. Our study demonstrated that oxidized phosphotidylcholine stimulated the sPLA2-IIA activity. By contrast, halogenated phosphotidylcholine suppressed the sPLA2-IIA activity in human blood serum. Thus, changes in the oxidized phosphotidylcholine/halogenated phosphotidylcholine concentration ratio may affect the regulatory mechanisms of sPLA2-IIA activity in human blood.

Control over the mechanisms of sPLA2-IIA regulation could be a promising strategy in inflammatory and cardiovascular therapy.

POSTER 56

MONITORING PGD2 PRODUCTION IN HUMANS AS AN OBJECTIVE BIOMARKER OF MAST CELL ACTIVATION

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Abstract: Introduction: Prostaglandin (PG) D2 is a major mast cell mediator of asthma and allergy. The urinary level of its early appearing metabolite 11β-PGF2α has been established for monitoring PGD2 production and thereby mast cell activation. Downstream metabolites of PGD2 such as 2,3-dinor-11beta-PGF2alpha (DN-11beta-PGF2alpha) and tetranor prostaglandin D (PGDM) are however more abundant in urine. Aims: To evaluate the pattern of urinary excretion of PGD metabolites at baseline and during asthmatic or allergic reactions. Methods: Urinary samples from healthy volunteers and from several clinical studies were investigated; viz. bronchoprovocations using allergen in atopic asthmatics or aspirin in aspirin-intolerant asthma, effect of the COX-2 inhibitors celecoxib and etoricoxib on baseline excretion, and levels in patients with mastocytosis or anaphylaxis during exacerbation. Urinary excretion of 11beta-PGF2alpha and PGDM were routinely measured by EIAs (Cayman Chemical) and UPLC-MS/MS was used to identify the metabolites in selected samples. All data are expressed per mmol excreted creatinine (Cr).

Results: The levels of PGDM (mean±SEM) before allergen challenge (n=9) were 1.3±0.3 and 1.3±0.2 microg/mmol Cr after ($p>0.05$), the corresponding values of 11beta-PGF2alpha were 40±4 and 77±10 nanog/mmol Cr ($p=0.002$), respectively. In another allergen provocation (n=16), it was found that DN -11beta-PGF2alpha increased more post challenge than 11beta-PGF2alpha. With aspirin challenge, the urinary levels of PGDM were 0.5±0.08 before and 0.6±0.1 microg/mmol Cr after ($p>0.05$; (n=11), whereas 11beta-PGF2alpha increased from 56±16 to 75±24 nanog/mmol Cr ($p=0.002$), respectively. In mastocytosis and anaphylaxis (n=15), the levels of PGDM and 11beta- PGF2alpha were 2.0±0.9 and 0.3±0.07 microg/mmol Cr, respectively, both being significantly increased above those in healthy subjects (n=25; $p<0.05$).

Baseline (n=35) and allergen-induced (n=16) excretion of all PGD2 metabolites was unaffected by treatments with celecoxib or etoricoxib that caused substantial inhibition of urinary PGE2 metabolites.

Conclusion: In humans, PGD2 is biosynthesised by the COX-1 pathway. Data suggest that the PGD2 urinary metabolites 11beta-PGF2alpha and DN-11beta-PGF2alpha may be the most suitable for following the dynamics of shortterm release during on-going reactions. Conversely, the higher levels of PGDM may be more appropriate to assess daily production of PGD2 and hence for monitoring of global differences in mast cell activation between patient populations.

POSTER 57

CHARACTERIZATION OF A NEW mPGES-1 INHIBITOR IN MURINE AND HUMAN MODELS OF INFLAMMATION

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Abstract: mPGES-1 inhibition in Microsomal prostaglandin E synthase-1 (mPGES-1) inhibition has been proposed as an alternative to cyclooxygenase (COX) inhibition in the treatment of pain and inflammation. This approach could potentially mitigate the gastro-intestinal and cardiovascular side-effects seen after long-term treatment with traditional non-steroidal anti-inflammatory drugs (NSAIDs) and Coxibs respectively. The aim of this study was to characterize a new selective mPGES-1 inhibitor, compound III, which has nanomolar potency, in murine and human experimental models of inflammation.

Compound III was assayed for potency and selectivity in vitro, using recombinant enzymes of the prostanoid pathway. Its potency was further investigated in the stringent LPS-stimulated whole-blood assay. To study mPGES-1 inhibition and its repercussion on prostanoid synthesis, compound III was investigated in a short-term inhibition assay (30mins) where A549 cells were stimulated with IL-1beta and a long-term assay (24h) where rat peritoneal macrophages were stimulated with LPS. Lastly, compound III was assayed in the carrageenan-induced air pouch model of inflammation. The resulting prostanoid profile was compared with that obtained in the mPGES-1 KO mouse.

POSTER 58

EXPRESSION OF FAAH AND CANNABINOID RECEPTOR 1 AND 2 IN THE EFFECT OF n- ACYLETHANOLAMINES IN BREAST CANCER

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Abstract: There is much to link fish oil that is high in EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) with a lower incidence of breast cancer. Dietary PUFAs are believed to have opposite effects on the molecular mechanisms in the proliferation of tumour cells. MDA MB 231 and MCF7 cells were treated with EPA, DHA and their ethanolamides EPEA and DHEA respectively. PUFAs dose dependently inhibited growth of both MDA MB231 and MCF7 cells. Both MDA MB231 and MCF7 cells expressed CB1, 2 and FAAH (fatty acid amide hydrolase) as determined by western blot. JNJ 1661010 (an inhibitor of FAAH) only enhanced the effect of DHEA after 48h incubation. CB receptor 1/2 inhibitors (AM281 and AM630 alone or combined) decreased the effect of PUFAs in breast cancer cell lines These results suggest that DHEA maybe is metabolised differently from EPA, DHA and EPEA or it that the anti-tumour effect of DHEA is due to a metabolite of DHEA when it is metabolised by FAAH. In addition as th e effect of PUFAs were decreased by the addition of CB ½ inhibitors, this suggests that anti-tumour effects of PUFAs work via CB1/2 receptors in breast cancer cell lines. These results indicate the role of cannabinoid receptors and FAAH in the anti-tumour effects of PUFAs in breast cancer.

POSTER 59

RESOLVIN D1, DERIVED FROM OMEGA-3 POLYUNSATURATED FATTY ACID, STIMULATES EFFEROCYTOSIS THROUGH p50/p50-MEDIATED SUPPRESSION OF TUMOR NECROSIS FACTOR-α

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Abstract: Phagocytosis of apoptotic neutrophils by macrophages, called efferocytosis, is critical to resolution of inflammation as this process prevents the exposure of surrounding tissues at the inflamed site to the toxic contents of lytic cells. Docosahexaenoic acid (DHA)-derived resolvin D1 (RvD1), endogenously generated during resolution of inflammation, is known to stimulate efferocytosis, but little is known about the mechanism of RvD1-mediated enhancement of efferocytosis. In the present study, we found that murine macrophage-like RAW264.7 cells treated with lipopolysaccharide (LPS) exhibited markedly decreased efferocytic activity. The addition of RvD1 restored the efferocytic ability of the cells, which appears to be mediated by down-regulating the LPS-induced upregulation of TNF-α. The inhibitory effect of RvD1 on LPS-induced TNF-α expression was associated with enhanced nuclear localization of p50/p50 homodimer and concomitant reduction of p65/p50 heterod imer in the nucleus. RvD1 triggered extracellular signal-regulated kinase (ERK)-mediated degradation of nuclear factor κB1 (NF-κB1) p105 to generate p50, which was subsequently translocated to the nucleus as p50/p50 homodimer. Knockdown of NF-κB p50 abolished RvD1-mediated suppression of TNF-α expression and restoration of efferocytosis. These findings suggest that the replacement of p65/p50 with p50/p50 homodimer in the nucleus is an essential event for RvD1-mediated stimulation of efferocytosis. In a murine peritonitis model, intraperitoneal administration of RvD1 abrogated the zymosan A-induced TNF-α production, thereby reactivating efferocytosis. In conclusion, RvD1 expedites the resolution of inflammation through induction of efferocytosis by p50/p50 homodimer-mediated repression of TNF-α.

POSTER 60

THE INCREASED EXPRESSION OF CYCLOOXYGENASE 2 INVOLVES PRODUCTION OF SPHINGOSINE-1-PHOSPHATE AND ITS RELEASE BY ATP-BINDING CASSETTE ABCC1 IN LATE RAT PREGNANT MYOMETRIUM AND UTERINE LEIOMYOMA CELLS

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Abstract: Cyclooxygenase (COX)2/prostaglandin axis plays a critical role in the physiology and pathophysiology of the uterus. We analyzed the mechanism involved in the production of prostaglandins. In late pregnant (day 19) rat myometrium, sphingosine kinase1 (SphK1) activity is upregulated and is associated with the increased expression of COX2. In rat uterine leiomyoma cells (ELT3), SphK1/Sphingosine-1-phosphate (S1P) axis controls cell survival and proliferation. The expression of COX2 is increased by exogenous S1P. In myometrium and in leiomyoma cells the activation of SphK1 activation requires the stimulation of PKC and ERK1/2. In addition, the activated SphK1 produces S1P that is released in the medium. S1P export is abolished by PKC inhibitors (Ro-318220 and BIM), MEK inhibitors (U0126 and PD98059). The S1P release is due to the activation of SphK1 because S1P export is inhibited by SKI-II (a SphK1/2 inhibitor) and SphK1-siRN. The release of S1P is insensitive to inhibitors of ATP Binding Cassette (ABC) A1 and ABCB1 transporters, but is abolished when ABCC1 transporters are inhibited by MK571 or down-regulated by ABCC1-siRNA. The increase of COX2 expression is abolished by the inhibition of PKC, ERK1/2, SphK1, and when cells are treated with MK571 or transfected with ABCC1-siRNA. The induction of COX2 by the S1P release due to SphK1 activation in the presence of PDBu or by exogenous S1P, involves S1P2 receptors coupled to Gi. The induction of COX2 is accompanied by the increase of PGE2 in the medium. Data show that the increase of COX2 expression and subsequent prostaglandin production is regulated by SphK1/S1P axis. This cross-regulation between two lipid bioactive mediators plays a pivotal role in the physiology of parturition as well as in leiomyoma growth.

POSTER 61

PPAR-gamma AND –delta ARE IMPLICATED IN PGE2 RELEASE AND PERILIPIN2 (PLIN2) EXPRESSION INDUCED BY A SNAKE VENOM PLA2 IN MACROPHAGES

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Abstract: MT-III, a secreted phospholipase A2 (sPLA2) isolated from snake venom, up-regulates COX-2 and PLIN2 protein expression, which are involved in arachidonic acid metabolism and uptake of fatty acids, respectively. Peroxisome proliferator-activated receptors (PPAR-gamma and -delta) are transcription factors with key roles in lipid metabolism and inflammation. However, the involvement these receptors in MT-III-induced effects are unknown. This study aimed to evaluate the effects of MT-III on PPAR-gamma and -delta expression and activation in murine macrophages and the role of these receptors in MT-III–induced COX-2 and PLIN2 protein expression and PGE2 release. Thioglycolate-elicited macrophages from male Swiss mice (Butantan Institute Ethical Committee 744/10) were incubated with MT-III (0.4 μM) or culture medium (control) from 1 up to 24 h. Protein expression of PPAR-gamma and –delta and COX-2 was determined by Western blotting and PPARs activation by immunofluorescence assays. Participation of PPAR-gamma and -delta in PLIN2 and COX-2 protein expression and PGE2 release was evaluated by pharmacological intervention. PGE2 concentration was quantified by EIA. Incubation of macrophages with MT-III significantly increased protein expression of PPARgamma (45% increase) and -delta (74% increase) from 1 h up to 24 h compared with control (0.36 ± 0.06 AU PPAR-gamma or -delta/Beta-actin). Moreover, MT-III activated both PPAR-gamma and - delta after 3 h incubation. Pretreatment of cells with GSK660, a PPAR-delta antagonist, but not with GW9662, a PPAR-gamma antagonist, abrogated MT-III-induced PLIN2 expression, reduced PGE2 release (16.67%), but did not modify COX-2 protein expression. This is the first demonstration that a sPLA2 is able to up-regulate expression and activity of the transcription factors PPAR-gamma and - delta in macrophages. Moreover, PPAR-delta, but not PPAR-gamma, is involved in the venom PLA2- induced PLIN2 expression and PGE2 release.

Support: FAPESP, CNPq, INCTOX

POSTER 62

PRODUCTION AND SECRETION OF sPLA2-IIA FROM PNEUMONOCYTES TYPE II AFTER STIMULATION WITH LPS

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Abstract: Phospholipases A2 (PLA2s) catalyse the hydrolysis of ester bonds at the sn-2 position of glycerophospholipids producing free fatty acids and lysophospholipids. Locally in the lung they can disrupt lung surfactant, a lipoprotein that lines lungs and reduces the surface tension at the air-liquid interface of the alveoli. Surfactant deficiency is associated with serious lung disorders, such as acute lung injury/ARDS. The aim of the study was to investigate sPLA2-IIA production and secretion pathway from alveolar type II (AT-II) cells under LPS treatment.

A549 cells, a model of AT-II cells, were treated with LPS in dose- and time-dependent experiments to induce sPLA2-IIA production and secretion. Intracellular and extracellular sPLA2-IIA levels were determined by western blotting. The localization and secretion of the enzyme was tracked by confocal laser scanning microscopy

Our results indicate that LPS induces sPLA2-IIA expression and secretion from A549 cells: For LPS concentrations greater than 1 μg/mL, sPLA2- IIA was detected intra- and extracellularly. Interestingly, in the extracellular medium, the enzyme was detected at even lower LPS concentrations, 0.4 μg/mL, even after 1 min-incubation. Moreover, the blots revealed increasing sPLA2-IIA concentrations in both the cells and cell supernatants for concentrations greater than 1 and 0.4 μg LPS /mL, respectively, as a function of time. The delay in the appearance of the enzyme intracellularly could in part be attributed to the lower sensitivity of western blotting technique compared to the more sensitive fluorescence microscopy.

After successful preparation of the fusion protein sPLA2-IIA–GFP and the high efficiency transfection it was shown that secretion of the endogenous enzyme, induced by LPS, followed the typical biosynthetic route through the ER and Golgi apparatus. In addition, it was accumulated in cytoplasmic vesicles close to the cell periphery.

Under overexpression of sPLA2-IIA–GFP, high amounts of the enzyme were colocalized with ER and Golgi markers, but it was also secreted from the cells and observed, in non-permeabilized cells, at the external site of the plasma membrane of A549 cells.

Finally, our results cannot exclude the existence of two different pools of sPLA2-IIA, one pre-existing (constitutive) and one newly formed, induced by LPS.

POSTER 63

NON-ESTERIFIED FATTY ACIDS INCREASE INTRACELLULAR CALCIUM ON BOVINE UMBILICAL VEIN ENDOTHELIAL CELLS (BUVEC)

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Abstract: Growing evidence shows an association between the increase of nonesterified fatty acids (NEFAs) levels and an augmented incidence of inflammatory diseases such as mastitis in cattles. It has been shown that certain NEFAs are able to activate the endothelium, which participates actively in inflammatory response. However, the modulator role of NEFAs on intracellular calcium in BUVECs is until now unknown.

For this reason, the effect of NEFAs on calcium fluxes in BUVECs was studied. The changes elicited by saturated NEFAs (myristic (C14:O), stearic (C16:O), palmitic (C18:O) and unsaturated NEFAs (oleic (C18:1) and linoleic (C18:2)) were measured on the intracellular calcium (Cai+2) using the dye fluorescent Fura-2 AM. Time courses of Cai+2 in suspension cells were registered using a spectrofluorometer. The contribution of Cai+2 release or calcium influx was evaluated using BAPTA AM and EGTA respectively. Likewise, we assessed the expression of GPR40, a putative receptor associated to medium and long chain fatty acids, by western blot and immunofluorescence. Finally, we evaluated the effect of GW1100, a well - known GPR40 antagonist, on Cai+2.

We observed that myristic, stearic and palmitic acid caused a fast and sustained Cai+2 increase. On the contrary, oleic and linoleic acids induced a slower Cai+2 increase. The myristic acid response was reduced partially in BAPTA AM or GW1100 pre-incubated cells, and was abolished with EGTA. On the other hand, the Cai+2 increase induced by linoleic acid was abolished by BAPTA AM and EGTA, only partially by GW1100 a well-known GPR40 antagonist.

Additionally, we demonstrated the presence of bGPR40 in BUVEC by western blot and immunofluorescence, the observed molecular weight was similar to the human isoform detected in LOVO cells.

All results suggest that unsaturated NEFAs increase the Cai+2 from intracellular stores mediated partially through GPR40.

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POSTER 64

QUANTUM MECHANICAL STUDY OF THE LIPID DECOMPOSITION IN PRESENCE OF LOW-MOLECULAR WEIGHT ORGANIC COMPOUNDS

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Abstract: Decomposition of lipids is a key process of metabolism of living matter also lipids serve for signal transduction in the range of biological signal systems and they can be involved in inflammation and in several diseases. Understanding of biochemistry and of molecular mechanisms of lipid decomposition gives deeper insight into realization of the natural organism functioning and serves as a base of development of more effective some up-to-day biotechnological applications. Hydrolytic enzymes provide hydrolytic cleavage both ester and peptide bonds and are a useful catalysts of biotechnological hydrolysis and synthesis of wide range compounds. It is well known that some lipids and peptides are hardly water-soluble so replacement of water surrounding a protein by organic solvents can be a solution of this problem. Moreover in such non-natural environment of proteins preserve their structure and activity and also show unique enantio- and regioselectivity. At the same time organic compounds regulate catalytic rate constants and correct choice of the solvent can improve efficiency of the process.

Here we report comparative study of nonenzymatic hydrolysis of both ester and peptide bonds in presence of organic solvents using methods of quantum chemistry. Understanding of nonenzymatic degradation of lipids in solution is necessary to evaluate the power of enzymes. Computational investigation of catalytic systems is a useful tool for study of the reaction pathway: free energy profile, structures of activated complexes and intermediates and allows one to conclude favorable mechanism of the reaction. We compare four mechanisms of nonenzymatic cleavage – concerted and step-wise ones that are catalyzed or not by low-molecular-weight organic compounds (molecules of alcohols, nitriles, sulfoxydes and carbon acids). We performed analysis of the potential energy profile of the hydrolytic reaction and evaluated activation energies and rate constants of all stages of reaction and the alternation on these parameters in dependence on organic compound nature. Data reveal that organic compound-assistant step-wise mechanism is more favorable than concerted one for nonenzymatic hydrolysis in solution. Calculated rate constants are in good agreement with available experimental data.

POSTER 65

EVALUATION OF DIFFERENT FISH PRODUCTS AS A SOURCE OF ESSENTIAL PUFA, AND BENEFIT-RISK RATIO OF FISH INTAKE IN HUMAN NUTRITION.

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Abstract: We analyzed fatty acid content of food fish under different ways of cooking. Boiled humpback salmon and trout appeared to be more valuable fish dishes for obtaining the officially recommended appropriate daily intake of eicosapentaenoic acid + docosahexaenoic acid, the essential fatty acids (EFA) for humans. Herring and sole had intermediate values, while boiled cod had a comparatively low value. Contents of EFA in all studied canned fish were very high. The canned fish appeared to be highly valuable products for human nutrition. However consumption of fish provides not only benefits for human health due to its high contents of EFA, but harm due to containing toxic organic compounds and heavy metals. We derived a formula for quantification of benefit-risk ratio (hazard quotient) for the intake of a product containing EFA vs. heavy metals. The quotient was used in a case study of the contents of EFA and heavy metals in Siberian grayling (Thymallus arcticus). As found, in general the fish intake was potentially very beneficial for human health, except on a few occasions, when the risk overweighed the benefit. The data demonstrated the necessity for regular monitoring of the hazard quotients for food fish in wild conditions.

POSTER 66

FLEXIBILITY ENHANCES SUBSTRATE SPECIFICITY AND CATALYSIS IN COX-2

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Abstract: The cyclooxygenases (COX-1 and COX-2) are homodimers that catalyze the committed step in the biosynthesis of prostaglandins. Each monomer of the homodimer houses two separate, but functionally linked active sites, which generate the product prostaglandin H2 from the polyunsaturated fatty acid substrate arachidonic acid (AA) in sequential reactions. Both COX-1 and COX-2 oxygenate AA and other fatty acid substrates via a half-of-sites reactivity mechanism, such that at any given time, only one monomer of the homodimer is functional. Although COX- 1 and COX-2 share a similar three-dimensional fold and the catalytic mechanism is conserved between isoforms, understanding the similarities and differences associated with COX-1 and COX-2 and the rationale for the existence of two isoforms has been the focus of much recent research. While AA is the preferred substrate for both isoforms, COX-2 can oxygenate a broad spectrum of substrates, which include an extensive array of de rivatives of AA such as the endocannabinoids arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG). These substrates are larger than AA due to the additional chemical constituent attached to the carboxylate group of the fatty acid. We utilized X-ray crystallographic methods and functional analyses of mutant COX-2 constructs to characterize the nuances involved in the binding of fatty acid and endocannabinoid substrates within the cyclooxygenase channel of COX-2 and the molecular determinants that govern isoform specificity. Fatty acid substrates bind in different conformations in each monomer constituting the homodimer in their respective structures, consistent with half-of-sites activity. Leu-531, located near the opening of the channel, exhibits a different side chain conformation when the non-productive and productive binding modes are compared. The different conformations observed for the Leu-531 side chain results in an increase in the volume available at the opening of the cyclooxygenase channel. Overall, our structure-function studies provide the first molecular insight into the productive binding of different fatty acid and endocannabinoid substrates to COX-2 and clarify the roles that Arg-120, Arg-513, Leu-531, and Gly-533 play in substrate binding and specificity.

POSTER 67

LINOLEIC ACID STIMULATES THE RELEASE OF MMP-9 AND CD11b EXPRESSION VIA GPR40 RECEPTOR IN BOVINE NEUTROPHILS

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Abstract: GPR40 (FFA1) is a G-protein coupled receptor which is mainly expressed in the brain and pancreas. This receptor is activated by medium and long chain saturated and unsaturated free fatty acids. Recent studies have shown that mRNA and the protein of GRP40 are expressed in bovine neutrophils. Moreover, it has been demonstrated that oleic acid, a long chain monounsaturated free fatty acid, activates responses in the bovine neutrophils such as release of MMP-9 and expression of CD11b in the cell membrane. The mechanisms involved in the activation of these responses stimulated by fatty acids in the bovine neutrophils are not yet clear. The goal of this work was to demonstrate that the increase in the release of MMP-9 and expression of CD11b by linoleic acid in bovine neutrophils is, in part, controlled by GPR40 and subsequent activation of phospholipase C (PLC).

To evaluate whether activation of GPR40-PLC increased the release of MMP-9 and expression of CD11b, bovine neutrophils were stimulated with linoleic acid and GW9508, an agonist of GPR40. In another set of experiments, bovine neutrophils were incubated with GW1100, an antagonist of GPR40, and U73122, an inhibitor of PLC. To evaluate CD11b expression on membrane, the bovine neutrophils were incubated with anti-bovine CD11b and Alexa-488 secondary antibodies, and analyzed by flow cytometry. To study MMP-9 release, the supernatant of bovine neutrophils were analyzed by zymography.

GW9508 (10 μM) and 10 μM linoleic acid stimulated the CD11b expression and MMP-9 release in bovine neutrophils, at 5 min of treatment. These responses decreased significantly when bovine neutrophils were incubated with 10 μM of the antagonist of GPR40 GW1100 before stimulation with linoleic acid or GW9508. Furthermore, CD11b expression and MMP-9 release decreased when bovine neutrophils were incubated with the inhibitor of PLC U73122 and then stimulated with linoleic acid or GW9508.

These results suggest that the release of MMP-9 and CD11b expression in bovine neutrophils stimulated with linoleic acid is dependent of GPR40 and PLC.

POSTER 68

ANTI-INFLAMMATORY LIPID MEDIATOR 15d-PGJ2 MODULATES HUMAN ENDOTHELIAL CELLS ACTIVATION THROUGH INHIBITION OF 26S PROTEASOME FUNCTIONS

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Abstract: The expression of cell adhesion molecules on human endothelial cells (EC) is increased under inflammatory conditions, facilitating the infiltration of monocytes, a critical step in the pathogenesis of atherosclerosis. This may be regulated by 15-deoxy-delta 12, 14 prostaglandin J2 (15d-PGJ2) formed at site of inflammation in the atherosclerotic plaque. 15d-PGJ2 is an inhibitor of proinflammatory gene-expression, although the signaling mechanisms involved are unclear. One potential target is the proteasome system, which has been implicated in the pathogenesis of atherosclerosis.

Here we explored the anti-inflammatory effect of 15d-PGJ2 on EC expression of adhesion molecules VCAM-1, ICAM-1, e-selectin and of chemokines MCP-1, MCP-4 and IL-8. 15d-PGJ2 5μM significantly decreased mRNA expression of VCAM-1 (82% ± 7%, $p<0.001$), ICAM-1 (57% ± 3%, $p<0.001$) and E-selectin (78% ± 5%, $p<0.001$) on activated EC as well as MCP-1 (69% ± 5%, $p<0.001$), MCP-4 (42% ± 4%, $p<0.01$) and IL-8 (22% ± 2%, $p<0.01$) compared to control. The reduction in adhesion molecules and chemokines expression was accompanied by inhibition of adhesion of fluorescein-labelled monocytes to EC (89% ± 2%, $p<0.001$) and by decrease of monocyte migration (79% ±4%, $p<0.01$).

It is known that 15d-PGJ2 can covalently modify proteins, profoundly affecting their biological function. To identify potential targets of 15d- PGJ2, we incubated EC with biotinylated-15d-PGJ2. Modified proteins were bound to neutravidin beads and analyzed by SDS-PAGE, followed by LC-MS/MS. Bioinformatics pathway analysis of the 140 identified proteins showed the ubiquitin-proteasome-system to be disproportionally enriched (pvalue = 3.68E-07). 13 of these 15d-PGJ2 modified proteins were found in the 19S-regulatory-particle of the proteasome, a large protein complex involved in the degradation of cellular proteins and processing of inflammatory proteins. Moreover, 15d-PGJ2 reduced proteasome activity, assayed using a fluorogenic substrate (43% ± 4%, $p<0.01$). The reduction in proteasome activity was accompanied by inhibition of the activation of NFkappaB, a transcription-factor involved in the expression of proinflammatory genes. This was due to the suppression of degradation o f NF-kappaB-inhibitors (IkB-alpha and p105), and inhibition of the nuclear translocation of NF-kappaB.

Our findings indicate that 15d-PGJ2 suppresses the inflammatory phenotype of EC, possibly as 15d-PGJ2 covalently modifies proteasome proteins, acting as a proteasome inhibitor and blocking NF-kappaB transcriptional activity.

POSTER 69

EVALUATION OF RAT LIPID PARAMETERS AND OXIDATIVE DAMAGE BIOMARKERS TREATED BY DIBENZO SULFIDE (TTD) AND DIBENZO SULFOXIDE (TSD) MACROCYCLIC DIAMIDES

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Abstract: Two Tri-Aza macrocyclic as diamide derivatives of macrocyclic compounds including dibenzo sulfide (TTD) and dibenzo sulfoxide (TSD) pose the ability to transfer across membranes and interfere with different living systems without harming healthy cells. In this study we injected inter-peritonaly each compounds in range of 5 to 160 μMol to rat. After three weeks, the levels of cholesterol and LDL decreased 28% and 17% respectively at 40 μMol TTD. In this condition, triglyceride and HDL did not varied significantly with respect to control. Treatment with TSD at 80 μMol decreased triglyceride (14%) and elevated HDL (20%) without significant alterations in cholesterol and LDL levels in comparison with control treatment. The activity of Superoxide dismutase elevated 2.2 folds of control levels in treatment only with 80 μMol TTD, and there was no considerable variation in catalase activity.

Treatment with TSD at 80 μMol decreased triglyceride (14%) and elevated HDL (19%) without significant alterations in cholesterol and LDL levels in comparison with control treatment. There were significant decrease in malondialdehyd as lipid oxidative damage biomarker for TSD (34%) and TTD (22%) treatment with respect to control. There wasn\'t significant chanson dityrosine as protein oxidative damage biomarcker in the presence of each compound.

On the other hand, higher concentrations (160 μMol) of both compounds caused markedly elevation in MDA and cholesterol. As conclusion, administration of both compounds at ordinary levels can protect health by acting against atherosclerotic lipids metabolism.

Key words: atherosclerotic lipids, macrocyclic diamides, antioxidant enzymes, malondialdehyde.

POSTER 70

LYSOPHOSPHOLIPIDS LEVELS ARE CORRELATED WITH INFLAMMATION IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

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Abstract: Inflammatory infiltration has been recently emphasized in the demyelinating diseases of the central nervous system including multiple sclerosis. Experimental autoimmune encephalomyelitis (EAE), also called experimental allergic encephalomyelitis, is a commonly used experimental model for multiple sclerosis. EAE is an acute or chronic-relapsing, acquired, inflammatory and demyelinating autoimmune disease. We are interested in understanding the involvement of bioactive lipids in EAE pathophysiology, to identify bioactive lipids-related therapeutic targets. One approach is to study and correlate bioactive lipids variations, measured by HPLC-MS, with inflammatory markers.

In this preliminary study, EAE was induced in mice by rMOG35-55 administration in complete Freund's adjuvent. Mice clinical score was graded on a 0 to 5 scale. When comparing sulfatides levels in control mice and clinical score 4 mice, we found decreased levels for d18:1/22:0-sulfatide and d18:1/24:0-sulfatide, as previously reported by Marbois (1). We also found increased mRNA expression for several inflammatory markers (e.g. TLR4, ATF3, MIP1a). When comparing these markers with the sulfatides levels, we found a negative correlation between d18:1/22:0-sulfatide and both TLR4 ($r=-0.830$, $p=0.002$) and MIP1alpha ($r=-0.781$, $p=0.007$) and between d18:1/24:0-sulfatide and both TLR4 ($r=-0.781$, $p=0.007$) and MIP1a ($r=-0.769$, $p=0.009$).

Because PLA2 is involved in the EAE pathology, we next studied the correlations between PLA2 metabolites and ATF3 (a transcription factor that plays a regulatory role in inflammation).

We found positive correlations for some lysophospholipids and fatty acids: 16:0-lysophosphatidylinositol $r=0.769$, $p=0.009$; 16:0-lysophosphatidylethanolamine $r=0.903$, $p=0.0003$; docosahexaenoic acid $r=0.830$, $p=0.002$ and arachidonic acid $r=0.781$, $p=0.007$. Note that several other lysophospholipids levels were not correlated with ATF3: 16:0-lysophosphatidylserine $r=0.284$, $p=0.425$ and 16:0-lysophosphatidylglycerol $r=0.478$, $p=0.161$.

In conclusion, these correlations between inflammatory markers and lipids mediators, may implicate these lysophospholipids in EAE pathophysiology.

1 Marbois et al. Biochim. Biophys. Acta, 2000, 1484, 59

POSTER 71

REGULATION OF ENDOTHELIAL PROSTACYCLIN BIOSYNTHESIS BY A SNAKE VENOM SECRETORY PHOSPHOLIPASE A2: SIGNALING PATHWAYS

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Abstract: Prostacyclin (PGI2) is a member of the prostaglandin family and main lipid mediator released by endothelial cells (ECs). As with other prostaglandins, PGI2 is produced in vascular ECs in response to physiological/pathological stimuli by sequential enzymatic reactions initiated by phospholipases A2 (PLA2). The main toxin of Crotalus durissus terrificus snake venom is a secretory type IIA PLA2 (CB) that exerts neurotoxic, myotoxic and anti-inflammatory effects. PLA2 enzymes act on membrane phospholipids releasing AA, which is subsequently converted into prostanoids by an enzymatic cascade, with tissue-specific synthases as terminal components. Several reports have shown that both NF-κB and MAPK family are key signaling molecules of agonist-induced PGI2 biosynthesis. Therefore, the current study examined the signaling pathways involved in biosynthesis of prostacyclin induced by CB. To this purpose, rat primary microvascular ECs in culture were used. These cells we re stimulated by CB (0.4 μM) or culture medium (control) for 6 hours and then used for parameter analysis. Results showed that incubation of ECs with non-toxic concentration of CB (0.4 μM) induced a significant increase (221%) of prostacyclin levels after 6 hours in comparison with controls. Pre-treatment of ECs with VSA or PD98059 or U0126 compounds or tranylcypromine (COX-1, ERK1/2, MEK1/2 and PGIS inhibitors, respectively) abrogated the release of PGI2 induced by CB. In contrast, compounds NS- 398, SN50, JNK inhibitor II or SB202190 (COX-2, NF-kB, JNK and p38MAPK inhibitors, respectively) did not alter this CB-induced effect. In conclusion, these data indicate that COX-1, PGIS, ERK1/2 and MEK1/2, but not COX-2, NF-kB, JNK and p38MAPK, regulate biosynthesis of prostacyclin induced by CB in ECs. These findings indicate novel regulatory mechanism for snake venom secretory type IIA PLA2 in vascular endothelium.

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POSTER 72

LIPIN-1 IS ESSENTIAL FOR THE INFLAMMATORY RESPONSE OF MURINE MACROPHAGES TO BACTERIAL LIPOPOLYSACCHARIDE

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Abstract: Lipin-1 is a member of the phosphatidic acid phosphatases family of enzymes (PAP-1), which dephosphorylates phosphatidic acid (PA) to generate diacylglycerol (DAG). Mice lacking Lipin1, named fld (fatty liver dystrophy) are known to exhibit changes in lipid storage but up to date, there are no studies about lipid alterations in the macrophages of these animals. TLR4-stimulation of bone marrow derived macrophages from wt mice resulted in an increase of the total content of DAG at 5 min, reaching a maximum at 20 min, however in fld cells DAG levels were significantly lower. Despite this difference, the fatty acid composition of DAG for both groups was the same, with saturated fatty acids like myristic (14:0), palmitic (16:0) and stearic (18:0) being the major constituents. After exposure to LPS, PA levels increased in wt cells reaching the plateau at 10 min, but this was decreased in fld cells. In both cases, there were no differences in the PA profile. The following PA species were found by using LC/MS: PA(16:0/18:2), PA(16:0/18:1), PA(16:0/18:0), PA(O-18:0/18:2), PA(O-18:0/18:1), PA(O-18:0/18:0), PA (18:0/18:1) and PA (18:0/18:0). Because both DAG and PA have essential signaling roles in macrophage function, we studied the signaling consequences of the lack of lipin-1 during LPS stimulation. Activation of the ERKs p42/p44 and JNK was diminished in fld macrophages, as also was the activation of the transcription factors NFκB and AP-1. We also found differences in the relative mRNA expression of some important cytokines such as Il6, Il12p40 and proinflammatory enzymes such as Nos2 and Cox2. All together, these results suggest a proinflammatory role for lipin-1 that is due to the generation of signaling lipids.

POSTER 73

SUPERCRITICAL FLUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY FOR LIPIDOMIC ANALYSIS

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Abstract: Nowadays, it is necessary to tend towards analytical technics with a low environmental impact. Supercritical fluid chromatography or SFC is a 'green' technique using CO2 in its supercritical state as the main eluting solvent. CO2 is non-flammable, miscible with common organic solvents and involving a low toxicity towards environment compared to normal-phase solvents.

In terms of system performance, supercritical CO2 has a low-viscosity and a high diffusivity, which lead to shortened equilibration and analysis times if compared with classical liquid chromatography (HPLC).

The analyses are performed on a new commercial SFC system from Agilent Technologies including an HPLC Agilent 1260 equipped with an « Aurora Fusion » module, generating CO2 in its supercritical state, and an UV visible detector.

The SFC system will be coupled to a hybrid quadrupole - time of flight (QTOF) mass spectrometer, allowing high mass accuracy (below 1 ppm) and high mass resolution (higher than 30 000). Three different sources will be tested: atmospheric pressure photoionization (APPI), atmospheric pressure chemical ionization (APCI), and electrospray ionization (ESI).

The first developments with SFC-UV detection were realized with molecules from vitamin A and E families, which are highly soluble in organic solvents. The aim of this work is mainly to compare the performance of each ion source for the analysis of lipids, which represent a great interest for the elucidation of biological mechanisms of some diseases. Each ion source will be compared in terms of sensitivity, repeatability and linearity. These results will be extended to other class of lipids such as phospholipids, sphingolipids, sterols or triglycerides, which are not detected by UV, and for which mass spectrometry appears to be a more adequate detection system.

POSTER 74

EFFECT OF A MODE HYPERGRAS ON THE COMPOSITION IN LIPIDS OF THE BODIES AT THE RAT WISTAR DURING GESTATION AND LACTATION (LIVER, FAT FABRIC, MUSCLE, INTESTINE, BRAIN AND HEART)

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Abstract: The objective of this work is to determine the effects of the mode cafeteria (hyperlipidic and hypercaloric) on the metabolism of the lipids in rats during pregnancy and lactation.(1) the study was undertaken on rats of the wistar type. The rats pregnant were divided into two great groups, a pilot batch and an experimental batch which consumes the mode cafeteria. Samples taken on tube EDTA are used to proportion glucose, triglycerides, cholesterol. Aliquot parts of bodies were preserved for lipidic, proteinic proportionings and determination of their composition in fatty-acids.In addition, the increase in the serum total cholesterol and triglyceride rates is related to an increase in synthesis and secretion of the lipoproteins(2).Our results show that during the period of gestation-breast feeding, the polyinsaturés fatty-acids of the series omega-6 behave in vitro like powerful agents "adipogenic" and in vivo like factors supporting the development of fat fabric. For the periods of gestation and breast feeding, there exists an effect-amount between the quantities of omega-3 in the food and the accumulation of these compounds in the brain, until the optimum is reached.

POSTER 75

PLASMA OMEGA3 FATTY ACIDS AND THE RISK FOR AGE-RELATED MACULAR DEGENERATION IN FRENCH ELDERLY SUBJECTS: THE ALIENOR STUDY

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Abstract: Objective High dietary intakes of omega3 polyunsaturated fatty acids (PUFA) and fish have been consistently associated with a decreased risk for age-related macular degeneration (AMD), a degenerative disease of the retina, responsible of half of the cases of blindness in industrialized countries. We aimed at assessing the associations of AMD with plasma omega3 PUFA, which represent a more objective assessment of omega3 PUFA status.

Design Population-based cohort study in residents of Bordeaux (France). Participants 963 adults (≥ 73 years), included in the Alienor cohort in 2006-2008 and followed for 31 months on average.

Methods Associations of AMD with plasma omega, adjusted for potential confounders, were estimated using Generalized Estimating Equation (GEE) logistic regressions.

Main Outcome Measures AMD (neovascular AMD and/or geographic atrophy) was graded according to the international classification, from non mydriatic colour retinal photographs at all examinations, and spectral domain optical coherence tomography at follow-up. Plasma fatty acids were measured by gas chromatography from fasting blood samples collected in 1999-2001. Persons implicated in AMD classification had no access to plasma fatty acid data, which were assessed several years before.

Results Of 963 subjects, 84 (8.7%) had no gradable eye examinations, 167 (17.3%) had missing data for plasma fatty acids and 107 (11.1%) for confounders, leaving 605 subjects (1170 gradable eyes) for the statistical analysis, including 64 subjects with AMD (96 eyes). After adjustment for age, follow-up time, gender, smoking, education, physical activity, plasma HDL-cholesterol, plasma triglycerides, CFH Y402H, ARMS2 A69S and apoE4 polymorphisms, high plasma total omega3 PUFA was associated with a reduced risk for late AMD (OR=0.62 for 1-standard deviation increase, 95% confidence interval (CI): 0.44-0.88, p=0.008). Associations were similar for plasma alpha-linolenic acid (OR=0.62, 95% CI: 0.43-0.88, p=0.008) and omega3 long-chain PUFA (OR=0.65, 95% CI: 0.46-0.92, p=0.01).

Conclusions This study gives further support to the potential role of omega3 PUFA in the prevention of AMD and highlights the necessity of randomized clinical trials to determine more accurately the value of omega3 PUFA as a means of reducing AMD incidence.

POSTER 76

B1-PHYTOPROSTANES PROTECT IMMATURE NEURONS BUT NOT OLIGODENDROCYTES FROM OXIDANT INJURY

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Abstract: Phytoprostanes (PhytoPs) are formed in higher plants from alinolenic acid (ALA), via a non-enzymatic free radical catalyzed pathway analogous to that leading to isoprostane formation in animal cells (Durand et al., 2009). The development of a new chemical strategy, based on a furan approach has led to the synthesis of enantiomerically pure B1- PhytoP, F1-PhytoP and E1-PhytoP (El Fangour et al., 2004; Pinot et al., 2008) thus allowing to fully assess the physiological activities of each of these compounds.

Experimental evidence strongly suggests that in plants B1-PhytoP acts as an endogenous mediator capable of protecting cells from damage under various conditions, particularly those related to oxidative stress. Since human are potentially exposed to PhytoPs, which can be absorbed after oral ingestion of vegetable food or by inhalation of pollen, it is important to identify their potential activities in animal cells (Durand, et al. 2011). On this basis we investigated the possible effect of B1-PhytoP on cells of the central nervous system, which are particularly susceptible to oxidative stress. In a human neuronal model (neuroblastoma SH-S5Y5 cell line) B1-PhytoP increased mitochondrial activity of the cells (as indicated by the increased ability of SH-SY5Y cells to reduce MTT) and significantly protected them from death caused by H2O2. When cells were induced to differentiate towards a more mature phenotype, they became resistant to B1-PhytoP activities. Interestingly B1-Phyto P facilitated the process of differentiation. In primary culture of oligodendrocyte progenitors, B1-PhytoP increased the mitochondrial activity but it did not protect cells from H2O2 damage. In addition, B1- PhytoP strongly accelerates the differentiation of the progenitors to immature oligodendrocytes. These data suggest that B1-PhytoP can beneficial effects on cells of the central nervous system and in particularly on neuronal cells not yet differentiated, which are found in the adult brain in the so-called neurogenic niche.

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POSTER 77

EFFECT OF LEUKOTRIENES IN IMMUNOPATHOGENESIS OF RHEUMATOID ARTHRITIS

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Abstract: Rheumatoid arthritis (RA) is a chronic inflammatory disorder of joints that there is no any strict cure for it, however conventional medications are able to reduce inflammatory reactions, relieve the pain and slow joint damage. Leukotrienes are a family of paracrine agents derived from the oxidative metabolism of arachidonic acid. Lipid mediators synthesis and subsequent induction of receptor activity are tightly regulated under normal physiological conditions, so that, enzyme and/or receptor dysfunction can lead to a variety of disease clinical signs and symptoms, such as local pain and tissue edema. In these tissues, immunocompetent cells accumulate at the site of injury, which contribute to tissue damage and perpetuation of the disease process. Leukotrienes (often leukotriene B4) as a potent chemotactic agent can provoke most of signs and symptoms in rheumatoid arthritis by initiation, coordination, sustain and amplifying the inflammatory response, through recruitment of leukocytes. A number of evidences reported that the pharmacological modulation in this field can significantly attenuate the clinical manifestations associated with different inflammatory pathologies and could be an appropriate target for further investigations.

POSTER 78

ENHANCED LIPID PEROXIDATION AND INFLAMMATION DURING HEAT EXPOSURE IN RATS OF DIFFERENT AGES: ROLE OF α-TOCOPHEROL

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Abstract: To investigate early events possibly related to the development of heat shock, we examined whether inflammatory and peroxidative markers are altered during acute heat exposure and aging. We also studied the relationship between inflammatory-(IL-6, TNF-α and 15-keto-13,14-dihydro-PGF2α and peroxidative-(8-iso-PGF2α and MDA) markers in this setting. In order to prevent these reactions developed as a consequence of conditions mentioned above, we tested the effects of α-tocopherol.

Methods: The levels of IL-6, TNF-α and 15-keto-13,14-dihydro-PGF2α in the plasma and 8-iso-PGF2α and 15-keto-13,14-dihydro-PGF2 α in the liver were analyzed by a newly developed ELISA for quantitative analysis. The lipid peroxides were estimated in the plasma and liver homogenates using a modified thiobarbituric acid test.

Our results demonstrated that the liver 15-K-DH-prostaglandin F2α and malondialdehyde, were altered during acute heat exposure in young and aged rats and could be predicted by changes in the levels of circulating cytokines. Regardless of age, α-tocopherol induced prevention of the plasma cytokine, liver 15-K-DH-prostaglandin Fα and liver malondialdehyde rising during acute heat exposure.

This study notably emphasized the ability of α-tocopherol to prevent different heat induced mechanisms, involved in induction of inflammatory or peroxidative reaction.

POSTER 79

LIPID LOWERING EFFECT OF PIPER SARMENTOSUM WATER EXTRACT ON OVARIECTOMY-INDUCED OBESE RATS

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Abstract: Introduction: Cholesterol is a waxy steroid metabolite found in the cell membranes and transported in the blood plasma. The synthesis and utilization of cholesterol are tightly regulated in order to prevent over-accumulation and abnormal deposition within the body. This present study was conducted to characterize the mechanisms which control the cholesterol synthesis as effects of Piper sarmentosum (PS) water extract supplementation in ovariectomized-induced obese rats.

Methods: Fourty-two female Sprague-Dawley rats were randomly divided into six groups; four treatments (PS, GCA, CTRL and SHM) groups and two basal (B-CTRL and B-SHM) groups. All groups underwent ovariectomy except for the SHM and B-SHM which underwent sham operation. Basal groups were sacrificed on the first day of treatment, while the ovariectomized groups were given PS water extract (0.125g/kg), glycyrrhizic acid (GCA) (0.120g/kg) or water (CTRL) respectively, while the SHM group received only water. After five months of treatment, the rats were killed; blood samples were taken for lipid profile and liver tissue was taken for gene expression analysis.

Results: All three ovariectomized groups had a significant increased in the plasma total cholesterol (TC) ($p\approx0.035$), triglyceride (TG) ($p\approx0.000$) and low-density lipoprotein (LDL) ($p\approx0.005$), and significant reduction ($p\approx0.000$) in high-density lipoprotein (HDL) level compared to the SHM group. After 5 months of treatment, both PS and GCA treated group showed a significant reduction in the TC ($p\approx0.000$), TG ($p\approx0.000$) and LDL ($p\approx0.000$) level. Meanwhile for HDL level only PS treated group showed significant ($p\approx0.013$) increment. Both PS and GCA treated group showed a significant up-regulation of the LDL receptor gene but no significant effects on HMGCR, SREBP-2 and APOB gene expression.

Conclusion: PS water extract possessed lipid lowering effects as shown by the reduction of TC, TG and LDL and increment of HDL in ovariectomy-induced obese rats. The mechanism for the lipid lowering effect is possibly due to its ability to up-regulate the LDL receptors in ovariectomized-induced obese rats.

POSTER 80

LIPIDOMIC ANALYSIS OF HUMAN PLATELETS REVEALS A DIVERSITY OF UNIQUE SPECIES THAT FORM ON THROMBIN ACTIVATION.

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Abstract: Platelets play a central role in haemostasis through aggregation to form a plug, and providing a pro-coagulant surface for clotting factors. On agonist activation, their plasma membrane undergoes significant remodeling, including, biophysical changes (shape change, vesicle formation) and hydrolysis to release substrates for lipid signaling pathways (phosphoinositides, eicosanoids, diacylglycerides, etc). In addition, activated platelets sustain arterial thrombosis and cancer metastasis through interaction with endothelial and other immune cells. How platelets regulate these processes is not fully understood, and may involve as yet undiscovered lipid signaling mediators released during cell activation. Here we report characterization of novel lipid species generated by thrombin-activated platelets using an untargeted lipidomic approach with ultra-performance liquid chromatography-MS (hybrid Orbitrap Elite). Total lipid extracts of human washed platelets, activated us ing 0.2 U/ml thrombin for 15 min, were analysed in both positive and negative ion mode over m/z 100-1800. Data processing and statistical analysis revealed that 1,046 lipid species were upregulated (>2-fold), and 2,859 were downregulated on activation of platelets, in at least 3 out of 4 genetically-unrelated donors. Putative up-regulated lipids included eicosanoids (thromboxane, HETEs, etc), mono- and diglycerides, lysophospholipids, acyl carnitines, oxidised phospholipids, ceramides, gangliosides and cardiolipins. Several of these have never been described as generated by platelets before, and several have known potent signaling actions relevant to cancer and atherosclerosis. Over 75% of all up- or downregulated lipids were unknown and potentially represent novel lipid families of relevance to human disease. Further studies will target biologically active novel species for in-depth structural charactisation using MSn and NMR.

POSTER 81

PROTECTIVE EFFECT OF DIETARY GAMMA-LINOLENIC ACID IN A MURINE MODEL OF GENERALIZED CHRONIC PAIN AND IMMUNOINFLAMATORY STATE

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Abstract: Most research has been done on the role of diet in acute pain tests, while there is only sparse evidence on the effect of diet in attenuating chronic pain in experimental animals. GLA have been applied in the treatment of diseases as multiple sclerosis, rheumatoid arthritis and pre-menstrual syndrome. In order to examine the effect of GLA in generalized chronic pain including fibromyalgia syndrome (FMS) induced by intermittent cold stress (ICS) (Nishiyori and Ueda, 2008). Fifty mice of five week old, female Swiss, weighing 18-22g were used for the experiments. The animals were allocated in five groups, each comprising of 10 mice. The mice included in the control group (Group 1) were not subjected to any model, for a period of 8 weeks group 2 and group 3 were fed diets containing 10g GLA/100 g lipids (Group 2), Group 3 received 20g GLA/100 g lipids, Group 4 was treated with Gabapentin 1mg/ mL/ Kg (drug used to relieve neuropathic pain) and Group 5 was subjected to any treatment. At the end of 8 weeks, ICS was induced to all groups except group 1. When in vivo tests were completed, we proceeded to isolation and culture of peritoneal macrophages in order to determine the effects on the release of proinflammatory mediators by sandwich immunoassay (Fernandez- Arche, et al., 2009). The results indicate that the higher dose dietary- GLA is suitable to improve mechanical allodynia and thermal allodynia and hyperalgesia (Paw pressure test, $p < 0.05$; Hot plate test, $p < 0.01$; and Tail immersion test, $p < 0.05$) and it is also being able to improve behavioural changes related to cognitive disturbances, anxiety and depression (Hole board test, $p < 0.01$; and traction and evasion test, $p < 0.01$). Besides, GLA reduces significantly the inflammatory response in LPS - macrophages stimulated (5 μg/mL) at 10g GLA/ 100g lipids (NO and PGE2, $p < 0.05$; TXB2 and IL-1β, $p < 0.01$) and at 20g GLA/100g lipids (NO and PGE2, $p < 0.01$; TXB2 and IL-1β, $p < 0 .001$). In conclusion, this study demonstrates that dietary-GLA can modify the nociceptive response and other symptoms associated with chronic pain and FMS, as well as reduce the inflammatory state.

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POSTER 82

DOCOSAHEXAENOIC ACID (DHA) UPREGULATES Fndc5 AND Aadac GENE EXPRESSION IN SUBCUTANEOUS ADIPOCYTES OF OVERWEIGHT SUBJECTS

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Abstract: The n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic (EPA) and DHA have been reported to have protective effects in obesitylinked metabolic disorders. In fact, these n-3 PUFAs have been shown to ameliorate low-grade inflammation in white adipose tissue (WAT) and the dysregulation of adipokine secretion associated with obesity, as well as to up-regulate mitochondrial biogenesis and induce beta-oxidation in WAT in mice. Resolvins and protectins are potent bioactive lipid mediators derived from long-chain n-3 PUFAs. Recently, it has been demonstrated the ability of Resolvin D1 (RvD1) to promote resolution of adipose tissue inflammation and improve insulin sensitivity in obese-diabetic mice.

The Fndc5 gene encodes a membrane protein that is cleaved and secreted as a newly identified hormone termed irisin. Irisin secretion from muscle is induced by exercise. Irisin upregulates genes typifying brown fat phenotype in subcutaneous fat, and causes a significant increase in total body energy expenditure and resistance to obesity-linked insulin resistance. FNDC5 also promotes the induction of arylacetamide deacetylase (Aadac) gene, a major esterase that could potentially participate in the regulation of lipid metabolism.

The main objective of the present study was to analyze the effects of EPA, DHA and RvD1 on Fndc5 and Aadac gene expression in human adipocytes. For this purpose, human subcutaneous preadipocytes from overweight females (BMI: 28.1-29.8 kg/m2) were differentiated according to the manufacturer's procedures. Fully differentiated human subcutaneous adipocytes were treated with EPA (100-200 μM), DHA (100 μM) or RvD1 (10 nM-100 nM) for 24 hours. mRNA levels of Fndc5 and Aadac were determined by real-time-PCR. The obtained data showed that DHA significantly increased both Fndc5 ($p<0.001$) and Aadac ($p<0.05$) mRNA expression. However, no significant changes were observed on Fndc5 mRNA levels in EPA and RvD1-treated adipocytes.

These preliminary data suggest that the upregulation of Fndc5 and Aadac in WAT could contribute somehow to the beneficial effects of DHA in obesity-related metabolic disorders. Further studies are needed to both confirm this possibility and better understand the underlying mechanisms.

POSTER 83

MICROPARTICLES CONTAINING PRECURSORS FOR SPECIALIZED PRO-RESOLVING LIPID MEDIATORS ARE EFFECTORS IN RESOLUTION OF INFLAMMATION.

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Abstract: Microparticles (MPs) are small vesicles shed from the plasma membrane that retain unique markers and mediators of their parent cell, enabling them to exert independent functions. Elevated levels of circulating MPs are associated with a number of cardiovascular and inflammatory pathologies, yet their functions remain uncertain. An emerging notion is that MPs originating from different cell types or due to differential stimuli can exert deleterious or protective effects. In this study, endogenous MPs were systematically profiled during the time course of self-limited inflammation. Precursors for specialized pro-resolving lipid mediators (LM) were identified in MPs from inflammatory exudates utilizing LC-MS/MS-based metabolomics. The levels of MP-associated hydroxydocosahexaenoic acids; namely 14-HDHA and 17-HDHA were high during the initiation phase of the acute inflammatory response, decreased during the peak of inflammation and accumulated in resolution, the phase in which potent anti-inflammatory and pro-resolving LM are biosynthesized. Esterified precursors could be liberated from MPs using secretory PLA2, an enzyme induced during the resolution of inflammation. These results implicate endogenous MPs as intercellular communicators that can deliver pro-resolving LM precursors to inflammatory loci. We postulated that formation of anti-inflammatory and pro-resolving LM could underlie the beneficial effects attributed to neutrophil-derived MPs and that further enrichment of MPs with these lipids could enhance their beneficial properties. Using human neutrophil-derived MPs as scaffolds, we constructed novel nanoparticles (NPs) containing aspirin-triggered resolvin D1 (AT-RvD1) or a lipoxin A4 (LXA4) analog. Enriched NPs exerted potent inhibitory actions in experimental peritonitis, accelerated resolution and produced protective effects in the temporomandibular joint. A novel therapeutic approach is proposed, whereby MPs can be fortified with anti-inflammatory lipid mediators producing innovative nanomedicines, which would reduce acute inflammation and protect from joint disease.

POSTER 84

EICOSANOID REGULATION OF BONE REGENERATION

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Abstract: The molecular and cellular pathways that regulate bone regeneration after a fracture are poorly understood. We investigated whether the prostaglandin and leukotriene synthesis enzymes, cyclooxygenase-1 (COX-1), COX-2, and 5-lipoxygenase (5-LO) affect fracture healing. Studies were conducted in relevant mouse knockout models and in rats treated with selective COX-2 inhibitors or 5-LO inhibitors. A standard femur fracture model was employed in both species and healing was assessed by x-rays, histology, and mechanical testing. The data show that COX-1 has no major regulatory function during fracture healing. However, COX-2 and 5-LO have significant, diametric effects. Loss or inhibition of COX-2 significantly impairs healing while loss or inhibition of 5-LO significantly enhances healing. Histological and supporting molecular data indicate that COX-2 and 5-LO affect cartilage formation during the fracture healing process, which significantly influences the healing outcome. These studies indicate that eicosanoids are significant regulators of fracture healing and suggest that pharmacological agents affecting these pathways can be developed to accelerate this tissue regeneration process.

POSTER 85

ALL-ROUND IN VITRO ANTIOXIDATIVE ACTIVITY BIOASSAY OF THE PERSPECTIVE PHISIOLOGICALLY ACTIVE COMPOUNDS

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Abstract: The antioxidative activity study of novel compounds by various methods is the widespread method for physiological activity determinination. Usually only one or two methods of antioxidant activity determination are applied for each compound under investigation. This approach allows one to evaluate physiological activity in general, but do not give a complete undestanding of a substance antioxidant activity nature that is determined by the specific molecular mechanism of interaction with molecular targets.
The combination of various methods and approaches for the antioxidant activity evaluation allows one to estimate the integral contribution of different activity types towards considered molecular targets. When using a network structure of applied methods the quantitative antioxidant activity characteristics take into account mechanism detailes of the each compound action.

The following antioxidant candidates were studied: ferrocenes and dipicolylamines, porphyrines and phosphonates, bearing antioxidative 2,6-dialkylphenol fragments, and their complexes of biometals (Fe, Co, Cu, Mn, Ni, Zn). The results obtained open up a possibility to identify structure-activity relationship and to determine the mechanism of antioxidant action.

POSTER 86

EFFECT OF PERIVASCULAR ADIPOSE TISSUE ON THE VASOREACTIVITY OF HUMAN SAPHENOUS VEIN: A ROLE OF PROSTAGLANDIN E2

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Abstract: Perivascular adipose tissue which surrounds systemic vessels, is a modulator of vascular function and attenuates vasoreactivity to various vasoconstrictor agonists.

In this study, we aimed to investigate the effect of perivascular adipose tissue on the vasoreactivity of human saphenous vein (SV) and the involvement of prostaglandin E2 (PGE2) in the effect of perivascular adipose tissue.

In organ bath experiments, the presence of perivascular adipose tissue significantly attenuated the contractile response to noradrenalin comparing to SV without perivascular adipose tissue. Incubation of SV with indomethacin (10-5M, 30 min) caused a significant decrease in the vasorelaxant effect of perivascular adipose tissue. This result suggested the participation of prostanoids in the perivascular adipose tissue, induced vasodilation. Moreover, It has been shown that perivascular adipose tissue of SV released PGE2 which was abolished in the presence of indomethacin. On the other hand, the expression of mPGES1 which is mainly responsible for the synthesis of PGE2 in perivascular adipose tissue of SV was determined by Western Blot.

In conclusion, our results have shown that the synthesis and the release of PGE2 from perivascular adipose tissue might diminish the vasoreactivity of SV. Finally, we suggest that the presence of perivascular adipose tissue in SV may have additional benefits on the prevention of vasospasm and graft patency.

POSTER 87

IN VIVO EXPRESSION OF MARKERS OF OSTEOCLASTOGENESIS AND GENES INVOLVED IN BONE METABOLISM ARE MODULATED BY INDOMETHACIN IN RESPONSE TO BACTERIAL LIPOPOLYSACCHARIDE

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Abstract: Considering that gram-negative bacterial lipopolysaccharide (LPS) is an important stimuli for apical periodontitis development, the aim of this study was to evaluate the expression of messenger RNA (mRNA) for the enzymes involved in arachidonic acid metabolism, cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO), and the osteoclastogenesis mediators (RANK, RANKL and OPG) in bone tissue after inoculation of LPS in murine dental root canals. Then the effect of pharmacological block with Indomethacin in the expression of RANK, RANKL, OPG and others genes involved in bone metabolism were investigated. Apical periodontitis was experimentally induced in the first molars of 144 C57BL/6 mice following inoculation of a solution containing LPS from E. coli (0.1 or 1.0 mg/ml) into root canals. After 7, 14, 21 and 28 days the animals were euthanized and the tooth-and-bone blocks were removed for total RNA extraction. Evaluation of gene expression was performed by real time reverse transcription and polymerase chain reaction (qRT-PCR). Global analysis of mRNA expression for proteins involved in bone metabolism was performed using PCR arrays. Statistical analysis was performed using analysis of variance (ANOVA) followed by Bonferroni post-test or one-way ANOVA followed by Dunnett\'s test (alpha=0.05). In vivo inoculation of LPS into root canals induced periapical inflammation and bone resorption. Enzymes 5 -LO and COX-2 mRNAs were upregulated and reached higher expression at 7 and 14 days, respectively ($p<0.05$). At 7 days, LPS upregulated RANKL mRNA expression while OPG mRNA expression was downregulated ($p<0.05$). At 14 days, OPG was upregulated and reduced afterwards reaching expression lower than the control at 28 days ($p<0.05$). On the other hand, RANKL was downregulated at 14 days, then expression increased at 21 days and reduced at 28 days. RANK expression increased at 14 days and decreased after that ($p<0.05$). Indomethacin treatment inhibited RANK and RANKL and stimulated OPG gene expression, a signalling against osteoclastic formation. Expression of genes involved in LPS-induced bone catabolism was also abrogated by Indomethacin, indicating that cyclooxygenase inhibition in earlier phase of LPS-induced apical periodontitis might prevent bone resorption.

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POSTER 88

ROLE OF GROUP IVA PHOSPHOLIPASE A2 IN ADIPOCYTE DIFFERENTIATION

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Abstract: Phospholipases A2 (PLA2s) are enzymes that catalyze the hydrolysis of the sn-2 fatty acid of glycerophospholipids, generating a free fatty acid and a 2-lysophospholipid. In mammalians, more than 20 PLA2 forms have been identified to date. One of the better characterized is group IVA PLA2, also known as cytosolic PLA2alpha, an enzyme responsible for the release of the arachidonic acid that is used for prostaglandin synthesis. To define the contribution of this enzyme to adipocyte differentiation, we have performed in vitro experiments using the hormoneinduced 3T3-L1 cell model of adipocyte differentiation. Down-regulation of cPLA2alpha in 3T3-L1 preadipocytes using siRNA technology results in a decreased accumulation of neutral lipids in differentiated adipocytes, as measured by Oil Red O staining. Moreover, a reduction in the expression of key transcription factors during adipocyte differentiation process such as PPARgamma and C/EBPalpha, was also observed. As a consequence, expression of the adipocyte-related gen aP2/FABP4 had a low expression in cells treated with cPLA2alpha siRNA. Taken together these results suggest that cPLA2alpha is a key component of the regulation of transcriptional programs involved in the adipocyte differentiation in 3T3-L1 cells.

POSTER 89

ROLE OF PROSTAGLANDINS IN HOST DEFENCE DURING HISTOPLASMA CAPSULATUM INFECTION

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Abstract: Prostaglandins are lipid mediators released during inflammation and infection. Histoplasmosis is a pulmonary disease caused by inhalation of conidial or mycelium fragments of Histoplasma capsulatum. Previously, we showed the role of leukotrienes in this infection, but nothing is known about the role of the prostaglandins. Our objective was to investigate the role of prostaglandins in H. capsulatum infection in mice. C55bl6 mice were infected intratraqueally with 5 x 105 (sub lethal inoculum) of H. capsulatum yeast and daily treated or not with celecoxib (1 mg/kg). At 2, 14 and 28 days post infection, bronchoalveolar lavage fluids and lung tissue were collected in order to investigated local immune response. We observed that inhibition of cyclooxygenase 2 with celecoxib reduced the total fungal burden in the lungs; PGE2 and cytokine concentration. Also lymphocytes, neutrophils and mononuclear cell numbers in the bronchoalveolar space and lung parenchyma were decreased. Furthermore, the synthesis of nitric oxide, IFN-γ, leukotriene B4 and the phagocytic capacity of alveolar macrophages increased with celecoxib treatment. Moreover, celecoxib treatment improved mouse survival after infection with a lethal inoculum of H. capsulatum. Ours results showed that prostaglandins have a relevant role during histoplasmosis. In this manner, inhibition of prostaglandins in association with antifungal therapy may be a new strategy to histoplasmosis treatment.

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POSTER 90

PROTECTIVE EFFECT OF GLYCOLIPIDS AT INHIBITION OF HEME OXYGENASE IN CHOLESTASIS

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Abstract: (Glyco)sphingolipids (GSLs) are involved in stabilization of plasma membrane and are important receptor molecules of cell surface. Simple sphingolipid metabolites: ceramide or sphingosine-1-phosphate are important mediators in the signaling cascades involved in apoptosis, proliferation, stress responses, necrosis, inflammation, and differentiation.

The role of GSLs in increased membrane rigidity in estrogen induced cholestasis was published by our research team. It was found an increase of total gangliosides and B-serie of gangliosides in rat liver (1). Histochemically, increased shift of GM1 as a representative of GSLs from cytoplasma into sinusoidal membrane was found in liver of rats. An increased content of gangliosides in plasma membrane may protect lipid bilayer against detergent attack of bile acids (2).

The objective of present study was to find if similar changes of GLS composition can be induced by hem oxygenase-1(HMOX-1), inducible enzyme involved in the catabolism of heme. The products of the catabolism have antioxidant, antiapoptotic, cytoprotective and anti-inflammatory properties and protect liver against oxidative stress.

Cholestasis was induced in adult female Wistar rats by ethinylestradiol (EE) and control animals received propanediol (vehicle for EE) for 18 days. Activation of HMOX was induced by hemin and inhibition by tinmesoporphyrin. Bile acids and gangliosides were determined spectrophotometrically, HMOX activity was determined by gas chromatography and changes in the composition of gangliosides were established by thinlayer chromatography (TLC). Bilirubin, ALP activity were analyzed by routine assays.

Significant increase of total liver gangliosides against controls was caused by induction of EE cholestasis as well as by inhibition of HMOX ($p<0.05$). Activation of HMOX in control and cholestatic samples does not cause changes in total gangliosides. TLC shows a significant increase in GD1b and GT1b gangliosides in cholestatic liver samples as well as inhibition and activation of HMOX when compared to controls. We can hypothesize that lower hepatoprotection due to HMOX inhibition might be compensated by increase of gangliosides.

(1) Majer F, et al., Biomedical Chromatography, 21, 446-450, 2007
(2)Jirkovska M, et al., Glycoconjugate Journal, 24, 231-241, 2007

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POSTER 91

REDUCED LIPOXIN A4 / LEUKOTRIENE B4 RATIO IN EARLY CYSTIC FIBROSIS BAL - AIRWAY EPITHELIAL LIPOXIN A4 SYNTHESIS CAPACITY IS IMPAIRED

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Abstract: Cystic Fibrosis (CF) is characterised by impaired muco-ciliary clearance, persistent neutrophilic inflammation and bacterial infection. Normal resolution of inflammation occurs through an active switch in mediators that predominate in exudates. Early in inflammation, Leukotriene B4 (LTB4) plays a prominent role in neutrophil activation. Resolution and return to tissue homeostasis is signalled by the trans-cellular synthesis of Lipoxin A4 (LXA4) by the action of Lipoxygenase enzymes (LO) expressed in cells such as neutrophils and airway epithelial cells (Serhan C.N. 2005). The aims of this study were to quantify LXA4 production in the airways of children with CF and characterise LXA4 synthesis by airway epithelial cells in CF.

Lipoxin A4 and Leukotriene B4 were measured by ELISA in bronchoalveolar lavage samples from children with CF and controls. Non CF (NuLi-1) and CF (CuFi-1 Homozygous ΔF508) bronchial epithelial cell lines were cultured as differentiated epithelia at air / liquid interface. We quantified the capacity of Non CF and CF cells to synthesize LXA4 by the action of epithelial 15-LO on 5(S),6(R)-DiHETE, (a precursor of LXA4). Expression of 15-LO was compared between NuLi-1 and CuFi-1 cells by Western Blot.

Relative production of Lipoxin A4 is significantly depressed in children with CF versus controls when compared to Leukotriene B4. The ability of CuFi-1 cells to convert 5(S), 6(R)-DiHETE to LXA4 was reduced as compared with NuLi-1 cells. Inhibition of CFTR activity did not significantly affect LXA4 production by Nuli-1 cells. The expression of 15-LO2 was reduced in CuFi-1 compared with Nuli-1 cells.

The ratio of Lipoxin A4 to Leukotriene B4 in the airway of young children with Cystic Fibrosis is depressed. Our results indicate that the contribution of airway epithelial cells to Lipoxin A4 synthesis is reduced in CF. This may contribute to the persistence of acute inflammation and consequent lung damage in CF.

POSTER 92

RESOLVIN D1 PRIMES THE RESOLUTION PROCESS INITIATED BY CALORIE RESTRICTION IN OBESITY-INDUCED STEATOHEPATITIS.

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Abstract: Background and aims: Obesity entails a chronic state of low grade inflammation that leads to metabolic abnormalities including the development of steatohepatitis, a condition characterized by a combination of hepatic lipid deposition and inflammation. Both obesity and steatohepatitis can be effectively attenuated by weight loss. Resolvins of the D-series (RvDs) are endogenous anti-inflammatory and pro-resolving lipid mediators derived from the omega-3 fatty acid docosahexaenoic acid (DHA) that promotes timely resolution of inflammation and the return to tissue homeostasis. The hypothesis of the current study was that RvD1 could potentiate and accelerate the resolution process initiated by calorie restriction in an experimental model of high-fat diet (HFD)-induced obesity and steatohepatitis.

Methods: Male C57BL/6 mice (n=27) were fed with a HFD for 12 weeks and then randomly assigned into three study groups. One group continued on HFD for 3 more weeks (n=13) (Control group) and the rest were switched to a low-fat diet (40% calorie restriction) and received either RvD1 (300 ng/mouse, i.p., n=7) (RvD1 group) or placebo (n=7) (Placebo group) for the last 3 weeks. At the end of the intervention period, weight loss, hepatic steatosis, inflammation and insulin resistance were assessed. A model of hypoxia-induced inflammation in precision cut liver slices (PCLS) was used to test the in vitro actions of RvD1 (10 nM) on liver cells.

Results: Calorie restriction was associated with weight loss in adipose and liver tissues, reduced serum leptin and resistin levels and lower hepatic steatosis and insulin resistance, reflected by decreased JNK phosphorylation compared to the HFD control group. In addition to these anti-obesogenic and anti-steatotic effects, RvD1-treated animals showed significantly reduced serum insulin and glucose levels and a reduction in hepatic inflammatory infiltrate as assessed by positive F4/80 immunostaining and down-regulation of MCP-1 and IL-1beta. Moreover, a differential expression in CCR7, STAT3 phosphorylation and the macrophage M2 marker Arg1 was observed between the placebo and the RvD1 groups. Antiinflammatory actions of RvD1 in liver cells were confirmed in PCLS experiments.

Conclusion: RvD1 primes the resolution process initiated by calorie restriction through reducing the hepatic inflammatory component of obesity-induced steatohepatitis.

POSTER 93

PROLONGED EXPOSURE TO OMEGA 3 FATTY ACIDS IMPROVES INSULIN SENSITIVITY AND MODULATES GENES INVOLVED IN INSULIN SIGNALING PATHWAY IN MICE

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Abstract: A growing body of evidence suggests that the amount and type of fat included in the diet contribute to the development of insulin resistance and that the reduction of the tissue n-6/n-3 fatty acid ratio could effectively suppress the development of many chronic diseases. The fat-1 transgenic mouse is capable of converting omega-6 (n-6) to omega-3 (n-3) fatty acids, leading to an increase in n-3 fatty acid content with a balanced n-6/n-3 fatty acid ratio in all tissues, independent of diet. In the present study we investigate the effects of n-3 on insulin sensitivity as well as the expression of insulin pathway-related genes in aging mice. C57BL/6 mice were divided in four groups: wild-type and fat-1 at age 8 weeks, wild-type and fat-1 at age 8 months. After the treatment period, body mass, food intake and the plasma concentration of glucose and insulin were measured. Glucose tolerant testing (GTT), insulin tolerant testing (ITT) and heart gene expression by Real Time PCR were performed in all groups. Lipid analysis confirmed that n-3 tissue levels of fat-1 mice are much higher than that of wild type mice. Fat-1 mice at 8 months of age showed decreased body weight and food intake, decreased basal glucose and insulin levels and displayed an improvement in glucose tolerance and insulin sensitivity during GTT and ITT, respectively, compared to wild-type mice of the same age. Evidence for increased insulin related genes in the heart tissue of fat-1 mice (age 8 months) was obtained by a 2-fold increase in mRNA levels for insulin receptor, protein kinase B, phosphatidylinositol 3-kinase and nitric oxide synthase compared with all other groups. In conclusion, aged fat-1 mice are more insulin sensitive despite the age-related insulin resistance observed in the aged wild type mice. This suggests that a prolonged increase in n-3 fatty acid tissue content may be an important approach for the prevention and treatment of insulin resistance and related chronic diseases.

POSTER 94

INDUCTION OF COX-2 SIGNALING BY S. MUCILAGINOUS HIGHLIGHTS THE PATHOGENIC POTENTIAL OF AN ORAL COMMENSAL

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Abstract: Bronchiectasis is primarily a disease of the bronchi and bronchioles and has the potential to cause devastating illness by predisposing susceptible individuals to recurrent respiratory infections. We found that 12% of patients with bronchiectasis were colonized with Stomatococcus mucilaginosa (SM), in their bronchoalveolar lavage (BAL). SM is a gram positive human pathogen found in oral cavity which has been reported to cause severe infections in immunocompromised patients. Similar to P.aeruginosa (PA), SM is known to form biofilms however little is known about the pathogenic role of SM in chronic airway infections. We hypothesized that SM is a low grade pathogen which may predispose individuals with bronchiectaiss to other infections such as PA. In vitro studies in RAW cells and bone marrow derived macrophage (BMDM) from wild type (WT) mice showed that treatment of cells with SM (MOI of 100) resulted in induction of pro-inflammatory mediators including COX-2 and prostaglandin mediators. Induction of COX-2 and production of PGE2 in macrophages by SM was dependent on p38 and ERK1/2 MAP Kinase pathway. In vivo studies showed that SM induces COX-2 with production of PGE2 in vivo in lungs of mice treated with SM (MOI 1010). Furthermore mice treated with SM showed an increase in neutrophils, cytokines and chemokines in BAL similar to mice treated with PA. Interestingly mice that were treated with SM and PA showed an increased mortality compared to mice treated with PA or SM alone. We performed additional experiments to determine the effects of inhibition of COX-2. Mice that were treated with NS-398 (specific inhibitor of COX-2) showed an enhanced clearance of SM with improved survival. Together these data suggest that SM has the potential to exaggerate the inflammatory response and increase the pathogenic profile of other co-habitant microbes. We show that SM induces COX-2 in vitro and in vivo with increased production of PGE2 in a MAP Kinase dependent manner. In conclusion our data for the first time provide novel insights into the pathogenic role of SM and suggest that SM from lower respiratory tract should not be considered an innocent bystander.

POSTER 95

PROSTAGLANDIN E2 MAINTAINS THE TONE OF THE GUINEA PIG TRACHEA THROUGH A BALANCE BETWEEN ACTIVATION OF CONTRACTILE EP1 RECEPTORS AND RELAXANT EP2 RECEPTORS

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Abstract: Introduction: Prostaglandin (PG) E2 is a central messenger molecule that exerts its action via four different G-protein coupled receptors (EP1-EP4), mediating both contraction and relaxation in airway smooth muscle. However, the mechanisms involved in the actions of PGE2 in the airways are not yet clear.

Aim: Using guinea pig specific primers for the enzymes and receptors in the PGE2 pathway, new selective inhibitors and antagonists we aimed to establish which receptor(s) mediate the contractions and relaxations to exogenous PGE2 as well as assessed the role of endogenous PGE2 for regulation of tone in the guinea-pig trachea (GPT)

Methods: Expression of mRNA for EP receptors and key enzymes in the PGE2 pathway were assessed by real-time PCR. Isometric responses were assessed in tracheal segments from male guinea pigs. The effects of the ONO-8130 (EP1), PF-04418948 (EP2), ONO-AE5-599 (EP3) or ONO-AE3-208 (EP4) on responses to PGE2 were assessed. Furthermore, the tone was investigated using several isotype selective COX-inhibitors ($n \geq 7$ for each intervention and concentration of drug).

Results: Expression of mRNA for the four EP receptors was found in airway smooth muscle. PGE2 displayed a bell-shaped concentration-response curve, where the initial contraction was inhibited by the EP1 receptor antagonist, and the subsequent relaxation by the EP2 receptor antagonist. Neither EP3 nor EP4 selective receptor antagonists affected the response to PGE2. Expression of COX-2 was greater than COX-1 in GPT, and the spontaneous tone was most effectively abolished by selective COX-2 inhibitors. Furthermore, the EP1 antagonist and a specific PGE2 antibody eliminated the spontaneous tone, whereas the EP2 antagonist increased it. Antagonists of other prostanoid receptors had no effect on basal tension. The relaxant EP2 response to PGE2 was maintained after long-term culture, whereas the contractile EP1 response showed homologous desensitisation to PGE2 which was prevented by COX-inhibitors.

Conclusion: Endogenous PGE2, synthetized predominantly by COX-2, maintains the spontaneous tone of GPT by a balance between contractile EP1 receptors and relaxant EP2 receptors.

POSTER 96

MECHANISMS OF ACTION OF PUFA IN MACROPHAGE-MEDIATED IMMUNE DEFENSE

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Abstract: We recently demonstrated that polyunsaturated fatty acids (PUFA) exert anti-inflammatory and immune suppressive actions on macrophage immune defense. The underlying mechanisms, however, are largely unknown. Therefore, the objective of the present study is to elucidate the mechanisms of action which underlie the immune modulatory effects of the fatty acids. Fluorescence microscopic examinations show that protein kinase C (PKC) is not affected by PUFA enrichment of macrophages. There was no influence of PUFA supplementation on the dynamic PKC trafficking in response to stimulation. In contrast, the toll-like receptor (TLR) signaling as well as the MAP kinase pathway emerged to be affected by the enrichment of the macrophages with PUFA. The modulation of the TLR and MAP kinase pathways seems to be partially mediated by a down-regulation of TLR4 gene expression. Furthermore, the disruption of lipid rafts due to PUFA supplementation is likely to perturbate the interaction of TLR4 with its adapter protein CD14. Hence, our data support the idea that the immune suppressive actions of PUFA are mediated on the membrane level.

POSTER 97

COMPARISON OF FREE SERUM OXYLIPIN CONCENTRATIONS IN HYPER- vs. NORMOLIPIDEMIC MEN

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Abstract: Omega-6 polyunsaturated fatty acids (n-6 PUFA) such as arachidonic acid (AA) and linoleic acid (LA) and n-3 PUFAs such as α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to affect lipid metabolism and inflammation. Many actions of n-6 and n-3 PUFAs are mediated by their oxidation products, so called oxylipins. These potent lipid mediators, formed from their PUFA precursors by enzymes (COXs, LOXs, CYP, sEH) and autoxidation, regulate various biological processes such as inflammation, pain, blood clotting, among many other functions. While AA-derived oxylipins, i.e. eicosanoids, formed by COXs and LOX are well characterized, the regulation of endogenous formation and biological role of most oxylipins is poorly understood.

Hyperlipidemia and chronic inflammation are often linked together and play pivotal roles in atherogenesis and cardiovascular disease. Due to diverse effects of oxylipins in lipid metabolism and inflammatory pathways, we hypothesized that differences in n-6 and n-3 oxylipin levels between normo- and hyperlipidemic subjects might explain an underlying cause for these conditions.

In the present study, we compared oxylipin patterns of twenty hyperlipidemic (total cholesterol >200 mg/dl; LDL-C >130 mg/dl; TG >150 mg/ml) and twenty normolipidemic men. Levels of 45 free hydroxy-, dihydroxy- and epoxy-FAs were analyzed in serum by liquid chromatography mass spectrometry (LC-MS) and compared among hyper- and normolipidemic men. Additionally, we compared oxylipin levels with their parent FA (LA, AA, ALA, EPA, DHA) levels in erythrocyte membranes; the most suitable marker for assessing PUFA status.

Differences in oxylipin levels between the hyperlipidemic and control group were minor. Levels of 8,9-DiHETrE, 5-HEPE, and 10,11-DiHDPE were slightly higher in the hyperlipidemic group, while 12,13-DiHOME, 9,10-DiHODE, and 12,13-DiHODE were lower compared to the control group. Interestingly, we observed a strong correlation between n-3 PUFAs erythrocyte membrane concentrations and levels of their corresponding oxylipins in both normo- and hyperlipidemic men. Particularly the levels of EPA oxylipins in serum were positively correlated with its precursor FA. Further studies should investigate the influence of n-3 PUFA supplementation on oxylipin patterns in normo- and hyperlipidemic subjects to understand their formation and role in lipid metabolism and inflammation.

POSTER 98

INTERACTION OF CELEXOCIB, NITRIC OXIDE AND REACTIVE OXYGEN SPECIES IN ISCHEMIC ACUTE RENAL FAILURE IN RATS

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Abstract: Objective: Renal ischemic injury is a major cause of acute renal failure which is associated with a high mortality rate in humans. During renal ischemia, there is an increase in the production of inflammatory mediators and vasoactive molecules, an increased production of free radicals as well as an increase in renal prostaglandin production. Growing evidence suggests that COX-2 may act as a source of reactive oxygen species, which are responsible for nitric oxide (NO) breakdown. NO plays a major role in renal hemodynamics and tubular sodium transport and to participate in the pathophysiology of acute renal failure. The interaction of COX-2 and NO, and particularly their putative synergistic or antagonistic roles in ischemic acute renal failure have not been clearly defined. The major goal of the present study is to investigate the possible interaction between NO availability, changes in COX-2 activity and oxidative stress and their role in the pathogenesis of renal dysfunction secondary to renal ischemia-reperfusion injury (IRI) in rats. Methods: Suprarenal aortic clamping is performed, followed by reperfusion at different time intervals in rats pre-treated with celecoxib (10mg/Kg), L-arginine (125mg/Kg) or a combination of both. In all rat groups, renal NO, malondialdehyde (MDA), reduced glutathione (GSH) levels, superoxide dismutase (SOD) activity, as well as plasma urea and creatinine levels are measured. Results: IRI caused a significant inhibition of GSH and SOD as well as NO levels. It was clearly noticed that these deleterious effects were more significant 24 hrs after reperfusion than 48 hrs after. In line, MDA, creatinine and urea levels were significantly increased by IRI; the effect was maintained for 48 hrs except for creatinine where control values were almost restored at the end of the experiment. Celexocib significantly ameliorated the effect of IRI on oxidative stress parameters, NO, creatinine & urea levels. However, the effect of celexocib was significantly lower than that of L-arginine. Kidney MDA levels were 0.79±0.03, 1.97±0.08, 1.53±0.07 and 1.11±0.05 nmoles/mg protein for sham-operated, IRI, celexocib and L-arginine groups respectively at 24 hrs. Combination of celexocib and L-arginine showed additive effect but only sham-operated values of MDA, SOD and creatinine levels were restored. On the other hand, control urea levels were far from being restored; at 24 hrs, serum urea concentrations were 97.08±1.95, 93.94±1.69 and 73.97±1.98 mg/ml for celexocib, L-arginine and their combination respectively, compared to 33.58 ± 1.38 mg/ml for the shamoperated group. Conclusions: COX-2 inhibition could reverse symptoms of renal failure following IRI by inhibiting the formation of reactive oxygen species and elevating kidney NO level. Combining L-arginine –which exhibits a significant antioxidant activity- with celexocib showed an effect superior to each drug alone.

POSTER 99

ELUCIDATION OF HUMAN FPR2/ALX GENE REGULATORY MACHINERY AND EVIDENCE FOR A HERITABLE MUTATION THAT REDUCES PROMOTER ACTIVITY

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Abstract: Lipoxin (LX) A4 is a potent regulator of the immune-inflammatory response and its resolution. FPR2/ALX, a G-protein-coupled receptor specific for lipoxin A4, is highly expressed in PMNs, monocytes, lymphocytes, and endothelial cells, and it is recognized by a number of peptides, including the anti-inflammatory annexin A1. Knowledge FPR2/ALX regulatory mechanisms is important to investigate new approaches for the treatment of inflammatory diseases. We mapped FPR2/ALX promoter region at 307 bp upstream the initiator sequence. This core sequence is essential for the transcription of FPR2/ALX gene. Chromatin immunoprecipitation and site direct mutagenesis revealed that specific protein 1 (Sp1) directly bind FPR2/ALX promoter and its overexpression is tightly involved in the FPR2/ALX promoter activity. Further, methylation of this region significantly reduced promoter activity, while treatment with 5-aza-2\'- deoxycytidine, increased FPR2/ALX mRNA and protein. LXA4 enhanced FPR2/ALX promoter activity (+74%) and mRNA expression (+87.5%) in MDAMB231 cells. A single nucleotide polymorphism, (A/G), was identified in FPR2/ALX promoter region at -220 bp from trascription start site in one subject with history of cardiovascular disease and of his two daughters. This mutation reduced by ~35-90% the promoter activity in vitro. Moreover, neutrophils from individuals carrying the A/G variant displayed ~10- and 3-fold reduction in FPR2/ALX mRNA and protein, respectively, compared with cells from their relatives or healthy volunteers expressing the wild-type allele. Together, these results indicate that FPR2/ALX is transcriptionally regulated by specific mechanisms that act on its core promoter region and provide the first evidence of mutations that influence FPR2/ALX transcription thus opening new opportunities for the understanding of the LXA(4)-FPR2/ALX axis in human disease.

POSTER 100

EFFECTS OF POLAR LIPIDS OF NIGELLA SATIVA SEEDS ON THE REDUCTION OF ATP AND ON THE INHIBITION OF ALPHA GLUCOSIDASE ACTIVITY

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Abstract: Experimentally, it has been demonstrated that Nigella sativa (Ns) seed extracts possess antioxidant and hepatoprotective activities. In addition to the hypoglycaemic and lipid lowering effects, this study was carried out to evaluate, in vitro, the toxicological effect of lipid extracts from the Ns seeds. The tested fractions were: (i) defatted methanolic extract, (ii) total lipid extract obtained by hexane extraction from methanolic extract and (iii) neutral and polar lipid fractions of the total lipid extract. Toxicological evaluation was carried out on precision-cut rat liver slices (PCLS). The fractions were assessed, in vitro, for their inhibitory potential on the activity of alpha-glucosidase because suppressing this enzyme activity is one of the therapeutic approaches for decreasing postprandial hyperglycaemia.

On PCLS, lipid extracts reduced ATP levels by 27-35 %. High inhibition of alpha-glucosidase by the two polar lipid fractions (F6 & F7) was reflected by their IC50 values (0.51 ± 0.04 mg/ml and 0.55 ± 0.09 mg/ml, respectively), compared to those of acarbose (0.53 ± 0.06 mg/ml) and thymoquinone (0.65 ± 0.05 mg/ml). The inhibitory effect on alphaglucosidase activity could result from inhibition of the early steps of carbohydrate metabolism and so could explain the anti-hyperglycaemic effect of this medicinal plant.

POSTER 101

A ROLE FOR CYTOSOLIC PHOSPHOLIPASE A2 IN TNF SIGNALING IN SYNOVIOCYTES

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Abstract: Rheumatoid arthritis (RA) is a systemic, chronic inflammatory disorder characterized by chronic synovitis causing pain, stiffness, swelling, and cartilage and bone destruction. The persistent inflammation is in part mediated by aberrant cytokine signaling, tumor necrosis factor (TNF) being a key participator. TNF-blocking therapy revolutionized the treatment of RA-patients, but the side effects of these drugs are up for discussion and the search for alternative therapeutic targets is of great importance. PLA2 enzymes are regulators of inflammation, hydrolyzing the sn-2 ester bond of membrane phospholipids to release free fatty acids. The cytosolic phospholipase A2 group 4A (cPLA2-IVa) is an important coordinator of both inflammatory and destructive processes in the RA joint, and the development of agents that specifically target cPLA2-IVa to inhibit downstream destructive effects is a promising therapeutic strategy. We aimed to investigate the role of cPLA2-IVa in TNF mediated signaling in fibroblastlike synoviocytes (FLS) by utilizing the novel cPLA2-IVa inhibitor AVX002. We found that AVX002 effectively diminished TNF-induced AA release and PGE2 production to basal levels. Furthermore, AVX002 inhibited TNF-induced gene expression and protein production of key mediators in RA pathology representing bone and cartilage destruction, angiogenesis, chemoattraction and AA metabolism, namely IL-8, MMP-3 and COX-2. We show for the first time an autoregulation of cPLA2-IVa, as AVX002 normalized transcription of the PLA2G4A gene. Our results demonstrate that TNF-mediated inflammatory signaling events depend on cPLA2-IVa activity in SW982 FLS, and that AVX002 may have the potential to decrease the inflammatory state of RA synovium. In conclusion, our results sustain the potential for cPLA2-IVa as an attractive therapeutic target for inflammatory diseases, and nominate AVX002 as a potential antiinflammatory drug for RA treatment.

POSTER 102

ISOPROSTANE EXCRETION IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

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Abstract: As part of a larger study to measure the effect of DHA supplementation for children with Autism Spectrum Disorders we are measuring the effect DHA supplementation on urinary isoprostane excretion. The Isoprostane 8-iso-PGF2α is a well accepted marker for oxidative stress. 24 children with ASD were randomized into two groups, group 1 treated with 200 mg DHA/day and group 2 were given placebos. All analyses with done by Isotope dilution liquid chromatography mass spectrometry- mass spectrometry using a modification the method of Saenger et al (Clin. Biochem. 40:1297, 2007) for 8-iso-PGF2α. 2H4 8-iso-PGF2α; and 13C Methyl ethylhexyl phthalate (MEHP) were used as internal standards. Urines were treated with glucuronidase prior to LC-MSMS assay to convert any conjugated isoprostanes to free isoprostanes so that total isoprostane could be measured. Results (ng/ml urine): DHA treated: pre-treatment 0.40 + 0.06, post treatment 0.35 + 0.02 (n=9), placebo pre 0.27 + 0.04, post 0.34 + 0.05 (n=9). There were two outliers with very high levels of oxidative stress in the ASD group. Their oxidative stress levels were reduced with 6 weeks of DHA supplementation. Excluding these two children from the cohort shows that DHA supplementation had no effect on oxidative stress in children with Autism.

POSTER 103

INTESTINAL EPITHELIAL DIFFERENTIATION AFFECTS CYCLOOXYGENASE PATHWAY ENZYMES AND PROSTAGLANDIN RECEPTORS EXPRESSION

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Abstract: Diverse evidences suggest that prostaglandins (PG) are important players in the control of intestinal epithelial cell growth and intestinal barrier functions. Colorectal cancer is caused by defects in the homeostasis balance between intestinal epithelial cell proliferation, differentiation and death. Thus, PGs can influence many of the hallmarks of colorectal cancer (1). Using Caco-2 cells, derived from a human colon adenocarcinoma, in different stages of differentiation, we studied the expression of cyclooxygenases (COX) and PGE2 receptors (EP1-4) as well as PGE2 synthesis and PGE2 action on intracellular calcium and AMPc concentrations.

Our results show that Caco-2 cell differentiation reduces COX-2 expression and consequently PGE2 synthesis. Moreover, we observed a marked decrease of EP1-4 expression when Caco-2 cells were differentiated. Thus, PGE2 induced rapid and important changes in intracellular calcium and AMPc concentrations in non-differentiated Caco-2 whereas PGE2 a higher concentration (10 fold high) was not able to induce appreciable changes of intracellular calcium and AMPc concentrations.

In conclusion, our findings suggest that intestinal epithelial differentiation modulates COX pathway enzymes and PGE2 receptors expression, events that can play important role in the homeostasis of the intestinal epithelium and colorectal cancer.

(1) R Ferrer, JJ Moreno. Role of eicosanoids on intestinal epithelial homeostasis. Biochem Pharmacol 2010;80:431-438

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POSTER 104

IS THE INCREASED N-6 FATTY ACID INTAKE CONTRIBUTING TO THE INCREASE OF OBESITY AND DIABETES WORLDWIDE?

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Abstract: The prevalence of obesity and diabetes are increasing worldwide and the causes are multifactorial, including life-style changes related to physical activity, food availability and quality. Obesity is increasing worldwide parallel with the extension of Western diet, characterized by exchange of saturated fat for vegetable oils, rich in n-6 fatty acids. The increase of obesity and diabetes occurs despite still high prevalence of starvation, suggesting that the quality of food might be of great importance. The quality of fat influences gene expression in the developing child both in uteri, and postnatally by the breast milk, and might thereby program for diseases later in life.

In series of animal experiments with modulation of the intake of essential fatty acids, we have found that later development of obesity was related to the early time period of essential fatty acid deficiency as well as the ratio of n-6 to n-3 fatty acids [1-4]. We also noticed that development of insulin resistance in adult animals was related to a n-6/n-3 ratio around 10:1 [3,4] and that animals with very low intake of essential fatty acids postnatally - but high intake of saturated fat - had an increased glucose tolerance [4] and changed PPAR expression in liver [5] , changes less clear if intervention only before birth [6]. In this context is is interesting to note that breast milk has high concentrations of saturated fat.

Our studies imply that high intake of saturated fat with low essential fatty acids during pregnancy or postnatal period has different impact on long-term programming related to obesity and insulin-glucose homeostasis. The data support the view by Holman and others (see review by Lands in Progr Lipid Res 2008) that our need for essential fatty acids are low and the hypothesis by Ailhaud et al. (Progr Lipid Res 2006) that high intake of n-6 fatty acids early in life might be hazardous for later health in adults.

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POSTER 105

OXYLIPIN PROFILING IN RESPIRATORY DISEASE

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Abstract: Oxylipins, including eicosanoids such as prostaglandins, leukotrienes and thromboxanes, act as lipid mediators involved in inflammation and cell signaling. Since Oxylipins are generated by oxidation of (poly-) unsaturated fatty acids via different pathways, including cylcooxygenase (COX), cytochrome P450 (CYP450) and lipoxygenase (LOX), a large and diverse group of target compounds exists. It encompasses various chemical classes including fatty acid-hydroperoxides, -alcohols, -epoxides, and -diols.
In order to detect and to quantify oxylipins, a targeted method is developed based on high performance liquid chromatography coupled to electrospray triple quadrupole mass spectrometry. To detect the large variety of oxylipins, individually optimized MRM transitions in the negative ion mode were monitored during a gradient elution of 22 minutes.
In order to obtain, both quantitatively and qualitatively, a full profile of inflammation related markers, viz. pro- and anti-inflammatory, we enlarged our target library to include eicosanoids not only produced by arachidonic acid, but also linoleic acid, dihomo-γ-linolenic acid as well as from α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid. Our analytical approach allows for the detection and quantification of more than 100 oxylipin compounds down to nanomolar level. Our methodology is applicable to a variety of bio-fluids such as plasma and serum, and was recently applied to bronchoalveolar lavage fluid (BALF) and exhaled breath condensate (EBC).
BALF and EBC are mainly used for diagnostics, but came more and more into perspective for research. BALF is collected from the lower respiratory tract and represents the local site of inflammation. EBC with its non invasive method of sampling is of high interest.
It is already known that oxylipins, as lipid mediators, are involved in inflammation processes in respiratory diseases. It is assumed that especially the 5-LOX derived compound leukotriene B4, as chemoattractant for polymorphonuclear leukocytes, plays a major role and is target of antagonists used for therapy. Our findings showed that besides 5-LOX derived oxylipins also 15-LOX derived oxylipins were highly abundant.
A variety of respiratory disorders are evaluated and latest results in conjunction with their biological rationale are discussed in this paper.

POSTER 106

TLR2 AND MyD88 SIGNALING ARE REQUIRED FOR RELEASE OF LIPID MEDIATORS AND FORMATION OF LIPID DROPLETS BY MACROPHAGES STIMULATED WITH MT-III, A SNAKE VENOM PHOSPHOLIPASE A2

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Abstract: Toll-like receptors (TLRs) are major components of the innate immune system and primary sensors for noxious stimuli. Upon activation these receptors initiate an immediate inflammatory response through the myeloid differentiation factor 88 (MyD88) adaptor protein signal transduction. TLRs are highly expressed on macrophages, which are key cells in inflammation. MT-III is a group II-A secreted phospholipase A2 (PLA2) with potent inflammatory action. This enzyme induces inflammatory events and activates macrophage functions, including release of inflammatory mediators and lipid droplets (LD) formation. However, the role of the TLR system in the inflammatory actions of venom PLA2s are unknown. This study examined the role of TLR2 and MyD88 adaptor protein in the release of eicosanoids (PGE2, PGD2, LTB4), cyclooxygenase-2 (COX-2) expression and LD formation induced by MT-III in macrophages. To this purpose, peritoneal macrophages were obtained from C57BL/6 wild type (WT) , TLR2-/- and MyD88-/- male mice and stimulated with MT-III (0.4 μM) or RPMI (control) for 6 hours. PGE2, PGD2 and LTB4 concentrations were determined by EIA and COX-2 protein expression by Western Blotting. LDs were stained with osmium tetroxide (1%) and counted under phase contrast microscopy. Results showed that stimulation of WT macrophages with MT-III caused a marked release of PGE2, PGD2 and LTB4, and increased numbers of LDs in comparison with controls. In MT-III-stimulated TLR2-/- macrophages, release of PGE2, LTB4 and PGD2 and formation of LDs were abrogated. In MyD88-/- macrophages, release of PGE2 induced by MT-III was abrogated, but release of LTB4 and PGD2 was maintained in comparison with WT macrophages. Moreover, the ability of MT-III to induce COX-2 protein expression seen in WT macrophages was significantly reduced in both TLR2-/- and MyD88-/- macrophages. In conclusion, TLR2 and MyD88 participate in the innate immune response to MT-III through activation of macrophages, since the absence of these pathways resulted in deficiency in release of lipid mediators and LD formation by these cells. TLR2 via MyD88 molecule signals to PGE2 biosynthesis, COX-2 expression and LD formation induced by MT-III. However, another adaptor molecule is involved in TLR2- mediated LTB4 and PGD2 biosynthesis induced by the PLA2.

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POSTER 107

CYP2J2 REGULATE PHAGOCYTOSIS IN HUMAN MONOCYTES

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Abstract: Epoxyeicosatrienoic acids (EETs) are generated by the activity of both selective and also more general cytochrome p450 (CYP) enzymes on arachidonic acid, and inactivated largely by soluble epoxide hydrolase (sEH), which converts them to their corresponding dihydroxyeicosatrienoic acids (DHETs). EETs have been shown to mediate diverse biological effects including relaxation of vascular tone, cellular proliferation and angiogenesis. However, their role in inflammation is less well defined. Although several CYP enzymes possess epoxygenase activity, the family member CYP2J2 is considered to be one of the most important epoxygenases in humans. Here we show that treatment of primary human peripheral blood mononuclear cells with 10ng/ml of lipopolysaccharide (LPS) caused significant induction of CYP2J2 mRNA that peaked around 4 hours and subsided by 24 hours, as measured by real-time RT-PCR. LPS treatment also led to induction of CYP2J2 mRNA and protein levels in the human monocytic cell line THP-1. We show that both the general epoxygenase inhibitor SKF525A, and the more specific CYP2J2 inhibitor C4, significantly inhibited the uptake of E.coli particles by THP-1 cells differentiated in to macrophages by PMA. Furthermore, this inhibition was attenuated by exogenous addition of both 11,12-EET and 14,15-EET respectively. Taken together, these data suggest that endogenous epoxygenase products produced by CYP2J2 play an important role in regulating phagocytosis in human monocytes. Potential mechanisms underlying this regulation will be discussed.

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POSTER 108

ENZYMES CHANGED BY SITE-DIRECTED MUTAGENESIS FOR NOVEL OXYLIPINS PRODUCTION

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Abstract: At the present time special attention is paid to question of providing biologics for correction of human metabolism, particularly fight against obesity and related disorders (for example, atherogenesis and diseases of the musculoskeletal system). Novel group of such perspective compounds comprise of conjugate linoleic acid and its derivatives. Since plant oxylipins are physiologically active derivatives of conjugated linoleic acid, research in this area will create a foundation for the development of competitive analogues of conjugated linoleic acid.

The lipoxygenase pathway of fatty acids metabolism plays a fundamental role in plant development and resistance. Crucial enzymes of this pathway are lipoxygenases and cytochromes P450 of CYP74 family comprised of allene oxide synthases (AOSs), hydroperoxide lyases (HPLs) and divinyl ether synthases (DESs). It was long thought that the substrates for the enzymes of this cascade are only endogenous fatty acids and hydroperoxides. Recently, the range of possible substrates of lipoxygenases has grown considerably. Necessary condition for conversion of the substrate is the presence of unsaturated fatty acid with conjugated double bond. In turn, features of the chemical structure determine the structure and physiological properties of the reaction products. Study of the catalytic properties of enzymes and change of their properties in order to obtain enzymes capable of converting complex substrates may expand our knowledge in this area. One of such methods is site-directed mutagenesis. We obtained number of different CYP74 mutant forms with drastically changed catalytic mechanisms compared with wild-type enzymes.

POSTER 109

MASS SPECTROMETRY IMAGING: TOWARDS A « LIPID MICROSCOPE »?

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Abstract: Biological imaging techniques are the most efficient way to locally measure the variation of different parameters on a tissue sections. These analyses allow observing extremely complex biological phenomena at lower and lower time and resolution scale. Nevertheless, most of them only target very few compounds of interest, which are chosen a priori, due to their low resolution power. New chemical imaging techniques have to be introduced in order to overcome these limitations, leading to more informative and sensitive analyses for biologists and clinicians. Two major mass spectrometry methods can be efficiently used to generate the distribution of biological compounds over a tissue section. Matrix- Assisted Laser Desorption/Ionisation-Mass Spectrometry (MALDI-MS) needs the co-crystallization of the sample compounds with a matrix before to be irradiated by a laser, whereas the analyte is directly desorbed after an ion bombardment for Secondary Ion Mass Spectrometry (SIMS) experiments. In both cases, the desorption/ionization probe is focused meaning that small areas over the surface sample can be separately analyzed. Step by step analysis allows acquisition of spectra over the tissue sections and the data are treated by modern software in order to plot the intensity of any specific ion versus the (x,y) position.

Due to their high amount and diversity in tissues, lipids are perfect candidate for the development and first biological applications of mass spectrometry imaging. Various applications, such as the distribution of glycosphingolipids in kidney sections from Fabry disease patients, fatty acid distribution in non-alcoholic fatty liver at a micron scale or the cholesterol distribution from Alzheimer's disease brains, will illustrate the complementarity of MALDI and SIMS analysis.

Mass Spectrometry imaging can thus be considered as a method of choice for studying the structure, composition and distribution of lipids directly from a tissue sections and with a minimal and reproducible sample preparation.

POSTER 110

CONVERSION OF THE LIPOXYGENASE OXYGENATION MECHANISM FROM SUPRAFACIAL TO ANTARAFACIAL

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Abstract: There are only two manganese lipoxygenases (LOX) known today, 13R-MnLOX and 9S-MnLOX; both are secreted by filamentous fungi. 13R-MnLOX abstracts the bis allylic hydrogen of alfa-linolenic acid and allows suprafacial attack of oxygen on the alkyl radical, forming 13R-hydroperoxy - and 11R-hydroperoxy metabolites. In contrast to mammalian and plant LOXs this oxygenation occurs in a suprafacial manner, allowing oxygen to be inserted from the same direction as the hydrogen abstraction.

In this study we can show that by replacing Phe337 of 13R-MnLOX with the corresponding Ile of soybean LOX-1, the oxygenation mechanism can be switched from suprafacial (100%) to antarafacial (75%). This mutant metabolized 18:2n-6 and 18:3n-3 to 13S- and 13R-hydroperoxides in a ratio of 3:1, without altering the pro-S hydrogen abstraction of C-11 of 18:2n-6.

Site-directed mutagenesis has previously been reported to switch the oxygenation specificity in LOXs as the hydrogen abstraction remains invariant. One way to is to change the substrate alignment in the active site, this has been demonstrated by swapping a Gly to an Ala in the Coffa- Brash determinant and by replacement of residues in the bottom of the active site in the Sloane determinant. However, the direction of oxygenation and hydrogen abstraction was unaltered. Different pH can also change the orientation of the substrate in the active site; acidic pH can allow the fatty acid carboxyl to enter to the bottom of the active site, resulting in oxidation in reversed head-tail orientation. In a similar way, unmasking of a positively charged amino acid in the bottom of the hydrophobic pocket of the active site can stabilize the carboxyl in reverse head-tail orientation.

In conclusion, we report for the first time how a modification in the LOX active site can switch the reaction mechanism from suprafacial to antarafacial by replacement of a single Phe.

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POSTER 111

EFFICACY OF ALTERNATIVE LIPID LOWERING THERAPY IN PATIENTS WITH STATIN INTOLERANCE

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Abstract: Cardiovascular disease is a very common and serious problem in the western world; first line management relates to lifestyle changes and addressing risk factors such as hypertension and hypercholesterolemia. Statin drug therapy is used in both primary, secondary prevention and in familial hypercholesterolemia. However, these are frequently associated with side effects ranging from muscle problems, elevated liver enzymes and neurological problems; causing poor adherence and thus putting patients at risk for future cardiovascular events. We identified 50 patients who had statin intolerance and assessed the affect of alternative lipid lowering therapy on lipid profile (total cholesterol or TC and LDL-cholesterol) and clinical outcome in the form of cardiovascular events. In patients with secondary prevention and familial hypercholesterolemia the recommended target is TC<4mmol/L and LDL-cholesterol <2 mmol/L where as in primary prevention there are no recommended targets. Pravastatin was the least intolerant where as Rosuvastatin most intolerant in our cohort; with the most common cause of discontinuation being muscle problems. Average duration of therapy for intolerance to statin was within a year. The patients intolerant were either commenced on alternative statin only (30%), alternative statin plus non-statin lipid lowering drug (50%) or on non-statin lipid lowering therapy only (20%). In secondary prevention group only 9.5% achieved the recommended target in lipid profile although there was reduction in TC and LDL. In primary prevention TC and LDL-cholesterol reduced by 16% and 19% from baseline respectively. The most effective therapy was statin plus ezetimibe in lowering the TC and LDL-cholesterol in our cohort. The most ineffective therapy was ezetemibe alone, blue acid sequestrate alone or with statin and omacor alone in lowering TC and LDL-cholesterol. None of the patients in secondary prevention had cardiovascular events where as 2 patients in primary prevention group had cardiovascular event whilst on alternative lipid lowering therapy.

Conclusion: The alternative lipid powering therapy in patients with statin intolerance was able to lower TC and LDL-cholesterol although the recommended target was not achieved in secondary prevention. This may be due to shorter duration of alternative therapy in these patients.

POSTER 112

A ROLE OF PULMONARY CYTOSOLIC PHOSPHOLIPASE A2 IN MOUSE MORTALITY BY PSEUDOMONAS AERUGINOSA INFECTION

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Abstract: Pseudomonas aeruginosa induces lung injury in numerous diseases including cystic fibrosis (CF). The cytosolic phospholipase A2α (cPLA2 α) releases arachidonic acid (AA) but its role in lung injury by P. aeruginosa infection is unknown. Here, we examined this role in mouse model of lung infection by P. aeruginosa PAK and CHA strains. The results showed that CHA caused mouse mortality and lung injury at higher levels than PAK with similar intensity of lung inflammation except for IL6 and LDH whose levels were higher in CHA-infected mice. cPLA2α -null mutation reduced animal mortality and LDH secretion without interfering with lung inflammation. In lung epithelial cells CHA triggered cPLA2α phosphorylation in parallel to AA, PGE2 and LDH at higher levels than PAK, which were reduced by cPLA2α inhibitors. MAPK p38 was proved to be involved in these processes. Finally, we examined the role of cPLA2α in CHA-induced mortality in CF mice previously shown to display increased pulmonary cPLA2α activity. CHA infection induced higher mortality in CF mice compared to their littermates. Inhibition of cPLA2α attenuated CHA-induced CF mouse mortality without interfering with lung inflammation. We conclude that cPLA2α plays a role in P. aeruginosa-induced lethality independently from lung inflammation and in part via cPLA2α -induced toxicity.

POSTER 113

ROLE OF FLAXSEED OIL IN REDUCING INFLAMMATION IN AN EXPERIMENTAL MODEL OF LUNG FIBROSIS INDUCED BY BLEOMYCIN IN RATS

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Abstract: Objectives: Our study aims to investigate whether Flaxseed (Linum usitatissimum) Oil (FO), used as a preventive treatment, can inhibit bleomycin induced lung fibrosis in rats.

Methods: Twenty Wistar rats, weighting 180–220g, were divided randomly into 2 groups: control group (G1, n = 10) and a treated group (G2, n = 10). All rats received a distilled water in G1 at dose of 2mL/kg bw and flaxseed oil in G2 at dose of 2mL/kg bw by daily gavages during 60 days. After this period of preventive treatment, pulmonary fibrosis was induced in all rats by bleomycin (4 mg/kg, single dose, intratracheally). Three days later, all rats were sacrificed and lungs were extracted for histological analysis (Inflammatory Index and Score Fibrosis). FO composition was analyzed by Gas Chromatography (GC). Total Polyunsaturated Fatty Acids (PUFAs) in the lungs and in the red cells (RC), into both groups, were measured also by GC. Data are presented as mean ±standard deviations (S.D), $P< 0.05$ was considered to be significant.

Results: Lung sections showed a significant reduction of inflammation (G1 = 3,3±0,48, G2 = 1,9±0,87, P= 0,001) and fibrosis (G1= 3,7±0,948, G2= 2,2±1,39, P = 0,012). Analysis by GC revealed that more than 50% of Flaxseed oil was C18:3, a gamma-linolenic acid. PUFAs in red cells are (16,56%) in G1 and (26 ,12%) in G2 and on the lungs are (24,96%) in G1 and (18,91%) in G2. We noticed that Arachidonic acid was the major component in RC in G2 (14,14±5,04%), decreased almost half in lungs (8,41±3,33%) on the same group compared to control group (RC: 7,96±6,95%, in lungs: 14,03±3,63%).

Conclusion: Based on these results: increasing intake of gamma-linolenic acid leads to an increasing production of arachidonic acid, the major fatty acid precursor of ecosanoids, modulators of inflammatory response observed in the lung fibrosis airways.

Key worlds: flaxseed oil, lung fibrosis, Inflammation, Fatty acids, Lipid mediators, rats.

POSTER 114

THE ROLE OF CDP-CHOLINE IN CENTRAL CARDIOVASCULAR AND RESPIRATORY SYSTEM: THE MEDIATION OF CENTRAL PROSTAGLANDINERGIC SYSTEM

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Abstract: Cytidne-5'-diphosphatecoline (CDP-choline) is an endogenously synthesized mononucleotide and intermediate product of membrane phosphatidylcholine. Recently we demonstrated that centrally or peripherally administrated CDP-choline caused pressor effect and cholinergic system mediated these effects of it. Also central prostaglandinergic system leads to pressor and bradycardic response by activating totally central prostaglandin receptors and partly central cholinergic nicotinic receptors in normotensive rats. The main object of the present study was to determine the effect of centrally injected CDPcholine on cardiovascular and respiratory system and the mediation of the central prostaglandinergic system on CDP-choline-induced cardiovascular and respiratory effects in normotensive rats.

Experiments were carried out in male Spraque Dawley rats. Intracerebroventricularly (i.c.v.) injected CDP-choline (0.5, 1 and 2 μmol) caused dose- and time-dependent pressor and bradycardic effect on cardiovascular system and increased respiratory rates, tidal volume and minute ventilation of normotensive rats. Also centrally injected CDP-choline accompanied by 62 % increase in extracellular prostaglandin concentration in the posterior hypothalamus, as shown in microdialysis studies. Moreover, nonselective COX inhibitor ibuprofen (250 mg; i.c.v.) almost completely and thromboxane A2 (TXA2) synthesis inhibitor furegrelate (250 mg; i.c.v.) partly blocked CDP-choline-evoked cardiovascular and respiratory effects,

In conclusion, results show that centrally administered CDP-choline causes pressor and bradycardic response on cardiovascular system and increases in conscious rats. Moreover, according to our findings, there is an involvement of the central prostaglandinergic system in CDP-choline-induced cardiovascular and respiratory.

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POSTER 115

EPA DERIVED PGE3 INHIBITS PROLIFERATION AND ANGIOGENESIS OF NON-SMALL CELL LUNG CANCER A549 CELLS THROUGH INHIBITION OF MTOR PATHWAY

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Abstract: The beneficial effects of EFA are believed to be due, in part, to selective alteration of arachidonic acid metabolism that involves cyclooxygenase enzymes (i.e. decrease formation of PGE2 and increase formation of PGE3). We previously reported that the anti-proliferative effect of EPA in human non-small cell lung cancer (NSCLC) A549 cells is associated with formation of PGE3, a COX-2 metabolite of the fish oil derived EPA (J. Lipid Res. 45: 1030-1039, 2004). Here we report the antiproliferative and antiangiogenic activity of EPA derived PGE3 in A549 cells and its associated mechanisms. At physiologically achievable concentration, PGE3 (0.1 to 0.5 μM) moderately inhibited the proliferation of A549 and H596 cells by approximately 30%. The reduction of colony formation has also been observed in PGE3 treated A549 and H596 cells in soft agar assay. Interestingly, there was no inhibition of normal human bronchial epithelial cells by PGE3. While the effect of both PGE2 and PGE3 on the cell cycle in A549 and H596 cells showed no significant changes, determination of cell migration resulted in a slower migration of HUVEC cells treated with PGE3 compared to PGE2. Additionally, PGE3 exhibited a dose dependent inhibition of tubular formation. Mechanistically, proteomic analysis suggested that PGE3 inhibited the phosphorylation of both PRAS40 and S6 proteins in A549 cells in a time and concentration dependent manner. Treatment with PI3K inhibitor (LY294002) enhanced the antiproliferative effect of PGE3 in A549 cells, suggesting PGE3 might also act downstream of PI3kinase pathway. Furthermore, the results of inflammation gene and proteomic arrays demonstrated that PGE3 has the ability to reduce the expression of the COX-2 gene and protein in A549 cells. Taken together, the data suggest that the antiproliferative effect of PGE3 might be mediated through inhibiting both mTOR and PI3kinase pathways in A549 cells. Given that we and other investigators have shown that EPA can inhibit mTOR signaling in NSCLC (A549) and human prostate cancer (LNCaP) cells (Friedrichs W, Nutr Cancer, 63: 771-777, 2011), we are currently investigating whether EPA-elicited mTOR inhibition is mediated through PGE3.

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POSTER 116

QUANTIFICATION OF NEUROPROSTANES BY LC-MS METHOD IN RAT BRAIN EXTRACTS

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Abstract: Docosahexaenoic acid (DHA) is an abundant fatty acid in the brain.1 Free-radical induced peroxidation of DHA results in the formation of "neuroprostanes" (NeuroP)2 which may be biomarkers of inflammation/oxidative stress in brain tissue. We describe here an LC-MS method for NeuroP in rodent brain tissue, using several synthetic NeuroP and applied in a study of rat brains.

Standards of F2-, F3-, and F4-NeuroP were synthesized using our flexible strategy, recently developed.3,4 Analysis was by UPLC interfaced to a QTof mass spectrometer with separation using reverse-phase chromatography and accurate mass detection5 to identify NeuroP. Preparation of the brain extracts was done using modification of a literature method where homogenized tissue was extracted into organic solvent and the phospholipids were cleaved by hydrolysis. NeuroP were isolated from an acidified solution using SPE, dried, and dissolved in mobile phase.

Preliminary results from rat brain extracts indicated numerous F4-NeuroP isomers but very little of the F2- or F3-NeuroP compounds which is consistent with previous literature reports. Thus, quantitation of F4- NeuroP isomers was done. Initial work looking at fragmentation patterns indicates 10-, 13-, and 17-F4-NeuroP isomers as the major species present in these rat brain extracts; however, it is unclear whether these are artifacts from the base hydrolysis step. Such a biomarker can be used as a tool in studies of brain tissue where determination of oxidative stress/inflammation is desired. This analytical method provides the sensitivity and isomer resolution that may identify any unique compounds related to oxidation in the brain.

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POSTER 117

ARE OXYGENATED METABOLITES OF n-3 POLY UNSATURATED FATTY ACIDS (EPA, DHA) NEW ANTIARRHYTHMIC AGENTS?

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Abstract: Some fishes are rich in particular fatty acids, the n-3 poly-unsaturated fatty acids (n-3 PUFA).
The effects of n-3 PUFA on cardiac function are still debated, notably because of the lack of information on underlying mechanisms. For example, it is not really known which the active lipid is: the PUFA or one of their oxygenated metabolites. A prospective study on a large number of patients showed that the most marked effect of PUFAs is a reduction of sudden cardiac death in the months following a cardiac infarction (GISSI-Prevenzione Investigators 1999). This benefit has been explained by a reduction of arrhythmias and of systolic cardiac failure, in parallel with other cardioprotective effects of n-3 PUFA (Saravanan 2010).
Experiments on single cardiac cells have shown that EPA and DHA can modulate the activity of ion channels, the transmembrane proteins responsible for the electrical activity of the heart (Judé 2006), which allowed establishing a mechanistic basis for the antiarrhythmic (AA) effects of n-3 PUFA. However, concomitantly, we have also shown that the effects of DHA on some rat cardiac ion channels are correlated with the oxidation of the fatty acid and not with the fatty acid itself (Judé 2003). Because the n-3 PUFA (EPA, DHA) are highly peroxidable, they undergo lipid peroxidation to form isoprostanes, neuroprostanes and neurofurans (Morrow 1990; Jahn 2008). We will present the strategy to perform the total synthesis (Oger 2010, 2012) in large quantities, of such oxygenated metabolites of EPA and DHA. Then, we will present our preliminary results on their effects on ionic currents and calcium homeostasis of cardiomyocytes.

POSTER 118

BIOTINYLATED AND PHOTOACTIVATABLE OMDM1 ANALOGS TO PROBE THE PUTATIVE ANANDAMIDE CARRIER

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Abstract: Endocannabinoids (eCB) are endogenous polyunsaturated fatty acids that bind and activate the same receptors as Delta9- tetrahydrocannabinol (THC) ie CB1R and CB2R. They are involved in a multitude of health and disease processes.1
To date, despite extensive investigations, the eCB system remains elusive.2
The anandamide cellular uptake is a current controversial topic, with results in favor of both passive and facilitated energy-independent diffusion.3 Clearly, pharmacological studies of the eCB system would benefit from the availability of chemical tools which would assist in the detection, isolation and characterization of this putative carrier.

Thus, in order to shed light on the anandamide transport process, we developed the synthesis of several novel probes containing:

* a photoactivatable group such as an aryl azide group or a diazirin moiety which may be converted, upon light irradiation, to highly reactive nitrene or carbene, respectively.
* and/or a biotin unit which can be detected by complexation with a supported streptavidin
* or a fluorescent group
* or a terminal alkyne function which can serve in some ‘in situ clickchemistry’ experiments with some commercially available fluorescent or biotinylated azides.

We have then examined the effect of the novel compounds on anandamide uptake from RBL-2H3 cells, where a putative anandamide transporter has been preliminary characterized.4

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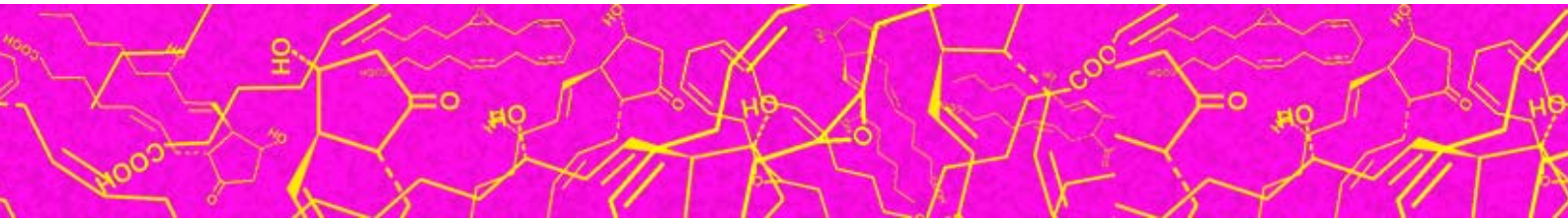

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