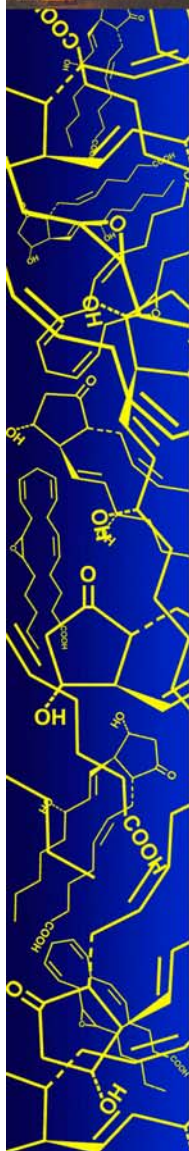


# Third European Workshop on Lipid Mediators Pasteur Institute, Paris June 3-4, 2010

Organising Committee:  
Jesús Balsinde, Gerard Bannenberg, Joan Clària,  
Francis Berenbaum, Xavier Norel, Lhousseine Touqui  
and



<http://workshop-lipid.eu>

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Under the auspices of



**Laurent Baud, INSERM U702, Tenon Hospital, Paris.**

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

Scientific Program - June 3, 2010

**SCIENTIFIC PROGRAM**

**JUNE 3**

**8:30-10:00 Registration, coffee and poster set-up**

**10:00-10:05 Welcome by the Organizers**

**Opening lecture**

**10:05-10:45 Charles N. Serhan (Brigham & Women's Hospital, HMS, Boston, USA)**  
Resolution of inflammation; A novel genus of anti-inflammatory and pro-resolving lipid mediators.

**Session 1. The Pharmacology of Lipid Mediators**

**Part 1: "Targets and Bullets in cardiovascular and respiratory diseases"**

**Chairpersons: Joan Clària & Xavier Norel**

**10:45-11:10 Jane A. Mitchell (Imperial College, London, UK)**  
Prostanoids in respiratory and vascular inflammation.

**11:10 -11:35 Ingrid Fleming (Goethe University, Frankfurt, Germany)**  
Epoxyeicosatrienoic acids & vascular function.

**11:35-11:55 Ignacio Garcia-Verdugo\* (Pasteur Institute, Paris, France)**  
A role for 12*R*-lipoxygenase in mucus production by respiratory epithelial cells.

**11:55-12:15 Gokce Topal\* (Istanbul University, Turkey)**  
Involvement of prostaglandin F<sub>2</sub>α in preeclamptic human umbilical vein vasospasm: a role of FP and TP receptors.

**12:15-12:35 Angelo Sala\* (University of Milan, Italy)**  
Transcellular biosynthesis of leukotrienes in vivo.

**12:35-14:35 Lunch**

**Part 2: "Targets and Bullets in inflammation, cancer, musculoskeletal and neuronal diseases"**

**Chairperson: Francis Berenbaum & Joan Clària**

**14:35-15:00 Sylvain Doré (Johns Hopkins University, Baltimore, USA)**  
Prostaglandins receptors in stroke.

**15:00-15:25 Oliver Werz (University of Frankfurt, Tübingen, Germany)**  
5-Lipoxygenase in health and disease.

**15:25-15:50 Leslie J. Crofford (University of Kentucky, Lexington, USA)**

mPGES-1 as a novel target for inflammation in musculoskeletal diseases.

**15:50-16:10 Marjolaine Gosset\* (Paris VI University, France)**

Inhibition of MMP-3 and MMP-13 synthesis induced by IL-1beta; in chondrocytes from mice lacking microsomal prostaglandin E synthase-1.

**16:10-16:30 Saul Yedgar\* (Hebrew University - Hadassah Medical School, Israel)**

Inclusive control of lipid mediator production: a multi-factorial approach to treatment of inflammatory/allergic diseases.

**16:30-17:00 Coffee Break: Poster session and Exhibition visit**

**Session 2. Non-mammalian Lipid Mediators**

**Chairperson: Gerard Bannenberg & Francis Berenbaum**

**17:00-17:25 Ernst Oliw (Uppsala University, Uppsala, Sweden)**

Fungal fatty acid dioxygenases.

**17:25-17:50 Ivo Feussner (Georg-August University, Göttingen, Germany)**

Oxylipins & host-pathogen interactions in plants.

**17:50-18:10 Kjetil Berge\* (Haukeland University Hospital, Bergen, Norway)**

Krill and fish oil: differences in composition and anti-inflammatory properties.

**18:10-18:30 Ivan Chechetkin\* (Kazan Institute of Biochemistry and Biophysics, Russia)**

Linolipin biosynthesis as a new kind of plant defense strategy.

**18:30-18:50 Maite Sanmartin\* (Centro Nacional de Biotecnología CSIC, Madrid, Spain)**

Increasing omega-3 desaturase expression in tomato results in altered aroma profile and enhanced resistance to cold stress.

**Closing Remarks for Day 1 Gerard Bannenberg**

**Cheese and Wine Session and Poster session**

**JUNE 4**

**9:00-9:05 Morning address by Francis Berenbaum**

**Session 3. Young Investigator Session**

**Part 1: 9:05 – 10:40 Chairperson: Charles Brink & Lhoussaine Touqui**

**8 Junior speakers, 8+4 min. selected from abstract submissions**

**Erika Villanueva (Irving K. Barber School of Arts and Sciences, Univ. British Columbia, Kelowna, Canada):** *Characterizing the role of secretory phospholipase A<sub>2</sub> group IIA in glial cell-mediated neurotoxicity.*

**David Balgoma (Instituto de Biología y Genética Molecular/CSIC, Valladolid, Spain):** *Markers of monocyte activation revealed by lipidomic profiling of arachidonic acid-containing phospholipids.*

**Sophie Ayciriex (Membrane Biogenesis laboratory, CNRS UMR5200, Bordeaux, France):** *Lipid species profiling of yeast mutants defective for putative glycerolipid acyltransferases by mass spectrometry.*

**Michela Rigoni (Dept. of Biomedical Sciences, University of Padova, Italy):** *Inverted cone-shaped lipids cause blockade and degeneration of nerve terminals.*

**Jeremy Bellien (Dept. Pharmacology, Rouen University Hospital & INSERM / University of Rouen, France):** *Cytochrome-derived eicosanoids and nitric oxide regulate arterial wall viscosity in vivo in humans.*

**Nicolas Cenac (INSERM U563, CHU Purpan, Toulouse, France):** *TRPV4 expression and activation in animal model of colitis.*

**Beatriz Díez-Dacal (Centro de Investigaciones Biológicas, CSIC, Madrid, Spain):** *Electrophilic prostaglandin 15d-PGJ<sub>2</sub> induces irreversible GSTP1-1 oligomerization and apoptosis in Jurkat T cells.*

**Olivier Beaslas (Minerva Foundation Institute for Medical Research, Helsinki, Finland):** *Decrypting Oxysterol Binding-protein Related Protein 8 (ORP8) functions.*

**10:40 – 11.05 Coffee Break: Poster session and Exhibition visit**

**Part 2: 11:05 – 12.30 Chairperson: Charles Brink & Xavier Norel**

**7 Junior speakers, 8+4 min. selected from abstract submissions**

**Manuela Oraldi (Dipartimento Medicina ed Oncologia Sperimentale, Univ. Torino, Italy):** *Effect of n-3 polyunsaturated fatty acids on cachexia in advanced lung cancer: study “in vivo” and “in vitro”.*

**Esther Titos (Dept. Biochemistry and Molecular Genetics, Hospital Clínic, Barcelona, Spain):** *Omega-3 fatty acids promote the resolution of adipose tissue inflammation by inducing M2 (alternative) macrophage polarization.*

**Valia Verriere (Departments of Molecular Medicine & Respiratory Medicine, Beaumont Hospital Dublin, Ireland):** *Lipoxin A<sub>4</sub> increases airway surface liquid height in cystic fibrosis and non-CF human bronchial epithelia.*

**Éva Ruisanchez. (Semmelweis University, Faculty of Medicine, Budapest, Hungary):** *Vascular effects of sphingomyelinase and sphingosine-1-phosphate.*

**Andy Liedtke (A.B. Hancock Jr. Memorial Laboratory for Cancer Research, and Vanderbilt University School of Medicine, Nashville, TN, USA):** *Development of cyclooxygenase (COX)-1-selective inhibitors derived from 2'-des-methyl sulindac sulfide.*

**Ingrid Gomez (Eicosanoids and Vascular Pharmacology, INSERM U698, CHU X. Bichat, Paris 13 University, Paris, France):** *Metabolism of PGE<sub>2</sub> in human varicose veins.*

**Sven-Christian Pawelzik (Department of Medicine, Karolinska University Hospital, Stockholm, Sweden):** *Identification of key residues determining species differences in inhibitor binding of microsomal prostaglandin E synthase 1.*

**12:30-14:15 Lunch**

#### **Session 4. Lipid Mediators in Innate Immunity**

**Chairperson: Lhousseine Touqui & Lisardo Boscá, CSIC, Madrid**

**14:15-14:40 María A. Balboa (Spanish Research Council, Valladolid, Spain)**  
Innate immune signaling & phospholipases.

**14:40-15:05 Jesper Z. Haeggström (Karolinska Institutet, Stockholm, Sweden)**  
Leukotrienes & innate immunity.

**15:05-15:30 Martin Thurnher (University of Innsbrück, Austria)**  
Dendritic cells and innate immunity.

**15:30-15:50 Janos Filep\* (University of Montreal, Canada)**

Aspirin-triggered lipoxins facilitate resolution of inflammation by promoting neutrophil apoptosis.

**15:50-16:10 Jonas Bystrom\* (William Harvey Research Institute, London, UK)**

Epoxygenases in control of macrophage phenotype.

**16:10-16:30 Pierre Borgeat\* (Centre de recherche du CHUQ, (CHUL), Quebec, Canada)**

The extra domain A of fibronectin primes leukotriene biosynthesis through Toll-like receptor-4 and stimulates human neutrophil migration.

**16:30-17:00 Coffee Break : Poster session and Exhibition visit**

**Session 5. Lipidomics**

**Chairperson: Jesús Balsinde & Gerard Bannenberg**

**17:00-17:25 Anthony Postle (University of Southampton, UK)**

The specificity of hepatic phosphatidylcholine synthesis in vivo by the CRP: Choline and PEMT pathways.

**17:25-17:50 Michel Lagarde (INSERM, Lyon, France)**

Poxotrans; a new family of bioactive triene derivatives.

**17:50-18:15 Michel Record\* (INSERM U563, Toulouse, France)**

The bioactive exosome vesicles involved in the immune response are transcellular vectors of lipolytic enzymes and prostaglandins.

**18:15-18:40 Gerard Lambeau (CNRS UMR 6097, Université Nice-Sophia Antipolis, France)**

Emerging and diverse roles of secreted phospholipases A<sub>2</sub> in physiology and pathophysiology: a particular focus on the group X enzyme.

**Workshop closing address by organizers**

\* indicates selected speakers from the abstracts



# **SELECTED ORAL SESSION**

**June 3, 2010**

**O-1 : A role for 12R-lipoxygenase in mucus production by respiratory epithelial cells**

IGNACIO GARCIA-VERDUGO 1,2,3, Sonja Tattermusch 1,2, Dominique Leduc 1,2 , Gilles Charpigny 4, Michel Chignard 1,2, Mario Ollero 5 and Lhousseine Touqui 1,2 #  
Institute address: From the 1Unité de Défense Innée et Inflammation, 2Unité Inserm U. 874, Institut Pasteur, Paris; the 3UFR Sciences du Vivant, Université Paris-Diderot; the 4Unité Biologie du Développement et Reproduction, INRA, Jouy en Josas and the 5INSERM U806, Paris, France.

Cytosolic phospholipase A2alpha (cPLA2alpha) is a key enzyme controlling the release of arachidonic acid (AA) from membrane phospholipids. Conversion of AA by cyclooxygenases (COX) and lipoxygenases (LOX) generates prostaglandins and leukotrienes, respectively. Mucus provides a protective barrier against pathogens and toxins and contributes to the innate defensive system in mucosal immunology. However, in chronic airway diseases such as asthma, chronic obstructive pulmonary diseases (COPD), or cystic fibrosis (CF), mucus is overproduced in the airways, which greatly contributes to airway obstruction in patients. Mucus is composed by water, ions, mucins, and lipids. We have previously observed that cPLA2alpha is involved in the mucus overproduction and mucin MUC5AC expression in CF mice. The aim of the present work was to identify AA metabolites and signalling pathways involved in mucin expression in the bronchial epithelial cell line NCI-H292. Our results showed that PMA-, LPS-, and TGFalpha-induced MUC5AC production was inhibited by cPLA2alpha inhibitors and mimicked by AA. MUC5AC expression was inhibited by a general LOX inhibitor (nordihydroguaiaretic acid or NDGA) but not by COX inhibitors (aspirin, NS398). Inhibitors of 12-LOX (cinnamyl-3,4-dihydroxy-alpha-cyanocinnamate or CDC and baicalein), but not those of 5-LOX or 15-LOX, reduced MUC5AC expression. These inhibitors also abrogated the expression of the mucins MUC5B and MUC2 and the production of whole mucus by cell monolayers. However, they failed to interfere with IL-8 secretion. 12-HETE, the first AA metabolite by 12-LOX, stimulated MUC5AC production. PMA stimulated ERK activation and SP-1 translocation, which were enhanced by 12-HETE and inhibited by baicalein. Two forms (R and S) of 12-LOX exist in mammals and 12R-LOX represents the only AA metabolizing enzyme producing compounds with R-chirality. Both 12-R-LOX and 12-S-LOX forms were expressed in NCI-H292. Using siRNA targeting 12-R-LOX or 12-S-LOX we showed that 12-R-LOX, but not 12-S-LOX, was involved in PMA-induced MUC5AC secretion. The latter was induced by the addition of 12-R-HETE. A recent study showed an increased expression of 12-LOX in sinonasal mucosa of CF patients. We conclude that 12-LOX may represent an interesting molecular target in the treatment of mucus overproduction in chronic obstructive lung diseases.

**O-2 : Involvement of prostaglandin F2alpha in preeclamptic human umbilical vein vasospasm: a role of FP and TP receptors**

GOKCE TOPAL<sup>1</sup>, Nabil Foudi<sup>2</sup>, B.Sonmez Uydes-Dogan<sup>1</sup>, Thierry Cachina<sup>2</sup>, Mine Kucur<sup>3</sup>, Altay Gezer<sup>4</sup>, Riza Madazli<sup>4</sup>, Osman Ozdemir<sup>1</sup> and Xavier Norel<sup>2</sup>

Institute address: <sup>1</sup>Istanbul University, Faculty of Pharmacy, Department of Pharmacology, Istanbul, Turkey, <sup>2</sup>INSERM U698: Haemostasis, Bio-engineering and Cardiovascular Remodeling, CHU X, Bichat, Paris, France, <sup>3</sup>Fikret Biyal Central Biochemistry Laboratory, <sup>4</sup>Department of Obstetrics and Gynecology, Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey.

**Objective:** Preeclampsia is characterized by hypertension and proteinuria developing after 20 weeks of gestation. The increased vasoconstriction can be one of the major underlying pathophysiological event in this syndrome. We examined the role of the vasoconstrictor prostanoid, prostaglandin F2alpha (PGF2alpha) in preeclamptic and normotensive human umbilical veins.

**Methods:** Umbilical veins were set up in organ bath. The concentration-response curves induced by PGF2alpha (endogenous agonist of FP receptor) and fluprostenol (FP receptor selective agonist) were determined in the absence or presence of BAY u3405 (TP receptor selective antagonist). PGF2alpha release and its concentration in maternal and umbilical cord serum were measured by enzyme immunoassay kit. The expression of vasoconstrictor prostanoid receptors was determined by western blot.

**Results:** The concentration-response curves to PGF2alpha and fluprostenol were significantly greater in human umbilical vein preparations derived from preeclamptic women compared to those of normotensives. BAY u3405 (10 µM) didn't modify the concentration-response curves induced by PGF2alpha in preparations obtained from normotensive women whereas reduced that contraction in the presence of preeclampsia. No difference was observed between preeclamptic and normotensive groups concerning its major metabolite, 13,14-dihydro-15-keto-PGF2alpha concentration in maternal serum. Nevertheless, it was significantly increased in umbilical cord serum of the preeclamptic group. Its release from human umbilical vein was also significantly augmented in the presence of preeclampsia. FP receptor protein expression was increased significantly whereas EP3 and TP protein expressions were unaltered in preeclamptic umbilical vein preparations.

**Conclusion:** FP and TP receptors activation by PGF2alpha could be involved in umbilical vasospasm observed in preeclampsia.

**O-3 : Transcellular biosynthesis of leukotrienes in vivo**

ANGELO SALA, Simona Zarini\*, Miguel Gijon\*, Robert C. Murphy\*

Institute address: \*Department of Pharmacology, University of Colorado Denver, Denver, CO, U.S.A.; Dipartimento di Scienze Farmacologiche, Università di Milano, Milano, Italy

Leukotrienes (LTs) are lipid mediators of inflammation formed by enzymatic oxidation of arachidonic acid. One intriguing aspect of LT production is transcellular biosynthesis: cells expressing 5-lipoxygenase (5LO) form LTA<sub>4</sub> and transfer it to cells expressing LTA<sub>4</sub> hydrolase (LTA<sub>4</sub>H) or LTC<sub>4</sub> synthase (LTC<sub>4</sub>S) to produce LTB<sub>4</sub> or LTC<sub>4</sub>. This process has been demonstrated in vivo for LTB<sub>4</sub>, but not for cysteinyl LTs (cysLTs). We examined transcellular cysLT synthesis during zymosan-induced peritonitis, using bone marrow transplants with transgenic mice deficient in key enzymes of LT synthesis and analyzing all eicosanoids by LC/MS/MS. WT mice time-dependently produced LTB<sub>4</sub> and cysLTs (LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>). 5LO<sup>-/-</sup> mice were incapable of producing LTs. WT bone marrow cells restored this biosynthetic ability, but 5LO<sup>-/-</sup> bone marrow did not rescue LT synthesis in irradiated WT mice, demonstrating that bone marrow-derived cells are the ultimate source of all LTs in this model.

Total levels of 5LO-derived products were comparable in LTA<sub>4</sub>H<sup>-/-</sup> and WT mice, but were reduced in LTC<sub>4</sub>S<sup>-/-</sup> animals. No differences in prostaglandin production were observed between these transgenic or chimeric mice. Bone marrow cells from LTC<sub>4</sub>S<sup>-/-</sup> mice injected into 5LO<sup>-/-</sup> mice restored the ability to synthesize cysLTs, providing unequivocal evidence of efficient transcellular biosynthesis of cysLTs. These results highlight the potential relevance of transcellular exchange of LTA<sub>4</sub> for the synthesis of LTs mediating biological activities during inflammatory events in vivo.

AS was the recipient of a W. Fulbright Research Scholarship.

**O-4 : Inhibition of MMP-3 and MMP-13 synthesis induced by IL-1beta; in chondrocytes from mice lacking microsomal prostaglandin E synthase-1.**

MARJOLAINE GOSSET, Audrey Pigenet, Colette Salvat, Francis Berenbaum, Claire Jacques.

Institute address: UR4 Paris Universitatis - University Pierre & Marie Curie Paris VI Aging, Stress and Inflammation Bat A 5ème étage ,7 Quai Saint-Bernard 75252 Paris Cedex 5

Joint destruction in arthritis is in part due to the induction of matrix metalloproteinases (MMP) expression, especially MMP-13 and -3, which directly degrade the cartilage matrix. Although IL-1beta is considered as the main catabolic factor involved in MMP-13 and -3 expression, the role of prostaglandin (PG)E2 remains controversial. The goal of this study was to determine the role of PGE2 on MMP synthesis in articular chondrocytes using mice lacking microsomal Prostaglandin E Synthase (mPGES)-1, which catalyses the rate limiting step of PGE2 synthesis.

MMP-3 and MMP-13 mRNA and protein expressions were assessed by real-time RT-PCR, immunoblotting and ELISA in primary cultures of articular chondrocytes from mice with genetic deletion of mPGES-1.

IL-1beta-induced PGE2 synthesis was dramatically reduced at 24h in mPGES-1<sup>-/-</sup> (-96%) and mPGES-1<sup>+/-</sup> (-64%) compared to mPGES-1<sup>+/+</sup> chondrocytes. 10ng/ml IL-1beta increased MMP-3 and MMP-13 mRNA, protein expression and release in mPGES-1<sup>+/+</sup> chondrocytes in a time-dependent manner. IL-1beta-induced MMP-3 and MMP-13 mRNA expressions decreased in mPGES-1<sup>-/-</sup> and mPGES-1<sup>+/-</sup> chondrocytes (respectively -75% and -80% for MMP-13; -58% and -65% for MMP-3 at 24h) compared to mPGES-1<sup>+/+</sup> chondrocytes. Moreover, IL-1beta-induced MMP-13 and MMP-3 protein expression and release decreased in mPGES-1<sup>+/-</sup> and mPGES-1<sup>-/-</sup> chondrocytes compared to mPGES-1<sup>+/+</sup> chondrocytes from 8h up to 24h. Finally, MMPs inhibition was partially reversed by addition of 10ng/ml PGE2 in mPGES-1<sup>-/-</sup> chondrocytes.

These results demonstrate that PGE2 plays a key role in the induction of MMP-3 and MMP-13 in an inflammatory context. Therefore, mPGES-1 could be considered as a critical target to counteract cartilage degradation in arthritis.

**O-5 : Inclusive control of lipid mediator production: A multi-factorial approach to treatment of inflammatory/allergic diseases.**

SAUL YEDGAR, Miron Krivsky, David Shosayov, Arie Ingber

Institute address: Department of Biochemistry; Hebrew University-Hadassah Medical School

Jerusalem, Israel 91120.

Arachidonic acid (AA)-derived prostaglandins and leukotrienes, produced by the COX and LOX pathways, are considered potent mediators of inflammatory/allergic diseases, and the control of their production has been a target of extensive efforts aimed at developing anti-inflammatory drugs. However, both COX and LOX pathways produce mediators of the same pathology, and inhibition of one pathway diverts the AA pool to the other pathway, and often exacerbates the disease. Thus, selective targeting of eicosanoid production pathways (e.g. COX-2) has produced disappointing results, as it overlooked the complex balance between eicosanoids from different pathways, some of which exhibit both pro- and anti-inflammatory activities (depending on the organs and disease stage) and may have essential physiological roles. In addition, the focus on eicosanoids ignores the concomitant production of lyso-phospholipids (Lyso-PL) that are potent pathogenic mediators. Therefore, the upstream control of phospholipase A2 (PLA2), producing AA and Lyso-PL, is considered a preferable approach. However, the PLA2s include the secretory (sPLA2s) and the intracellular (i/cPLA2) iso-enzymes. While sPLA2 is a key enzyme in induction of inflammatory/allergic processes, and should therefore be suppressed to treat these conditions, interference with the intracellular PLA2s might interfere with the vital phospholipid metabolism and cell viability. Accordingly, it has long been suggested that cell-impermeable sPLA2 inhibitors should provide the desired treatment. In addition, enrichment of cell-surface glycosaminoglycans (GAG) is desirable for cell protection, as their stripping exposes the cell to the action of exogenous sPLA2s and other inflammatory agents. To address both needs (sPLA2 inhibition and GAG enrichment), we linked GAG to PLA2-inhibiting (PLA2I) lipids. While the PLA2I incorporates into the cell membrane, its internalization is prevented by the GAG. At the same time, PLA2I anchors the GAG to the membrane, thereby enriching the cell surface protective layer. These lipid-conjugates, administered by different routes, have been found to be effective in the amelioration of diverse inflammatory/allergic conditions in animal models, including: sepsis, intestinal injury (IBD), encephalomyelitis (EAE) and asthma, as well as in two recent positive clinical studies in contact dermatitis and in allergic rhinitis. These multi-functional anti-inflammatory drugs introduce a novel strategy for the treatment of inflammatory/allergic diseases.

**O-6 : Effects of fish oil, krill oil and a modified thia fatty acid on DSS-induced inflammation, ROS-production, and mitochondrial dysfunction in rat colon**

Bodil Bjorndal 1, Tore Grimstad 2, Daniel Cacabelos 3, Natalya Vigerust 1, KJETIL BERGE 4, Trygve Hausken 1, Rolf Berge 1

Institute address: 1 Institute of Medicine, University of Bergen, Norway. 2 Stavanger University Hospital, Norway. 3 Department of Experimental Medicine, School of Medicine, University of Lleida, Spain. 4 Department of Heart Disease, Haukeland University Hospital, Bergen/Aker BioMarine, Oslo, Norway.

The anti-inflammatory effects of dietary n-3 fatty acids has been frequently reported, and is believed to be due to the inhibition of arachidonic acid-derived pro-inflammatory eicosanoids as well as effects on transcription factors that regulate inflammatory gene expression.

In this study we wished to compare the effect of EPA/DHA from different marine sources, as well as the effect of an artificially made sulphur-modified fatty acid on a rodent model of inflammatory bowel disease. Male Wistar rats were fed diets containing 5% fish oil, 5% krill oil or 0,8% TTA for 30 days, and the intake of 5% dextran sodium sulphate (DSS) in the drinking water during the last week induced colitis. During DSS-treatment, the weight of the animals and the disease activity index (DAI) were recorded, and after sacrifice, colon length was measured in all animals. The level of inflammation, ROS-production and the lipid profile of the colon were measured, and mitochondrial dysfunction was studied using transmission electron microscopy. We found that although all groups experienced weight loss, colonic shortening and elevated DAI following DSS intake, these parameters were significantly improved in the krill oil vs. the fish oil and TTA group. Equimolar amounts of EPA/DHA from different marine sources seemed to have different effects on DSS-induced colonic inflammation.

**O-7 : Linolipin biosynthesis as a new kind of plant defense strategy**

CHECHETKIN I.R., Blufard A.S., Yarin A.Y., Antsygina L.L., Mukhitova F.K., Grechkin A.N.

Institute address: Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Kazan, Russia

Oxygenated fatty acids and their metabolic derivatives, collectively termed oxylipins, play important roles in plant signaling and defense. They can be toxic or cytostatic compounds for plant pathogens and/or signal molecules for defense gene expression. Some oxylipins (jasmonates, traumatin and related C12 metabolites) are also known as phytohormones. Recently we found a new family of oxylipins, named linolipins, in leaves of flax (*Linum usitatissimum*) and meadow buttercup (*Ranunculus acris*). According to our data, linolipins represent an interesting case of chemical evolution of regulatory and antipathogenic molecules. This family consists of galactolipids containing the esterified residues of divinyl ether ( $\omega$ 5Z)-etherolenic acid. Unstressed flax plants possess only linolipin A. The inoculation of flax plants with *Pectobacterium atrosepticum* induces the biosynthesis of linolipins B and C and the accumulation of all mentioned linolipins in the leaves. To understand why flax and meadow buttercup plants produce linolipins, we studied properties of these oxylipins. It was found that linolipins were much less stable than ( $\omega$ 5Z)-etherolenic acid or its methyl ester at pH 5.5 and could be easily degraded to wound hormone traumatin and volatile oxylipin hexenal. Both metabolites are known to be products of lipoxygenase (LOX)-hydroperoxyde lyase (HPL) pathway in damaged plant tissues. We also observed the hexenal emission from the leaves of flax and meadow buttercup that had been injured by freezing, but they possessed no HPL activity. In this case, hexenal was obviously originated from linolipins. Our findings indicate that linolipins are a storage form of the compounds involved into defense responses to wounding and pathogen attack. The linolipin decomposition to traumatin ester and hexenal has apparent advantage in comparison with LOX-HPL pathway: it can proceed nonenzymatically and thus can occur even in the presence of enzyme inhibitors or proteases.

This work was supported by Grant 09-04-01023-#1072; from Russian Foundation for Basic Research.



**O-8 : Increasing omega-3 desaturase expression in tomato results in altered aroma profile and enhanced resistance to cold stress**

Teresa Domínguez<sup>1</sup>, M. Luisa Hernández<sup>2</sup>, Joyce C. Pennycooke<sup>3</sup>, Pedro Jiménez<sup>1</sup>, José Manuel Martínez-Rivas<sup>2</sup>, Carlos Sanz<sup>2</sup>, Eric J. Stockinger<sup>3</sup>, José J. Sánchez-Serrano<sup>1</sup> and MAITE SANMARTÍN<sup>1</sup>

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One of the drawbacks in improving the aroma properties of tomato fruit is the complexity of this organoleptic trait with a great variety of volatiles contributing to determine specific quality features. It is well established that the oxylipins hexanal and (*Z*)-hex-3-enal, synthesized through the lipoxygenase pathway, are among the most important aroma compounds and impart in a correct proportion some of the unique fresh notes in tomato. Here, we confirm that all enzymes responsible for the synthesis of these C6 compounds are present and active in tomato fruit. Moreover, due to the low odor threshold of (*Z*)-hex-3-enal, small changes in the concentration of this compound could modify the properties of the tomato fruit aroma. To address this possibility, we have over-expressed the  $\omega$ -3 fatty acid desaturases FAD3 and FAD7 that catalyzed the conversion of linoleic (18:2) to linolenic acid (18:3), the precursor of hexenals and its derived alcohols. Transgenic OE-FAD tomato plants exhibit altered fatty acid composition with an increase in the 18:3/18:2 ratio in leaves and fruits. These changes provoke a clear variation in the C6 content that results in a significant alteration of the (*Z*)-hex-3-enal/hexanal ratio that is particularly important in ripe OE-FAD3FAD7 fruits. In addition to this effect on tomato volatile profile, OE-FAD tomato plants are more tolerant to chilling. However, the different behavior of OE-FAD plants underscores the existence of separate FA fluxes to ensure plant survival under adverse conditions.

# **YOUNG SESSION**

**Selected oral**

**June 4, 2010**

**YS -1 : Characterizing the role of secretory phospholipase A2 group IIA in glial cell-mediated neurotoxicity.**

Authors: ERIKA VILLANUEVA, Dr. Andis Klegeris

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Both astrocytes and microglia are capable of initiating and maintaining inflammation in the brain. Though inflammation is necessary to ensure destruction of hazardous foreign bodies, the abnormal prolongation of inflammation can have detrimental effects on healthy neurons. For example, it is thought that the accelerated formation of amyloid-beta plaques in Alzheimer's disease (AD) induces chronic inflammation and results in gradual neuronal death.

Phospholipases constitute a large family of enzymes capable of producing inflammatory mediators from membrane phospholipids. It has been previously shown that astrocytes are capable of producing secretory phospholipase A2 group IIA (sPLA2IIA) under the conditions of cerebral ischemia. In addition, elevated levels of pro-inflammatory sPLA2IIA have been found in post-mortem brain tissues of AD patients. Therefore, the primary purpose of this study is to investigate the role sPLA2IIA may have in the chronic inflammatory process inherent to neurodegenerative disorders such as AD.

Using in vitro pharmacological approaches, this study has: 1) confirmed the neurotoxic effect of sPLA2IIA via exogenous application to neurons; 2) identified pro-inflammatory cytokines that initiate glial cell production and secretion of sPLA2IIA using reverse transcriptase polymerase chain reaction (RT-PCR) and an enzyme-linked immunosorbent assay (ELISA); 3) investigated the effects of removing sPLA2IIA from stimulated glial supernatants before their application to neuronal cells; and 4) explored the toxicity of sPLA2IIA after inhibiting its enzymatic activity. Promonocytic THP-1 cells, U373-MG astrocytoma cells and primary human astrocytes are used as glial cell surrogates, while human SH-SY5Y neuroblastoma cells model neurons.

RT-PCR results have confirmed that promonocytic THP-1 cells do not express sPLA2IIA mRNA unless stimulated with pro-inflammatory cytokines. Non-specific sPLA2 inhibitors did not reduce the neurotoxicity of stimulated microglial secretions; however, the removal of sPLA2IIA from stimulated microglial cell culture supernatants resulted in significantly decreased neurotoxicity. We are to propose a novel mechanism of sPLA2IIA involvement with glial cell neurotoxicity. Further experiments will establish whether the pro-inflammatory effect of sPLA2IIA is due to its enzymatic or non-enzymatic activity. Information from this research may aid in the discovery of specific targets for therapeutic intervention, which may lead to effective and much-needed treatment for patients suffering from a number of neurodegenerative diseases.

**YS -2 : Markers of monocyte activation revealed by lipidomic profiling of arachidonic acid-containing phospholipids**

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Stimulated human monocytes undergo an intense trafficking of arachidonic acid (AA) among glycerophospholipid classes. Utilizing high performance liquid chromatography coupled to electrospray ionization mass spectrometry, we have characterized changes in the levels of AA-containing phospholipid species in human monocytes. In resting cells, AA was found esterified into various molecular species of phosphatidylinositol (PI), choline glycerophospholipids (PC) and ethanolamine glycerophospholipids (PE). All major AA-containing PC and PI molecular species decreased in zymosan-stimulated cells; however, no PE molecular species was found to decrease. On the other hand, the levels of three AA-containing species increased in zymosan-activated cells compared to resting cells. These were 1,2-diarachidonyl-glycero-3-phosphoinositol (PI(20:4/20:4)), 1,2-diarachidonyl-glycero-3-phosphocholine (PC(20:4/20:4)), and 1-palmitoleoyl-2-arachidonyl-glycero-3-phosphoethanolamine (PE(16:1/20:4)). PI(20:4/20:4) and PC(20:4/20:4), but not PE(16:1/20:4), also significantly increased when platelet activating factor or phorbol myristate acetate were used instead of zymosan as stimulants of the monocytes. Analysis of the pathways involved in the synthesis of these three lipids suggest that PI(20:4/20:4) and PC(20:4/20:4) were produced in a deacylation/reacylation pathway via acyl-CoA synthetase-dependent reactions, while PE(16:1/20:4) was generated via a CoA-independent transacylation reaction. Collectively, our results define the rises of PI(20:4/20:4) and PC(20:4/20:4) as lipid metabolic markers of human monocyte activation.

**YS -3 : Lipid species profiling of yeast mutants defective for putative glycerolipid acyltransferases by mass spectrometry**

Authors: AYCIRIEX S., Ejsing C.S., Le Guedard M., Bessoule J.J., Lessire R., Shevchenko A., Schmitter J.M. and Testet E.

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In addition to their structural properties as main constituents of biological membranes, lipids play a multitude of roles such as in cell signalling, energy storage, and protein transport. Their biological importance has led to an increasing focus on analytical methods for the characterisation of their individual molecular species.

Improvements in mass spectrometric technology has provided a great advantage for the characterisation and quantification of molecular lipid species in total lipid extracts (Han and Gross, 2005; Murphy et al., 2001). For instance, phospholipid molecular species can be identified on the basis of a characteristic fragment of the lipid class, the nature of the acyl chains and their positions on the glycerol backbone. A method allowing the quantitative profiling of the yeast lipidome was developed in a recent study using automated shotgun infusion strategy (Ejsing et al., 2009). We applied this method to characterise several lysophospholipid acyltransferase yeast mutants produced using reverse-genetics. These enzymes are involved in essential biological processes like de novo synthesis or remodelling of the phospholipid membrane component (Testet et al., 2005; Le Guedard et al., 2009). The comparative analysis of phospholipid molecular species from the wild-type strain and the corresponding deletion mutants has allowed us to identify lipid compositional changes, and has given us significant indications about the in vivo function of the encoded lysophospholipid acyltransferases.

**YS -4: Inverted cone-shaped lipids cause blockade and degeneration of nerve terminals.**

Authors: MICHELA RIGONI, Paola Caccin, Marco Paoli, Ornella Rossetto, Erik Tedesco and Cesare Montecucco

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The venom of some Elapid snakes contains toxins endowed with enzymatic phospholipase A2 (PLA2) activity (SPANs) that paralyze the neuromuscular junction (NMJ) by depleting synaptic vesicles pools and cause degeneration of nerve terminals (1). In the last ten years our studies focussed in elucidating SPANs molecular mechanism of action and in particular the involvement of the PLA2 activity in the neurotoxic effects observed (2,3). Using in vitro and ex-vivo models, we found that lysophospholipids (LysoPLs) and fatty acids (FAs), generated by the PLA2 activity of the toxins on neuronal membranes, are the biochemical mediators of their neurotoxicity (4). Lysophospholipids are amphipatic molecules with an inverted-cone shape that remain confined to the outer plasma membrane monolayer, whereas fatty acids redistribute in both leaflets. LysoPLs on the outer layer of the presynaptic membrane induces a positive curvature of the bilayer, thus promoting the complete fusion with pore formation of those vesicles that are in a hemifused state, i.e. those vesicles whose outer membrane layer is already fused with the cytosolic leaflet of the presynaptic membrane. For the same biophysical reason, such membrane configuration inhibits the inverse process of membrane fission that is required for vesicle retrieval, thus impairing the exo-endocytic balance of the synapse. Moreover, we found that LysoPLs and FAs released by SPANs cause a large influx of extracellular calcium, which accounts for the extensive synaptic vesicle release (5). Similarly to mammalian NMJs, lysophospholipids cause an early increase followed by an inhibition of both spontaneous and evoked neurotransmitter release at *Drosophila* NMJs (6), indicating that LysoPLs are common agonists of membrane fusion, and inhibitors of membrane fission, at nerve terminals. In addition, at higher concentrations they form transient lipidic pores that allow calcium entry and calcium induced toxicity. Alterations in neurotransmitter release can be achieved by diverse molecules sharing the overall molecular shape of an amphipatic inverted cone but of entirely different chemical nature and biological properties (7). We are currently investigating different signalling cascades triggered by the massive calcium influx induced by SPANs or by lysophospholipids that ultimately lead to degeneration of nerve terminal.

1. Rossetto, O., Morbiato, L., Caccin, P., Rigoni, M., and Montecucco, C. (2006). *J. Neurochem.* 97, 1534–1545.
2. Rigoni, M., Schiavo, G., Weston, A. E., Caccin, P., Allegroni, F., Pennuto, M., Valtorta, F., Montecucco, C., and Rossetto, O. (2004). *J. Cell Sci.* 117, 3561–3570.
3. Bonanomi, D., Pennuto, M., Rigoni, M., Rossetto, O., Montecucco, C., and Valtorta, F. (2005). *Mol. Pharmacol.* 67, 1901–1908.
4. Rigoni, M., Caccin, P., Gschmeissner, S., Koster, G., Postle, A. D., Rossetto, O., Schiavo, G., and Montecucco, C. (2005). *Science* 310, 1678–1680
5. Rigoni, M., Pizzo, P., Schiavo, G., Weston, A. E., Zatti, G., Caccin, P., Rossetto, O., Pozzan, T., and Montecucco, C. (2007). *J. Biol. Chem.* 282, 11238–11245
6. Megighian, A., Rigoni, M., Caccin, P., Zordan, M.A., and Montecucco, C. (2007). *Neurosci. Lett.* 416, 6-11.
7. Caccin, P., Rossetto, O., and Montecucco, C. (2009). *Neurotoxicol.* 30, 174-181.

**YS -5 : Cytochrome-derived eicosanoids and nitric oxide regulate arterial wall viscosity in vivo in humans**

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Although the viscoelasticity of conduit arteries has been extensively investigated, few studies have focused on arterial wall viscosity (AWV) itself and its regulation by the endothelium in vivo. This is of particular importance since AWV is a major source of energy dissipation through the vascular system reducing cardiovascular coupling efficiency.

We simultaneously measured radial artery diameter and arterial pressure (NIUS02) in healthy volunteers before and during local infusion of pharmacological inhibitors of endothelial pathways. L-NMMA (8 micromol/min) was used as NO-synthase inhibitor, tetraethylammonium (TEA: 9 micromol/min), as blocker of calcium-activated potassium (KCa) channels, the target of endothelium-derived hyperpolarizing factors (EDHF) and fluconazole (0.4 micromol/min), as inhibitor of cytochrome epoxygenases which promote the synthesis of epoxyeicosatrienoic acids synthesis, identified as EDHF in human conduit arteries. AWV was estimated from the ratio of the area of the hysteresis loop of the pressure-diameter relationship to the area representing the whole energy exchanged during each cardiac cycle.

L-NMMA paradoxically reduced AWV (n=5: 27.6±0.7 to 23.4±0.7%, P=0.053). Conversely, AWV was increased by TEA (n=6: 25.5±0.5 to 31.3±0.7%, P=0.040) and fluconazole (n=5: 26.6±0.6 to 30.6±0.6%, P=0.047). This increase was more marked with the association of L-NMMA+TEA (n=6: 27.6±0.9 to 41.0±0.7%, P=0.002) and L-NMMA+fluconazole (n=6: 26.1±0.7 to 36.3±0.3%, P=0.001) showing a synergistic effect of both combinations on AWV. In parallel, we verified in isolated mice coronary arteries that fluconazole reduced the endothelium-dependent relaxations to acetylcholine as well as the specific inhibitor of cytochrome epoxygenases MS-PPOH. Moreover, fluconazole did not affect the relaxations to the openers of calcium-activated potassium channels of small and intermediate conductance NS309 and of large conductance NS1619 excluding a direct effect on these channels. At last, tetraethylammonium reduced the relaxations to NS1619 but not to NS309 suggesting that the cytochrome-related EDHF involved mainly acts on large conductance KCa channels.

These results demonstrate that the endothelium contributes in vivo in humans to the regulation of AWV through an interaction between NO and cytochrome-related EDHF. Therefore, the prevention of endothelial dysfunction appears a critical target to improve cardiovascular coupling and thus may help to limit the development of complications in cardiovascular diseases.

**YS -6 : TRPV4 expression and activation in animal model of colitis**

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In a previous study we demonstrated that local activation of TRPV4 provoked colonic inflammation. Arachidonic acid metabolites such as 5, 6-epoxyeicosatrienoic acid (5, 6-EET) have been suggested to act as endogenous agonists for TRPV4 activation. We thus investigated TRPV4 expression and the release of EETs and their metabolites hydroxy-eicosatetraenoic acid (HETE) and dihydroxy-eicosatetraenoic acid (diHETE) in a mouse colitis model.

Methods: Four groups of 10 mice were treated during 7 days with DSS 3% in drinking water. After 7 days, colons were removed in order to evaluate inflammation by micro and macroscopic damages scores and myeloperoxidase activity (MPO). TRPV4 expression was quantified by quantitative Rt-PCR. Its localization was evaluated by co-labeling of TRPV4 and cytokeratine 18 (marker of epithelial cells) or CD45 (marker of hematopoietic cells except erythrocytes and platelets). Lipids from mouse colon were extracted and EET, HETE and diHETE quantified by mass spectrometry after HPLC. In a second set of experiments, by calcium flux quantification, agonist properties of these metabolites and their commercially available standard (10µM) were studied on HEK-TRPV4 transfected cells or on human epithelial cells line (Caco2 cells) treated or not by a silencer RNA against TRPV4.

Results: 7 days treatment by DSS provoked an inflammatory reaction characterized by an increase in MPO activity, macro and microscopic damage score. TRPV4 mRNA expression was increased in the colon of inflamed mice compared to control. TRPV4 was localized predominantly on epithelial cells and was not observed on immune cells (CD45 positive cells). By LC-MS we observed a different profile of EET, HETE and diHETE expression between control and inflamed colon characterized by an increase in EET concentration. Exposure of HEK-TRPV4 transfected cells to lipid extracts only from inflamed mice colon provoked an increase in calcium flux compared to none transfected cells. Moreover, in Caco-2 cells, 5, 6-EET induced calcium signal through TRPV4 activation.

Conclusions: We have demonstrated in a mouse model of colitis that TRPV4 expression was increased. Moreover, we show an increase of TRPV4 agonist activity in lipid extracts from inflamed colon. These results position this receptor channel as a new potential therapeutic target for colonic inflammation.



**YS -7 : The electrophilic prostaglandin 15d-PGJ2 induces irreversible GSTP1-1 oligomerization and apoptosis in Jurkat T cells**

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The reactive lipid mediator 15-deoxy-Delta<sup>12,14</sup>-prostaglandin J<sub>2</sub> (15d-PGJ<sub>2</sub>) is generated during inflammation and tissue injury by dehydration of PGD<sub>2</sub>. 15d-PGJ<sub>2</sub> plays an important role in the modulation of inflammatory processes through the inhibition of the expression of pro-inflammatory genes, the induction of apoptosis in macrophages and the clearance of leukocytes from the site of inflammation, among other mechanisms. In fact, protective effects of 15d-PGJ<sub>2</sub> have been evidenced in various experimental models of inflammation. 15d-PGJ<sub>2</sub> and related cyclopentenone prostaglandins (cyPG) possess an alpha,beta-unsaturated carbonyl group that confers them the ability to form covalent adducts with cellular nucleophiles, including thiol groups in proteins.

Glutathione-S-transferases are ubiquitous enzymes which play a key role in the detoxification of both physiological and xenobiotic electrophilic compounds by catalyzing their conjugation with glutathione (GSH). GSTP1-1 is a member of this family, known to be over-expressed in several human tumors, which has been related to chemoresistance. In addition, GSTP1-1 may block stress signalling cascades by sequestering kinases involved in these processes, including JNK and TRAF-2.

We have recently shown that 15d-PGJ<sub>2</sub> binds to GSTP1-1 covalently and inactivates this enzyme irreversibly both in vitro and in cells. Here we show that 15d-PGJ<sub>2</sub> and other cyPG possessing a dienone structure induce the appearance of GSTP1-1 oligomers in cells. In contrast with GSTP1-1 oligomers produced after treatment with other oxidants or GSTP1-1 inhibitors, dienone cyPG-induced oligomeric species are irreversible. GSTP1-1 oligomers accumulate over time in 15d-PGJ<sub>2</sub>-treated cells. This is associated with a sustained activation of JNK, c-jun phosphorylation and induction of apoptosis. Moreover, cysteine 101, a residue present in the human but not in the murine enzyme, is essential for cyPG-induced GSTP1-1 crosslinking.

Taken together our results show a novel evidence for protein crosslinking by inflammatory mediators and delineate a hypothetical pathway in the modulation of inflammation, involving GSTP1-1 oligomerization, disruption of its interaction with stress kinases, JNK activation, and induction of apoptosis.

**YS -8 : Decrypting Oxysterol Binding-protein Related Protein 8 (ORP8) functions**

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ORP8 is a member of the OSBP-related protein family implicated in lipid metabolism, vesicle transport and cell signaling, and is highly expressed in macrophages. To understand the function of ORP8, we investigated the impact of ORP8 silencing on macrophage gene expression.

Microarray analysis of the transcriptome in mouse RAW 264.7 macrophages, silenced for ORP8 by shRNA lentiviruses, using Affymetrix GeneChipMouse Genome 430A 2.0 Arrays. The analysis was carried out on cells cultured in normal growth medium supplemented with 10% foetal calf serum. We used two independent shRNAs against ORP8 with a high (>90%) silencing efficiency. Data analysis was done with Genespring GX software, combined with a gene ontology pathway analysis.

Upon silencing of ORP8, the major effects detected were up-regulation transcripts for components involved in a number of nuclear and microtubule-associated functions. The nuclear functions affected include nucleosome assembly, DNA strand elongation, initiation of DNA replication, DNA unwinding, and DNA repair/homologous recombination. The microtubule-related functions involved spindle organization as well as centrosome organization and biogenesis. The most prominent mRNA changes were validated by quantitative PCR.

To search for protein interaction partners of ORP8, a yeast two-hybrid screen was carried out. Full length ORP8 cDNA in pGBKT7 was used as a bait, and human fetal kidney Matchmaker cDNA library (Clontech) as prey. Major interaction partners discovered were nucleoporin 62 (NUP62), a component of the nuclear pore complex, and SPAG5/Astrin, protein associated with the mitotic spindle apparatus. The finding was confirmed by specific pull-down of NUP62 by GST-ORP8 from HepG2 cell lysate.

We created a global knock-out mouse model for ORP8. Preliminary results (in mixed background animals) show gender specific modifications of plasma lipid levels.

The results suggest that ORP8 acts as a lipid-sensing factor involved in the control of nuclear and centrosome-associated functions, with specific impacts on lipid metabolism.

**YS -9 : Effect of n-3 polyunsaturated fatty acids on cachexia in advanced lung cancer: study “in vivo” and “in vitro”.**

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Cancer-related weight loss is different from simple starvation, in which refeeding restores normal nutritional status. In cancer patients, tumor-associated metabolic abnormalities prevent restoration of muscle mass by nutrient provision. Consequently, cancer-related malnutrition can evolve to cancer cachexia due to interactions between pro-inflammatory cytokines and host metabolism. Therefore nutritional support must have the aim to reduce inflammatory status. Polyunsaturated fatty acids (n-3) can modulate inflammation, reducing cytokine production. About this topic there are several, but contradictory, studies. Aim of this research was to evaluate: 1) on patients with lung cancer the effect of n-3 supplementation, determining oxidative status, pro-inflammatory cytokines, PGE<sub>2</sub>, nutritional status; 2) the effect of “in vitro” n-3 supplementation in a neoplastic cachexia model.

The “in vivo” study was a randomized double-blind placebo-controlled trial. Daily supplementation with n-3 in n-3 group or placebo in control group was given for 66 days. At different times the following parameters were evaluated: C-reactive protein, pro-inflammatory cytokines, PGE<sub>2</sub>, glutathione, ROS, HNE; body weight; the n-3 content in plasma and erythrocytes to evaluate the adherence to treatment.

The “in vitro” study evaluated the effect of n-3 on differentiation of muscle C2C12 cells grown in medium conditioned by human tumour lung cells A427.

As regards patient study, it was evidenced that some inflammatory parameters in n-3 group did not change during the time, whereas they increased in control group; IL-6 and PGE<sub>2</sub> decreased in n-3 group in comparison with control. Moreover, there was an improvement of oxidative status and body weight, and the level of n-3 increased in patients treated with these fatty acids, confirming the compliance to treatment.

As regards “in vitro” experiments, medium conditioned by A427 prevented myotube formation in muscle C2C12 cells; by contrast, when A427 cells were grown in the presence of n-3 for 24 or 48 hours, their conditioned medium induced the differentiation of C2C12.

Results from both “in vivo” and “in vitro” studies evidenced a beneficial effect of n-3 in reducing cachectic status.

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**YS -10 : Omega-3-polyunsaturated fatty acids promote the resolution of adipose tissue inflammation by inducing M2(alternative) macrophage polarization**

Authors: ESTHER TITOS, Ana González-Pérez, Cristina López-Vicario, Raquel Horrillo, Marta López-Parra, Eva Morán-Salvador, Marcos Martínez-Clemente, Vicente Arroyo, Joan Clària.

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The presence of a subclinical state of inflammation in adipose tissue is a key pathogenic factor in the development of insulin resistance and hepatic complications associated with obesity. In a recent study, we demonstrated that omega-3 polyunsaturated fatty acids (PUFA) prevent insulin resistance and hepatic steatosis in obese mice through modulation of adipose tissue function. To address mechanisms by which omega-3-PUFA exert these beneficial actions, in the current study we assessed the effects of the omega-3-PUFA docosahexaenoic acid (DHA) on adipocytes and stromal vascular cells (SVC). Male C57BL6/J mice were rendered obese by feeding a high-fat diet (60% kcal from fat) for 13 weeks and randomly divided into two groups receiving either DHA (4 micrograms/g/day via i.p., n=20) or placebo (saline, n=17) for 10 days. Adipocytes and SVC fractions were isolated from adipose tissue by collagenase digestion and subsequent centrifugation and the macrophage population was immunophenotyped by flow cytometry and the gene expression was analyzed by real-time PCR. The high-fat diet induced an increase in the number of double F4/80(+)/CD11b(+) cells, indicative of the presence of a larger population of tissue macrophages, associated with an increased expression of the pro-inflammatory adipokines IL-6 and MCP-1 in adipose tissue. The administration of DHA to these high-fat diet-induced obese mice did not modify the percentage of double F4/80(+)/CD11b(+) cells in adipose tissue, but it induced a change in the macrophage marker distribution associated with the alternative activation of these cells towards the M2 phenotype. This shift in macrophage polarization was characterized by increased expression of established markers of inflammation resolution such as IL-10, CD206 (mannose receptor C type 1), arginase 1, RELMalpha (resistin-like alpha) and Ym1 (chitinase-3-like protein 3). This anti-inflammatory scenario was associated with down-regulation of pro-inflammatory adipokines (TNFalpha and MCP-1) and up-regulation of anti-diabetic adipokines and insulin-sensitizing factors (i.e. adiponectin and PPARgamma). Finally, mice receiving DHA showed lower serum glucose and triglyceride concentrations and reduced hepatic steatosis. Taking together, these findings indicate that omega-3-PUFA exert anti-inflammatory actions on adipose tissue by mechanisms involving a phenotypic switch in macrophage polarization towards the alternative activation (M2) phenotype, thus promoting the resolution of inflammation.

**YS -11 : Lipoxin A4 increases airway surface liquid height in cystic fibrosis and non-CF human bronchial epithelia**

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Lipoxin A4 (LXA4) is an endogenous anti-inflammatory lipid mediator which is reduced in Cystic Fibrosis (CF) airway<sup>1</sup>. The altered Cl<sup>-</sup> secretion and Na<sup>+</sup> hyperabsorption in CF affects the Airway Surface Liquid (ASL) homeostasis and leads to a defective mucociliary clearance, chronic infection, inflammation and progressive lung destruction. In a previous study, we have shown that LXA4 stimulated Ca<sup>2+</sup>-activated Cl<sup>-</sup> secretion in airway epithelium<sup>2</sup>. The aim of this study was to investigate the potential role of LXA4 in modulating ion transport and ASL height in CF and non-CF airway epithelia. CF (CuFi-1) and non-CF (NuLi-1) bronchial epithelial cell lines were grown into well-differentiated polarised epithelia. LXA4 effects were explored using confocal fluorescence microscopy to measure ASL height, short-circuit current and whole cell patch-clamp to investigate ion transporter activity, and Fura2-AM for intracellular Ca<sup>2+</sup> imaging.

The spontaneous steady-state ASL height was lower in CF than non-CF epithelium. LXA4 (1nM) treatment for 15 minutes, increased ASL by a third in Nuli-1 epithelia (n=18) and doubled ASL in CuFi-1 epithelia (n=19). This effect was sustained over 24 hours in the CF epithelia. The increase in ASL height by LXA4 was inhibited by Boc-2 (FPR2 receptor inhibitor), bumetanide (NKCC1 co-transporter inhibitor), amiloride (ENaC inhibitor), reactive blue (P2Y receptor antagonist) and extracellular hexokinase (ATP hydrolysis). In addition, LXA4 stimulated an intracellular Ca<sup>2+</sup> mobilization, activated a Ca<sup>2+</sup>-dependent Cl<sup>-</sup> secretion and inhibited Na<sup>+</sup> absorption in the non-CF and CF epithelia.

Taken together, our results provide evidence for a novel effect of LXA4 involving the FPR2 receptor, ATP secretion and purinoreceptor activation, inhibition of Na<sup>+</sup> absorption and stimulation of Cl<sup>-</sup> secretion in CF and non-CF epithelia to finally increase ASL height. These novel pro-resolving effects of LXA4 open up a new therapeutic avenue in the treatment of CF.

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References.

1: Karp CL et al, 2004 Defective lipoxin-mediated anti-inflammatory activity in the cystic fibrosis airway. *Nat Immunol.* 2004 Apr;5(4):388-92.

2. Bonnans et al. 2003. Lipoxin A4 stimulates a cytosolic Ca<sup>2+</sup> increase in human bronchial epithelium. *J Biol Chem.* 278(13):10879-84.

**YS -12 : Vascular effects of sphingomyelinase and sphingosine-1-phosphate**

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Sphingolipids, derived from sphingomyelin metabolism, have been implicated as important mediators in the cardiovascular system. Sphingomyelinase (SMase) catalyzes the conversion of sphingomyelin to ceramide, which is the precursor of other sphingolipid mediators, e.g. sphingosine-1-phosphate (S1P). Both the endothelium and the vascular smooth muscle express receptors of S1P which mediate diverse vascular effects. On the other hand, sphingolipid mediators may have biological effects independently of the activation of S1P receptors. In the present study we show that the vascular effects of SMase are only partly related to S1P receptors.

Segments of the thoracic aorta have been isolated from adult male C57Bl6 (WT) as well as endothelial nitric oxide synthase deficient (eNOS-KO) and cyclooxygenase-1 deficient (COX1-KO) mice. Vascular effects of SMase and S1P have been analyzed by isometric tension recording after precontraction with phenylephrine. Both agents induced biphasic vascular responses. However, while the effect of SMase was a transient contraction followed by sustained relaxation, S1P induced a transient relaxation followed by a strong, tonic contraction. The relaxant effect of both SMase and S1P could be blocked by the NOS-inhibitor L-NAME in WT and was absent in eNOS-KO vessels. On the other hand, the constrictor effect of SMase but not that of S1P could be blocked by the COX-inhibitor indomethacin in WT and was absent in COX1-KO vessels. In L-NAME-treated COX1-KO as well as in indomethacin-treated eNOS-KO vessels SMase failed to induce any tension change while S1P evoked strong, tonic contraction.

These results demonstrate that both SMase and S1P induce endothelial NO-mediated vasorelaxation indicating that these effects may be mediated by the same S1P receptor(s). However, while the vasoconstrictor effect of S1P is independent of prostanoids, SMase induced contraction is mediated by COX1. Therefore, a sphingolipid mediator independent of S1P and its receptors appears to induce the release of vasoconstrictor prostanoid(s). Furthermore, in contrast to exogenous S1P, which induce sustained contraction, endogenously produced S1P appears to be rather vasorelaxant in nature.

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**YS -13 : Development of cyclooxygenase (COX)-1-selective inhibitors derived from 2'-des-methyl sulindac sulfide**

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Cyclooxygenase(COX)-1, but not COX-2 is highly expressed in human malignant ovarian cells and represents a potential target for the prevention and treatment of ovarian cancer.[1] COX enzymes are broadly addressed by non-steroidal anti-inflammatory drugs (NSAIDs). The pharmacological profiles of the available NSAIDs are complex and may include both COX-dependent and COX-independent actions, which leads to direct and indirect effects against tumor cells. Thus, the use of COX-1-selective inhibitors may be more plausible than a therapy with unselective COX inhibitors or even COX-2-selective compounds.[1]

Sulindac sulfide (SS), a benzylidene-indene derivative, is a potent, time-dependent inhibitor of COX-1 and -2. Removal of the 2'-methyl group from the indene ring not only dramatically reduces time-dependent inhibition of both COX isozymes but also changes the geometry of the benzylidene double bond from Z to E.[2] The recent discovery that (E)-2'-des-methyl SS (LM-4503) is a weak (IC<sub>50</sub> = 1.8 μM) but selective COX-1 inhibitor represents the first report of selective COX-1 inhibition by a member of the arylacetic acid class of inhibitors.[2] Against this background and the possible non-COX related therapeutic potential of the des-methyl sulindac analogues, the objective of our study is to optimize the anti-COX-1 potency and at the same time completely eliminate the COX-2 inhibitory activity. By following a concrete derivatization strategy, several new compounds are being generated and evaluated for their COX-1/-2 inhibitory potential in a routine COX assay. The most promising compounds are intended for additional experiments, e. g. in intact cells, and/or state-of-the-art animal models. The biochemical screening of a first series of free acid analogues of 2'-des-methyl SS already led to the identification of a promising inhibitor candidate (LM-4624) bearing a biphenylidene substituent instead of the benzylidene residue in LM-4503, which was selected for follow-up trials and currently serves as our lead structure. The COX-1-IC<sub>50</sub> value of LM-4624 was found to be as low as 565 nM and this compound did not markedly inhibit COX-2 under the test conditions used. Furthermore, we presume that the E-isomer of 2'-des-methyl SS and its benzylidene analogs bind in a completely different orientation within the active site of COX, compared to their origin SS, which is presently under investigation by X-ray crystallography.

**Acknowledgements:** This work is supported by the National Cancer Institute (CA89450) and the National Foundation for Cancer Research

**References:**

1. Daikoku, T.; Wang, D.; Tranguch, S.; Morrow, J. D.; Orsulic, S.; DuBois, R. N.; Dey, S. K. Cyclooxygenase-1 is a potential target for prevention and treatment of ovarian epithelial cancer. *Cancer Res.* 2005, 65, 3735-3744.
2. Walters, M. J.; Blobaum, A. L.; Kingsley, P. J.; Felts, A. S.; Sulikowski, G. A.; Marnett, L. J. The influence of double bond geometry in the inhibition of cyclooxygenases by sulindac derivatives. *Bioorg. Med. Chem. Lett.* 2009, 19, 3271-3274.

**YS -14 : Metabolism of PGE2 in human saphenous varicose veins.**

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Varicose veins are elongated and dilated veins. They are characterized by venous backflow and blood stagnation. In western countries, this pathology affects approximately one-third of the adult population, however, the pathogenesis of varicosities disorder is unclear.

AIMS: The aim of the present work was to study the role of Prostaglandin E2 (PGE2) in varicose veins formation. PGE2, a lipid mediator, is synthesized from arachidonic acid metabolism. Different enzymes are involved: cyclooxygenase (COX), microsomal PGES (mPGES) -1, -2 and cytosolic PGES (cPGES). PGE2 is a vasodilator and could be the cause of the dilatation of varicose veins. During inflammation, mPGES1 and COX-2 are mainly responsible for PGE2 synthesis. Several studies showed that PGE2 production is upregulated in inflammatory vascular disorders (such as atherosclerosis).

METHODS: In order to study the different COX and PGESs expressions, proteins were extracted from human varicose veins (small and large diameter) or from healthy saphenous veins obtained at Bichat hospital (Paris). The PGESs expression were detected and compared by Western blot analysis. In addition, PGE2 production by the different venous preparations was measured and compared by ELISA.

RESULTS: COX-2, mPGES -1 and -2 are expressed in varicose and healthy saphenous veins. However, we observed a significant decreased expression of mPGES1 in varicose veins (large diameter) with regard to the healthy and less pathological preparations (small diameter).

CONCLUSION: In contrast to our expectations, mPGES1 is decreased, that could be due to a negative feed-back, in the more pathological veins. These results show that PGE2 and PGES play a role during varicose vein formation.

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**YS -15 : Identification of key residues determining species differences in inhibitor binding of Microsomal Prostaglandin E Synthase 1**

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Microsomal Prostaglandin E Synthase-1 (MPGES1) is induced during an inflammatory reaction from low basal levels by pro-inflammatory cytokines and subsequently involved in the production of the important mediator of inflammation, prostaglandin (PG) E<sub>2</sub>. Non-steroidal anti-inflammatory drugs (NSAIDs) prevent PGE<sub>2</sub> production by inhibiting the upstream enzymes Cyclooxygenase (COX)-1 and COX-2. In contrast to these conventional drugs, a new generation of NSAIDs targets the terminal enzyme MPGES1. Some of these compounds potently inhibit human MPGES1 but do not have an effect on the rat orthologue. We investigated this interspecies difference in a rat/human chimeric form of the enzyme as well as in several mutants and identified key residues Thr-131, Leu-135, and Ala-138 in human MPGES1 that play a crucial role as gate keepers for the active site of MPGES1. These residues are situated in transmembrane helix (TM) 4, lining the entrance to the cleft between two subunits in the protein trimer, and regulate access of the inhibitor in the rat enzyme. Exchange towards the human residues in rat MPGES1 was accompanied with gain of inhibitor activity, while exchange in human MPGES1 towards the residues found in rat abrogated inhibitor activity.

Our data give evidence for the location of the active site at the interface between subunits in the homotrimeric enzyme and suggest a model of how the natural substrate PGH<sub>2</sub>, or competitive inhibitors of MPGES1, enter the active site via the phospholipid bilayer of the membrane.

# **SELECTED ORAL SESSION**

**June 4, 2010**

**O-9 : Aspirin-triggered lipoxins facilitate resolution of inflammation by promoting neutrophil apoptosis**

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Neutrophils play a central role in innate immunity. Their timely removal from inflamed tissues is essential for efficient resolution of inflammation. Recent data indicate that the acute-phase protein serum amyloid A (SAA) and the neutrophil azurophilic granule constituent myeloperoxidase (MPO) signal through the formyl peptide receptor 2 (FPR2)/lipoxin receptor and the beta2-integrin Mac-1 (CD11b/CD18), respectively to rescue neutrophils from apoptosis and to parallel with prolonging tissue injury. Since aspirin-triggered 15-epi-lipoxin A4 (15-epi-LXA4) and its metabolically stable analog 15-epi-16-p-fluorophenoxy-LXA4 also bind to FPR2 and inhibit Mac-1 expression, we studied their impact on neutrophil apoptosis and the resolution of inflammation. In human neutrophils, 15-epi-LXA4 and 15-epi-16-p-fluorophenoxy-LXA4 attenuated MPO-induced upregulation of Mac-1 expression and overcame the powerful anti-apoptosis signal from SAA and MPO. 15-epi-LXA4 promoted neutrophil apoptosis by attenuating MPO or SAA-induced ERK and Akt activation and by reducing expression of the anti-apoptotic protein Mcl-1. These led to collapse of mitochondrial transmembrane potential, cytochrome c release and subsequent activation of caspase-3. The pro-apoptotic action of 15-epi-LXA4 was predominant over MPO or SAA-mediated effects even when 15-epi-LXA4 was added at 4-hour post-MPO or SAA. In mice, treatment with 15-epi-LXA4 accelerated the resolution of established carrageenan plus MPO-evoked and live *E. coli*-induced pulmonary inflammation through redirecting neutrophils to caspase-3-mediated cell death. 15-epi-LXA4 reduced pulmonary neutrophil accumulation, edema formation and IL-6 release, and enhanced recruitment of monocytes/macrophages and phagocytosis of apoptotic neutrophils. The pan-caspase inhibitor zVAD-fmk fully abolished the beneficial actions of 15-epi-LXA4. These results show that aspirin-triggered 15-epi-LXA4 enhances resolution of acute lung inflammation by redirecting neutrophils to apoptosis, and identify a new mechanism by which aspirin promotes resolution of neutrophil-mediated inflammation. (Grant support: CIHR MOP-64283).

**O-10 : Epoxygenases in control of macrophage phenotype**

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Epoxygenases, cytochrome p450 enzymes, of the CYP2 family are producers of the anti-inflammatory lipids epoxyeicosatrienoic acids. These enzymes are increasingly recognized as being expressed by inflammatory cells but their functions there are unknown. We analysed macrophage epoxygenases expression and found CYP2J2, CYP2C8 and CYP2C9 mRNA present in the cells. Dependent on exogenous stimulation macrophages display an inflammatory or alternatively activated phenotype. We wanted to determine whether epoxygenase activity contributes to macrophage phenotype. Human peripheral blood mononuclear cells (PBMCs) were differentiated for four days with LPS and interferon gamma resulting in COX2 and TNF alpha expressing M1 macrophages. In contrast differentiation using IL-4 resulted in DC-SIGN expressing M2 macrophages. mRNA for the three epoxygenases CYP2J2, CYP2C8 and CYP2C9 were found in both M1 and M2 macrophages. IL-4 stimulation of THP-1 macrophages caused upregulation of CYP2J2 while TNF production was reduced. The influence of epoxygenases on macrophage phenotypes was assessed by the addition of the pan-epoxygenase inhibitor SKF525-A. Addition of SKF525A (10 uM) induced further TNF alpha mRNA in PBMC M1 and THP-1 macrophages. Addition of the epoxygenase product 14,15 epoxyeicosatrienoic acid reversed this increase in TNF alpha. In contrast, in anti-inflammatory M2 and IL-4 treated THP-1 macrophages addition of SKF525-A (10uM) resulted in a further decrease in TNF alpha mRNA and protein production, and in the case of M2 macrophages a reduction in CD36 mRNA expression. In conclusion, CYP2 family epoxygenases are expressed in M1 and M2 macrophages and their activities appear to limit the extent of the phenotype development in each of the cells.

**O-11 : The extra domain A of fibronectin primes leukotriene biosynthesis through Toll-like receptor-4 and stimulates human neutrophil migration**

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A fibronectin isoform containing an extra domain A (EDA) is generated by alternative splicing under pathologic conditions such as rheumatoid arthritis. EDA has been identified as an endogenous TLR4 ligand but its impacts on lipid mediator biosynthesis and neutrophil trafficking remain to be investigated. Leukotriene(LT)B<sub>4</sub> and polymorphonuclear neutrophils (PMN), however, were shown to play a critical role in inflammatory arthritis in animal models. We therefore aimed to elucidate the effect of EDA on leukotriene biosynthesis and PMN migration. Incubation of freshly isolated human PMN with 100-300 nM recombinant EDA for 30 minutes efficiently primes LT biosynthesis stimulated by formyl-methionyl-leucyl-phenylalanine(f-MLP) and platelet-activating factor. This priming effect was not blocked by polymyxin B (up to 25 µg/ml) whereas priming with lipopolysaccharides(LPS) was completely blocked with 1 µg/ml polymyxin B demonstrating that the observed effects of EDA were not due to a contamination of EDA by LPS. EDA caused Ser-505 phosphorylation of the cytosolic phospholipaseA<sub>2</sub>-alpha (as assessed by band shift in SDS-PAGE) and primed PMN for the release of arachidonic acid by f-MLP, effects which likely account for the observed ability of EDA to upregulate LT biosynthesis. EDA also stimulated PMN transendothelial migration in vitro, which was inhibited by 50-60% by the LTB<sub>4</sub> receptor(BLT1) antagonist CP105,696 or the cytosolic phospholipase A<sub>2</sub> alpha inhibitor pyrrophenone. The TLR4 signalling inhibitor CLI-095 (also known as TAK-242) inhibited LT biosynthesis in PMN stimulated with EDA or LPS and f-MLP, but not by other priming agents such as TNF-alpha, GM-CSF, the TLR7/8 ligand Resiquimod, the TLR2/6 ligand Pam2CSK4, or the ionophore A23187 and thapsigargin. Moreover EDA induced PMN recruitment to the mouse dorsal air pouch in C3H/HeO<sub>u</sub>J mice expressing TLR4, whereas it did not stimulate PMN migration in C3H/HeJ mice naturally lacking TLR4. Altogether, these results suggest an important role of EDA in promoting leukotriene biosynthesis and PMN migration through the activation of TLR4. These results provide new insights on putative mechanisms of regulation of LTB<sub>4</sub> biosynthesis and PMN recruitment in arthritis.

**O-12 : The bioactive exosome vesicles involved in the immune response are transcellular vectors of lipolytic enzymes and prostaglandins**

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Exosomes are bioactive vesicles of 50-100 nm released from multivesicular bodies by intact cells and which participate to intercellular signalling. A network of circulating exosomes in the human body has been revealed by their characterization in biological fluids. They appear as stable vesicles able to remain for long periods of time in lymph nodes to maintain antigen presentation. Phase I clinical trials using dendritic cell-derived exosomes loaded with antigenic tumor peptide have been successfully performed in patients bearing melanoma, and exosomes appear as promising tools for cancer immunotherapy. Prostaglandins are involved in the differentiation and maturation of dendritic cells (DC). We have therefore investigated the role of exosomes as potential vehicles of lipid mediators.

Exosomes derived from the mast cell line RBL-2H3 displayed a specific lipid composition and an unusual membrane organisation characterized by the absence of phospholipid asymmetry subsequent to the presence of a phospholipid scramblase. They exhibit the phospholipase D/ Phosphatidate phosphatase pathway (PAP1) leading to the formation of diglycerides. RBL-2H3 exosomes also carried members of the three phospholipase A2 classes, i.e. the calcium-dependent cPLA2-IVA, the calcium-independent iPLA2-VIA and the secreted sPLA2-IIA and V. Interestingly, almost all members of the Ras G-Protein superfamily were present, and unexpectedly, incubation of exosomes with GTP $\gamma$ S triggered activation of all the PLA2s classes. A large panel of free fatty acids, including arachidonic acid, and derivatives such as prostaglandin PGE2 and 15-deoxy- $\Delta$ 12,14-prostaglandin J2 (15d-PGJ2) were detected. We observed that exosomes were internalized by resting and activated cells, and that they accumulated into an endosomal compartment. Endosomal concentrations were in the micromolar range for prostaglandins, i.e concentrations able to trigger prostaglandin-dependent biological responses such as PPAR $\gamma$  activation in target cells. Therefore exosomes are able to shuttle between cells and to carry GTP-activable phospholipases and prostaglandins from cell-to-cells, therefore possibly accounting for the transcellular metabolism of eicosanoids.

# **POSTERS**

**P -01 : Leptin production and lipid release by bone marrow adipocytes regulate in vitro the evolution of a myeloid leukemic cell line**

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Bone marrow (BM) adipocytes are described as negative regulators of hematopoiesis and could also influence malignant hematopoietic cells. To test this hypothesis we realized co cultures of a leukemic myeloid precursor cell line with BM adipocytes or with a BM stromal cell line (HS-5) as control. BM adipocytes, but not HS-5, produce high levels of leptin (100pg/ml) and induce accumulation of lipids in leukemic cells (LC). They increase OB-R (leptin receptor) and TLR-4 expression in LC, activate NFKB pathway, decrease BCL-2 expression and partially inhibit LC proliferation. Lipids added to the culture medium of LC exert the same effects except that they induce an increase in BCL-2 expression.

Leptin alone does not have any influence on LC but restores the decrease in this anti-apoptotic protein expression observed in the presence of BM adipocytes. Adipocytes seems thus to negatively regulate leukemic cells. However, adipocytes, but not HS-5, display morphological evidence of cell death after one week in co-culture: the presence of pro-inflammatory cytokines produced in the culture medium as a consequence of the activation of NFKB pathway induced by the binding of fatty acids to TLR-4 could explain this effect. In conclusion, we think that BM adipocytes could negatively regulate myeloid malignant cells in the first stage of the disease; their disappearance in leukemic BM in response to pro-inflammatory cytokines could favour the evolution of the disease.



**P -02 :Temporal profiles show differential contribution of lipid mediators in UV-induced inflammation in sun-reactive skin types I and IV**

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Exposure to sunlight is a major environmental factor contributing to skin ageing and cancer development. Skin pigmentation has photoprotective properties, as illustrated by lower incidence of skin cancer in individuals who tan readily without suffering sunburn. The objective of this study was to undertake a detailed examination of cutaneous lipid mediator production in sun-reactive skin types I and IV and link the outcome to the individuals' susceptibility to burn (phototype I) or tan (phototype IV) following UV exposure.

Healthy adult white Caucasian subjects (17 subjects skin type I and 17 skin type IV) were recruited. Suction blister fluid and skin punch biopsies were sampled from unirradiated skin and skin at intervals to 72h following irradiation with a 120mJ/cm<sup>2</sup> UVB. Lipid mediators were assessed in blister fluid using LC/ESI-MS/MS; cyclooxygenase (COX) and lipoxygenase (LOX) expression were assessed in skin sections by immunohistochemical staining.

Individuals with skin phototype I developed erythema within 4 h post-UV; this response was slower for the phototype IV subjects. However, for both groups, erythema peaked at 24h and reached the same level at 72h post-UV. Prostaglandins (PG) E<sub>2</sub> and E<sub>1</sub> were found to be upregulated at 24h in both groups. Nonetheless, in skin type I, PGE<sub>1</sub> and PGE<sub>2</sub> were still elevated at 72h post-UV, whilst in skin type IV they were not significantly different from baseline levels. UVR significantly upregulated COX-2 in the epidermis of skin phototype I but not in phototype IV subjects. 12- and 15-HETE were upregulated post UVR. While 12-HETE levels were similar in both groups, 15-HETE was produced at higher levels in skin phototype I at 72h.

Our results suggest that sun-reactive skin type I shows greater inflammatory response at the earlier time point of the study thus indicating a greater susceptibility of this skin phototype to solar damage. At the later time point, increased production of pro-inflammatory prostanoids in skin type I appears balanced by increased expression of the anti-inflammatory eicosanoid 15-HETE.

**P -03 : Contact sensitizers modulate the arachidonic acid metabolism induced by PMA/LPS in the U937 myeloid cell line.**

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Contact sensitizers are defined as reactive molecules (electrophilic) which have the ability to modify skin proteins to form an antigen (hapten). In addition to the haptentation mechanism, inflammatory signals (danger signals), leading to the activation of dendritic cells, are described to be crucial for the effective induction of an antigen-specific T cell immune response. However, some of well known contact sensitizers (Eugenol, Cinnamaldehyde...) are also described to possess anti-inflammatory properties. We thus investigated if these properties are common to other contact sensitizers and more specifically, if contact sensitizers have the capacity to modulate the arachidonic acid (AA) metabolism.

A PMA/LPS-induced U937 model was used to assess the effect of 6 contact sensitizers on the AA metabolic profile. Our results show that all tested sensitizers prevent the production of PMA/LPS-induced COX-2 metabolites (PGE<sub>2</sub>, TxB<sub>2</sub> and PGD<sub>2</sub>). Tested chemicals, however, did not inhibit the release of arachidonic acid (AA) from membrane phospholipids of [<sup>3</sup>H]AA-prelabelled U937 cells. We further demonstrated that sensitizers indeed inhibit COX-2 gene expression and/or interfere with its enzymatic activity.

Although these results add a new insight into the multiple biochemical effects described so far for sensitizers, further investigations are needed to better understand the relation between the eicosanoïd metabolism and early mechanisms of skin sensitization.

**P -04 : Protection by D609 through cell cycle regulation after stroke**

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Expressions of cell cycle regulating proteins are altered after stroke. Cell cycle inhibition has shown dramatic reduction in infarction after stroke. Ceramide can induce cell cycle arrest by up-regulation of cyclin-dependent kinase (Cdk) inhibitors p21 and p27 through activation of protein phosphatase 2A (PP2A). Tricyclodecan-9-yl-xanthogenate (D609) increased ceramide levels after transient middle cerebral artery occlusion (tMCAO) in spontaneously hypertensive rat (SHR) probably by inhibiting sphingomyelin synthase (SMS).

D609 significantly reduced cerebral infarction and up-regulated Cdk inhibitor p21 and down-regulated phospho-retinoblastoma (pRb) expression after tMCAO in rat. Others have suggested bFGF-induced astrocyte proliferation is attenuated by D609 due to an increase in ceramide by SMS inhibition. D609 also reduced the formation of oxidized phosphatidylcholine (OxPC) protein adducts. D609 may attenuate generation of reactive oxygen species and formation of OxPC by inhibiting microglia/macrophage proliferation after tMCAO. It has been proposed that D609 provides benefit after tMCAO by attenuating hypoxia inducible factor-1 (HIF-1 $\alpha$ ) and Bcl2/adenovirus E1B 19kDa interacting protein 3 (BNIP3) expressions. Our data suggest that D609 provides benefit after stroke through inhibition of SMS, increased ceramide levels, and induction of cell cycle arrest by up-regulating p21 and causing hypo-phosphorylation of Rb (through increased protein phosphatase activity and/or Cdk inhibition). Alternatively, D609 may prevent mature neurons from entering the cell cycle at the early reperfusion, however may not interfere with later proliferation of microglia/ macrophages that are the source of brain derived neurotrophic factor (BDNF) and insulin-like growth factor (IGF-1) in offering protection).

**P -05 : Regulation of Peroxisome Proliferator-Activated Receptor-gamma (PPAR-gamma) expression in human monocyte/macrophages**

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Different experimental evidences indicate that PPAR-gamma regulates inflammatory and immune diseases, its anti-inflammatory potential largely residing in the ability of PPAR-gamma agonists to inhibit monocyte/macrophage activation and expression of inflammatory molecules.

We previously reported that monocyte/macrophages from healthy smokers present a significantly enhanced constitutive PPAR-gamma expression, as compared to healthy non-smokers, and that this expression is up-regulated by 15-deoxy-delta<sup>12,14</sup>-Prostaglandin J<sub>2</sub> (PGJ), a major metabolite of PGD<sub>2</sub> and an important endogenous PPAR-gamma ligand, and by different thiazolidinediones (TZD), the oral antidiabetics acting as exogenous agonists of the PPAR-gamma receptor (Amoruso et al, Life Sci. 81: 906, 2007).

This study was aimed to evaluate: 1) PPAR-gamma expression (and its function) in human monocytes and monocyte-derived macrophages (MDM) isolated from patients with coronary artery disease (CAD), as compared to healthy donors; 2) the ability of different compounds, either polyphenols identified in cocoa beans (clovamide) and olive oil (minor polar compounds- MPC- e.g., oleocanthal, deacetoxy-oleuropein aglycone etc), or relevant inflammatory mediators (e.g., Substance P), to affect PPAR-gamma expression.

Monocyte and MDM were prepared as described (Amoruso et al., 2007); the release of pro-inflammatory cytokines and NF-kappaB nuclear translocation were evaluated as functional parameters of cell activity.

Our results indicate that PPAR-gamma expression in CAD patients is significantly higher (about 10-fold) than in healthy donors, and suggest PPAR-gamma expression and cytokine release to be gender-related, CAD women having the highest PPAR-gamma expression and the lowest cytokine release. Substance P stimulates PPAR-gamma protein expression in monocytes and MDM, with maximal effects similar to those evoked by PGJ. SP-induced PPAR-gamma expression is receptor-mediated, as it is reproduced by a NK1 selective agonist and reverted by the competitive NK1 antagonist GR71251. An olive oil extract, particularly rich in MPC, dose-dependently inhibits PMA-induced NF-kappaB translocation and TNF-alpha release in human monocyte/macrophages, maximal effects being similar to those exerted by PGJ and TZD, but does not significantly affect PPAR-gamma expression. On the contrary, clovamide, enhances PPAR-gamma expression and activity, besides inhibiting cytokine release and NF-kappaB translocation. These data indicate that PPAR-gamma expression in human monocyte/macrophages is enhanced in CAD and can be modulated by different compounds.

**P -06 : Signaling Role for Lysophosphatidylcholine Acyltransferase 3 in Receptor-Regulated Arachidonic Acid Reacylation Reactions in Human Monocytes**

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Cellular availability of free arachidonic acid (AA) is an important step in the production of pro- and anti-inflammatory eicosanoids. Control of free AA levels in cells is carried out by the action of phospholipase A2s and lysophospholipid acyltransferases, which are responsible for the reactions of deacylation and incorporation of AA from and into the sn-2 position of phospholipids, respectively. In this work, we have examined the pathways for AA incorporation into phospholipids in human monocytes stimulated by zymosan. Our data show that stimulated cells exhibit an enhanced incorporation of AA into phospholipids that is not secondary to an increased availability of lysophospholipid acceptors due to phospholipase A2 activation but rather reflects the receptorregulated nature of the AA reacylation pathway. In vitro activity measurements indicate that the receptor-sensitive step of the AA reacylation pathway is the acyltransferase using lysophosphatidylcholine (lysoPC) as acceptor, and inhibition of the enzyme lysoPC acyltransferase 3 by specific small interfering RNA results in inhibition of the stimulated incorporation of AA into phospholipids. Collectively, these results define lysoPC acyltransferase 3 as a novel-signal-regulated enzyme that is centrally implicated in limiting free AA levels in activated cells.

**P -07 : Mass spectrometry based approaches in the identification of oxidized phospholipid-peptide adducts**

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Oxidized phospholipids have important biological activities, exhibiting pro- and anti-inflammatory properties, and thought to be involved in the initiation and development of atherosclerotic conditions. In the past decade, oxidized phospholipids have been found in atherosclerotic plaques at different developmental stages, where the core aldehydes played an important role in the development of atheroma. The biological activity attributed to oxidized phospholipids with terminal aldehyde groups may derive from its reactivity towards amino groups, present in proteins, to form phospholipid-protein adducts. Oxidized phospholipid-protein adducts found in atherosclerotic plaques have been identified based on immunoassays though limited in the structural information provided. The use of mass spectrometry based approaches to the identification of phospholipid-protein adducts in atherosclerotic plaques has not yet been applied.

In view of the reactivity of terminal aldehyde groups formed during oxidation of phospholipids, oxidized phosphocholine and ethanolamines (PC and PE) were incubated with model peptides. Phospholipid-peptide adducts in the form of Schiff and Michael adducts were identified through the use of mass spectrometry as the detection technique. Structural characterization of phospholipid-peptide Schiff and Michael adducts by tandem MS showed characteristic fragments, namely i) losses typical of phospholipids involving the polar head, ii) loss of peptide chain, iii) cleavages involving the peptide chain and iv) cleavages involving the fatty acid chains. This fragmentation pattern is very distinct from the widely studied lipid-peptide adducts.

Through the identification of fragmentation patterns characteristic for phospholipid-peptide adducts, the best MS strategic approaches suitable for the profiling of phospholipid-protein adducts in the scrutiny of phospholipid adducts from atherosclerotic samples will be presented and discussed.

**P -08 : Cytosolic Phospholipase A2-alpha (cPLA2) Enhances Induction of Endoplasmic Reticulum (ER) Stress**

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Accumulation of misfolded proteins in the ER initiates a stress response that upregulates the capacity of the ER to process abnormal proteins. The ER stress response allows cells to recover from stress, but prolonged ER stress may lead to apoptosis. Induction of ER stress by the complement membrane attack complex is enhanced by activation of cPLA2, and plays an important role in kidney glomerular epithelial cell injury (JBC 277:41342, 2002; JBC 280:24396, 2005). To address mechanisms by which cPLA2 enhances ER stress, we compared effects of wild type (wt) cPLA2 with a mutant, in which we deleted the calcium-dependent lipid binding (CaLB) domain of cPLA2-wt, and fused the cPLA2 catalytic domain with the ER targeting domain of cytochrome b5 (cPLA2-ERmut). After transfection and fractionation of COS-1 cells, cPLA2-ERmut was present mainly in the membrane fraction, whereas cPLA2-wt was principally cytosolic. By fluorescence microscopy, cPLA2-ERmut was enriched in a perinuclear distribution under basal conditions, colocalizing with the ER protein, calnexin; cPLA2-wt was mainly cytosolic, but shifted to the perinuclear region after treatment of cells with calcium ionophore. Both forms of cPLA2 transiently expressed in COS cells showed basal phosphorylation at serine-505, which correlates with catalytic activity. Expression of cPLA2-wt was ~5-fold greater, compared with cPLA2-ERmut, but both enzymes increased free arachidonic acid comparably, implying that cPLA2-ERmut effectively hydrolyzed membrane phospholipids, most likely at the ER. Although transfection of cPLA2-ERmut or wt did not induce ER stress independently, cPLA2-ERmut and wt enhanced the induction of ER stress by tunicamycin, dithiothreitol and ionomycin (monitored by induction of GRP94 and C/EBP homologous protein-10), and the effect was dependent on the catalytic activity. In contrast, expression of the cPLA2 CaLB domain did not affect tunicamycin-induced ER stress. cPLA2-ERmut enhanced production of superoxide. Induction of ER stress in tunicamycin-treated cells expressing cPLA2-ERmut was attenuated in the presence of the antioxidant, N-acetyl cysteine, and reduced glutathione, and was exacerbated by DL-buthionine-(S,R)-sulfoximine (which depletes glutathione). Expression of cPLA2-ERmut exacerbated tunicamycin-induced apoptosis. Thus, induction of ER stress is enhanced by the activation of cPLA2 at the ER. The mechanism involves ER membrane phospholipid hydrolysis, and accumulation of reactive oxygen species.

**P -09 : Influence of pregnancy on leukotriene product formation**

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Pregnancy is accompanied by tremendous changes of the immune system to maintain a successful outcome of gestation. Thus, downregulation of cellular mediated immunity and upregulation of humoral immunity and of certain components of the innate immune system are observed. Leukotrienes (LT) and other lipoxygenase (LO) products are powerful lipid mediators with major roles in inflammation and innate immunity. Although it is known that the course and characteristics of LT-related diseases (like asthma and allergic rhinitis) change during pregnancy, the regulation of LT biosynthesis has not been investigated. Here, we show that LT synthesis in blood and isolated neutrophils is different during pregnancy compared to non-pregnant females. Thus, product formation in stimulated (Cationophore A23187 or LPS/fMLP) whole blood from pregnant donors is significantly higher (about 1.7-fold) than in blood from female controls. Although increased neutrophil numbers may explain the increased LT formation in blood of pregnant donors, isolated neutrophils from pregnant donors have lower capacities (about 1.7-fold) of 5-LO product synthesis. Interestingly, plasma from pregnant donors upregulates 5-LO product formation (about 2.3-fold) in neutrophils from control females. Taking together, complex mechanisms (neutrophilia, upregulating effects of plasma, downregulation of LT synthetic capacity in neutrophils) are involved in regulation of 5-LO product formation during pregnancy resulting in higher LT-biosynthesis in blood from pregnant donors. This higher LT formation in blood from pregnant donors might explain the higher incidence of asthma and allergic rhinitis during pregnancy.



**P -10 : Olive oil and nuts in the Mediterranean diet enhance the plasmatic antioxidant capabilities in metabolic syndrome patients**

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Reactive oxygen species cause oxidative damage to cellular components and reacts with nitric oxide generating the toxic peroxynitrite. Oxidative stress is involved in the metabolic syndrome pathologies and its attenuation could ameliorate the symptoms. Both the amount and quality of dietary lipids affect the risk of cardiovascular and inflammatory diseases. The aim of this work was to study the effects of the introduction of olive oil or nuts in the diet on the plasmatic activities and levels of some antioxidant enzymes in patients with metabolic syndrome. The subjects were included in the PREDIMED study. Three groups of twenty paired matched subjects were analyzed. All subjects presented almost two indicators of metabolic syndrome and their diet was controlled for 5 years. The control group consumed a low fat diet, the olive oil group consumed a high fat diet with olive oil as the main source of fat, and the nuts group also consumed a high fat diet but with nuts as the main responsible for the high fat intake. Ten millilitres of blood were extracted in basal conditions after overnight fasting. Plasma and blood cells were obtained after blood centrifugation at 900g, frozen immediately and stored at -20°C until analysis in the two next days. The plasmatic activities of extracellular superoxide dismutase (ec-SOD), catalase (CAT), and myeloperoxidase (MPO), and the blood cell activity of lipoxygenase (LPO) were measured using standardized methods. The protein levels of xanthine oxidase and superoxide dismutase were also determined in plasma by western blot techniques.

Plasmatic activities of catalase and superoxide dismutase were higher in the olive oil and nut groups than in controls, with no differences between nuts and olive oil groups. MPO and LPO activities were similar in all groups. The protein levels of ec-SOD presented a similar trend than the ec-SOD activities, i.e., higher levels in the oil olive and nut groups than in controls. The protein levels of xanthine oxidase (XDO) were similar in all groups. A Mediterranean diet enriched with either olive oil or nuts enhances the plasma antioxidant capabilities in patients with the metabolic syndrome but it did not change the levels of inflammatory and the prooxidant markers.

**P -11 : Prostaglandin E1 analog promotes esophageal ulcer healing through activation of EP2 receptor/cAMP/protein kinase A and P-CREB signaling pathways.**

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Previous studies indicate that prostaglandins E (PGEs) promote healing of gastrointestinal ulcers while COX inhibitors (NSAIDs) delay ulcer healing (New Engl J Med 1992; J Physiol & Pharmacol 2005). The roles of PGEs, their EP 1-4 receptors, signaling pathways and their relation to VEGF gene activation and angiogenesis during esophageal ulcer healing remain all unknown. We examined whether esophageal ulceration alters expression and activity of COX2, EP1-4 receptors; cAMP response element-binding protein (CREB); and whether PGE1 analog - misoprostol (MPGE1) affects CREB activity, VEGF expression, angiogenesis and ulcer healing. **METHODS:** Esophageal ulcers were induced in rats by focal application of 100% acetic acid. Rats were treated with either placebo or MPGE1 i.g. 50 µg/kg b.i.d. **STUDIES** at 3, 6 and 9 days after ulcer induction: 1) ulcer size; 2) quantitative histology; 3) cell proliferation; 4) microvessel density; 5) VEGF mRNA and protein and 6) EP1-4, CREB, P-CREB expression by Western blotting. In cultured human esophageal epithelial HET-1A cells we examined expression of EP1-4 receptors and the effects of MPGE1, Rp-cAMP (PKA inhibitor) and Sp-cAMP (PKA activator) on cAMP levels, P-CREB and VEGF expression. **RESULTS:** Esophageal ulceration triggered increases in COX2 and EP2 (but not EP1, 3 or 4) receptor expression at day 3 (>200% p<0.01), increases in cAMP, P-CREB (4.5-fold increase; p <0.01) and VEGF mRNA and protein by ~ 2- and 6-fold, respectively at day 3 (both p < 0.01). Treatment with MPGE1 further increased in ulcerated esophageal mucosa P-CREB and VEGF expression, increased angiogenesis in granulation tissue (by >60%) and accelerated ulcer healing. In cultured HET-1A cells, MPGE1 increased cAMP levels (by 163-fold; p<0.001) and significantly increased P-CREB and VEGF expression. PKA inhibitor abolished all these MPGE1 actions. **CONCLUSIONS:** 1) Esophageal ulceration triggers increased expression of COX2, EP2 (but not EP1, 3 or 4) receptors, CREB phosphorylation and VEGF upregulation; 2) MPGE1 activates CREB through EP2 receptor/cAMP/protein kinase A signaling pathway; 3) P- CREB activates VEGF gene promoter and VEGF expression, which in turn initiates angiogenesis essential for delivery of oxygen and nutrients to the healing site and ulcer healing.

**P -12 : Prostaglandin E2 (PGE2) transactivates in gastrointestinal mucosa EGF receptor (EGFR) in epithelial cells and VEGF receptor 2 (VEGFR2) in endothelial cells. implications for PGE2 trophic, ulcer healing and cancer growth promoting actions.**

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PGE2 analogs protect gastrointestinal (GI) mucosa against injury by necrotizing agents – phenomenon termed cytoprotection (Gastroenterology 1979 & 2008). PGE2 also exerts a trophic action on normal GI mucosa, on GI ulcers and promotes growth of colonic cancers, but the underlying mechanisms are not fully elucidated. Growth and proliferation of GI epithelial cells is triggered by activation of EGF receptor (EGFR) on mucosal progenitor cells by its ligands (e.g EGF, TGF $\alpha$ , or HBEGF) . Mucosal growth is also dependent on angiogenesis that is initiated and regulated by VEGF and its receptor 2 (VEGFR2). We investigated whether PGE2 may induce mucosal growth via transactivation of EGFR and /or VEGFR2. **METHODS:** In vivo experiments: rats were treated with PGE2 or vehicle, 1-10mg/kg bw and expression of EGFR , P-EGFR2 and Erk2 activity were determined. In vitro experiments: cultured normal gastric epithelial RGM-1 cells were pretreated with either: vehicle, EGFR and MEKK inhibitors, neutralizing antibodies against EGF, HB-EGF and TGF $\alpha$  and treated with PGE2, 1-10  $\mu$ M or vehicle. **STUDIES:** EGFR phosphorylation, Erk2 activation, c-fos and cell proliferation. In separate in vitro study human microvascular endothelial cells (HMVEC) were we treated with either vehicle or PGE2 1-10  $\mu$ M and expression of VEGFR2 and P-VEGFR2 and nuclear translocation of P-VEGFR2 were determined.

**RESULTS:** PGE2 treatment in vivo increased EGFR and Erk2 phosphorylation in gastric mucosa by 215% and 230% (p<0.01 vs. vehicle). In vitro in RGM1 cells PGE2 increased EGFR phosphorylation 185% , ERK2 activity 290% and cell proliferation by 195% (all p< 0.01). These actions of PGE2 were dependent on activation of c-src, matrix metalloproteinases (MMPs) and release of the EGFR ligand, TGF $\alpha$ . PGE2 also in a similar manner transactivated EGFR, activated Erk2 and cell proliferation in two colon cancer cell lines - Caco2 and LoVo. In human microvascular endothelial cells PGE2 induced phosphorylation of VEGFR2 and its nuclear translocation. **CONCLUSIONS:** 1) PGE2 transactivates EGFR and induces mitogenic signaling in normal gastric mucosa and in colon cancer cells, 2) the mechanisms include activation of s-src and MMPs, which release TGF $\alpha$  that binds to and activates EGFR, 3) PGE2 also transactivates VEGFR2 in endothelial cells.

**P -13 : Could aspirin-triggered lipoxins explain the beneficial effects of low-doses of aspirin in prevention of some gestational alterations?**

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Low-doses of aspirin are widely used in the prevention and the treatment of diverse alterations of gestation such as abortion and preeclampsia. Although there is controversy on the true efficiency and empirical administration of this medicine, its use is very common in pregnancy; additionally its cost is low, it is relatively safe and easily accessible to all.

The traditional mechanism of action of aspirin is to inhibit the synthesis of prostaglandins but this by itself does not explain the repertoire of anti-inflammatory actions of aspirin. Recently, another mechanism was described that is the induction of the production of 15-epilipoxins from arachidonic acid by acetylation of the enzyme COX-2. The aspirin-triggered 15-epilipoxins act as immunomodulators rather than immunosuppressors, attenuating the network of pro-inflammatory cytokines/chemokines among other effects. If we consider that in preeclampsia and abortion there is an underlying inflammatory process, aspirin might be used more rationally based on the induction of epilipoxins.

In our Abortion Program, aspirin has been used alone or combined with other therapies in the treatment of habitual abortion: in women with three or more abortions that have included treatment with aspirin during their pregnancies, the gestational success is greater (71.6%, 48/67) than in patients who did not include this therapy (44.2%, 14/32), and is greater than the results obtained with the use of lymphocyte immunotherapy alone (35%, 7/20).

These results encourage us to search for other mechanisms of action of aspirin, which would lead to a more rational use of this compound in gestational alterations such as to select the patients who could benefit from aspirin, the period at which to begin the treatment, and the dose to be used.

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**P -14 : Effect of quercetin and alpha-tocopherol on age-related heart and liver sphingolipid turnover**

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The aging process appears to contribute to degeneration of heart tissue. Apoptosis-associated loss of cardiomyocytes in aging heart is a well-known phenomenon that can lead to the myocardial dysfunction. In recent years ceramide has attracted attention as a potential inducer of apoptosis. The increased sphingolipid turnover and ceramide accumulation in the liver is associated with elevation of free radical production and state of chronic inflammation at old age. Only the ceramide, but not the other lipid second messengers, mimics the effect of pro-inflammatory cytokines. The neutral sphingomyelinase (SMase) has been suggested to mediate the interleukin-1beta signaling in the liver and brain. Plant polyphenols are reported to exhibit antioxidant and anti-inflammatory effects. In the present paper, the sphingolipids levels and ceramide production in the heart and liver tissues have been investigated as well as the correction of sphingolipid metabolism at old age by using the quercetin and alpha-tocopherol. To study the sphingolipids turnover, the [14C]palmitate and [14C-methyl]sphingomyelin (SM) were used. The content of ceramide was higher in the liver and heart of 24-month-old animals as compared to adult 3-month-old Wistar rats. Ceramide accumulation in aged heart and liver was accompanied by a drop in the SM levels and increase in the neutral and acid SMase activity. The administration of flavonoid or alpha-tocopherol to old rats decreased both the elevated ceramide mass and the newly synthesized ceramide content as well as the elevated neutral and acid SMases activities in the tissues. The significant decrease in the ceramide/SM ratio has been observed in the heart and liver of quercetin- or alpha-tocopherol-treated old rats as compared with the control animals. The data above evidence that the quercetin is powerful modulator of sphingolipid metabolism in the heart and liver of the old animals. Taking into account that the quercetin mimics the alpha-tocopherol effects on sphingolipid turnover in the tissues of old rats, it cannot be excluded that flavonoid prevents ceramide accumulation, at least in part, due to its antioxidant properties.

**P -15 : Is there a novel endocannabinoid receptor in endothelial cells?**

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Endocannabinoids are endogenous polyunsaturated fatty acids involved in a multitude of health and disease processes. Recently, several lines of evidence suggest the presence of a novel non-CB1/CB2 anandamide receptor in endothelial cells. Thus, we synthesized two types of photoaffinity probes which contain either an aryl azide group or a diazirin moiety, together with a fluorescent analog. The key steps rely on selective hydrogenation of skipped tetrayne backbones, and copper-mediated cross-coupling reactions between diynic precursors. In biological functional assays, we found that both the arylazide and the fluorescent probes induced robust increases in matrix metalloprotease activity and produced positive angiogenic responses in in vitro endothelial cell tube formation assays. Irradiation of the arylazide probe nicely enhanced this effect in both HUVEC and CB1-KO HUVEC. These results suggest that the arylazide and the fluorescent probes can be used to identify “non-CB1/CB2 anandamide receptor” from endothelial cells.

**P -16 : Ultrafine carbonaceous particles induce lipid mediator synthesis in healthy mice and in an allergic mouse model for asthma: in-vitro and in-vivo studies.**

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Exposure to ultrafine particles exerts diverse harmful effects including aggravation of pulmonary diseases like asthma. We have recently shown that ultrafine carbonaceous particles (UfCP) induced augmentation of oxidative stress in allergen-induced lung inflammation. In a follow-up study we hypothesized that UfCP may enhance inflammatory reactions concerning induction of lipid mediator production in: i) in-vitro model with primary rat alveolar macrophages (AM), and ii) in-vivo study with an allergic mouse model. The investigated lipid mediators include immune-modulating prostaglandin E2 (PGE2), anti-inflammatory 15(S)-hydroxy-eicosatetraenoic acid (15(S)-HETE), pro-resolving lipoxin A4 (LXA4), pro-inflammatory leukotriene B4 (LTB4) and 8-isoprostane as oxidative stress marker. For in-vitro study, rat AM were incubated with UfCP (10 µg/1 x 10<sup>6</sup> cells/ml) for 1h, and lipid mediators were determined by specific enzyme immunoassays. For in-vivo study, the same parameters were measured in lungs of: a) non sensitized mice exposed for 24 h to UfCP (504 µg/me<sup>3</sup>) by inhalation (NS-UfCP); b) ovalbumin-sensitized and challenged mice (S/OVA); c) ovalbumin-sensitized mice exposed for 24 h to UfCP (504 µg/me<sup>3</sup>) prior to ovalbumin challenge (S/UfCP/OVA). Directly (0 h), 24 h and 168 h after exposure lungs were examined for lipid mediator formation. In-vitro, UfCP induced a two-fold increased generation of all lipid mediators (P<0.05). PGE2 and 15(S)-HETE dominated the mediator profile because of high absolute baseline values compared to the others. In-vivo, UfCP inhalation induced in healthy mice significant increased levels of all mediators at 24 h and 168 h after exposure compared to controls (P<0.05). In NS/UfCP mice PGE2 and 15(S)-HETE also dominated the mediator response, comparable with that found in-vitro. In allergic mice (S/OVA) all mediator levels were elevated at 24 h and 168 h with PGE2 and 15(S)-HETE being the most (P<0.05). UfCP inhalation aggravated these levels even higher at 0 h, 24 h and 168 h after exposure (P<0.05). These findings were confirmed by immunohistochemical analyses for PGE2 and 8-isoprostane. Concluding, UfCP induced increased lipid mediator production in-vitro and in-vivo with dominating anti-inflammatory PGE2 and 15(S)-HETE response. However, in susceptible lungs of allergic mice (S/OVA), particle inhalation aggravated the inflammation suggesting worsening of the inflammatory response in asthmatic patients.

**P -17 : Effects of conjugated fatty acids on TNBS induced colon inflammation in rats**

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We analyzed the effect of punicic acid the major fatty acid of pomegranate seed oil, and conjugated linoleic acid (CLA) on ROS production and on TNBS-induced rat colon inflammation. Punicic acid and CLA intake decreased neutrophil-activation and ROS/MPO-mediated tissue damage as measured by F2-isoprostane release and protected rats from TNBS-induced colon inflammation.

Conclusions/Significance: These data show that conjugated linoleic, and linolenic acid, which have a trans double bond on C11 exerts a potent anti-inflammatory effect through inhibition of TNF $\alpha$ -induced priming of NADPH oxidase by targeting the p38MAPKinase/Ser345-p47phox-axis and MPO release. These natural dietary compounds may provide a novel alternative therapeutic strategy in inflammatory diseases such as inflammatory bowel diseases.



**P -18 : Lipid metabolism regulation in histiocytosis**

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Chronic inflammation plays an important role in the pathogenesis of cancers, autoimmune and allergic diseases, as well as in rare diseases called histiocytosis. In these latter, accumulation of myeloid cells, then associated to secondary recruitment of other cells of the immune system, form aggressive granuloma inside tissues. Foamy myeloid mono- and multi-nucleated giant cells are associated to Sea-Blue histiocytosis and Juvenile Xanthogranuloma but have not been investigated in other histiocytosis such as Langerhans Cell Histiocytosis (LCH). LCH accumulates dendritic cells (DC) partly exhibiting skin DC (Langerhans cells) phenotype but developing a larger rounded shape. Our laboratory recently involved IL-17A in DC fusion leading to giant cells *in vitro*, as observed in LCH granuloma *in vivo* (Coury et al., *Nat Med*, 2008). We now explore the new characteristics and functions of the subsequent multinucleated giant cell (MGC).

Our preliminary data show a complete remodeling of lipid metabolism in IL-17A-treated DC transcriptome: expression of enzymes, transporters and other proteins involved in lipid storage and metabolism is profoundly affected. Moreover, electronic microscopy pictures of both DC from LCH patient and IL-17A-treated DC from healthy donors present a cytoplasm enriched in lipid droplets, one of the principal reservoirs for storing cellular energy.

All IL-17A-related diseases exhibit a strong inflammatory profile. In the case of LCH, we cannot explain the inflammatory syndrome by detection of the classical pro-inflammatory cytokines (IL-6, IL-1beta and TNFalpha), thus production of lipid inflammatory mediators could drive the inflammation process. Our study of IL-17A-dependent lipid metabolism regulation of myeloid cells may also be useful to understand the role of the recently described foamy myeloid cells in tuberculosis granuloma, (Russell et al., *Nat Immunol*, 2009) since *Mycobacterium Tuberculosis* induces an IL-17A-dependent immune response (Umemura et al., *J. Immunol*, 2007).

**P -19 : Gender influences the efficiency of leukotriene synthesis inhibitors**

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Leukotrienes (LTs) are powerful lipid mediators of immune and inflammatory responses derived from phospholipase(PL)A<sub>2</sub>-released arachidonic acid by the enzyme 5-lipoxygenase (5-LO), aided by the 5-LO-activating protein (FLAP). LT-related diseases include bronchial asthma and allergic rhinitis and have a higher incidence in females than in males. We have recently shown gender-related differences in LT biosynthesis and in 5-LO cellular biology in stimulated human whole blood and neutrophils, due to down-regulation of 5-LO by androgens (1). Here we addressed the efficiency of inhibitors of LT biosynthesis in male and female human whole blood and in an experimental model of inflammation in rats. Thus, direct 5-LO inhibitors efficiently reduced LT biosynthesis in male and female blood stimulated with LPS/fMLP, with only slight (for the redox-type inhibitor AA861) or no significant differences (for the iron-ligands BWA4C and zileuton and the non-redox type 5-LO inhibitor ZM230487) in the potency. Also, no significant gender-differences were observed for the cPLA<sub>2</sub> inhibitor pyrrolidine. In contrast, FLAP inhibitors as MK886, BayX-1005 and licofelone showed a significant higher potency in female than in male blood (IC<sub>50</sub> differed by a factor of 4 to >10). Addition of 5 $\alpha$ -dihydrotestosterone (30 nM) to female blood impaired the potency of MK886 and licofelone, but not of the direct inhibitor zileuton, and abolished gender-dependent differences. Finally, in a model of carrageenan-induced pleurisy, MK-886 (0.5 mg/kg, i.p.) effectively inhibited LTB<sub>4</sub> pleural levels of female but not of male rats, while zileuton (10 mg/kg) was effective in both genders. In conclusion, the gender-bias in 5-LO product formation may not only affect pathophysiology but also alters the susceptibility of the 5-LO pathway against certain pharmacological inhibitors, and suggests to call for “gender-tailored therapy” in LT-related diseases

References

1. Pergola, C. et al., ERK-mediated regulation of leukotriene biosynthesis by androgens: a molecular basis for gender differences in inflammation and asthma. Proc Natl Acad Sci U S A. 105(50):19881-6 (2008).

**P -20 : Inhibition of 5-lipoxygenase by derivatives of pirinixic acid: molecular pharmacology and efficacy in vivo**

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5-Lipoxygenase (5-LO) is a promising target for the treatment of diseases related to conditions with excessive leukotriene formation, such as asthma and allergic reactions. We have previously shown that alpha-substituted derivatives of pirinixic acid (PA) inhibit 5-LO product formation in intact human neutrophils as well as in cell-free assays with IC<sub>50</sub> values in the low micromolar range. Here, we present further structural optimization of PA derivatives and we provide insights into the molecular mechanism of 5-LO inhibition. Regarding the molecular mode of action the compounds inhibit 5-LO in a reversible and uncompetitive manner. Modulation of the inhibitory potency by different regulatory factors of 5-LO activity such as Ca<sup>2+</sup>, phosphatidylcholine, diacylglycerols, and coactosine like protein was addressed. Our data point to the C2 like domain as possible site of interference. Finally, we show that the alpha-(n-hexyl) PA derivative YS121 (1.5 mg/kg, i.p.) significantly reduced exudate volume, infiltration of inflammatory cells and LTB<sub>4</sub> levels in the carrageenan-induced pleurisy in rats, implying in vivo effectiveness of the compound. In summary, alpha-substituted PA derivatives offer a novel therapeutic approach for intervention with inflammatory diseases and are of value for further investigations.

**P -21 : Covalent modification of H-Ras C-terminus by inflammatory mediators: role in membrane association**

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Cyclopentenone prostaglandins (cyPG) are inflammatory mediators which elicit varied biological effects, ranging from the modulation of cell viability to the regulation of pro-inflammatory genes. An essential mechanism of action of cyPG is the covalent modification of proteins through the formation of Michael adducts with nucleophilic residues. We recently showed that Ras proteins are targets for the covalent binding of cyPG, which is associated with activation of Ras-dependent pathways.

Here we have characterized the structural aspects of this modification.

We have found that cyPG with dienone structure bind simultaneously to the two cysteine residues located near the C-terminal end of H-Ras, C181 and C184, which normally serve as sites of palmitoylation in cells. This implies the formation of an intramolecular crosslinking in H-Ras.

This phenomenon can be mimicked by small molecule bifunctional cysteine reagents, including dibromobimane and phenylarsine oxide (PAO), a widely used tyrosine phosphatase inhibitor. Moreover, PAO competes for the binding of biotinylated 15-deoxy-Delta12,14-PGJ2 (15d-PGJ2) to H-Ras both in vitro and in cells. However, whereas dienone cyPG binding to H-Ras is irreversible, binding of PAO is reversible under reducing conditions.

Intramolecular cross-linking by cyPG or PAO would drastically affect the conformation of H-Ras C-terminus, potentially altering the interaction of H-Ras with membranes and/or effectors. Interestingly, our results indicate that reactive prostanoids and small molecules may exert differential effects on Ras membrane association and activation of Ras-dependent pathways. These observations support the role of reactive inflammatory mediators in the regulation of the specific localization of key signalling proteins through covalent modification.

**P -22 : Oxidation of glycated phosphatidylethanolamine's: evidence of oxidation in glycated polar head**

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Phosphatidylethanolamines (PEs) glycation were found to occur in diabetic patients with hyperglycemia, and their levels have correlation with the ones of glycated hemoglobin. Oxidized phospholipids are important mediators in biological processes such as immune response, inflammation, apoptosis and age-related diseases. Some studies provide evidence that glycated PEs promote lipid peroxidation, increasing LDL oxidation, which could be related with oxidative stress and with diabetic complications, particularly atherosclerosis, cardiovascular diseases and retinopathies. Furthermore glycated PEs seems to increase oxidation of other molecules, however the reason why is not understood. Until now, there is no report about oxidation of glycated PEs or even the probable mechanism that justify its possible relation with diabetes. The aim of this work is to study the modifications of glycated PEs that occur under oxidative stress, induced by the hydroxyl radical, under Fenton Reaction condition, using a lipidomic approach. LC-ESI-MS and ESI-MS/MS in both positive and negative modes were used for detecting and identifying the oxidation products of glycated PEs (1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylethanolamine; PLPE and 1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine; dPPE). We were able to identify several oxidation products with oxidation in unsaturated sn-2 acyl chain of PLPE, as long and short chain products. Other products were identified in both glycated PLPE and glycated dPPE, revealing, for the first time, that oxidation also occurs in the glycated polar head. Oxidation of glycated PEs occurred more quickly than the oxidation of non-glycated PEs probably because of the existence of more oxidation sites derived from glycation of polar head group. Considering the biological effects of glycated PEs and probably of their oxidation products, and taking in consideration the scarce knowledge on their relation structure-activity, an effort to study the modifications at the molecular level of glycated aminophospholipids, using a sensitive methodology, such as mass spectrometry, is essential to be aware of their role in biological environment systems. Monitoring glycated polar head oxidation of glycated PEs could be important to evaluate oxidative stress modifications that occur in diabetic patient and may be a key step to identify possible biomarkers for the early detection and to evaluate the prognosis of diabetic complications.

**P -23 : Plasma F4-Neuroprostanes isomers as potential lipid biomarkers of oxidative neuronal injury – Application to human Rett syndrome**

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**BACKGROUND:** Lipid peroxidation plays a key role in the pathogenesis and/or progression of several neurological diseases. High concentrations of polyunsaturated fatty acids (PUFA), such as docosahexaenoic acid (DHA) (highest levels in neuronal membranes, present mostly as cell bodies and dendrites in gray matter and as axons in white matter) adrenic acid (AdA) (highest levels in myelin) and arachidonic acid (AA) (more evenly distributed in the brain), together with the self-propagating nature of PUFA peroxidations, make the primate brain especially vulnerable to free radical attack. While F4-Neuroprostanes (F4-NeuroPs) are biologically active markers for neuronal membrane peroxidation *in vivo*, their diagnostic value in sites outside of the CNS has not been tested. **AIMS:** Here, we firstly tested the diagnostic value of F4-NeuroPs isomers plasma measurements as markers of neuronal injury. Rett syndrome (RTT), a neurodevelopmental disorder caused in about 80% the cases by the X-linked MeCP2 gene mutations, was used as a human model of oxidative stress-mediated neuronal damage.

**METHODS:** Classic RTT (n=13), Preserved Speech Variant (PSV) (n=6), the mildest RTT clinical form, and control subjects (n=9) were examined. RTT severity was measured using severity scales and PaO<sub>2</sub> was determined by blood gas analysis. Plasma F4-NeuroPs isomers were measured by gas chromatography/negative ion chemical ionization tandem mass spectrometry. Plasma F2- isoprostanes (F2-IsoPs) and non-protein-bound iron (NPBI) were also determined. The diagnostic value of F4-NeuroPs isomers in classic RTT was tested by ROC curve analysis.

**RESULTS:** Plasma levels of F4-NeuroPs isomers were increased (x 10.5) in classic RTT as compared to controls (p<0.0001) while PSV patients showed intermediate values (x 2.96, p=0.0166). Increased F2-IsoPs (x 2.1) and NPBI (x 3.54) with significantly decreased PaO<sub>2</sub> (-10.6%) were associated with classic RTT (p<0.001). Intermediate NPBI levels were observed in PSV (p=0.0363). Plasma F4-NeuroPs isomers levels were positively related to clinical severity and inversely related to PaO<sub>2</sub>, with plasma levels >9.4 pg/mL identifying classic RTT with 100% sensitivity and 92.3% specificity.

**CONCLUSIONS:** The findings indicate that (1) DHA is a key target for systemic oxidative stress in RTT; and (2) plasma F4-NeuroPs isomers are promising lipid biomarkers for detecting oxidative neuronal damage.

**P -24 : Omega-3 long chain polyunsaturated fatty acids (PUFAs) decrease oxidative stress and severity in Rett syndrome**

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**Abstract: BACKGROUND:** To date, no effective therapy is available for Rett syndrome (RS), a progressive severe neurodevelopmental disorder mainly caused by de novo loss-of-function mutations in the MeCP2 gene. Recently, a hypoxia-related oxidative stress process in patients with classic RS has been demonstrated (De Felice C, Ciccoli L, Leoncini S, Signorini C, Rossi M, Vannuccini L, Guazzi G, Latini G, Comporti M, Valacchi G, Hayek J. Systemic oxidative stress in classic Rett syndrome. *Free Radic Biol Med* 47:440-448; 2009).

**AIMS:** As dietary supplementation with omega-3 polyunsaturated fatty acids ( $\omega$ -3 PUFAs) is known to mitigate oxidative stress and/or improve oxygenation in several experimental and clinical scenarios, here we hypothesized that oral administration of  $\omega$ -3 PUFAs could reduce oxidative stress and clinical severity in classic RS.

**METHODS:** Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (30 to 40 mg/kg b.w./day) were administered orally for 6 months to patients with classic RS (n=22). Healthy controls (n=21) and  $\omega$ -3 PUFAs-not supplemented classic RS patients (n=8) were also examined. Measured oxidative stress markers included intraerythrocyte non-protein-bound iron (NPBI; i.e., free iron), plasma NPBI, and F2-isoprostanes (F2-IsoPs, as free form). Clinical severity was assessed using the Percy scale.

**RESULTS:** Supplementation with  $\omega$ -3 PUFAs resulted in a significant reduction of the Percy score (-16.41, SD: 7.28%), decreased plasma NPBI (-60.74%, SD: 19.28%), intraerythrocyte NPBI (-61.39, SD: 18.39%), and plasma free F2-IsoPs (-37.05, SD: 10.86%) concentrations, and increased (+9.63, SD: 6.9%) partial arterial pressures of oxygen (PaO<sub>2</sub>). The degree of decrease in severity was positively related to the degree of decrease in free F2-IsoPs (r=0.92) plasma NPBI (r=0.54), intraerythrocyte NPBI (r=0.66), and inversely related to the increase in PaO<sub>2</sub> (r=-0.87). Conversely, no changes in oxidative stress markers levels or severity were observed in the untreated group.

**CONCLUSIONS:** These findings indicate that omega-3 PUFAs supplementation decreases clinical severity in classic RS, likely by decreasing the degree of oxidative stress and improving oxygenation.

**P -25 : Phospholipase A2 as a control point in the diversity of lipid mediators**

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Lipid mediators are involved in numerous cellular processes, ranging from apoptosis to distinct medical conditions, being the biosynthesis of these molecules controlled by phospholipases. Eicosanoids represent an important group of lipid mediators due to its direct participation in inflammation. The superfamily of phospholipases directly responsible for their production is the one comprising phospholipase A2 (PLA2), a water-soluble enzyme that acts at the beginning of the inflammatory cascade and catalyses the sn-2 ester bonds of phospholipids and by this way controls the release of arachidonic acid from membranes and the formation of prostaglandins, leukotrienes and thromboxanes. Drugs that are able to modulate PLA2 activity represent a possible target for new anti-inflammatory agents. The inhibition of this enzyme right at the early stage of the development of an inflammatory condition is of special interest due to the implications that it might have in the production of pro-inflammatory mediators.

In the present work, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) were considered as potential therapeutic inhibitors of PLA2 enzyme and the interaction of several drugs belonging to this group with PLA2 enzyme was evaluated. The aim of this study is to research possible mechanisms by which these drugs can modulate PLA2 activity and consequently affect the production of eicosanoids. Dynamic and physical-chemical techniques were applied: fluorescent probes, and intrinsic fluorescence of the protein for studying the activity/inhibition of PLA2 in the absence and presence of the drugs, and circular dichroism analysis for observing conformational changes on the secondary structure of the enzyme following the contact with NSAIDs which can render PLA2 unable to perform its normal enzymatic activity and finally perturb the biosynthesis of chemical messengers.

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**P -26 : Partial characterization of the lipases from different tissues of *Bombus terrestris***

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Lipases are enzymes of interest in different disciplines in natural science: medicine, biochemistry, biology, the food industry, and organic chemistry. Lipases from insects, their properties and importance are poorly investigated area. In bumblebees, lipases can participate in addition to the hydrolysis of storage lipids, in the biosynthesis of different fatty acids, which serve as pheromones for very specific sexual communication of this species. We detected lipase activity in the fat body, labial gland and midgut of *Bombus terrestris* and performed characterization of these novel lipases. The pH optimum of crude lipase extracts from labial gland, fat body, and midgut, detected with p-nitrophenyl laurate, was 8.3. Lipases from labial gland and fat body displayed temperature optimum at 50 °C and the activity of the midgut lipase was not dependent on temperature in the range from 4 °C to 55°C. Decreasing activity of the enzyme was observed at 60 °C. According the SDS electrophoresis, the molecular weight of lipases from labial gland and in fat body was 67 kDa and that from midgut was 30 kDa.

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**P -27 : Monocyte-derived microparticles (MP) affect monocyte/macrophage responsiveness**

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Microparticles (MP), also referred to as ectosomes, are phospholipid vesicles shed by cells upon activation or during apoptosis. Once considered *in vitro* artifacts or, at most, cell debris generated during necrosis and devoid of physiological significance, MP have been more recently shown to participate in several processes (e.g., atherosclerosis, diabetes, rheumatoid arthritis), but little is known on their possible role in lung pathophysiology. Recent data demonstrate that MP obtained from human monocyte/macrophages induce IL-8 and MCP-1 release from human airway epithelial cells (Cerri et al., *J. Immunol* 177: 1975, 2006), so supporting their role in the pathogenesis of airway inflammatory diseases.

This study was aimed to evaluate the role of MP in human monocytes, monocyte-derived macrophages (MDM), alveolar macrophages (AM) and macrophage cell lines (RAW 264.7 cells, Mono-Mac cells). MP were obtained from A23187-treated monocytes, as described (Cerri et al, 2006). Monocyte and MDM were prepared according to Amoruso et al. (*Brit J Pharmacol* 154: 144, 2008); AM were recovered by broncho-alveolar lavage as described (Bardelli et al., *Brit J Pharmacol* 145: 385, 2005). Superoxide anion (O<sub>2</sub><sup>-</sup>) production, pro-inflammatory cytokine release and NF-kappaB activation were evaluated as functional parameters, as described (Bardelli et al, 2005).

In our hands, MP activated, in a concentration-dependent manner, all the cell types evaluated. In human monocytes, MDM and AM, MP induced O<sub>2</sub><sup>-</sup> production and pro-inflammatory cytokine (TNF-alpha, IL-6) release, maximal effects being similar to those evoked by phorbol 12-myristate 13-acetate (PMA). Some minor differences were observed among cell types, human AM evoking the highest O<sub>2</sub><sup>-</sup> production, whereas monocytes released the highest amounts of TNF-alpha and IL-6 following MP stimulation. In both macrophage cell lines and human cells, MP induced a rapid and potent NF-kappaB nuclear translocation, as PMA did.

These data indicate that MP have the potential to sustain monocyte/macrophage activation, as well as to contribute to the pathogenesis of inflammatory diseases in the lung.

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**P -28 : A mitochondrial targeted fatty acid analogue induces mitochondrial fatty acid oxidation with selective increased n-3 polyunsaturated fatty acids in heart muscle**

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Background: Polyunsaturated fatty acids (PUFAs), like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are well-known bioactive components present in fish oil. Tetradecylthioacetic acid (TTA) belongs to a group of sulphur-substituted fatty acids (FAs) (3-thia FAs). Despite the fact that TTA is not able to undergo  $\beta$ -oxidation, it has both chemical and physiological properties that are almost similar to natural FAs. TTA is known to reduce plasma lipids, probably due to hepatic proliferation of mitochondria and an increased  $\beta$ -oxidation of FAs through a PPAR-dependent and -independent mechanism.

Objectives: We hypothesized that dietary treatment with TTA changes mitochondrial function in the heart, accompanied with changes in the lipid metabolism and FA composition.

Methods: Two hundred male Wistar rats (aged 8-10 weeks) were assigned to one out of five different diets: low fat (7% fat); high fat (25% fat); high fat combined with TTA (0.375% TTA); high fat combined with fish oil (10.4% fish oil); or high fat combined with fish oil and TTA, for a 50 week period. Heart muscle tissue was collected and stored at -80°C. FA oxidation was measured as previously described, and FA composition was determined using gas chromatography. Gene expression analyses were done by microarray, and selected genes were analysed using Real-time PCR.

Results: This long-term study demonstrated an increased FA oxidation in the heart muscle after TTA treatment. There was no accumulation of lipids, but dietary TTA lead to an enrichment of n-3 PUFAs (EPA and DHA, % of total FA) (mean  $\pm$  SD:  $19.0 \pm 3.8$  %) in the heart muscle compared to a high fat diet alone ( $16.0 \pm 2.0$  %),  $p=0.028$ . Furthermore, TTA lowered n-6 PUFAs compared to high fat alone, with mean  $\pm$  SD  $34.9 \pm 2.1$  % in the TTA group and  $41.8 \pm 2.4$  % in the high fat group ( $p<0.001$ ). This was accompanied by an upregulation of carnitine palmitoyltransferase I (CPT1) at the mRNA level in the TTA treated group.

Conclusions: A mitochondrial targeted FA induces an enrichment of n-3 PUFAs in the heart. Gene analyses suggest that TTA behaves like an n-3 PUFA in certain biological processes.

**P -29 : CRBM-0244 prevents and reverses inflammation and hyperresponsiveness in mammalian lungs.**

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The effects of CRBM-0244, a newly synthesized DHA derivative, were determined on lung inflammation and airway hyperresponsiveness in an in vitro model of TNF $\alpha$ -stimulated human bronchi as well as in an in vivo model of chronic asthma. Mechanical tension measurements revealed that CRBM-0244 prevented bronchial hyperresponsiveness in TNF $\alpha$ -pretreated human bronchi. Moreover, CRBM-0244 treatments resulted in a decrease in NF $\kappa$ B activation and COX2 over-expression triggered by TNF $\alpha$ . Inhibition of PPAR $\gamma$ ; with GW9662 abolished CRBM-0244 mediated anti-inflammatory effects. CRBM-0244 reduced Ca<sup>2+</sup> sensitivity of bronchial smooth muscle through a decrease in CPI-17 phosphorylation and expression level. Results also revealed an over-expression of CPI-17 protein in lung biopsies derived from asthmatic patients. In vivo anti-inflammatory properties of CRBM-0244 were also investigated in a guinea pig model of chronic asthma. After oral CRBM-0244 administration, airway leukocyte recruitment, airway mucus, ovalbumin-specific IgE and pro-inflammatory markers such as TNF $\alpha$  and COX2 were markedly reduced. Hence, CRBM-0244 treatment prevents airway hyperresponsiveness, Ca<sup>2+</sup> hypersensitivity and CPI-17 over-expression in lung tissues. Furthermore, the presence of specialized enzymes such as 5- and 15-Lipoxygenases in the lung may convert CRBM-0244 in active mediators leading to resolution of inflammation. Together, these findings provide key evidence regarding the mode of action of CRBM-0244 in lung and point to new therapeutic strategies for modulating inflammation in asthmatic patients.

**P -30 : Lipin1-alpha localizes in lipid droplets in human macrophages**

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Lipins are bifunctional proteins involved in lipid metabolism and adipogenesis. Through their phosphatidate phosphatase activity they can influence the cellular levels of diacylglycerol/phosphatidic acid, but also the production of triacylglycerol. Three different genes coding for Lipins have been described, Lpin1, Lpin2 and Lpin3. Alternative mRNA splicing of the Lpin1 gene can generate two different Lipin1 proteins, Lipin1-alpha and Lipin1-beta. We have previously shown that phosphatidate phosphatase activity is important for arachidonic acid release in macrophages. However, the identification of the protein displaying such an activity was not possible at that time. Now, we have studied the subcellular localization and role of human Lipin1 in macrophages. Our results show that constructs of EGFP with either Lipin1-alpha or Lipin1-beta exhibit different subcellular localization patterns in transfected cells. EGFP-Lipin1-beta is located mainly in the cytosol, whereas EGFP-Lipin1-alpha is located in the membranes of organelles that get stained with the neutral lipid dye Nile red and that also express the adipose differentiation-related protein. These features are markers of lipid droplets (LDs), the major cellular stores of triacylglycerol. Subcellular fractionation and western-blot analysis also confirmed that the endogenous Lipin1-alpha is located in LDs. Mutation of the active site does not change Lipin1-alpha attachment to LDs. Moreover, cellular depletion of Lipin1 by siRNA technology changes the amount and pattern of LDs generated by cellular treatment with oleic acid. This phenomenon also occurs in macrophages from lipin1 mutant mice. Analysis of the capacity of Lipin1-deficient macrophages to release AA, demonstrate that Lipin1 is important for the activation of AA-releasing enzymes. Altogether, these results suggest that Lipin1-alpha; may be involved in the biogenesis of LDs and in the inflammatory response of macrophages.

**P -31 : Conjugated linoleic acid (CLA) regulates phosphorylation of PPAR $\gamma$  receptor by modulation of ERK 1/2 and /p38 signaling in human macrophage/foam cells**

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Atherosclerosis is a progressive inflammatory disease characterise by macrophage infiltration into the artery wall. Activation of macrophages by different agents (e.g. ox - LDL) leads to up - regulation of MAP kinases and limitation activity of the peroxisome proliferator activated receptor; (PPAR $\gamma$ ). PPAR $\gamma$  are the receptors that control expression of various genes crucial for lipid and glucose metabolism, as well as the expression of CD 36 scavenger receptor. CD 36 is a multi-ligand scavenger receptor present on the surface of a number of cells such a monocytes/macrophages. CD 36 play role in the development of atherosclerosis. Ligands that can control of PPAR $\gamma$  activation are natural free fatty acids and its derivatives.

Conjugated linoleic acids (CLAs) are stereoisomers of linoleic acid (cis -9, cis-12 C 18:2 ) that originate from food. Many studies showed positive role of this fatty acids in animals (as anti-atherosclerotic, anticancer and anti-diabetic) however studies in human have been shown less unequivocal role of CLA. We investigate the influence of CLA isomers on MAP kinase phosphorylation (ERK 1/2, p 38), phosphorylation of PPAR $\gamma$ ; as well as expression of scavenger receptor CD 36 in the macrophage cultured with CLAs.

Expression of PPAR $\gamma$  and phosphorylation MAP kinases and PPAR $\gamma$  were determined by real-time PCR and Western blot. Expression CD 36 was estimated by flow cytometry. We demonstrate that CLAs attenuated MAP kinase phosphorylation and PPAR $\gamma$  activation. Phosphorylation of ERK1/2 (but not p 38 kinases) were diminished in cells cultivated with cis-9, trans-11 CLA, whereas phosphorylation of p 38 (but not ERK1/2) were reduced by trans-10, cis-12 CLA. PPAR $\gamma$  phosphorylation was suppressed by cis-9, trans -11 isomer in the macrophages /foam cells. This isomer slightly elevated expression of CD 36 on the macrophages surface. cis-9, trans -11 CLA may be acknowledged as PPAR $\gamma$  activator.

**P -32 : Linoleate 9R-dioxygenase and allene oxide synthase activities of *Aspergillus terreus***

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**BACKGROUND:** *Aspergillus terreus* is of medical importance as a producer of the cholesterol lowering drugs lovastatin and simvastatin, and as one of the causative agents of the serious lung disease aspergillosis. Since oxylipins have been shown to be of biological importance in many aspergilli, oxygenation of linoleic acid by *A. terreus* was studied with LC-MS/MS. The genome of *A. terreus* contains five putative linoleate diol synthases (The *Aspergillus* Website: [www.aspergillus.org.uk](http://www.aspergillus.org.uk)).

**AIM:** Determine the oxylipin profile of *A. terreus* and investigate the mechanism of formation of novel metabolites.

**RESULTS:** The fungus produced metabolites formed by two previously characterized enzymes, 10R-dioxygenase and 5,8-linoleate diol synthase. In addition, a novel metabolite 9(R)-hydroperoxy-10(E),12(Z)-octadecadienoic acid (9R-HpODE) was identified. 9R-HpODE was formed from [11S-2H]18:2n-6 with loss of the deuterium label, suggesting antarafacial hydrogen abstraction and oxygenation. Two polar metabolites were identified as 9-hydroxy-10-oxo-12(Z)-octadecenoic acid (alpha-ketol) and 13-hydroxy-10-oxo-11(E)-octadecenoic acid (gamma-ketol), likely formed by spontaneous hydrolysis of an unstable allene oxide, 9(R),10-epoxy-10,12(Z)-octadecadienoic acid. Allene oxides are well known in plant biochemistry, mainly as a precursor in the biosynthetic pathway of jasmonic acid, an important signaling compound involved in defence responses. Jasmonates have previously been described as products formed by *Lasioidiplodia theobromae*, and a few other fungi. Plant allene oxide synthases use hydroperoxides with S configuration formed by lipoxygenases as their natural substrates. The allene oxide synthase activity in *A. terreus* is specific for 9R-HpODE and is present in microsomal preparations, while the 9R-dioxygenase activity is soluble. The genome of *A. terreus* lacks lipoxygenases, and the 9R-dioxygenase activity could be encoded by any of the three unidentified linoleate diol synthase homologues present. Transcripts of two of these genes, ATEG\_03171 and ATEG\_02036, were readily detected by quantitative PCR. Cloning and expression of ATEG\_03171 and ATEG\_02036 are now underway in an attempt to identify the 9R-dioxygenase.

**CONCLUSIONS:** Our results give the first biochemical demonstration of a fungal allene oxide synthase activity that utilizes 9R-HpODE.

**P -33 : Exploration of the role of leukotriene B4 in atherothrombosis**

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Atherothrombosis is an inflammatory disease resulting from the unpredictable rupture of the fibrous cap delimiting the atherosclerotic plaque. This event is often followed by occlusive thrombosis leading to myocardial infarction, stroke or limb ischemia.

Leukotriene B4 (LTB4) is an inflammatory mediator, generated from arachidonic acid via the 5-LO pathway. LTB4 is a powerful chemoattractant for neutrophils. We hypothesized that 1) plaques produce LTB4, 2) LTB4 attract neutrophils in the plaques, 3) neutrophils release their granule content in the plaque, and 4) neutrophils subsequently degrade the fibrous cap which encapsulates the atherosclerotic lesion and thereby favour atherothrombosis. SNP studies suggest a potential link between the enzymes expression leading to LTB4 synthesis and myocardial infarction; however these data are still controversial.

We show here that murine atherosclerotic plaques produce significant amounts of LTB4. We then delivered high amounts of fluorescent neutrophils at the adventitial side of the carotid artery in order to show whether they can enter the plaque. However, autofluorescence of plaques impedes the clear demonstration that neutrophils can enter in the plaque. To circumvent this problem, we used fluorescence life time imaging microscopy (FLIM), because this technique differentiates autofluorescence from the specific fluorochrome signal. We established that neutrophils can enter the plaque after periadventitial delivery and that they are detectable in plaques for less than 6 hours. Therefore, either neutrophils are degraded on site and release their enzymes, or neutrophils leave the plaque back to blood flow. Using immunochemistry, we currently show that delivering high number of neutrophils into the plaque increases the plaque content of myeloperoxidase. In addition, in situ zymography, assessing Gelatinase activity, is strikingly increased after periadventitial neutrophil delivery, showing that neutrophil release functional enzymes in situ. Finally, we showed that neutrophils injected by IV route can home in the plaque. Our data suggest that neutrophils can be involved in plaque vulnerabilization.



**P -34 : Cigarette smoke impairs Fatty Acid Binding Protein 5-mediated bacterial clearance in human airway epithelial cells**

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Numerous studies indicate that cigarette smoke compromises the immune system. Inflammation is critical for the host defense against bacterial infection and the clearing process. However, ineffective microorganism clearance elicits prolonged inflammation, which may contribute to the pathogenesis of smoking-related diseases, such as chronic obstructive pulmonary disease (COPD). Thus, understanding the mechanisms underlying the increased susceptibility of COPD patients to airway bacterial infection is critical to develop new therapeutic approaches. The Fatty Acid Binding Protein 5 (FABP5) is highly expressed in airway epithelial cells, and is reduced in smokers with COPD as compared to smokers without COPD. However FABP5 function and regulation in airway epithelial cells are still unknown. We determined that, in primary normal human airway epithelial cells, FABP5 protein is increased following *Pseudomonas aeruginosa* infection, but decreased following cigarette smoke exposure. Furthermore, primary normal human airway epithelial cells knocked down for FABP5 and exposed to cigarette smoke, exhibit higher bacterial load and higher inflammatory cytokine (e.g., IL-8) secretion. Taken together, these results suggest that cigarette smoke decreases airway epithelial FABP5 expression, thus impairing its anti-microbial and anti-inflammatory function, resulting in a more sustained bacterial infection.

**P -35 : Generating the functional olive oils by hybridization**

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The aim of this research to produce olive oil which has high health benefits for consumer and long stability during cooking and shelf life conditions. For this purposes domestic and foreign olive types were crossed to generate the new crossed olive trees in Ataturk Horticultural Central Research Institute/Turkey. The oils of the crossed olive tree's fruits were evaluated by some chemical analysis. Also sensory analyses were applied to determine the consumer acceptance. Cooking and shelf life stability was important for olive oil because olive oils has high health benefits then the other vegetable oils but his own properties can be change dramatically in cooking and in some improper storage conditions. To determine the potential of health benefits of crossed olive oils; phenolic acids (Hydroxytyrosol, Rutin, Luteolein and Oleuropein) and E vitamin were detected by HPLC. Total antioxidant activities, total phenolic compounds and total flavonoids were determined by spectrophotometer, fatty acid compositions were detected by GC. To determine the cooking and shelf life stability of oils rancimat method was applied. Result show that some crossed olive oils have higher health benefit potential and stability than domestic and foreign standard types and analysis should be continue to determine the all physical, chemical and functional properties of crossed olive oils.

**P -36 : Modified lipoproteins and magnesium in children with attention deficit hyperactivity disorder (ADHD)**

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Objective: ADHD is supposed to be a heterogenous disorder with a complex etiology. Polyunsaturated long chained fatty acids (PUFAs) have been reported to play a role for a wide range of learning and mood disorders, including ADHD. Furthermore, various additional factors might be beneficial to the buffering effect of PUFAs, including zinc (Zn) and magnesium (Mg). Zn protects lipids against peroxidation by its antioxidative force, as well as it is necessary for the maintenance of fatty acid metabolism enzyme activity. Mg is known to be an indispensable element for fatty acid enzymes and neuronal activities.

In this study, lipid, Zn and Mg concentrations were analysed to investigate the pathological processes within the lipid metabolism in ADHD. We assumed that children displaying symptoms of ADHD would have lower concentrations of Mg, Zn and/or different lipoproteins, compared to controls.

Procedure: Blood serum concentrations of Zn, Mg, total cholesterol (Chol), Apolipoproteins a and b (Apo a and Apo b), High density lipoprotein-Cholesterol (HDL) and Low density lipoprotein-Cholesterol (LDL) were analysed in nine boys with ADHS ( $8.2 \pm 0.6$  years) and 11 controls ( $7.9 \pm 0.87$  years), at three times under different stressful conditions

Results: Mg and HDL concentrations were significantly higher as Apo b concentrations were lower in ADHD compared to the control group. None of the other parameters did show differences between both groups.

Conclusion: Mg and lipoproteins may play a role in the etiopathogenesis of ADHD. Further investigations should prove the controverse findings about the interrelationships between Mg concentrations and ADHD symptoms.

**P -37 : N-3/n-6 polyunsaturated fatty acid ratios differently affect cytokine release in human lung cells**

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**Abstract:** Recent both epidemiologic and experimental results evidenced a strong correlation between inflammation process and cancer induction. The inflammation effect seems to be due to the production of oxygen and nitrogen radicals, and of growth-promoting cytokines, to the inhibition of tumour suppressor and to the stimulation of signal transduction pathways leading to cell proliferation. This correlation was confirmed by the observation that agents modulating the production of molecular mediators of inflammation possess anti-carcinogenic activity. In turn, tumour induces in host a chronic inflammation status.

In physiological conditions, n-6 polyunsaturated fatty acids (PUFAs) and n-3 PUFAs possess opposite influence upon inflammatory processes: n-6 PUFAs induce it, whereas n-3 decrease it. Since both PUFAs n-3 and n-6 are taken via diet in different amount, the objective of this research was to determine whether different n-3/n-6 PUFA ratios affect lipopolysaccharide (LPS)-induced cytokine release in human lung A549 cells. Docosahexaenoic acid (DHA, n-3) plus arachidonic acid (AA, n-6) in four different n-3/n-6 ratios (1:1, 1:2, 1:4 and 1:7) were added to A549 cells 3 hours after LPS administration. Both pro- (TNF- $\alpha$ , IL-6 and IL-8) and anti-inflammatory (IL-10) cytokine levels were measured in culture media after further 4 hours.

The release of pro-inflammatory cytokines was reduced by 1:1 and 1:2 n-3/n-6 ratios, but increased by 1:4 and 1:7 n-3/n-6 ratios. Moreover, the 1:1 and 1:2 n-3/n-6 ratios increased the release of IL-10 ( $p < 0.001$ ) more than other ratios tested. The results of this study firstly evidenced that pro-inflammatory cytokine release is reduced by increasing the n-3 share in n-3/n-6 PUFA ratio, whereas after the administration of an n-6 predominance is increased. These results support the biochemical basis to shift the dietary PUFA uptake from n-6 to n-3 in order to prevent the induction of cancer. Moreover, they can also represent an important starting point to shift the PUFA content from n-6 to n-3 in nutritional supports for neoplastic patients.

This study was supported from Compagnia di San Paolo, Italy, Regione Piemonte, Italy and University of Turin, Italy.

**P -38 : Fatty acids might increase ROS synthesis and apoptosis in macrophages treated NaF**

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A long-term exposure to fluorides leads to changes in amount and catalytic properties of many enzymes and to an increased ROS production that can enhance the inflammatory and proliferation reactions, in which a significant role is played by macrophages, cells participating in the formation of atherosclerotic plaque. Several intracellular sources contribute to ROS generation in monocytes, including cyclooxygenases, lipoxygenases, mitochondrial respiration, and NADPH oxidase. ROS formation in mitochondria occurs mainly in a respiratory chain and the substrate to that process derived for example from lipids, carbohydrates and proteins metabolism through the Krebs Cycle.

We investigate the influence of NaF in concentrations determined in human serum on the intracellular synthesis of ROS and the activity of the respiratory chain.

Incubation of monocytes/macrophages with NaF caused significantly decreased the amount of synthesized cellular ATP and significant increase ROS synthesis in cells, which may suggest uncoupling of mitochondria. The addition of the respiratory chain inhibitors to the culture significantly (94%) decreased the ROS synthesis. The addition of apocynin (an inhibitor of NADPH oxygenase) caused only an insignificant decrease in ROS synthesis in macrophages vs. cultures without inhibitors. Our results indicate that the respiratory chain is the main site of ROS synthesis in macrophages. Because NaF is the inhibitor of aconitase (through fluorocitrate), probably the Krebs Cycle as the source of H<sup>+</sup> for the increased ROS synthesis may be limited. This fact would imply that fluor may elevate fatty acids metabolism and beta-oxidation process in macrophages. Activated fatty acids catabolism may be a source of protons on mitochondrial respiratory chain by the electrons transport flavoprotein.

**P -39 : Evaluation of urinary prostaglandin E2 metabolite as a biomarker of inflammation**

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**Objective:** Prostaglandin E2 (PGE2) is an important signaling mediator of pain and fever in inflammatory reactions. High levels of PGE2 have been found in smokers, patients with lung cancer and neonatal infants with apneas. The chemical instability of PGE2 makes it unsuitable as a biomarker in biological fluids. Therefore, tetranor PGE2 metabolite (tetranor PGEM) was evaluated as a potential biomarker of inflammation. Tetranor PGEM is the major urinary metabolite of PGE2 and the aim in this study was to develop a specific and sensitive method for its detection in urine. This method was then used to study normal levels, fluctuations and kinetics of tetranor PGEM in human urine. In a pilot study, we investigated the correlation of urinary tetranor PGEM levels to inflammatory reactions recorded in conjunction with the squalene-adjuvant containing H1N1/A influenza Pandremix vaccination.

**Methods:** Urine samples were collected from adults and children in association with vaccination for H1N1/A influenza virus. Samples were collected prior to vaccination and after three to seven consecutive days. Inflammatory reactions during the sample period were registered. Solid phase extraction (SPE) was used prior to separation and detection using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

**Results:** Tetranor PGEM levels increased with a factor of three and peaked after 1-4 days following vaccination for those individuals who reacted with fever and pain as well as flue like symptoms. After 3-5 days the peak levels of urinary tetranor PGEM decreased to the normal level of each individual (4-40 fmol/ $\mu$ l urine). Non-responders did not show a clear peak 1-4 days after vaccination and there tetranor PGEM levels were constant throughout the study.

**Conclusion:** The developed method, using SPE and LC-MS/MS, can be used to detect tetranor-PGEM in human urine and the results indicates that tetranor PGEM is a sensitive marker for inflammatory reactions.

**P -40 : Inorganic selenium positively influences the activity of particular eicosanoids synthesis pathways related enzymes in macrophages obtained from patients with environmental exposition to lead**

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Early and key changes related to atherogenesis are the implication of low density lipoproteins (LDL) oxidative modification. Macrophages are of the prime importance in this process because of the accumulation of cholesterol originating from LDL and subsequent transformation into foam cells, being the source of locally secreted proinflammatory factors. The abovementioned factors simultaneously with excessive reactive oxygen species (ROS) synthesis are the purpose of the inflammatory – proliferative process (atherogenesis) development. Selenium (Se) dependant glutathione peroxidase (GPx), by its reducing properties towards wide range of hydroperoxides, influences the activities of the arachidonic acid metabolism key enzymes; lipoxygenases (LOX), cyclooxygenases (COX). Selenium as the integral component of GPX, contributes to hydroperoxides detoxification and eicosanoids synthesis modulation. Despite positive Se influence on antioxidant enzymes actions, its particular chemical forms may accelerate and potentiate ROS dependant negative effects. Chronic exposition to lead (Pb) potentiates the intensity of free-radical reactions and changes in lipid metabolism leading to induction and stimulation of the atherosclerosis progression.

> The aim of the study was to investigate the influence of both Se forms: organic – selenomethionine and inorganic – sodium selenite, on the activity of enzymes involved in eicosanoids metabolism: COX-1, COX-2 (by measuring the products of the enzymes PGE2 and TXB2), 12/15LOX (by measuring the products of the enzymes – 12-HETE and 15-HETE) and GPX-1 activity in macrophages obtained from peripheral adults' blood. The blood was obtained from both genders volunteers, normolipemic, with the Pb concentration in whole blood (Pb-B) below the threshold level. Sodium selenite increased the COX-1 and COX-2 activities and reduced 12/15LOX activity in macrophages. That resulted in both pro-inflammatory factors synthesis (PGE2 and TXB2) and the reduction of arachidonic acid hydroxy derivatives generating proinflammatory properties. Positive correlation between the GPX-1 activity and the Pb-B was observed in both control and experimental (cultured with sodium selenite) macrophages. Sodium selenite activated GPX-1 in macrophage culture. Probably the observed changes of the enzymes activities were the implication of compensation mechanism based on protection against excessive ROS generation after exposition to Pb.

**P -41 : Expression of complete and partial sequences of recombinant 5,8- and 7,8-linoleate diol synthases and site-directed mutagenesis**

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Linoleate diol synthases (LDS) are expressed in aspergilli, e.g. 5,8-LDS in the human pathogen fungus *Aspergillus fumigatus*, and 7,8-LDS in the take-all fungus of wheat *Gaeumannomyces graminis*, and in the rice blast fungus *Magnaporthe oryzae*.

LDS contain two catalytic domains. The N-terminal domains (~600 amino acids) show sequence homology to the catalytic domains of mammalian cyclooxygenases and plant  $\alpha$ -dioxygenases. These enzymes belong to the family of “animal haem peroxidases”. A characteristic feature is substrate activation by hydrogen abstraction, catalyzed by a tyrosyl radical in the conserved sequence YRWH. By this mechanism, the 8R-dioxygenase activities of 5,8- and 7,8-LDS transform linoleic acid to (8R)-hydroperoxylinoleic acid (HPODE) and small amounts of 10R-hydroperoxylinoleic acid. The 8R-hydroperoxide is transformed to (5S,8R)- or (7S,8S)-dihydroxylinoleic acids (DiHODE) by the hydroperoxide isomerase activities of the C-terminal domains, which contain conserved YXXEXXR and GXHXCL sequences in analogy with P450 enzymes.

Our objective was to compare the catalytic properties of 7,8-LDS of *G. graminis* with 5,8-LDS of *A. fumigatus*. We first determined the catalytic importance of two key residues, Y in YRWH and C in GXHXCL, by site directed mutagenesis. Substitutions of the catalytic tyrosines by phenylalanines in the N-terminal domains abolished 8R-DOX activities, whereas replacements of cysteines by serines in the isomerase domains abrogated hydroperoxide isomerase activities [3]. We next investigated whether the dioxygenase and hydroperoxide isomerase activities could be expressed separately. Truncation of 5,8- and 7,8-LDS at the 5th and 4th intron, respectively retained 8R-DOX activities, whereas constructs of 600 N-terminal amino acids appeared to be inactive. Our results suggest that the two catalytic domains might be at least partly integrated. Finally, we investigated whether the ratio of oxygenation of 5,8-LDS at C-8 or C-10 could be changed by site-directed mutagenesis. In 10R-dioxygenase (10R-DOX) Leu-384 and Val-388 affect oxygenation of C8 and C10 of linoleic acid. The corresponding positions in 5,8-LDS were mutated but these positions had little influence on the position specificity.

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**P -42 : Measurement of urinary F(2)-isoprostanes by gas chromatography-mass spectrometry is confounded by interfering substances**

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Analysis of F(2)-isoprostanes in urine using gas chromatography-mass spectrometry is confounded by the presence of endogenous compounds interfering with the internal standard, 15-F(2t)-IsoP-d(4) (m/z 573). Previous efforts to resolve the 15-F(2t)-IsoP-d(4) from co-eluting peaks with different solid phase extractions were unsuccessful. This study has now used a highly-deuterated, d(9)-analogue of the derivatization agent N,O-Bis(trimethyl-d(9)-silyl) trifluoroacetamide (BSTFA-d(9)) yielding trimethylsilyl ethers, but this was not successful in resolving the 15-F(2t)-IsoP-d(4) from co-eluting peaks. It was hypothesized that interfering peaks at m/z 573 could be the tetrahydro analogue of 15-F(2t)-IsoP. However, using an authentic standard showed the interfering peaks are not due to this metabolite. In subsequent experiments good resolution was shown of the 15-F(2t)-IsoP peak using 8-F(2t)-IsoP-d(4) (m/z 573) as the internal standard. These data show that care must be taken when using GC-MS for quantitation of F(2)-IsoPs to prevent interfering substances affecting the results.

**P -43 : Eicosanoids derived from lipoxygenase pathway are involved in intestinal epithelial cell growth**

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Cyclooxygenase and 5-lipoxygenase (5-LOX) are simultaneously up-regulated in human colorectal cancer (1). Remarkable expression of leukotriene B4 receptor (BLT1) and cysteinyl LT receptor (CystLTR1) were also detected in human colon cancer tissues (2,3). We observed that intestinal epithelial Caco-2 cells cultured in the presence of 10% fetal bovine serum (FBS) produce LTB4 ( $2.01 \pm 0.17$  nM), 5-HETE ( $8.72 \pm 2.06$  nM), 12-HETE ( $4.89 \pm 1.23$  nM) and 15-HETE ( $7.96 \pm 1.53$  nM) but not LTD4 measured by HPLC-EM. Considering these findings we aimed to study the role of eicosanoids derived from LOX-pathway in the control of Caco-2 cell growth. Our results show that a 5-LOX inhibitor as zileuton (0.05-5  $\mu$ M), a FLAP inhibitor as MK 886 (0.1-10  $\mu$ M) and a 12-LOX/15-LOX inhibitor as baicalein (0.5-25  $\mu$ M) reduced Caco-2 cell growth induced by 10% FBS in a concentration dependent manner, and produced significant changes in the cell cycle. Similar effects were obtained when we incubated Caco-2 cells cultured in FBS with BLT1 antagonis (U75302, 0.1-1  $\mu$ M) and CystLTR1 antagonists (MK 571, 10-25  $\mu$ M; LY 171883, 10-25  $\mu$ M). Exogenous addition of LTB4 and LTD4 (1-10 nM) were able to induce Caco-2 cell proliferation and these effects were blocked by the respective antagonists, U75302 or MK571 and LY171883. 5-HETE, 12(S)-HETE and 15-HETE (10-100 nM) had also a proliferative pattern in absence of growth factors. HETEs receptors have not been identified but our results shown that U75302 and LY255283 (BLT2 antagonist) were able to inhibit Caco-2 cell growth induced by HETEs. These findings suggest the role of LTs and HETEs on intestinal epithelial cell growth through the interaction with BLT and cysteinyl LT receptors.

(1) Cianchi et al. *Mol. Cancer Ther.* 5:2716-2726; 2006

(2) Ihara et al. *J. Pharmacol. Sci.* 103:24-32; 2007

(3) Magnusson et al. *Cancer Res.* 67:9190-9198; 2007

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**P -44 : Upregulation of arachidonic acid cascade markers in frontal cortex from schizophrenic patients**

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Schizophrenia (SZ) is severe, debilitating psychiatric disorders. Increased neuroinflammatory markers have been reported in SZ. Such increases have been shown to be associated with upregulation of brain arachidonic acid (AA) metabolic markers. On this basis, we tested the hypothesis that SZ are associated with increased AA cascade markers. To do this, we used Western blotting and RT-PCR to compare protein and mRNA levels of AA cascade markers in postmortem frontal cortex from 10 SZ patients and 10 matched controls. Mean protein and mRNA levels of cytosolic and secretory phospholipase A2 (cPLA2 type IVA, sPLA2 type IIA), cyclooxygenase (COX)-2, and membrane prostaglandin E synthase (mPGES) were significantly elevated in postmortem compared to control cortex from SZ patients. Whereas levels of protein and mRNA of COX-1, cytosolic PGES (cPGES), Ca<sup>2+</sup>-independent iPLA2, lipoxygenase 5, 12, and 15, thromboxane synthase, cytochrome p450 epoxygenase were not significantly different. The present study indicates that SZ demonstrate upregulation of the brain AA cascade. Downregulation this cascade might thus be considers for new therapy. Acknowledgements: We thank the Harvard Brain Bank, Boston, MA for providing the postmortem brain samples to Dr JS RAO, under PHS grant number R24MH068855. This research was entirely supported by the Intramural Research Programs of the National Institute on Aging, NIH, Bethesda, MD 20892.

**P -45 : Arzanol, a dual inhibitor of 5-LO and mPGES-1**

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Prostaglandins (PGs) and leukotrienes (LTs) are powerful bioactive lipid mediators involved in inflammation. The dual inhibition of the LT-producing enzyme 5-lipoxygenase (5-LO) and of microsomal PGE2 synthase-1 (mPGES-1), which forms pro-inflammatory PGE2 from COX-2 derived PGH2, is a novel and promising strategy for the therapy of inflammation. The acylphloroglucinol arzanol has recently been suggested as an active ingredient responsible for the anti-inflammatory effects of the plant *Helichrysum italicum*. Here we analysed the effects of arzanol on LT and PGE2 formation in cell-free and cell-based models as well as in an experimental model of inflammation in rats. We find that arzanol inhibits 5-LO and mPGES-1 (IC50 = 5  $\mu$ M and 0.5  $\mu$ M, respectively) in cell-free assays and efficiently reduces mPGES-1-derived PGE2 formation in IL-1 $\beta$ -stimulated A549 cells (at 3  $\mu$ M) and LPS stimulated human whole blood (at 10  $\mu$ M) with efficacy comparable to that of the specific mPGES-1 inhibitor MD-52 (tested at 2 and 5  $\mu$ M). In human neutrophils arzanol inhibited 5-LO product formation with an IC50 = 5  $\mu$ M. The in vivo activity of arzanol (3.6 mg/kg i.p.) was demonstrated by the reduction of exudate volume (59%), number of inflammatory cells (48%) and PGE2 (47%) and LTB4 (31%) levels, in the pleural exudates of rats in a model of carrageenan-induced pleurisy. Taken together, these data qualify arzanol as a potent inhibitor of 5-LO and mPGES-1 in both cell-free and cell-based assays with significant anti-inflammatory effect in vivo.

**P -46 : Effect of mineral waters on prostacyclin synthesis in human varicose saphenous vein**

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**Objectives.** The Chronic venous insufficiency (CVD) affects 41% of the Italian population. The SPA therapy, in association with pharmacological and surgical treatments, can act as symptomatic and increase absorption of interstitial oedema. The aim of this study was to investigate the effect of mineral waters on the synthesis of prostacyclin (PGI<sub>2</sub>) a potent vasorelaxant on human varicose saphenous veins.

**Patients and Methods.** Human venous preparations were obtained after informed consent from 6 subjects (50% women and 50% males; mean age: 53±5 years; age range: 41-71 years) affected by CVD. Isolated venous preparations placed in physiological conditions were treated with three types of mineral waters: Solution A (Terme di Stabia in Castellammare, Naples-Italy) sulfate anion = 759 mg/L, hydrogen sulfide = 2.74 mg/L; Solution B (Terme of Telesse, Benevento-Italy) sulfate anion = 32.2 mg/L, hydrogen sulfide = 12.4 mg/L; Solution C (Terme Hotel Gran Paradiso, Casamicciola d'Ischia, Naples, Italy) sulfate anion = 1345 mg/L. The production of 2,3-dinor-6-keto-PGF<sub>1</sub>&#945; (stable metabolite of prostacyclin) were measured by ELISA.

**Results.** The stable metabolite of prostacyclin was detectable and measured in the varicose veins treated with the three Solutions. This preliminary study showed a greater production of PGI<sub>2</sub> after treatment with Solution B (hydrogen sulfide water): 4.53±0.81 pg/mg of vein in comparison with the measurements obtained after treatment with Solution C (sulfate water): 2.18±0.66 pg/mg of vein.

**Conclusion.** The angiogenic and vasodilator effects of PGI<sub>2</sub> could account for the improvement of clinical symptoms and quality of life observed in patients, after SPA therapy with these mineral waters. Our data demonstrate that SPA therapy may be an efficacious treatment to integrate with other principals therapeutic available in the care of varicose veins.

**Key Words:** mineral water, chronic venous insufficiency, PGI<sub>2</sub>.

**P -47 : Recent insights into the multiple molecular and functional properties of group X sPLA2**

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The human group X sPLA2 (hGX) was initially cloned in our lab (Cupillard et al, 1997. J. Biol. Chem. 272:15745). Northern blot analysis revealed at that time that the enzyme is expressed at high levels in circulating immune cells, immune tissues like spleen and thymus, as well as lung, pancreas and colon. The enzyme was later found in human and mouse testis. The enzyme has low identity with all other sPLA2s, but shares structural features with both group IB pancreatic and group IIA inflammatory-type sPLA2s, thus defining a novel group. Importantly, hGX sPLA2 is produced as a proenzyme and maturation of the propeptide is likely to be a key step in the regulation of its biological function. To determine the biological properties and functions of hGX sPLA2, we developed procedures to produce recombinant proenzyme and mature enzyme in *E. coli* and other cells. Interestingly, the matured hGX protein has 500-fold higher enzymatic activity than the proenzyme, demonstrating the importance of the 11 amino acids propeptide. We will present new data showing that the proenzyme is matured intracellularly in HEK293 cells by a furin-like convertase, but can also be matured extracellularly in diverse *in vitro* and *in vivo* situations. The complete maturation of the proenzyme is required for full enzymatic activity. Concerning its enzymatic properties, hGX sPLA2 efficiently hydrolyzes PC from lipid vesicles, cellular membranes and lipoproteins, a property which appears unique among the different sPLA2 members. hGX sPLA2 also degrades PAF. Concerning its biological roles, we embarked in a series of collaborative studies to identify the physiological and pathophysiological roles of group X sPLA2. Over the years, the enzyme was proposed to play a role in skin homeostasis, atherosclerosis, myocardial ischemia, neuritogenesis, asthma, host defense against bacteria and virus, and cancer. In line with its tissue and cellular expression, we will present our most recent data showing that group X sPLA2 plays multiple roles in cell proliferation, sperm capacitation and fertility, atherosclerosis and host defense against infection by *Plasmodium falciparum*, the parasite of malaria. The underlying mechanisms of action will be discussed.

**P -48 : Phospholipid-conjugated NSAIDs: A GI safer and highly effective anti-inflammatory**

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The phospholipid-based nanotechnology which is the focus of our research, originates from our original observation that NSAIDs form a non-covalent association with zwitterionic phospholipids and specifically PC (Nature Med 1: 154-8, 1995). Although the original impetus of this work was to elucidate the mechanism by which these drugs induce surface injury to the gastrointestinal (GI) mucosa and to develop a novel NSAID with reduced GI toxicity, subsequent studies revealed that pre-association of PC with NSAIDs leads to a platform of novel anti-inflammatory pharmaceuticals that possess high anti-inflammatory, analgesic, antipyretic and anti-platelet activity, that may even exceed that of the unmodified NSAID under certain conditions. Using a number of powerful techniques available in our laboratories including: Molecular Dynamic (MD) simulation; Dynamic Light Scattering; Nuclear Magnetic Resonance (NMR), Fourier Transformed InfraRed (FTIR); surface plasmon resonance; and fluorescent resonance energy transfer (FRET) spectroscopy, we have solidified our understanding of the electrostatic and hydrophobic interactions between NSAIDs and PC. These studies have been complemented by evaluating and comparing the biological activity and cytotoxicity of PC-NSAID nanoparticles vs unmodified NSAIDs on GI epithelia, both in culture and in laboratory animals, the latter using an established rodent model that allows one to simultaneously assess both the GI toxicity and anti-inflammatory/analgesic efficacy of our test formulations (Inflammopharmacology 17: 1-5, 2009; Br J Pharmacol 157:252-257, 2009). These laboratory studies have paved the way for the further development of this nanotechnology and its translation into the clinic, as we completed several Phase II clinical trials demonstrating the improved GI-safety of PC-NSAIDs (Aspirin-PC and Ibuprofen-PC) with evidence that the drugs' anti-platelet, analgesic and anti-inflammatory activity are fully maintained (Aliment Pharm Ther. 28: 431-442, 2008). In conclusion, PC-NSAIDs represent a novel class of phospholipid-conjugated anti-inflammatories, that markedly reduce the risk in patients with existing GI and cardiovascular disease. Supported by NIH and PLx Pharma.

**P -49 : Monitoring lipid metabolism in neurodegenerative disorder systems with SECARS and Stimulated Raman Scattering Microscopy**

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Recently, intensive efforts have been made on investigating the causes of abnormalities in lipid metabolism, monitored in some neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), and Alzheimer's disease. Previous findings reported an abnormal accumulation of some common lipids in motor nerve cells that may play a critical role in the development of amyotrophic lateral sclerosis. To determine what might cause these abnormalities in lipid metabolism, a novel application of Stimulated Raman Scattering (SRS) and Surface-Enhanced Coherent Anti-Stokes Raman Scattering (SECARS) microscopy has been used in imaging intravenously injected ultra small paramagnetic iron oxide (USPIO) nanoparticles in ALS rat model. The experiments are performed on Brain specimens, from transgenic rat model, expressing multiple copies of mutated (G93A) human SOD-1 gene (hSOD-1G93A), magnetically labelled with antibodies tagged with (USPIO) (1). Marked signal intensity enhancements have been observed in specific pathological regions of the (ALS) brain in compared with the wild type (WT) rat models. The results obtained correlated these significant enhancements to selective association of lipids to up-taken USPIO which shows high accumulation in the brainstem and midbrain region. The results presented show the promising potential of SRS and SECARS microscopy in investigating lipid metabolism in neurodegenerative disorders model systems.

1. Pavle R. Andjus, Danijela Batavelji , Greetje Vanhoutte et.al. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology, Vol. 292,12,1882 –1892 (2009).



**P -50 : Docosahexaenoic acid modulates pro-inflammatory and anti-neurogenic functions of activated microglial cells**

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The complex process of activation of microglial cells, the brain resident macrophages, encompasses several functional activation states associated with neurotoxic/anti-neurogenic or vice versa neurotrophic/pro-neurogenic properties, depending mainly on the nature of the activating stimuli and the extent of activation. One of the main goals of regenerative neuroscience is the proper manipulation of neuroinflammation and microglial activation in order to enhance endogenous self-repair mechanisms and limit neurodegeneration. Our group has recently demonstrated that the microglial cells can switch from an overt pro-inflammatory and anti-neurogenic phenotype to an anti-inflammatory and permissive for NPC survival and neuronal differentiation phenotype, when the exposure to the prototypical activating agent lipopolysaccharide (LPS) is persistent. .

In the present study we tested the hypothesis whether Docosahexaenoic acid (DHA), a n-3 polyunsaturated fatty acid highly incorporated in the brain and a potent immunomodulatory molecule, could dampen microglial pro-inflammatory functions and their anti-neurogenic action.

We found that DHA dose-dependently reduced the production of several pro-inflammatory mediators, including free radicals and cytokines, in activated primary rat microglial cells, by mechanisms involving the inhibition of the MAPkinase p38, a pivotal mediator for the synthesis of pro-inflammatory molecules in these cells, and the activation of PPAR-gamma, a nuclear receptor able to down-regulate microglial pro-inflammatory functions. DHA reduced the anti-neurogenic properties of activated microglial cells on NPC, suggesting a new important mechanism by which DHA may exert neuroprotective effects and enhance reparative processes in the brain.

**P -51 : 1,2-diarachidonoyl-glycerophosphoinositol, a major acceptor of arachidonic acid in human monocytes**

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Phosphatidylinositol (PtdIns) molecular species incorporate large quantities of exogenous arachidonic acid (AA) when inflammatory cells are exposed to this fatty acid. Liquid chromatography coupled to MS was used to characterize the incorporation of exogenous deuterated AA ([<sup>2</sup>H]AA) into specific PtdIns molecular species. When human U937 monocyte-like cells and peripheral blood monocytes were exposed to [<sup>2</sup>H]AA concentrations as low as 1  $\mu$ M, a PtdIns species containing two exogenous [<sup>2</sup>H]AA molecules (1-[<sup>2</sup>H]AA-2-[<sup>2</sup>H]AA-glycero-3-phosphoinositol) was detected. This specific phospholipid species showed an exponential time-dependent decrease and does not appear in human macrophages. Furthermore, Phorbol myristate acetate (PMA) and dimethyl sulfoxide (DMSO), two compounds that induce U937 cell differentiation, induced a time-dependent decrease in the cellular levels of 1-[<sup>2</sup>H]AA-2-[<sup>2</sup>H]AA-glycero-3-phosphoinositol. Bromoenol lactone, an inhibitor of Ca<sup>2+</sup> independent phospholipase A2 (iPLA2), inhibited the appearance of 1-[<sup>2</sup>H]AA-2-[<sup>2</sup>H]AA-glycero-3-phosphoinositol and diminished lyso-PtdIns levels, suggesting the involvement of a deacylation-reacylation pathway in the synthesis of this phospholipid. According to the results, 1-[<sup>2</sup>H]AA-2-[<sup>2</sup>H]AA-glycero-3-phosphoinositol is an important and a short-lived acceptor of arachidonic acid into PtdIns of human monocytes

**P -52 : Evaluation of inflammation and proresolving EPA and DHA mediators during peritonitis**

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**Background:**

Resolution of inflammation is a key step for the return to health and homeostasis. During this evolution, bioactive lipids mediators derived from polyunsaturated fatty acids (PUFA) are produced. Among them, arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) derivatives (termed lipoxin, resolvin and protectin) show anti-inflammatory and protective properties and are considered to actively participate to orchestrate resolution.

**Objectives:**

The objective of this study were first, to highlight the production of lipids mediators during inflammation. The impact of w-3 fatty acids (DHA/EPA) implementation on the production of this compounds was then assessed and their impact over inflammation resolution episode evaluated.

**Methods:**

We have developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology that quantitatively analyses lipids mediators production. This method was apply to an inflammation-resolution murine peritonitis model. Auto-resolutive inflammation exsudates were analysed at different important times (i.e: 2h, inflammation state and 18h, resolutive episode). The impact of w-3 fatty acids was assessed via i.p injection of a mixture containing EPA/DHA.

Cellular composition during peritonitis was assessed using FACS analysis.

Lipids mediators of interest were highlighted using LC-MS/MS. After solid phase extraction (SPE), compounds were separated using reverse phase chromatography on a C18 column and then the analysis were performed via Selected Reaction Monitoring (SRM) after electrospray ionization in negative mode. Metabolites were identified using commercially available standards and/or using literature's data (lipidmaps).

**Results:**

In this auto-resolutive inflammation, inflammatory process decreases along the time. FACS analysis show the appearance of granulocytes and the recruitment of F4/80<sup>lo</sup>/Gr1<sup>+</sup> macrophage consistent with their action in promoting resolution.

Using LC-MS/MS approach, we determined the levels of several lipids mediators at 2 times. Pro-inflammatory mediators (PGE<sub>2</sub>, LtB<sub>4</sub>, TxB<sub>2</sub>) decreased showing the appearance of a resolution phase. Interestingly, PUFA derivatives (LxA<sub>4</sub>, LxB<sub>4</sub>, pD1a, RvD1, 17-HDHA...) were present at 2h and their levels were very low (to non detectable) at 18h, showing a transient properties.

**P -53 : Involvement of lipin-2 in the inflammatory response of macrophages to fatty acids**

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Obesity and type 2 diabetes are characterized by elevated concentrations of free fatty acids (FAs) and increased macrophage infiltration in adipose tissue, suggesting that they might represent an important source of inflammation. Previous studies demonstrated that saturated but not unsaturated fatty acids can cause activation of macrophages through engagement of Toll-like receptors (TLR2/4) and activation of the NF-kappaB pathway leading to elevated levels of inflammatory cytokines, such as TNF alpha, IL-6 and MCP-1. The capacity of macrophages to incorporate FAs into TGs could serve to modulate this inflammatory response. The lipin protein family (lipin1, lipin2 and lipin3) has an important role in triacylglycerol and phospholipid biosynthesis due to its phosphatidate phosphatase activity. The diacylglycerol (DAG) generated can be metabolized into triacylglycerol, but it is also a key element in lipid-mediated signaling. In the current study, we have examined whether the inflammatory response induced by intracellular saturated FA is modulated by lipins in RAW264.7 macrophages. The data show that when lipin2 is downregulated by siRNA technology, the levels of inflammatory cytokines like IL-6 and MCP-1, increase after cellular treatment with palmitic acid. The activation of signaling kinases such as JNK was also affected, being higher in cells deficient in Lipin2. Moreover, cells treated with [3H]palmitic acid had increased levels of [3H]DAG, an effect that did not occur with oleic acid, a fatty acid that does not increase inflammatory gene expression.

**P -54 : Role of 12/15-lipoxygenase expression in mouse adipose tissue**

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Obesity, diabetes, and atherosclerosis are major health problems of ever increasing importance in developed countries. 12/15-lipoxygenase (12/15-LOX) has been implicated in vascular inflammation associated with early atherosclerosis and in development of insulin resistance, when mice were fed a Western-type lipid rich diet. Previous reports suggested expression of 12/15-LOX in adipose tissue and we confirmed this data by qRT-PCR. To shed light on the possible role of 12/15-LOX in the metabolism of adipose tissue we compared accumulation of visceral adipose of 12/15-LOX knockout mice of different ages with that of wild-type controls and observed interesting differences. Male 12/15-LOX knockout mice gained significantly less body weight than the wild-type controls, which was related to an impaired accumulation of visceral adipose tissue. Such differences were not observed in female individuals of identical genetic background. On the other hand, male knockout mice showed significantly higher food intake when compared with wild-type controls suggesting a less efficient energy turnover. Moreover, we found that compared with wild-type controls expression of leptin was significantly downregulated in 12/15-LOX knockout mice, which is consistent with an increased food intake of these individuals. It has been suggested before that 12/15-LOX plays a role in inflammation. When we quantified expression of EMR1, a specific macrophage marker, in adipose tissue of 12/15-LOX knockout mice and corresponding controls we found reduced expression in male knockouts. Here again, there were no differences observed between knockout mice and wild-type controls in female individuals. Similar results were obtained for inflammatory gene products such as COX2, 5-LOX and TNFalpha.

Taken together our data suggest that systemic knockout of 12/15-LOX reduced the accumulation of visceral adipose during ontogenesis of mice. This effect was gender specific for males, it could not be confirmed in female individuals. This data and our results on adipocytic leptin expression suggest that 12/15-LOX may play a role in the hormone homeostasis involved in regulation of adipocyte development.

**P -55: PGE2 interaction with EP1 and EP4 receptors is involved in intestinal epithelial cell growth**

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Up-regulated expression of cyclooxygenase-2 (COX-2) was observed in 40-50% of colorectal polyps and in up to 85% of colorectal cancer (1) with the subsequent elevation of prostaglandin (PG) levels (2). Considering these findings we aimed to study the role of arachidonic acid metabolites derived from COX-pathway in the control of intestinal epithelial Caco-2 cell growth. Our results show that ketoprofen (0.05-5  $\mu$ M), a COX inhibitor, and NS-398 (0.05-5  $\mu$ M), a specific COX-2 inhibitor, decreased cell proliferation induced by 10% fetal bovine serum (FBS). Interestingly, similar results were obtained using a specific COX-1 inhibitor as SC-560 (1-100 nM). In parallel, we confirmed that COX inhibition reduced PGE2 production ( $2.23 \pm 0.6$  nM vs  $0.61 \pm 0.2$  nM). However, we were not able to observe an appreciable effect of these treatments on cell cycle as analyzed by flow cytometry. Moreover, we demonstrated that the exogenous addition of PGE2 at concentrations reached in Caco-2 cultures had a proliferative effect in absence of other growth factors. This effect was reverted by SC 19220 (60 nM) and AH 23848 (20 nM), EP1 and EP4 antagonists, respectively. These treatments were also able to inhibit Caco-2 cell growth induced by FBS, but did not affect cell cycle. Finally, we observed that specific EP1 agonist such as carbacyclin (0.03-3  $\mu$ M) and an EP4 agonist as ONO 329 (0.1-10  $\mu$ M) were also able to induce Caco-2 cell growth. These findings suggest the role of PGE2 synthesized by COX-1 and COX-2 on intestinal epithelial cell growth through the interaction with EP1 and EP4 receptors.

(1) Eberhart et al. *Gastroenterology* 107:1183-1188; 1994

(2) Moran et al. *J. Biol. Chem.* 279:43261-43272; 2004

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**P -56: Inhibitory effect of prostaglandins on synthesis of other eicosanoids during inflammation**

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It is well known that prostaglandins (PGs) have an important roles during inflammation. Furthermore, it was found increased level of PGs in various inflammatory diseases and experimental models of inflammation. Besides their own effects, PGs can inhibit the synthesis of other eicosanoids which may also have functions in inflammation. This causes multifaceted interactions between PGs and other eicosanoids during inflammatory process.

In our study, indomethacin, a cyclooxygenase inhibitor, inhibited exudates leukotriene B<sub>4</sub> (LTB<sub>4</sub>) level dose dependently in carrageenan-induced air pouch inflammation in rats. In this model, misoprostol, a stabil analog of PGE<sub>1</sub>, inhibited exudates thromboxane B<sub>2</sub> level but did not change exudates LTB<sub>4</sub> level on your own. However, it inhibited indomethacin-induced increase in exudates LTB<sub>4</sub> level. In another study, the effect of misoprostol on cyclooxygenase-2 (COX-2) protein level was also investigated in this model. Misoprostol increased the level of COX-2 protein. Parellel to this finding, it increased exudates PGE<sub>2</sub> level. Indomethacin and SC-58236, a selective COX-2 inhibitor, partially inhibited misoprostol-induced increase in the level of COX-2 protein which indicate the modulatory roles of endogenous prostaglandins on the COX-2 expression.

In clinical study which was conducted on peripheral blood leukocytes of patients with aspirin induced asthma (AIA) and aspirin-tolerant asthma (ATA), aspirin (ASA) stimulation increased the cysteinyl leukotriene (Cys-LT) release comparing to the basal release. This increase was not determined in ATA group and healthy control group. In vitro PGE<sub>2</sub> treatment in this model caused the inhibition in ASA-induced increase in Cys-LT level in AIA group.

Results of these studies indicate the importance of regulatory role of PGs on the synthesis of other eicosanoids and their own synthesis when assessing their roles in inflammation.

These studies were supported by grants from Turkish Scientific and Technological Research Council (SBAG-1516 and SBAG-2103) and Ankara University Research Foundation (Project number: 98-09-00-06).

**P -57 : Atorvastatin treatment, its effect on certain biochemical markers of bone turnover in ovariectomized rats**

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IT has been reported that HMGCOA reductase inhibitors (statin ) demonstrated certain effect on bone metabolism, its mechanism here is not yet well illustrated. The aim of the present study is to evaluate the potential treatment of atorvastatin either orally or topically in gradient concentration. 40 wistar female rats ,4 month old were ovariectomized and allocated into 4 groups,first one ( control ), the second received oral dose 40 mg/kg body weight daily ,the third and fourth groups received topical formula 40 mg and 80 mg/kg body weight in consequences for 10 weeks. Atorvastatin Treatment increased Osteoprotegerin (OPG ),Bone morphogenic protein (BMP), Fibroblast growth Factor (FGF), IL-10, Alkaline phosphatase ( ALP) as compared to control group joined with decreased serum levels of cholesterol and Triacylglycerol(TG).Correlation coefficient of bone markers with ALP was positive and significant turned to be negative with cholesterol and TG.Histopathological examination of Bone (Tibia)was in agreement with the biochemical findings . In conclusion , atorvastatin might provide beneficial effect on bone regeneration process in ovariectomized rats . Results of topical application especially the higher concentration ( 80 mg/KG ) body weight was remarkable as compared to either the lower one or oral route



**P -58 : Oxidation stress and cardiolipin: Identification of specific structural modifications by LC-MS/MS**

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Cardiolipin (CL) is an important phospholipid found almost exclusively in the inner mitochondrial membrane where it plays an important role in energetic metabolism. CL gives support to proteins of the respiratory chain complexes, which are involved in the transduction of electrons and the production of ATP. Alteration of CL structure, namely by oxidative modifications, or CL amount or fatty acyl profile may lead to mitochondria dysfunction, which has been correlated with various pathological conditions, particularly in cardiac and neurodegenerative diseases. CL present a more complex structure, when compared with the other phospholipids, bearing four chains of fatty acids that can diversify in length and degree of insaturation. Similarly to the other phospholipids, CL is susceptible to oxidative damage by reactive oxygen species (ROS). Their location, in the mitochondria, makes them even more likely to be oxidized, since that there is a considerable production of ROS in the inner mitochondrial membrane. In spite of the importance of CL oxidation and its biological consequence, there is a limited knowledge about the oxidation products of CL. Further work is needed to develop methodology to determine CL status and to identify the nature of any oxidation products of CL, in order to understand the biological roles of each specific oxidized CL product.

In this study, LC-MS was used to identify the specific oxidative modifications of tetralinoleoyl CL induced by the OH generated under Fenton reaction conditions ( $H_2O_2/Fe^{2+}$ ). Long chain products formed by insertion of 2 to 8 oxygen atoms in CL were identified. Furthermore, short-chain products (with shortened fatty acyl chains) were also identified for the first time. In fact, these products were never reported to be formed during CL oxidation. The short-chain products identified resulted from beta-cleavage of oxygen-centered radicals and comprised terminal aldehydes, hydroxyaldehydes and carboxylic acids, yielding abundant  $[M-H]^-$  and  $[M-2H]^{2-}$  ions. They were further characterized by LC-ESI-MS/MS. Detailed identification of the fragmentation of these ions allowed the identification of specific product ions that may allow their unequivocal assignment, which may be useful for their detection in biological samples.

**P -59 : Valproic acid suppresses interleukin-1 $\beta$ -induced microsomal prostaglandin E2 Synthase-1 expression in chondrocytes**

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**INTRODUCTION:** Microsomal prostaglandin E2 Synthase (mPGES)-1 catalyzes the terminal step in the biosynthesis of PGE2. Early growth response factor-1 (Egr-1) is a key transcription factor in the regulation of mPGES-1. In the present study we examined the effects of valproic acid (VA), a histone deacetylase (HDAC) inhibitor, on interleukin (IL)-1 $\beta$ -induced mPGES-1-expression in human chondrocytes. **METHODS:** Chondrocytes were stimulated with IL-1 in the absence or presence of VA, and the level of mPGES-1 protein and mRNA expression were evaluated using Western blotting and real-time reverse-transcription polymerase chain reaction, respectively. The mPGES-1 promoter activity was analyzed in transient transfection experiments. Egr-1 recruitment to the mPGES-1 promoter were evaluated using chromatin immunoprecipitation (ChIP) assays. **RESULTS:** VA dose-dependently suppressed IL-1 $\beta$ -induced mPGES-1 protein and mRNA expression as well as its promoter activation. Treatment with VA did not alter IL-1-induced Egr-1 expression, nor its recruitment to the mPGES-1 promoter, but prevented its transcriptional activity. **CONCLUSION:** Our study demonstrates that VA inhibits IL-1-induced mPGES-1 expression in chondrocytes. The suppressive effect of VA was not due to reduced expression or recruitment of Egr-1 to the mPGES-1 promoter.

**P -60 : Prostaglandin D2 enhances interleukin-1beta-induced cyclooxygenase-2 expression in osteoarthritic synoviocytes**

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**OBJECTIVE:** To investigate the effects of prostaglandin D2 (PGD2) on interleukin-1beta (IL-1beta)-induced cyclooxygenase (COX)-2 expression in human synoviocytes and the signalling pathways involved in these effects.

**METHODS:** Synoviocytes were stimulated with IL-1 in the presence or absence of PGD2, and expression of COX-2 protein was evaluated by western-blotting. Messenger RNA (mRNA) expression was analyzed by real-time reverse transcription-polymerase chain reaction. The role of the PGD2 receptors D prostanoid receptor 1 (DP1) and chemoattractant receptor-like molecule expressed on Th2 cells (CRTH2) was evaluated using specific agonists.

**RESULTS:** PGD2 increased in a dose-dependent manner IL-1-induced COX-2 protein and mRNA expression. DP1 and CRTH2 were expressed and functional in synoviocytes. The effect of PGD2 was mimicked by DK-PGD2 and Indomethacin, selective agonists of CRTH2, but not by BW245C, a selective agonist of DP1. Furthermore, treatment with an anti-CRTH2 antibody reversed the effect of PGD2, indicating that the stimulatory effect of PGD2 is mediated by CRTH2. Activation of CRTH2 is consistent with the activation of a receptor coupled to a phosphoinositide-specific phospholipase, suggesting that the effect of PGD2 is mediated by the CRTH2/PIP2/PKC.

**CONCLUSION:** PGD2 enhances IL-1-induced production of COX-2 by synoviocytes through the CRTH2/PIP/PKC signalling pathway.

**P -61 : Interleukin-1-induced cyclooxygenase-2 and inducible nitric oxide synthase expression in human OA chondrocytes is associated with histone H3K4 methylation**

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**Objective;** Increased expression of inducible NO synthase (iNOS) and cyclooxygenase (COX)-2 plays a key role in the pathogenesis of inflammatory diseases. Methylation of lysine 4 on histone H3 (H3K4) was shown to be of fundamental importance in the regulation of gene expression. In the present study, we investigated the role of H3K4 methylation in interleukin-1 (IL-1)-induced COX-2 and iNOS expression in human OA chondrocytes.

**Methods;** Chondrocytes were stimulated with IL-1 for various time periods and the expression of iNOS and COX-2 mRNAs and proteins were evaluated using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting, respectively. H3K4 methylation at the iNOS and COX-2 promoters was evaluated using chromatin immunoprecipitation (ChIP) assays. The role of histone methylation was further evaluated using the methyltransferase inhibitor, 5'-deoxy-5'(methylthio) adenosine (MTA). **Results;** IL-1 induced iNOS and COX-2 mRNA and protein in a dose- and time-dependent manner. The induction of iNOS and COX-2 expression by IL-1 was associated with H3K4 di- and trimethylation at the iNOS and COX-2 promoters, whereas the levels of H3K4 monomethylation remained unchanged. Treatment with MTA inhibited IL-1-induced H3K4 methylation as well as IL-1-induced iNOS and COX-2 expression.

**Conclusion;** These results indicate that H3K4 methylation contributes to IL-1-induced iNOS and COX-2 expression and suggest that this pathway could be a potential target for pharmacological intervention in the treatment of inflammatory diseases.

**P -62 : Non-enzymatically oxidized free and phospholipid-esterified PUFAs are more potent antagonists of LPS than inducers of inflammation**

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Polyunsaturated fatty acids (PUFAs) are precursors of multiple pro- and anti-inflammatory molecules generated by enzymatic stereo- and positionally-specific insertion of oxygen, which is a prerequisite for recognition of these mediators by cellular receptors. However, non-enzymatically oxidized PUFAs also demonstrate activities relevant to inflammation. Oxidized phospholipids (OxPLs) that are generated by peroxidation of esterified PUFAs are increasingly recognized as lipid mediators relevant to chronic and acute inflammation. In particular, OxPLs were shown to induce proinflammatory changes in endothelial cells, but paradoxically also to inhibit inflammation initiated via Toll-like receptor 4 (TLR4). Here we show that half-maximal inhibition of LPS-induced elevation of E-selectin mRNA in endothelial cells developed at concentrations of OxPAPC approximately 10-fold lower than those required to induce proinflammatory response, e.g. upregulate IL-8. Similar concentration difference was observed for other classes and molecular species of OxPLs and non-esterified oxidized arachidonic acid. Upon injection into mice, OxPAPC did not elevate plasma levels of IL-6 and KC, but strongly inhibited LPS-induced upregulation of these inflammatory cytokines. Thus, both in vitro and in vivo, anti-LPS effects of OxPLs are observed at lower concentrations than their proinflammatory action. Quantification of OxPLs by HPLC coupled to tandem mass spectrometry showed that circulating concentrations of total OxPL species are close to the range where they demonstrate anti-LPS activity, but significantly lower than is required for induction of inflammation. We hypothesize that low levels of OxPLs in circulation serve mostly anti-LPS function and protect from excessive systemic response to TLR4 ligands, while proinflammatory effects of OxPLs are more likely to develop locally at sites of tissue deposition of OxPLs, e.g. in atherosclerotic vessels.

**P -63 : A native steroid triggers the resolution of inflammation**

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High doses of dehydroepiandrosterone and its 7-hydroxylated derivatives were shown to reduce oxidative stress and inflammatory responses in dextran sodium sulfate (DSS)-induced colitis in rats. Another endogenous steroid, 7beta-hydroxy-epiandrosterone (7beta-hydroxy-EpiA) was shown to exert neuroprotective effects at much smaller doses.

Our aims were to investigate in vivo and in vitro the effects of 7beta-hydroxy-EpiA on prostaglandin (PG) production and related enzyme gene expression. PGs levels and expression of cyclooxygenase (COX-2) and PG synthases were assessed during the course of these experiments.

First, we evaluated whether 7beta-hydroxy-EpiA pre-treatment prevented DSS-induced colitis and determined whether the effects were related to changes in anti-inflammatory prostaglandin PGD2 and 15-deoxy-Delta(12,14)-PGJ2 (15d-PGJ2) levels. Rats were administered 0.01, 0.1 and 1mg/kg 7beta-hydroxy-EpiA i.p. once a day for 7 days. Thereafter, colitis was induced by administration of 5% DSS in drinking water for 7 days. Administration of 7beta-hydroxy-EpiA caused a transient increase in COX-2 and PGE synthase expression within 6-15h, and starting at day 2, augmented 15d-PGJ2 colonic tissue levels. Treatment with DSS resulted in enhanced COX-2 and mPGES-1 synthase expression accompanied by increased PGE2, D2 and 15d-PGJ2 productions. Although all dose levels of 7beta-hydroxy-EpiA reduced PGE2 production, only the lowest dose (0.01mg/kg) of the steroid completely prevented colitis damage. 7beta-Hydroxy-EpiA pre-treatment prevented the occurrence of DSS-induced colitis through a shift from PGE2 to PGD2 production, associated with an early but transient increase in COX-2 expression and a sustained increase in the production of the anti-inflammatory prostaglandin 15d-PGJ2.

In vitro studies used human peripheral blood monocytes cultivated in the presence of 7beta-hydroxy-EpiA (1-100nM), with and without TNF-alpha (10ng/mL) for 4h and 24h.

TNF-alpha augmented PGs production and increased COX-2 and m-PGES1 expressions. In the presence of 7beta-hydroxy-EpiA, COX-2, m-PGES1 and PPAR-gamma expressions were decreased together with PGE2 levels while 15d-PGJ2 production was increased.

These results suggest that 7beta-hydroxy-EpiA is a native trigger for the resolution of inflammation through simultaneous activation of 15d-PGJ2 and depression of PGE2 synthesis. These effects may be mediated by a putative receptor and take place after inflammation-associated antigen presentation for cellular protection in the resolution of inflammation.

**P -64 : Protective effect of *Absinthium artemisia* on brain enzyme and behaviour activity in rat exposed to lead**

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Lead, a ubiquitous and potent neurotoxicant causes several neurophysiological and behavioural alterations. Considering the vulnerability of the developing brain to Pb neurotoxicity, this study was carried out to investigate the effects of Pb exposure on brain regions acetylcholinesterase (AChE) and mono-amino-oxidase (MAO) enzymes activities and on behavioural changes. Wistar rat were exposed to 750 ppm of Pb acetate in the drinking water for 11-weeks after weaning, and treated by *Artemisia Absinthium* L. (wormwood) extract (200 mg.kg<sup>-1</sup> body weight) for 4-weeks. The activities of AChE and MAOs were determined in the hypothalamus, hippocampus, cerebral cortex and striatum of male rats; and General/ Locomotor activity was evaluated in the open-field test. Results indicated that lead caused a significant decrease in AChE activity (hypothalamus: -12%, hippocampus: -57%, cerebral cortex: -18% and striatum: -43%) and MAOs activity (hypothalamus: -29%, hippocampus: -41%, cerebral cortex: -28% and striatum: -51%), respectively, compared to control with altered behavioural abnormalities in locomotor and stereotypic activity. After, wormwood extract administration, the activity of AChE and MAOs were significantly increased in all brain region compared to intoxicated group, but were significantly lower than control, the locomotor and stereotypic activity were reduced compared to intoxicated group. These data suggest that the administration of wormwood extract for 4-weeks protect against the lead acetate-induced change in behavioural and changes in neurobiochemical parameters.

**P -65 : Prophylactic effect of wormwood on brain oxidative stress after lead exposed**

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Effects of ROS generation have been postulated to be major contributors to lead-exposure related disease. The aim of the study was to investigate the effect of aqueous extract of wormwood (*Artemisia Absinthium*) on the occurrence of oxidative stress in the brain of rats protractedly exposed to lead at the dose of 750 ppm by measuring the extent of oxidative damage as well as the status of the antioxidant defence system. Aqueous extract of wormwood was administered orally (200 mg.kg<sup>-1</sup> body weight) for 4 weeks. The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GRase) and reduced glutathione (GSH) levels were determined in the hypothalamus, cerebral cortex, hippocampus and striatum of male rat at 11 weeks and after 4 weeks of treatment by wormwood extract. After 11-weeks, GSH level were significantly increased in all region brain in intoxicated group (Pb) compared to untreated (hypothalamus: 44%; cerebral cortex: 13%; hippocampus: 16% and striatum: 25%); the activity of GSH-Px and GRase were significantly increased compared to untreated, whereas SOD was decreased in the hypothalamus and striatum (-39% and -10%). After 4-weeks of treatment all treated groups show a decreased GSH level and GSH-Px, GRase activity in all brain region compared to intoxicated group at 11-weeks (Pb), and SOD activity was increased in hypothalamus and striatum compared to Pb (15% and 56%, respectively). The findings of this study suggest that wormwood extract possess antioxidant potential and can restored the enzymes activities perturbed by lead and that may be used for therapeutic purposes.



**P -66 : Mouse knockout models reveal an essential role of the lipoxygenases Alox12b and Alox3 for acquisition and maintenance of the epidermal barrier function in the skin.**

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12R-lipoxygenase (12R-LOX) and epidermal lipoxygenase-3 (eLOX-3) are part of a recently identified eicosanoid pathway critically involved in terminal differentiation in skin. Inactivating mutations in the genes of 12R-LOX and eLOX-3 (ALOX12B and ALOXE3) are causally linked to the development of autosomal recessive congenital ichthyosis (ARCI), an inherited skin disease associated with hyperkeratosis and impaired barrier function.

By using the Cre/LoxP system we have generated constitutive and conditional 12R-LOX- and eLOX-3-knockout mouse models.

In the constitutive knockout models, ablation of 12R-LOX or eLOX-3 leads to early neonatal death which is due to a severely impaired permeability barrier function of the skin. Disruption of the barrier function is associated with ultrastructural anomalies in the upper granular layers of the lesional skin, disordered composition of ester-bound ceramide species and impairment of profilaggrin processing. When transplanted onto the back of nude mice, 12R-LOX deficient mouse skin develops a severe adult phenotype that closely resembles that seen in ichthyosis patients, with thickening of the epidermis, hyperproliferation, hypergranulosis, focal parakeratosis and marked hyperkeratosis.

In the conditional knockout model, a similar ichthyosiform phenotype is observed upon tamoxifen-induced 12R-LOX ablation in the epidermis. The induced KO phenotype is associated morphologically with focal alopecia, scaling of the skin and palmoplantar keratoderma, and systemically with growth retardation, dramatic loss of body weight and premature death.

Our studies document a key role of the 12R-LOX/eLOX-3 pathway in the normal homeostasis of the epidermis and furthermore, provide useful mouse knockout models in which the molecular mechanism of LOX action can be investigated in order to develop novel strategies towards a therapy for ARCI.

**P -67 : Gastroprotective effect of lipoxin: role of nitric oxide (NO), p38(MAPK) signalling pathway and NFkappaB**

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Background and Aims: Lipoxins (LXs) represent a class of arachidonic acid (AA) metabolites that exhibit immunoregulatory and anti-inflammatory properties, LXA4 being the main component of these compounds series. LX epimers at carbon 15, the 15-epi-LXs, are formed by aspirin-acetylated cyclooxygenase-2 (COX-2) in cooperation with 5-lipoxygenase (5 LO). 15-epi-LXA4 is also termed aspirin-triggered LX (ATL). The aim of the study was 1) to study the effect of LPXA4 given i.p. or i.g. on the gastric mucosal lesions induced by aspirin and accompanying changes in gastric mucosal blood flow (GBF) 2) effect of LPXA4 on mRNA and protein expression of NOSes, activation of NFkappaB and p38 MAPK signalling pathway in the gastric mucosa. Methods: Wistar rats were exposed to acidified aspirin (200 mg/kg). 30 min before exposure to ASA rats were treated with vehicle (control) or increasing doses of lipoxins (0.1-5µg/kg i.p. or i.g). The lesion area and gastric mucosal blood flow were measured by planimetry and H<sub>2</sub> gas clearance method. Expression of proinflammatory cytokine IL-1β and NOSes (iNOS and cNOS) was analyzed by RT-PCR and Western blot. Expression of phosphorylated p38 in cytoplasmic fraction and NFkappaB in nuclear fraction was evaluated by Western blot. In separate experiments blockade of NOS by L-NNA ( 20 mg/kg i.p.) attenuated the protective effect of lipoxins on gastric mucosa. Results: LPXA4 given intragastrally and intraperitoneally significantly reduced dose-dependently the lesion area induced by acidified aspirin and this effect was accompanied by an increase in GBF. LXA4 caused significant down regulation of IL-1β expression, decrease in expression of phosphorylated p38 and activity of NFkappaB. LXA4 significantly expression of both iNOS and cNOS in the gastric mucosa. Conclusion: We conclude that LPXA4 show potent gastroprotective effect due to its anti-inflammatory action, inhibition of p38 MAPK signalling pathway and induction of NO by iNOS and cNOS.

**P -68 : Oleoyl Serine, an Endogenous Regulator of Skeletal Mass**

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Bone mass is determined by a continuous remodeling process whereby the mineralized matrix is being removed by osteoclasts and subsequently replaced with newly formed bone tissue produced by osteoblasts. Here we report the presence of endogenous N-acyl amides in mouse bone. Of these compounds N-oleoyl-L-serine (OS) had the highest activity in an osteoblast proliferation assay. In these cells OS triggers a Gi-protein coupled receptor and Erk1/2. It also mitigates osteoclast number by promoting osteoclast apoptosis through the inhibition of Erk1/2 phosphorylation and receptor activator of nuclear kappa B ligand (RANKL) expression in bone marrow stromal cells/osteoblasts. In a mouse OVX model for osteoporosis OS rescues bone loss by increasing bone formation and restraining bone resorption. Thus, OS is a novel endogenous lipid regulator of bone remodeling. It represents a new lead to anti-osteoporotic drug discovery, advantageous to currently available therapies, which are essentially either pro-formative or anti-resorptive.

**P -69 : Effect of Ephedrine (beta agonist) on growth performance and carcass characteristics of finishing lambs (Mazandaran Province).**

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The effect of the beta adrenergic agonist, ephedrine, on growth performance, carcass characteristics, blood metabolites of finishing lambs (Mazandaran Province) were examined. Lambs were randomly assigned to the three groups consisting of six animals each. Ephedrine was injected subcutaneously at 0, 4 (low dose) and 8 (high dose) mg. Most feedlot parameters were not influenced by ephedrine treatment ( $p < 0.05$ ). High dose of ephedrine had a better feed conversion efficiency. Carcass moisture, fat and protein percentage were not affected by ephedrine, but ash percentage was a significant difference. Total carcass protein was higher for lambs receiving the high dose of ephedrine (17.94). Ephedrine did not significantly affect longissimus muscle area (LM), hot weight, live weight, dressing percentage ( $p < 0.05$ ). But increasing level of ephedrine, increased carcass protein, longissimus muscle area, hot weight, live weight, gain: feed (linear component,  $p < 0.05$ ) and reduced kidney-pelvic fat and carcass fat (linear component,  $p < 0.05$ ). Plasma levels of glucose, cholesterol, triglycerid, total protein, BUN were not affected by treatment. But high dose of ephedrine had high protein and low triglycerid. The result showed that ephedrine increased the protein and decreased the fat content in the thin-tail lambs.

Keywords: Beta -agonist, ephedrine, carcass characteristics, Zel lambs.

**P -70 : 5-lipoxygenase activating protein signals adipose tissue inflammation and lipid dysfunction in experimental obesity**

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The presence of the so-called “low-grade” inflammatory state is recognized as a critical event in adipose tissue dysfunction leading to altered secretion of adipokines and free fatty acids (FFAs), insulin resistance and development of hepatic complications associated with obesity. This study was designed to investigate the potential contribution of the pro-inflammatory 5-lipoxygenase (5-LO) pathway to adipose tissue inflammation and lipid dysfunction in experimental obesity. Constitutive expression of key components of the 5-LO pathway as well as leukotriene (LT) receptors were detected in adipose tissue as well as in adipocyte and stromal vascular fractions. As compared to lean mice, adipose tissue from obese mice exhibited increased 5-LO activating protein (FLAP) expression and LTB<sub>4</sub> levels. Incubation of adipose tissue with 5-LO products resulted in NF-kappaB activation and augmented secretion of pro-inflammatory adipokines such as MCP-1, IL-6 and TNFalpha. In addition, LTB<sub>4</sub>, but not LTD<sub>4</sub>, reduced FFA uptake in primary adipocytes, whereas 5-LO inhibition suppressed isoproterenol-induced adipose tissue lipolysis. In mice with dietary obesity, elevated FLAP expression in adipose tissue was paralleled with macrophage infiltration, increased circulating FFA levels and hepatic steatosis, phenomena that were reversed by FLAP inhibition with Bay-X-1005. Interestingly, FLAP inhibition induced AMPK phosphorylation in parallel with decreases in HSL activity and the expression and secretion of TNFalpha and IL-6. Similar effects were observed in differentiated 3T3-adipocytes incubated with either Bay-X-1005 or the selective LTB<sub>4</sub> receptor antagonist U-75302. Taken together, these findings indicate that the 5-LO pathway signals the adipose tissue “low-grade” inflammatory state and steatogenic potential in experimental obesity.

**P -71 : Alpha-synuclein binding to oxidized polyunsaturated fatty acids promotes the formation of aggregates**

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Alpha-synuclein is a small cytosolic protein of unknown function and the major component of pathogenic Lewy bodies in Parkinson's disease. The mechanism by which alpha-synuclein deposits into Lewy bodies is still unclear and multiple factors have been implicated in the cause and progression of sporadic Parkinson's disease. Among these, alpha-synuclein malfunction, lipid metabolism and oxidative stress constitute a trio which may hold a key to the selective degeneration of dopaminergic nerve cells in this disease. Previous studies demonstrated that polyunsaturated fatty acids (PUFAs) change alpha-synuclein conformation, which adopts an alpha-helix conformation and form aggregates. Now, we report the effect of oxidation of PUFAs on their interaction with alpha-synuclein. The effect of fatty acid oxidation in alpha-synuclein aggregation was determined by incubation of purified protein with several concentrations of oxidized arachidonic acid. The presence of arachidonic acid is enough to produce alpha-synuclein aggregation but this effect is increased with non-enzymatic oxidation of this fatty acid. Moreover, this effect can be also achieved by the presence of dopamine and fatty acid in solution. These results suggest that an increase in cellular free fatty acids and oxidative stress contribute to increase alpha-synuclein aggregation and underline the importance of targeting lipid metabolism enzymes to combat Parkinson's disease progression. Finally, to explore the effect of PUFAs interaction with alpha-synuclein in vivo, RAW 264.7 cells were transfected with different constructs of wild-type and alpha-synuclein mutants. Interestingly, we found that overexpression of alpha-synuclein in RAW 264.7 affects cell viability, increasing cell death.

**P -72 : In vitro cytotoxic analysis of methanolic extract of *Berberis aristata* D.C. and *Hemidesmus indicus* R.Br.**

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The present study was carried out to evaluate the invitro cytotoxic activity of unexploited plants, *Berberis aristata* and *Hemidesmus indicus* indigenous to India. Different concentrations of the methanolic extracts of stems and rhizomes parts of the plant (1000, 500, 250, 125, 50, 25, 12.5 mg/ml) were subjected to cytotoxic study against MCF7 breast cancer cell lines. In addition, a phytochemical screening of the methanolic extracts was done. The phytochemical screening demonstrated the presence of different types of compounds like flavonoids, terpenoids, alkaloids, phenols and sterols, which could be responsible for the obtained activity. The maximum reducing power of the *Berberis aristata* and *Hemidesmus indicus* extract at 680nm was found to be  $0.997 \pm 0.081$  at 1000 mg/ml and  $0.956 \pm 0.067$  at 1000 mg/ml respectively. The inhibition percentage with regard to cytotoxicity was found to be 89 % at 1000  $\mu\text{g/ml}$  with IC<sub>50</sub> value of  $50 \pm 0.03$  mg/ml for *Berberis aristata* and 87 % at 1000  $\mu\text{g/ml}$  with IC<sub>50</sub> value of  $48 \pm 0.02$  mg/ml for *Hemidesmus indicus* respectively.

**P -73 : Synthesis of phytoprostanes B1 and E1: Bioactive lipids in plants and human**

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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) in vivo,<sup>1</sup> an interesting field of research has been developed.<sup>2</sup>

Higher plants are generally unable to synthesize AA and thus, neither form IsoPs. Instead, plants utilize  $\gamma$ -linolenic acid (LA) for the synthesis of prostaglandin-like compounds of the jasmonate type as dinor-IsoPs, termed phytoprostanes (PPs and PhytoPs).<sup>3</sup> These metabolites are also formed via a non-enzymatic free radical catalyzed pathway analogous to IsoPs.

In order to fully assess the physiological activities of each of the enantiomerically pure PPB1 and PPE1, we have developed a new chemical strategy, based on a furan approach<sup>4</sup>

In a variety of plant species PhytoPs induce phytoalexins<sup>5</sup> suggesting a possible function of PhytoPs as mediators of defense reactions in response to oxidative stress in plants. Similar to IsoPs in animals, plant PhytoPs represent reliable markers of oxidative stress in plants in vivo and can also serve as a quantitative measure of oxidative degradation of plant products such as vegetable oils and plant extracts used for nutrition or in pharmacotherapy.<sup>6</sup>

Interestingly, in a human neuronal model (neuroblastoma SH-SY5Y cell line),<sup>7</sup> B1-PhytoP increased the energetic metabolism of the cells (as indicated by the increased ability of SH-SY5Y cells to reduce MTT at mitochondrial levels) and significantly protected them from death induced by H<sub>2</sub>O<sub>2</sub>. These effects were observed at 0.1-1  $\mu$ M concentrations. The protective effect against H<sub>2</sub>O<sub>2</sub> was not due to an increase in the expression of scavenging enzymes such as catalase, CuZn- or Mn-SOD.

We will present the total synthesis of such PhytoPs together with our preliminary data on plant and human fields.

#### References

1. Morrow, J.D.; Hill, K.E.; Burk, R.F.; Nammour, T.M.; Badr, K.F.; Roberts II, L.J. Proc. Natl. Acad. Sci. USA 1990, 87, 9383-9387.
2. For a general review, see Chem. Phys. Lipids 2004, 128, 1-193. Jahn U.; Galano, JM; Durand, T. Angewandte chemie, 2008, 47, 5894-5955.
3. Imbusch, R.; Mueller, M.J. Plant Physiol. 2000, 124, 1293-12303. Durand, T.; Bultel-Ponce, V.; Guy, A.; Berger, S.; Mueller, M-J.; Galano, J-M. Lipids, 2009, DOI 10.1007/s11745-009-3351-1
4. a) El Fangour, S.; Guy, A.; Vidal, J-P.; Rossi, J-C.; Durand T. J. Org. Chem 2005, 70, 989-997. b) Pinot, E.; Guy, A.; Fournial, A.; Balas, L.; Rossi, J.-C.; Durand, T. J. Org. Chem. 2008, 73, 3063-3069.
5. Loeffler, C.; Berger, S.; Guy, A.; Durand, T.; Bringmann, G.; von Rad, U.; Durner, J.; Mueller M.J.; Plant Physiol. 2005, 137, 328-340
6. Karg K, Dirsch VM, Vollmar AM, Cracowski JL, Laporte F, Mueller MJ Free Radic Res. 2007,41, 25-37.
7. Ruffels, J.; Griffin, M.; Dickenson, J-M. Eur J Pharmacol. 2004, 483, 163-73.



**P -74 : Role for oxidized cholesterol nuclear receptors in airway allergic inflammation**

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Oxysterol-responsive Liver X Receptors (LXRs), members of the nuclear receptor family of transcription factors that regulate cholesterol, glucose and fatty acid homeostasis, have emerged as anti-inflammatory and anti-proliferative regulators of immune cells. In contrast, we and others have demonstrated that LXR-activation significantly contributes to the mouse Th1/Th17-mediated immune responses involved in the protection against pulmonary tuberculosis (Korf H. et al., 2009, JCI, 119(6):1626-1637) and in the articular inflammation in a murine collagen-induced arthritis model (Asquith D.L. et al., 2009, Arthritis Rheum., 60(9):2655-2665). Also, we observed a detrimental role for LXRs in the Th2-driven eosinophil inflammatory response to airway allergen challenge in an Alum-based asthma model. Mice deficient in both LXR-alpha and LXR-beta isoforms showed a significant, two-fold reduction in airway eosinophilia upon repeated allergen challenges as compared to wild-type animals, concurrent with an unaltered lung tissue Th-cell profile. This hypo-inflammatory phenotype could be ascribed to bone marrow derived leukocytes by the means of bone marrow chimeras. Preliminary results of experiments, aimed at disclosing the cellular mediators involved in LXR-dependent allergic inflammation, point towards a combined effect of eosinophil turn-over (hematopoiesis and recruitment) and of immune instruction by dendritic cells (migration and antigen presentation). By dissecting these pathways with whole-genome transcriptional and Chip-sequencing approaches, we expect to identify the molecular basis for LXR-supported allergic airway responses in time to present these exciting new data at the 3rd European Workshop on Lipid Mediators. Taken together, the importance of modified cholesterol hormones in hallmark immunopathological mechanisms underlying allergic inflammation presents a novel target of therapeutic intervention in human diseases such as mild-to-moderate asthma.

**P -75 : The impact of membrane active synthetic antioxidants on in vitro and ex vivo lipid peroxidation level and lipoxygenase activity.**

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It is considered that oxidative stress leads to damage of the intracellular and extracellular components, proteins, lipids, and nucleic acids, promoting the development of various diseases and accelerating the aging process. The potential of antioxidants to prevent oxidative processes in the living organism has therefore attracted much attention. The detection and measurement of lipid peroxidation (LP) level is most frequently used as a marker of oxidative stress degree. Usually only one or two methods of antioxidant activity determination are applied for each compound under investigation.

The aim of our study is the all-round evaluation of antioxidant activity of physiologically active compounds. The comparative analysis of various organic, organometallic and coordination compounds has been performed using following methods: DPPH-test, CUPRAC, scavenging activity of superoxide radical-anion generated by xanthine/xanthine oxidase, in vitro LP in liver homogenates and ex vivo LP in rat brain and rat liver mitochondria. A special attention has been paid to lipoxygenase inhibition activity of tested compounds.

The following antioxidant candidates were studied: ferrocenes and dipicolylamine (DPA) complexes of biometals (Fe, Co, Cu, Mn, Ni, Zn) bearing antioxidative 2,6-di-tert-butylphenol and 2,6-di-isobornylphenol fragments; and antimony(III) iodide complexes of the heterocyclic thioamides.

Antioxidant capacity assays may be broadly classified as electron transfer (ET) and hydrogen atom transfer assays according to the mechanism of antioxidant effect. DPPH-test is a model reaction of hydrogen atom transfer. Superoxide radical-anion scavenging activity and CUPRAC method show the capacity of an antioxidant to be involved in ET mechanism. In vitro LP inhibition includes hydrogen transfer to peroxy radicals of fatty acids. Conversion of arachidonic acid via the lipoxygenase pathway is associated with a production of ROS. Antioxidants may interact non-specifically with lipoxygenase by scavenging radical intermediates and/or reducing the active site. Thus, the impact of antioxidant on the lipoxygenase activity might be considered as a more sophisticated biotest.

Therefore, there is a strong necessity to consider all the mechanistic pathways responsible for the antioxidant effect. The most promising candidates were chosen according to the activity in all processes studied.

**P -76 : Modulation of eicosanoid biosynthesis by protein-membrane interactions**

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Leukotrienes constitute an important class of lipid-derived mediators involved in inflammation, allergy and related diseases. Biosynthesis of leukotrienes starts with phospholipase A2-mediated hydrolysis of membrane phospholipids and liberation of arachidonic acid, which then undergoes oxygenation by lipoxygenase enzymes, first of all 5-lipoxygenase. Phospholipase A2 (PLA2) and 5-lipoxygenase (5-LO) are interfacial enzymes that become activated by binding to cellular membranes and operate at the membrane-water interface. All aspects of protein-membrane interactions, such as lipid selectivity, membrane binding strength, depth of membrane insertion, and orientation of the membrane-bound protein are important determinants of the function of interfacial enzymes.

We have shown that membrane lipid composition modulates the activity of human 5-LO by changing membrane fluidity. Membrane binding strength, membrane insertion, and the activity of 5-LO increase with the degree of the lipid acyl chain unsaturation and reach a maximum value for membranes composed of 1-palmitoyl-2-arachidonoyl-phosphatidylcholine. These data suggest that subcellular localization of 5-LO at the nuclear membrane, which is rich in lipids with esterified arachidonic acid, may be determined by increased membrane fluidity, promoting a tighter membrane binding, deeper penetration, and a higher enzyme activity.

Studies on human secreted group IB and IIA PLA2s and bee venom PLA2 identified relatively deep membrane insertion of the former two enzymes but no appreciable insertion of the latter. This finding suggests that the human PLA2s use the membrane-residing phospholipid substrate whereas bee venom PLA2 can use both membrane-residing and aqueous phospholipid molecules as substrate. Polarized FTIR studies on membrane-bound PLA2 molecules that are labeled with stable isotopes ( $^{13}\text{C}$ ) at desired segments allowed determination of the precise configuration of the protein-membrane complex. These structures provide insight into the molecular mechanisms of the enzymes, such as the mode of substrate accession and product release. Our studies identify that a better understanding of the molecular events taking place at the protein-membrane interface may help develop new strategies to regulate the function of enzymes playing central roles in biosynthesis of lipid-derived mediators.

**P -77 : Novel derivatives of pyrazole-3-propanoic acid as 5-lipoxygenase inhibitors**

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5-Lipoxygenase (5-LO) converts arachidonic acid to leukotrienes (LT). The formation of LTs is associated with several inflammatory, cardiovascular and allergic diseases like asthma, atherosclerosis and allergic rhinitis. Therefore, the inhibition of LT formation is an interesting pharmacological strategy to treat these diseases. At present, there is no 5-LO inhibitor for therapeutic use available on the market in Europe. Here we show that derivatives of pyrazole-propanoic acids inhibit 5-LO product synthesis in intact human neutrophils when stimulated with ionophore A23187. Structural optimization led to compounds with IC<sub>50</sub> values between 1.2 and 4.8 µM. In a cell-free system, the compounds showed no or only moderate inhibition of 5-LO product formation. This suggests that the compounds may interfere with a regulatory mechanism of 5-LO in intact cells resulting in inhibition of LT formation rather than directly acting on the 5-LO enzyme. Ongoing studies aim on the identification of the exact mode of action underlying inhibition of cellular LT formation. Based on their high efficacy, these novel structures represent promising candidates for further development as suitable inhibitors of 5-LO product synthesis (This work is partly supported by a TÜBİTAK Research Grant 108S210).

**P -78 : Biphasic response in prostanoid breakdown during UVR-induced inflammation in human skin.**

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Sunburn is a self-resolving inflammatory process, evidenced clinically as an erythema reaching maximal expression at 24h, and histologically as a leucocytic infiltrate. Lipid mediators are pivotal in this response, following UVR-induced membrane release of precursor arachidonic acid and upregulation of cyclooxygenases and lipoxygenases. Prostaglandin (PG)E<sub>2</sub> is a key mediator of the vasodilatation, and modifies a range of cellular processes including apoptosis and cell proliferation. We aimed to identify pro-inflammatory prostanoids and their breakdown products in human sunburn.

Blister fluid was collected in vivo (n=10 subjects, aged 23-52yrs, phototypes II/III) from unirradiated skin and skin irradiated 24 hours earlier with 3 times the minimal erythema dose (MED, the sunburn threshold) of broadband UVB (Waldmann UV6; 280-400nm). Samples were analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), allowing identification of a range of pro- and anti-inflammatory eicosanoids and their breakdown products.

Elevated levels of 12-hydroxy eicosatetraenoic acid (HETE) and 15-HETE were observed in UV-exposed skin ( $p < 0.01$ ;  $p < 0.05$  respectively) compared with unexposed skin, with an apparent rise in 15-hydroxyeicosatrienoic acid (15-HETrE). In addition to these changes in pro- and anti-inflammatory lipoxygenase products, cyclooxygenase products PGE<sub>1</sub> and PGE<sub>2</sub> appeared raised, with considerable inter-subject variation. In contrast, a consistent trend for reduction in PG breakdown products was observed at 24h, characterised by a 90% fall (from 16.3pg/ $\mu$ l to 1.8pg/ $\mu$ l) in the PGE<sub>2</sub> breakdown product 13,14-dihydro 15-keto PGE<sub>2</sub> ( $p < 0.05$ ). This early reduction in 13,14-dihydro 15-keto PGE<sub>2</sub> is in contrast to our recent observation of significantly elevated levels of this and other PG breakdown products at later time points (48-72h) post UVR (Rhodes LE et al, FASEB J 2009; 23: 3947-3856).

Hence, a biphasic response in PG breakdown occurs following UVR exposure of human skin and this may contribute both to the early increase and later deactivation of pro-inflammatory prostanoids including PGE<sub>2</sub>.

**P -79 : Cardamonin inhibits IFN-gamma/LPS-induced inflammatory cytokines syntheses in macrophages mediated via ERK1/2 and p38 pathways**

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Anti-inflammatory activity of chalcones on cell signaling pathways linking to stress and inflammation have not been well elucidated. In this study, we investigated the mechanism(s) by which cardamonin, a chalcone derivative isolated from fruits of *Alpinia rafflesiana* inhibits IFN-gamma/LPS-induced inflammatory cytokines syntheses in macrophages through the mitogen activated protein kinase (MAPK) pathways. Cardamonin significantly inhibits IFN-gamma/LPS-induced interleukin (IL)-1beta, IL-6 and IL-10 syntheses in a dose-dependent manner and reduced the expression of ERK1/2 and p38 MAPK activation in RAW 264.7 cells. Our present results suggest that cardamonin inhibits IFN-gamma/LPS-induced IL-1beta and IL-6 syntheses through the inhibition of activation of ERK1/2 and p38 signaling pathways, respectively. However, the synthesis of anti-inflammatory cytokine (IL-10) has no direct effect on pro-inflammatory cytokines (IL-1beta and IL-6) syntheses upon cardamonin treatment. Comparable to PD98059 and SB203580, we suggest that suppression of phosphorylated ERK1/2 and p38 MAPK upon cardamonin treatment are due to the attenuation of MEK and MKK respectively, whereby disturb the activation of NF-kappaB to regulate the synthesis of IL-1beta, IL-6 and IL-10. Our present investigation showed that cardamonin might disturb the development of inflammation by attenuating the main inflammation signaling pathways, which are ERK1/2 and p38 pathways through the inhibition of IL-1beta, IL-6 and IL-10 syntheses.

**P -80 : Neuroprostanes Inhibit [<sup>3</sup>H]D-Aspartate Release in Bovine Retina, *in vitro***

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Although isoprostanes (IsoP) have been reported to regulate excitatory amino acid neurotransmitter release from mammalian neural retina, *in vitro* (LeDay et al. *Curr Eye Res* 28:367-372, 2004; Opere et al. *Neurochem Res* 30(1):129-137, 2005), the pharmacological/physiological role of IsoP-like compounds such as neuroprostanes (NeuroP) on the eye is unclear.

Purpose: In the present study, we investigated the effect of oxidant stress induced by peroxides on production of NeuroP. Furthermore, we studied the effect of NeuroP on K<sup>+</sup>-induced [<sup>3</sup>H]D-aspartate release in isolated bovine retina.

Method: Freshly isolated bovine retina were either exposed (a) to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and cumene hydroperoxide (cuOOH) for up to 6h, *in vitro* for measurement of NeuroP using the stable isotope dilution methods employing GC/NICI-MS or (b) incubated in oxygenated Krebs solution containing 200nM of [<sup>3</sup>H] D-aspartate for 60 mins and then prepared for studies of neurotransmitter release using the superfusion method. Release of [<sup>3</sup>H]D-aspartate was evoked by iso-osmotic concentration of K<sup>+</sup> (50mM)-stimuli applied at 80-88 mins (S<sub>1</sub>) and 116-124 min (S<sub>2</sub>) after the onset of superfusion.

Results: H<sub>2</sub>O<sub>2</sub> (1 mM) increased production of A<sub>4</sub>-nP levels by 15% and 56% after 1 and 6h periods of treatment, respectively. Similarly, cuOOH (1 mM) enhanced (p<0.001) A<sub>4</sub>-nP levels by 408% and 420% after 1h and 6h periods of exposure to the oxidant, respectively. In the concentration range, 1 nM to 3 μM, the docosapentaenoic acid-derived NeuroP, BA-12low and BA-12high inhibited [<sup>3</sup>H]D-aspartate release in a concentration dependent manner, with BA-12low achieving a maximum inhibitory effect of 16% at 0.1 μM while BA-12high achieved a maximal inhibitory effect of 37.2% (p<0.01) at 1 μM. Similarly, in the concentration range, 1nM to 1μM, the docosahexaenoic acid metabolite, CO3-475 exhibited a concentration-dependent attenuation of [<sup>3</sup>H]D-aspartate release, with a maximal inhibitory effect of 26.6% (p<0.01) being observed at the 0.1 μM concentration.

Conclusion: We conclude that oxidative stress can enhance endogenous NeuroP levels in bovine retina. Furthermore, NeuroP exhibit an inhibitory effect on excitatory neurotransmitter release in isolated bovine retina. Taken together, our findings support a pharmacological role for NeuroP on amino acid neurotransmission in the retina.

**P -81 : The assessment of the alterations in serum lipids and homocysteine in hemoglobinopathies**

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**Background:** Cardiovascular complications are common in hemoglobinopathies, mainly attributed to increased cardiac iron depositions. Homocysteine is a biochemical marker of premature atherosclerosis and cardiovascular disease. Early cardiovascular involvement in patients without cardiac symptoms and without cardiac iron overload has not been adequately investigated.

**Aim:** The aim of this study was to assess the lipid profile and total homocysteine (tHcy) serum levels in patients with hemoglobinopathies.

**Methods:** We evaluated in 45 patients (all women) with beta-thalassemia major (a-TM) and 52 with sickle cell anemia (SCA) the serum levels of total cholesterol (CHOL), triglycerides (TG), high-density lipoprotein (HDL) and low density lipoprotein (LDL) with a enzymatic colorimetric assay. Serum levels of total homocysteine (tHcy) were determined using Fluorescence Polarization Immunoassay (FPIA). A control group (CG) of 20 age-matched healthy women were studied simultaneously.

**Results:** Among patients with beta-thalassemia major and healthy control group there was statistically significant difference in serum levels of TG and HDL ( $p < 0,05$ ). It is remarkable that in patients with beta-thalassemia major serum levels of all lipids presented statistically significant difference ( $p < 0,05$ ) compared to the healthy group. The homocysteine levels are not significantly increased in any group but in the sickle cell anemia patients the mean value is higher than that of the normal individuals. The slightly increased levels of tHcy are probably due to the supplementary treatment of these patients with external intake of folic acid and  $\hat{A}6$

**Conclusions:** 1) Lipid profile of patients with beta-thalassemia major and sickle cell anemia differs from the one present in control group. 2) Patients with beta-thalassemia major and sickle cell present elevated levels of TG and low serum levels of HDL. 3) Low levels of total homocysteine may prevent atherosclerotic vessel damage.



**P -82 : Association between hyperglycaemia and hyperlipidaemia in women with diabetes mellitus type II.**

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Background: The alterations of lipids which are observed in Diabetes Mellitus type II (DM) contribute to the presence of atherosclerosis.

Aim: The aim of this study was to evaluate the existence of dyslipidemia in patients with type II Diabetes mellitus.

Methods: We studied 100 patients with DM (women), divided into two groups according to the percentage of Glycated hemoglobin (HbA1c). The first group comprised 50 patients with HbA1c > 8,00% and the second group comprised 50 patients with HbA1c < 8,00%. The third group included 40 normal individuals (N.I) (women).

HbA1c was measured by high-performance liquid chromatography (HPLC), while the levels of glucose (Glu) were measured by phasmatophotometric method. Cholesterol (CHOL), tricyclerides (Tg), high density lipoproteins (HDL) and low density lipoproteins (LDL) were determined by enzymatic colorimetric assay.

Results: There was a statistically significant difference between patients with HbA1c > 8,00% and patients with HbA1c < 8,00% concerning the values of Glu, Tg and HbA1c ( $p < 0.001$ ).

The values of serum levels of Glu, Tg and HbA1c in pathological groups were significantly higher in relation to the values of normal individuals ( $p < 0.001$ ), while the values of HDL were lower compared to the values of N.I. ( $p < 0.001$ ).

There was a positive correlation between the levels of Glu and Tg in the group with HbA1c > 8,00% ( $r = 0,419$   $p < 0,05$ ).

Conclusions: According to our study, in type II Diabetes Mellitus hypertriglyceridemia and low concentrations of HDL are observed. The increased levels of glucose and HbA1c induce increased levels of lipids.

**P -83 : The effects of lysophosphatidic acid on the vascular tone**

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Lysophosphatidic acid (LPA) has been known as an inflammatory mediator, but it also plays important roles in different cardiovascular diseases such as neointimal hyperplasia. LPA was described to cause vasoconstriction in newborn piglet pial arteries, but there is recent evidence that LPA induces nitric oxide (NO) production in bovine aortic endothelial cells. Our aim was to investigate the effects of LPA on the vascular tone and the signaling pathways involved.

Segments of the thoracic (TA) and abdominal aorta (AA) were dissected from male C57Bl/6J (WT) and endothelial NO synthase deficient (eNOS-KO) mice and mounted on a wire myograph. Effects of 10 microM LPA were tested under isometric conditions after precontraction by 0.1-1 microM phenylephrine. Pertussis toxin (PTX), wortmannin and edelfosine were administered to inhibit signaling via Galphai protein, PI3-kinase (PI3K) and phospholipase C (PLC), respectively.

WT TA segments responded to LPA by a transient relaxation ( $41.5 \pm 3.1$  %), which was absent in TA segments from eNOS-KO mice ( $2.5 \pm 0.3$  %). Relaxations of WT TA segments treated by PTX were significantly lower ( $14.5 \pm 2.9$  %) than relaxations of untreated WT TA segments. Interestingly wortmannin treated WT TA segments did not differ in relaxation ( $45.5 \pm 6.2$  %) compared with untreated WT TA segments. Administration of edelfosine lowered relaxation significantly ( $5.5 \pm 1.5$  %). AA segments showed similar results except of that LPA-induced relaxation was followed by a tonic contraction.

Our results indicate that LPA causes vasorelaxation by activation of eNOS. PTX-sensitive Gi protein mediates this effect of LPA. Surprisingly however, downstream of the Gi protein PI3K does not play any role in the vasodilation induced by LPA. PLC activity is involved in LPA induced vasodilatation probably via calcium mobilization, thereby activating calcium-calmodulin dependent NO production by eNOS.

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**P -84 : Expression of PAT proteins and lipid droplets accumulation during differentiation and dedifferentiation of the human adipose tissue progenitor cells (SVF)**

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The aim of the study was to evaluate lipid accumulation along with expression of the proteins associated with the lipid droplets (PAT proteins) and ER stress in progenitor cells SVFs (Stromal Vascular Fraction) treated with a selected free fatty acid (PA, OA, AA, EPA), PPAR $\gamma$  agonist (TTA, rosiglitazone), or TNF $\alpha$  to generate cellular stress.

Methods: SVF cells were cultured in the adipocyte differentiation medium 48hrs, out which the last 24hrs included incubation with non-toxic concentrations of FFA. The investigated cells were harvested after 15-day-long differentiation, and then after another 15 days of dedifferentiation induced by FCS. Changes in expression of the selected PAT proteins: perilipin (PLIN), adipophilin (ADFP) and TIP47 were monitored with specific antibodies visualized with immunofluorescent high throughput technique (BD Bioimager). Bodipy-produced signal corresponded to lipid accumulation and formation of lipid droplets (LD) in the cells. The red-oil-o staining for TG accumulation was also used. The parallel effect on the cell metabolic status was followed by changes in the mitochondrial membrane potential determined by TMRM staining, and ATP generation.

Results: Our findings indicate that FFA can modulate expression of the selected PAT proteins, but they only minimally correlate with intracellular TG accumulation. PLIN and TIP47 expression levels were decreased following differentiation period, while ADFP was mostly increased. Presence of serum during the entire procedure led to much stronger expression of both ADFP (mainly in the cells exposed to OA, EPA, AA and TTA) and TIP47. Incubation with serum to induce dedifferentiation phase led to a significant increase of PLIN, especially in OA and TTA-treated cells, and a slight decrease of ADFP and TIP47. At the same time, there was a significant Bodipy accumulation in the SVFs incubated with FCS for the entire length of the experiment (30 days), in particular in those treated with AA and EPA. Metabolism of the differentiating SVF cells initially increased, followed by its significant decrease after addition of FCS as demonstrated by reduction of ATP intracellular concentration.

Conclusions: mitochondrial membrane potential was reduced, hypopolarized, under all the analyzed conditions that indicated cellular stress.

**P -85 : Endothelial and adipose stromal vascular fraction (SVF) metabolism in presence of beta-carotene and fatty acids**

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**Background:** Adipose tissue consists of heterogenous population of cells called stromal vascular fraction (SVF). The mechanisms of differentiation of SVF has been extensively studied, but the influence of nutrients on the metabolism of SVF cells is still poorly understood. Endoplasmic reticulum (ER)-stress, connexin 43 (cx43) mitochondrial translocation cooperate with mitochondrial function in cell survival and protection from apoptosis.

**Aim.** The aim of the study concentrate on evaluation of the effect of dietary free fatty acids (FFA) and beta-carotene (BC) on mitochondrial function determining the proangiogenic or propadipogenic path of SVF differentiation.

**Methods.** HUVEC and human adipose tissue SVF were cultured with non-toxic concentrations of PA, AA, EPA, OA or BC for 24h. Mitochondria were isolated using Mitochondrial Isolation Kit for Cultured Cells with Protease Inhibitor (Halt<sup>TM</sup>), and stained by Mitotracker red CMXRos. Changes in gene expression was analyzed by microarray (Affymetrix) confirmed qRT-PCR. Western Blot method was used for estimating changes of amount of total and phosphorylated form of cx43. Metabolic activity of mitochondria were analyzed by ATP production and oxygen requirement (Oxygraph 2-K). The changes in mitochondrial inner membrane potential was followed by the fluorescence microscopy imaging in vivid cells (BioimagerBD)

**Results.** Proangiogenic VEGF, bFGF as well EPA and AA inhibited, when PA promoted differentiation SVF to adipocytes. SVF metabolism measured by consumed oxygen and ATP was higher than HUVEC. FFA as well as BC did not significantly change oxygen consumption, but ATP generation was decreased by PA and OA. Tendency to increase metabolism of lipids in SVF by EPA and in HUVEC, when glucose by OA in SVF was seen. BC and FFA inhibited cx43 genes in both investigated cell lines but neither induced translocation, mitochondrial membrane potential change nor influence oxygen consumption and ATP generation. Microarray analysis revealed an induction of intracellular substrate transporters by FFA, involvement of genes both in metabolism, angiogenesis as well as ER-shock chaperones protein induction.

**Conclusion.** The differentiation of SVF to adipose tissue is regulated by complex mechanisms involving not only metabolic regulation, but may activate ER-stress related apoptotic/autophagy pathways.

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**P -86 : Ulcerative colitis and its extraintestinal manifestations -as consequence of violation lipid an exchange**

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At dyslipidemia (an obliterating atherosclerosis, fatty infiltration a liver, a metabolic syndrome, etc.) occur expressed dysbiotic the intestines changes which consequence is endotoxemia, a bacterial translocation, infringement of function and liver structure. Violation of function of a liver at dyslipoproteinemia are caused, mainly, expressed dysbiosis the colon, expressed fall of the general level of flying fat acids and increase anaerobic an index, characteristic for oppression of resident microflora of intestines: violation of microecology of intestines, accumulation endotoxins – violation of enterohepatic circulation of bilious acids - violation of function of a liver – violation exchange of lipids - violation of structure of a liver (fatty infiltration, fibrosis) - violation exchange of lipids - maintenance (aggravation) broken intestinal of dysbiosis. It can be one of factors occurrence Ulcerative Colitis and its extraintestinal manifestations from outside skeletal-articulate system.

The most comprehensible to application in complex therapy Ulcerative Colitis and its manifestations may be is Lactulosa (Normase) on special scheme. In colon Lactulosa becomes a food substratum for microflorae: bifido - and lactobacterius that promotes its active growth and oppression of ability to live of putrefactive and is conditional-pathogenic bacteria. Oppressing pathogenic flora, Lactulosa thereby reduces penetration into blood of toxins (neurotoxins, carcinogens, endoantigens) - products of ability to live of pathogenic and putrefactive bacteria. In colon from 1 g Lactulosa it is formed nearby 0,5 g fat acids, thus reaction of environment in a colon varies with alkalescent (optimum for putrefactive bacteria) on subacidic. Regular reception Lactulosa (Normase) on special scheme promotes improvement or metabolism normalization lipids as on hematological to criteria (levels of cholesterol and other), and on hematological to indicators of a condition of function of a liver, decrease in activity Ulcerative Colitis.

**P -87 : Bactericidal effect of sPLA2-IIA on antibiotics multi-resistant *Pseudomonas aeruginosa***

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Abstract: Pulmonary infection by bacteria is a major cause of mortality in the world and discovery of antibiotics revolutionized medical care in the 20th century. However significant antibiotic resistance has now emerged and new methods of combating bacteria need to be pursued. Type IIA secreted phospholipase A2 (sPLA2-IIA) hydrolyzes phospholipids and has been shown to have bactericidal function particularly in killing G<sup>+</sup> bacteria. But limited studies examine its effect on G<sup>-</sup> bacteria such as *P. aeruginosa* which frequently colonize airways of patients with cystic fibrosis and are antibiotic multi-resistance. Here, we examine the role of sPLA2-IIA in pulmonary host defense against *P. aeruginosa*. Our results show rhsPLA2-IIA and bactericidal/permeability increasing protein (BPI) in vitro killed PAK, a laboratory strain of *P. aeruginosa*, in a dose-dependent manner and BPI enhanced the effect of rhsPLA2-IIA on this strain. Human BALFs efficiently killed PAK strain, which was abolished by the sPLA2-IIA specific inhibitor. Compared to their littermates, sPLA2-IIA transgenic mice were partially protected against pulmonary infection by PAK and CHA, a clinical strain of *P. aeruginosa*. Using chemiluminescent PAK strain, we showed that bacteria disseminated in a spatio-temporal manner in WT mice and that this dissemination was reduced in transgenic mice. Finally, intratracheal instillation of rhsPLA2-IIA improved the survival of PAK-infected WT mice. Based on these results, we concluded that sPLA2-IIA can be considered as an endogenous “antibiotic-like molecule” of the host and can be potentially used directly or as antibiotics complementary therapy during multiresistant bacteria infection.

**P -88 : Role of inflammatory mediators and their receptors in the crosstalk between colon cancer cells and macrophages**

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**Background**

Pro-inflammatory cysteinyl leukotrienes (CysLTs) play an important role in colon cancer. We have shown leukotriene D4 (LTD4) signaling via CysLT1 receptor is linked to tumorigenic processes, whereas LTC4 signaling via CysLT2 receptors is involved in cancer cell differentiation. Colon cancer patients with high CysLT1R and low CysLT2R expression demonstrate poor prognosis. Tumor-associated macrophages (TAMs) (M2 phenotype, CD68+) are not only a major source of CysLTs in tumoral tissues, but also promote cancer cell invasion. In many tumors, a high macrophage content is associated with poor prognosis of patients. It has been shown breast cancer cell invasion into a collagen matrix was increased by macrophage-synthesized epidermal growth factor (EGF). TAMs can produce protease enzymes as tumor-promoting factors. Matrix metalloproteinase (MMPs) are proteolytic enzymes linked to tumor invasion and progression. MMPs facilitate tumor cell invasion and tumor metastasis by degrading the extracellular matrix and basement membrane. However, little is known about a possible relation between leukotrienes and MMPs' expression in colon cancer. Our aim is to investigate how TAMs affect tumor behavior, and if EGF, which might be secreted by TAMs, changes the balance between CysLT1 and CysLT2 receptors, and to investigate the role of TAM derived MMPs in colon cancer. We show that TAMs are the source of EGF, which leads to a changed expression pattern of CysLT1R and CysLT2R. In vitro generated macrophages are shown to be the source of MMPs. These findings suggest TAMs play an important role in colon cancer progression.

**P -89 : Sterols depletion by antibiotic nystatin affects membrane functioning and glycosphingolipid composition in wheat roots**

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**Abstract:** Membrane sphingolipids and sterols that form detergent-resistant microdomains (lipid rafts) play key roles in stress signaling on the cell surface. Despite the abundance of biophysical evidence for the existence of interactions between sterols and sphingolipids in simple model systems, there is only scarce evidence for their interactions in living plant cells. In the present work, two contrasting approaches were used to elucidate the roles of sterols in the functioning of plant membranes. Sterol saturation induced by feeding wheat (*Triticum aestivum* L.) seedlings with exogenous cholesterol depolarized membranes, decreased membrane permeability for potassium and protons, and increased the level of extracellular superoxide radicals. By contrast, sterol depletion by binding endogenous sterols with the antibiotic nystatin increased membrane permeability for potassium and protons and decreased the level of extracellular superoxide radicals. Along with these changes, sterol binding with nystatin caused remarkable alterations in sphingolipids, specifically a 50% rise of the total level of glycosphingolipids and shifts in the ratios of their molecular species. Using ESI-MS/MS we showed that the biggest changes occurred in the ratios of molecular species of glycosphingolipids with short-chain fatty acids containing sphingosine, sphingodienine and phytosphingodienine as long-chain base moieties. Results obtained suggest that the interplay between raft-forming sterols and sphingolipids plays a crucial role in the signalling via plant membranes.



**P -90 : Therapeutic potential of cyclooxygenase-3 inhibitors in the management of glioblastoma**

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**Introduction:** Glioblastomas are the most common primary brain tumour and poorly responsive to current treatments. Previous studies have identified increased cyclooxygenase-2 (COX-2) expression in association with aggressive gliomas. Recently COX-3, a splice variant of COX-1 mRNA, has been isolated in brain tissues. Possibilities examined in the present study are whether the expression of COX-1, COX-2 or COX-3 mRNA involves progression of C6 glioblastoma, and whether these enzymes are potential targets of the antineoplastic action of inhibitor nonsteroidal anti-inflammatory drugs. We have therefore determined COX-1-2-3 mRNA expressions in C6 glioblastoma and the normal brain tissue, and the effects of indomethacin, acetaminophen or metamizol on them.

**Materials and method:** Glioblastoma cells (1x10<sup>6</sup>) were intracerebrally inoculated into the frontal lobe of adult male Wistar albino rats. 10 days after, rats were treated with 150 mg/kg acetaminophen, 10 mg/kg indomethacin or 150 mg/kg metamizol. Treatment lasted for 5 days. Tumour size was measured by histologically. Total RNA was isolated from the sample of tumour or normal brain tissue and reverse-transcribed and amplified using RT-PCR system with gene specific primers targeting COX isoforms.

**Results:** Our results demonstrated that the COX-1, COX-2 and COX-3 mRNA were expressed in both C6 glioblastoma and normal brain tissue. Tumour tissue showed the highest COX-3 expression. There was no difference between tumour and normal brain tissue for COX-1 and COX-2 level. Acetaminophen and indomethacin suppressed tumour COX-3 mRNA expression by 87 and 91% respectively. They also decreased tumour size by 71 and 43% respectively, whereas metamizol had no effect. Tumour COX-1 and COX-2 expression were not influenced by acetaminophen, indomethacine or metamizol treatment.

**Discussion:** These findings indicate that there may be potential for used currently available COX-3 inhibitors, such as acetaminophen and indomethacine, in the treatment of glioblastoma. However, the molecular events responsible for COX-3 effects on tumour development are still unresolved. Because of that these drugs can exert their anti-cancer effect via both COX-3 dependent and independent mechanism.

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



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