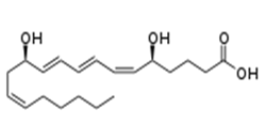
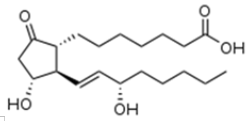
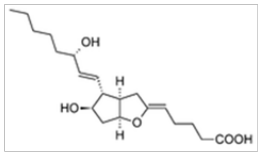
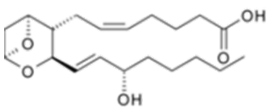
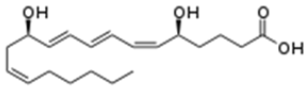
THE 15th

INTERNATIONAL

Winter Eicosanoid Conference

MARCH 9-12, 2014

Baltimore Marriott Inner Harbor Hotel At Camden Yards

110 South Eutaw Street
Baltimore, Maryland

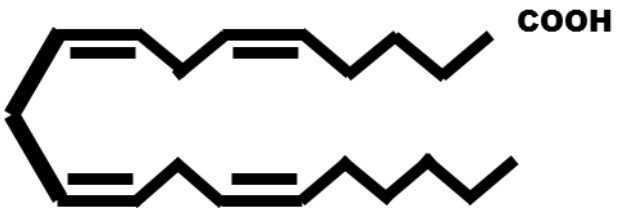
WELCOME TO

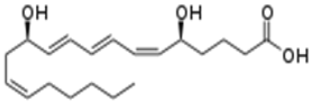
THE 15TH INTERNATIONAL WINTER EICOSANOID CONFERENCE

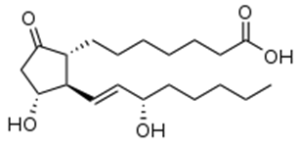
March 9-12, 2014

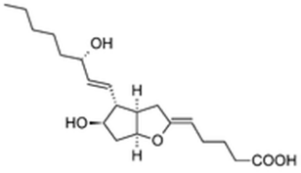
Baltimore Marriott Inner Harbor At Camden Yards

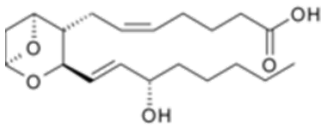
110 South Eutaw Street
Baltimore, Maryland

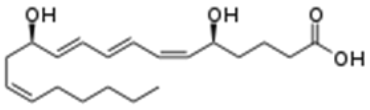












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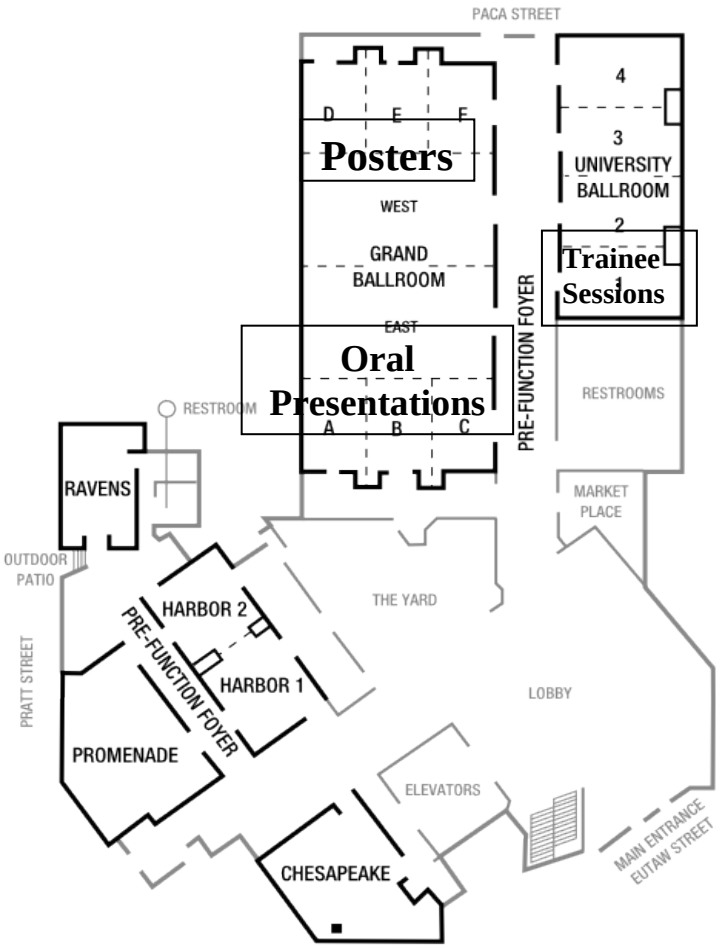
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EAST
Oral Presentations
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THE YARD
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PRATT STREET
HARBOR 1
LOBBY
PROMENADE
ELEVATORS
MAIN ENTRANCE EUTAW STREET
CHESAPEAKE

The 15th International Winter Eicosanoid Conference

March 9-12, 2014
Baltimore Marriott Inner Harbor at Camden Yards

ORAL PRESENTATIONS
GRAND BALLROOM ABC/EAST (Lobby Level)

Registration: **Sunday, March 9th, 4:00-8:00 pm**
Monday & Tuesday, 7:15 am; Wednesday, 7:45 am
Grand Ballroom ABC/East (Lobby Level)

Exhibitors: **Grand Ballroom West/DEF Foyer (Lobby Level)**

Continental Breakfast available daily 7:00 am-8:30 am – Grand Ballroom East/DEF Foyer

POSTER SESSIONS
GRAND BALLROOM WEST/DEF (Lobby Level)

Poster Session I
Sunday, March 9th
6:00 pm – 7:30 pm

Poster Session II
Monday, March 10th
6:35 pm – 8:00 pm

Poster Session III
Tuesday, March 11th
5:15 pm – 6:45 pm

TRAINEE ROUNDTABLES
UNIVERSITY ROOM 1 (Lobby Level)

Monday, March 10th
7:00-8:00 am

Tuesday, March 11th
7:15-8:15 am

CONFERENCE DINNER AND AWARDS
Tuesday, March 11th
8:00 pm
McCormick & Schmick's
Pier 5 Inner Harbor
711 Eastern Avenue

We gratefully acknowledge the following Organizations for their support of 15th International Winter Eicosanoid Conference.

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Mong-Heng Wang, Ph.D.
Guodong Zhang, Ph.D.

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Poster Awards will be presented for Outstanding Poster Presentations

Cardiovascular - Renal

Cancer - Diabetes

Inflammation

Mechanism-Structure-Genetics

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Oregon Health & Science University

Ming-Zhi Zhang
Vanderbilt University

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Honorable Mention

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Prostaglandins and Other Lipid Mediators (POLM)

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John C. McGiff, MD Memorial Award

Nune Markosyan
University of Pennsylvania

SUNDAY, MARCH 9, 2014

6:00-8:00 pm **Welcome Reception**
Salon West and Foyer F (Lobby Level)

6:00–7:30 pm **Poster Session I**
Grand Ballroom West/DEF (Lobby Level)

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MONDAY, MARCH 10, 2014

7:00-8:00 am **Trainee Roundtable: University Room 1 (Lobby Level)**
Women in Science/Industry/Senior Investigator (Academia)

ORAL PRESENTATIONS: GRAND BALLROOM ABC/EAST (Lobby Level)

8:00 am **Opening Remarks**
Darryl C. Zeldin, MD, NIH/NIEHS, Research Triangle Park, NC

SESSION I: PHOSPHOLIPASE A2 SUPERFAMILY
Chair: Edward A. Dennis, PhD, University of California, San Diego, CA

8:05 am **Keynote Lecture**
Edward A. Dennis, PhD, University of California, San Diego, CA
Phospholipase A_2 Structure/Function and Allosteric Regulation of Arachidonic Acid Release

8:40 am Christina C. Leslie, PhD, National Jewish Health, Denver, CO
Cytosolic Phospholipase A_2 ($cPLA_2$), Eicosanoids and Macrophages: Regulators of Inflammation and Immune Responses

9:05 am Makoto Murakami, MD, PhD, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
A New Era of Secreted Phospholipases A_2 ($sPLA_2$)

9:30 am Sasanka Ramanadham, PhD, University of Alabama at Birmingham, Birmingham
Ca^{2+}-Independent Phospholipase A_2 ($iPLA_2\beta$) and Type 1 Diabetes

9:55 am Nicolas Bazan, PhD, Louisiana State University Health Sciences Center, New Orleans, LA
Phospholipase A_2 Activation in Generating Neuroprotectin D1 on Demand upon Responses to Injury in the Brain and Retina

10:20 am **Coffee Break (15 min)**

SESSION II: LIPOXYGENASES
Co-Chairs: Alan Brash, PhD, Vanderbilt University, Nashville, TN
Peter Krieg, PhD, German Cancer Research Center, Heidelberg, Germany

10:35 am Claus Schneider, PhD, Vanderbilt University Medical Center, Nashville, TN
5-Lipoxygenase/COX-2 Interactions

11:00 am Jesmond Dalli, PhD, Brigham & Womens Hospital/Harvard Medical School, Boston, MA
Lipoxygenase pro-resolving pathways in myeloid cells: Resolvins and Maresins.

11:25 am Sabine Rosenberger, PhD, German Cancer Research Center, Heidelberg, Germany
The role of lipoxygenases in epidermal barrier function

11:50 pm Matthew Kobe, PhD, Louisiana State University, Baton Rouge, LA
New insights from the crystal structure of human 15-Lipoxygenase-2

12:15 pm Christopher Smyrniotis, University of California, Santa Cruz, Santa Cruz, CA
ATP Allosterically Activates the Molecular Mechanism of Human 5-Lipoxygenase

12:40 pm **Lunch Break (1 hr, 10 min)**

SESSION III: OMEGA-3 FATTY ACIDS/RESOLVINS: FROM BENCH TO BEDSIDE
Co-Chairs: Richard P. Phipps, PhD, University of Rochester, Rochester, NY
Nan Chiang, PhD, Brigham & Women's Hospital, Boston, MA

1:50 pm Richard P. Phipps, PhD, University of Rochester, Rochester, NY
Resolvin D1 Attenuates Cigarette Smoke-induced Lung Inflammation

2:15 pm Nan Chiang, PhD, Brigham & Women's Hospital, Boston, MA
Bacterial Infections: Dynamic Regulation of Pro-resolving Mediators That Lower Antibiotic Requirements

2:40 pm Joan Claria, PhD, Hospital Clinic-Barcelona University School of Medicine, Barcelona, Spain
ω-3 Fatty Acids and Resolvins in Metabolic Diseases

3:05 pm Matthew Spite, PhD, University of Louisville, Louisville, KY
Resolvin D1 and Wound Healing in Diabetes

3:30 pm Ann Skulas-Ray, PhD, Penn State University, University Park, PA
Omega-3 Fatty Acids and Inflammation: A Clinical Research Perspective

3:55 pm **Coffee Break (10 min)**

SESSION IV: EICOSANOIDS AND CANCER
Co-Chairs: Kenneth V. Honn, PhD, Wayne State University School of Medicine, Detroit, MI
Dipak Panigrahy, MD, Beth Israel Deaconess Medical Center/Harvard, Boston, MA

4:05 pm Kenneth V. Honn, PhD, Wayne State University, Detroit, MI
Role of TPα in Prostate Cancer Progression

4:30 pm Dipak Panigrahy, MD, Beth Israel Deaconess Medical Center/Harvard, Boston, MA
Regulation of Cancer through Cytochrome P450-derived Eicosanoids

4:55 pm Guodong Zhang, PhD, University of California, Davis, CA
Soluble Epoxide Hydrolase on Angiogenesis, Tumor Growth and Metastasis

5:20 pm Nune Markosyan, PhD, University of Pennsylvania, Philadelphia, PA
Deletion of Mammary Epithelial mPGES1 Suppresses Tumor Development in Mice: a Effect of Substrate Re-diversion

6:05 pm David Potter, MD, PhD, University of Minnesota, Minneapolis, MN
Roles of Cell Intrinsic Epoxygenase in Breast Cancer Progression

6:30 pm End of Monday Sessions

6:35-8:00 pm **Poster Session II (Grand Ballroom West/DEF – Lobby Level)**

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TUESDAY, MARCH 11, 2014

7:15-8:15 am **Trainee Roundtable: University Room 1 (Lobby Level)**
Government/Overseas/Junior Investigator

ORAL PRESENTATIONS: GRAND BALLROOM ABC/East (Lobby Level)

SESSION V: EETS AND 20-HETE IN CARDIOVASCULAR DISEASE

Co-Chairs: Michal L. Schwartzman, PhD, New York Medical College, Valhalla, NY
John D. Imig, PhD, Medical College of Wisconsin, Milwaukee, WI

8:30 am Kenneth D. Bloch, MD, Massachusetts General Hospital/Harvard Medical School, Boston, MA
EET and Pulmonary Hypertension

8:55 am Richard J. Roman, PhD, University of Mississippi Medical Center, Jackson, MS
Renoprotective Actions of 20-HETE in Hypertension and following Ischemia Reperfusion Injury

9:20 am Emanuel Buys, PhD, Harvard Medical School, Boston, MA
sGC-20-HETE

9:45 am Samuel Poloyac, PharmD, PhD, University of Pittsburgh, Pittsburgh, PA
CYP Eicosanoids and Stroke: Results from a Large Cohort of Aneurysmal Subarachnoid Hemorrhage Patients

10:10 am Wolf Schunck, PhD, Max-Delbrück for Molecular Medicine, Berlin, Germany
Role of CYP Eicosanoids in Cardiac Hypertrophy and Arrhythmia

10:35 am Bruce D. Hammock, PhD, University of California, Davis, CA
Tribute to a Biochemist Loved by All

10:50 am **Coffee Break (20 min)**

SESSION VI: ATHEROSCLEROSIS/VASCULAR BIOLOGY

Co-Chairs: Tilo Grosser, MD, University of Pennsylvania, Philadelphia, PA
John Hwa, MD, PhD, Yale University, New Haven, CT

11:10 am Emanuela Ricciotti, PhD, University of Pennsylvania
Vascular Response to COX-2 Inhibition

11:35 am Sylvain Galvani, PhD, Weill Cornell Medical College Center for Vascular Biology, New York, NY
S1P and S1P1 in inflammation and atherosclerosis

12:00 pm Matthew L. Edin, PhD, NIEHS
Role of Cell-Specific Soluble Epoxide Hydrolase Disruption in Cardiac Ischemia/Reperfusion Injury

12:25 pm Salam Ibrahim, PhD, University of Pennsylvania
Vascular Biology of Prostacyclin and Thromboxane

12:50 pm **Lunch Break (1 hr, 15 min)**

SESSION VII: THE PERSONALIZED NSAID THERAPEUTICS CONSORTIUM (PENTACON)
Co-Chairs: Garret A. FitzGerald, MD, University of Pennsylvania, Philadelphia, PA
Tilo Grosser, MD, University of Pennsylvania, Philadelphia, PA

2:05 pm Tilo Grosser, MD, University of Pennsylvania, Philadelphia, PA
A Systems Approach to Personalizing NSAID Therapy

2:30 pm Olga Troyanskaya, PhD, Princeton University, Princeton, NJ
Predicting the Biological NSAID Response Network Computationally

2:55 pm Simona Zarini, PhD, University of Colorado, Denver, CO
Lysophospholipid Acyltransferases: Backdoor Regulators of Eicosanoid Biosynthesis

3:20 pm John Hwa, MD, PhD, Yale University, New Haven, CT
Prostacyclin and NSAIDs; from structure/function to clinical studies

3:45 pm **Coffee Break (15 min)**

SESSION VIII: POLM YOUNG INVESTIGATORS
Co-Chairs: Austin M. Guo, PhD, New York Medical College, Valhalla, NY
Arthur A. Spector, MD, NIAAA/NIH, Bethesda, MD

4:00 pm Victor Garcia, New York Medical College, Valhalla, NY
20-Hydroxyeicosatetraenoic Acid (20-HETE) Induction of Angiotensin Converting Enzyme (ACE) Transcription is Mediated by NF-kB Binding Via EGFR/MAPK/IKK Pathway in Human Endothelial Cells

4:15 pm Dayna Mudge, Beth Israel Deaconess/Harvard
Maresin 1: A Novel Macrophage-derived Endogenous Proresolving Inhibitor of Tumor Growth and Metastasis

4:30 pm Jonathan Nelson, Oregon Health & Science University
Role of Soluble Epoxide Hydrolase in Age-related Vascular Cognitive Decline

4:45 pm Claudia Ramirez, Vanderbilt University, Nashville, TN
Arg287Gln Variant of EPHX2 and Epoxyeicosatrienoic Acids are Associated with Increased Insulin Sensitivity in Humans

5:00 pm End of Tuesday Sessions

5:15-6:45 pm Poster Session III (Grand Ballroom West/DEF – Lobby Level)

8:00 pm **Conference Dinner and Awards Presentations**
McCormick & Schmick's
Pier 5, 711 Eastern Avenue

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WEDNESDAY, MARCH 12, 2014

ORAL PRESENTATIONS: GRAND BALLROOM ABC/East (Lobby Level)

SESSION IX: EICOSANOIDS AND THE BRAIN
Co-Chairs: Dave R. Harder, PhD, Medical College of Wisconsin, Milwaukee, WI
Charles W. Leffler, PhD, University of Tennessee Health Science Center, Memphis, TN

8:35 am Raymond C. Koehler, PhD, Johns Hopkins School of Medicine, Baltimore, MD
Role of 20-HETE in Neuronal Injury

9:00 am Nabil J. Alkayed, MD, PhD, Oregon Health & Science University, Portland, OR
Role of EETs in Age-Related Vascular Cognitive Impairment

9:25 am Charles W. Leffler, PhD, University of Tennessee Health Science Center, Memphis, TN
Hydrogen Sulfide and Prostacyclin Regulation of Newborn Cerebral Microvascular Circulation

9:50 am David R. Harder, PhD, Medical College of Wisconsin, Milwaukee, WI
Protective Paracrine Action of Astrocyte-Derived EETs on Neuronal Cells

10:15 am **Coffee Break (15 min)**

SESSION X: EICOSANOIDS AND PAIN
Co-Chairs: Bruce D. Hammock, PhD, University of California, Davis, CA
Karen Wagner, PhD, University of California, Davis, CA

10:30 am Alonso Guedes, PhD, University of California, Davis, CA
Soluble Epoxide Hydrolase (sEH) Biology in Healthy and Laminitic Horses

10:55 am Scott Edwards, PhD, LSU Health Sciences Center, New Orleans, LA
Eicosanoids and Alcohol Dependence

11:20 am Karen Wagner, PhD, University of California, Davis, CA
Epoxides of omega 3 and 6 fatty acids and suppression of chronic pain

11:45 am Daniele Piomelli, PhD, University of California, Irvine, CA
Peripheral Gating of Pain Signals by Endogenous Analgesic Lipids

12:10 pm Closing Remarks

12:15 pm End of Conference

SPEAKERS' ABSTRACTS

(In order of presentation)

INVOLVEMENT OF iPLA2β-DERIVED SIGNALS IN IMMUNE RESPONSES LEADING TO ISLET INFILTRATION AND DIABETES DEVELOPMENT

Sasanka Ramanadham[1,2], Ying Gai[1,2], Victoria Magrioti[3], Hubert M. Tse[2,4], George Kokotos[3], Robert N. Bone[2,5], Xiayong Lei1,[2]
Departments of 1Cell, Developmental, & Integrative Biology, 4Microbiology, 5Pathology, and the 2Comprehensive Diabetes Center, University of Alabama at Birmingham, Birmingham, AL, USA and 3Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Athens, Greece.

Type 1 Diabetes (T1D) is a consequence of infiltration of islets by immune cells that generate inflammatory factors, which promote autoimmune destruction of beta-cells. The subsequent decrease in insulin availability leads to hyperglycemia and frank diabetes. We previously described a role for the Ca2+-independent phospholipase A2β (iPLA2β) in beta-cell apoptosis by a pathway that involves hydrolysis of sphingomyelins leading to accumulations in ceramide and triggering of intrinsic apoptotic processes. Activation of iPLA2β leads to hydrolysis of *sn*-2 substituent from membrane phospholipids and as beta-cells are enriched in arachidonate-containing lipids, this leads to increased generation of bioactive eicosanoids. Several eicosanoids are recognized to exacerbate immune responses by preventing clearance of cellular debris around the inflamed environment and also acting as chemoattractants. We find that betacell iPLA2β expression is increased in various models of diabetes and that inhibition of iPLA2β in a diabetes-prone rodent model reduces diabetes incidence and islet infiltration. Examination of the mechanism for reduced insulitis reveals that iPLA2β-derived signals enhance migration of inflammatory cells and that inhibition of iPLA2β reduces chemotaxis. These observations suggest that in addition to mediating ceramide-dependent beta-cell apoptosis, iPLA2β-derived lipid signals act as chemoattractants to promote islet infiltration, leading to autoimmune destruction of beta-cells. Collectively, our studies provide evidence for involvement of iPLA2β activation in the development of diabetes and that inhibition of iPLA2β may be beneficial in ameliorating T1D.

Acknowledgements: NIH R01-DK69455, The Iacocca Foundation, & the UAB Comprehensive Diabetes Center.

LIPOXYGENASE PRO-RESOLVING PATHWAYS IN MYELOID CELLS: RESOLVINS AND MARESINS.

Jesmond Dalli[1], Min Zhu[2], Bin Deng[1], Jesper Z Haeggström[3] Nicos Petasis[2] and Charles N. Serhan[1]
1Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative, and Pain Medicine, Harvard Institutes of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.; 2Loker Hydrocarbon Research Institute, University of Southern California, Los Angeles, California, USA; 3Department of Medical Biochemistry and Biophysics, Division of Chemistry 2, Karolinska Institutet, S-171 77 Stockholm, Sweden

Phagocytes are central in orchestrating the acute host response during inflammation and its timely resolution. Using lipid mediator (LM) metabololipidomcs, we assessed LM levels in neutrophils (PMN), apoptotic PMN and macrophages. During efferocytosis, macrophages upregulate the biosynthesis of specialized pro-resolving mediators (SPM), including resolvin (Rv) D1 and RvD2, that were further elevated by PMN microparticles (MP). Fresh PMN from peripheral blood with zymosan gave predominantly leukotriene B4, whereas apoptotic PMN displayed both elevated prostaglandin E2 and lipoxin (LX) B4. Using deuterium labeled precursors we found that both apoptotic PMN and MP donated precursors to macrophages that were utilized for transcellular SPM biosynthesis. Human macrophage 12-lipoxygenase (hm12-LOX) gave 14-hydro(peroxy)-docosahexaenoic acid (14-HpDHA), as well as several dihydroxy-docosahexaenoic acids, implicating an epoxide intermediate formation by this enzyme. Using a stereo-controlled synthesis, enantiomerically pure 13S,14S-epoxy-docosa-4Z,7Z,9E,11E,16Z,19Z-hexaenoic acid (13S,14S-epoxy-DHA) was prepared, and its stereochemistry was confirmed by NMR spectroscopy. When this 13S,14S-epoxide was incubated with human macrophages, it was converted to MaR1. MaR1 also accelerated surgical regeneration in planaria, increasing the rate of head reappearance. On injury of planaria, MaR1 was biosynthesized from deuterium-labeled (d(5))-DHA that was blocked with lipoxygenase (LOX) inhibitor. The synthetic 13S,14S-epoxide inhibited leukotriene B_4 (LTB_4) formation by human leukotriene A_4 hydrolase (LTA4H) ~40% ($P<0.05$) to a similar extent as LTA_4 (~50%, $P<0.05$) but was not converted to MaR1 by this enzyme.

13S,14S-epoxy-DHA also reduced (~60%; $P<0.05$) arachidonic acid conversion by hm12-LOX and promoted conversion of M1 macrophages to M2 phenotype, which produced more MaR1 from the epoxide than M1. Lineage differentiated macrophages showed distinct LM profiles where M2 macrophages gave higher SPM and lower prostaglandins then M1. These profiles were regulated during efferocytosis where M1 cells gave increased SPM including LXA4 while overall LM biosynthesis was reduced in M2. Together these findings establish the LM signatures of human phagocytes, their regulation during distinct stages of acute inflammation-resolution and suggest that chemical signals are shared in resolution cellular trafficking, a key process in tissue regeneration.

This work was supported by the NIH (grants GM038765 and GM095467).

A MOUSE ORGANOTYPIC TISSUE CULTURE MODEL TO STUDY MOLECULAR MECHANISMS OF LIPOXYGENASE ACTION IN SKIN AND TO TEST POTENTIAL THERAPEUTIC APPROACHES TO CONGENITAL ICHTHYOSES.

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12R-lipoxygenase (12R-LOX) and epidermal LOX-3 (eLOX-3) play an indispensable role in mammalian skin barrier formation. Loss-of-function mutations in the genes *ALOX12B* and *ALOXE3* have been found to represent the second most common cause of autosomal recessive congenital ichthyosis and targeted disruption of the corresponding LOX genes in mice resulted in neonatal death due to a severely impaired permeability barrier function. Here, we describe the generation of a mouse organotypic tissue culture model for autosomal recessive congenital ichthyosis. Primary epidermal keratinocytes were isolated from neonatal mice deficient for 12R-LOX and co-cultivated with mouse dermal fibroblasts embedded in a scaffold of native collagen type I obtained from rat tail tendon. Closely resembling the in vivo situation these mouse skin equivalents were grown air-exposed with nutrients being supplied from underneath by diffusion of medium of a defined composition. With this set up, the keratinocytes formed a well-organized multilayered stratified epithelium with proper epidermal morphogenesis. All epidermal layers were present and the keratinocytes within showed the respective characteristic morphological features. Several differentiation markers, such as keratin 10, involucrin, loricrin and filaggrin, and the major components of tight junctions and the basement membrane were expressed, processed and assembled properly. However, in contrast to their wildtype counterparts 12 R-LOX deficient skin equivalents consistently showed abnormal vacuole-like structures in the upper epidermal layers. The presence of these anomalies correlated with an increased transepidermal water loss and the loss of covalently bound ceramides suggesting a perturbed lipid metabolism resulting in an impaired epidermal barrier as found in 12R-LOX deficient mice. These results indicate that our organotypic mouse skin models are valuable tools to study the molecular basis for epidermal barrier defects due to LOX deficiency and to test potential therapeutic approaches.
ACKNOWLEDGEMENTS: This work was supported by grants from the Deutsche Forschungsgemeinschaft (KR 905/6-2, KR 905/7-1 and SCHN 569/4-1) and from the Intramural Funding Program of the DKFZ.

ATP ALLOSTERICALLY ACTIVATES THE MOLECULAR MECHANISM OF HUMAN 5- LIPOXYGENASE

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5-Lipoxygenase (5-LOX) and ATP have both been implicated in inflammatory diseases, such as chronic obstructive pulmonary disease (COPD) and emphysema. Since ATP is a known activator of 5-LOX, we investigated this relationship in greater detail. In the current work, we describe how ATP activates both hydroperoxidation and epoxidation by 5-LOX of multiple fatty acids, arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). For AA, a specific step in its molecular mechanism is changed, the dependency of the rate-limiting step on hydrogen-bond rearrangement. This change is the same for both hydroperoxidation and epoxidation, further supporting the hypothesis that both catalytic processes follow the same reaction trajectory. ATP activation also changes the product profile of the fatty acids mixture (AA, EPA and DHA). The products of these fatty acids, leukotrienes and resolvins, promote and inhibit inflammation, respectively, and thus changes in their relative concentrations in the cell could have implications in the regulation of inflammation. Additionally, the extensive mutations used to stabilize 5-LOX for crystallization also remove the ability of ATP to activate 5-LOX, raising the question whether the instability of 5-LOX is the structural cost of ATP activation.
Funding: *This work was supported by the National Institutes of Health, GM56062 (TRH) and S10-RR20939 (MS Equipment grant).*

RESOLVIN D1 BLUNTS CIGARETTE SMOKE-INDUCED LUNG INFLAMMATION AND INCREASES ALTERNATIVELY ACTIVATED MACROPHAGES (M2) WITH ANTI-INFLAMMATORY AND PRO-RESOLVING PROPERTIES

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Rationale: Cigarette smoke (CS) causes chronic obstructive pulmonary disease (COPD), which is a major global health problem affecting millions of people. An important concept is that COPD persists and lung function continues to deteriorate even after smoking cessation, indicating that COPD represents, in part, a failure to resolve the inflammatory process. Alveolar macrophages (AM) play an essential role in the clearance of inhaled particles, pathogens and apoptotic cells including neutrophils. AM from COPD patients have a reduced ability to clear apoptotic neutrophils by phagocytosis, suggesting a possible role of altered AM function in the pathogenesis of COPD. Resolution of inflammatory responses is an active process involving endogenous pro-resolving lipid mediators, derived from omega-3 polyunsaturated fatty acids, that control and limit inflammatory responses. Recently, we demonstrated that resolvin D1 (RvD1) dampens CS-induced acute lung inflammation in mice. The mechanism of the pro-resolving actions of RvD1 remains of high interest. We hypothesize that RvD1 promotes the resolution of inflammation, at least in part, by modulating AM phenotype and biological function. **Methods:** Mice were exposed to CS for 3 days to induce acute lung inflammation and were treated with RvD1 or vehicle by inhalation after the last smoke exposure, and euthanized the next day. Inflammatory cells and cytokines were determined in bronchoalveolar lavage fluid (BAL) and by Western blot and RT-PCR of cells

and tissues. AM were isolated from naive mice and from human subjects by bronchoscopy, treated with RvD1, incubated with LPS and FITC-conjugated *E. coli*, and phagocytic activity was determined by flow cytometry. **Results:** RvD1 stimulated resolution of CS-induced acute inflammation in mice, with 50% reductions in infiltrating neutrophils and pro-inflammatory cytokines IL-6, KC and MIP-2 ($p<0.05$). AM from RvD1-treated mice exhibited enhanced phagocytosis of apoptotic neutrophils and increased expression of *Arg-1* and *Mrc-1*, markers of alternatively activated macrophages (M2). Naive AM exposed to LPS expressed lower levels of pro-inflammatory cytokines when pre-treated with RvD1. RvD1 enhanced the phagocytic activity of human primary lung macrophages *ex vivo*. **Conclusions:** RvD1 has potent pro-resolving effects in both mouse and human lung macrophages, and promotes resolution of acute CS-induced inflammation by reducing production of pro-inflammatory mediators and by promoting differentiation and function of pro-resolving M2 lung macrophages. These new findings demonstrate the exciting translational potential of RvD1 and other pro-resolving lipid derivatives of omega-3 polyunsaturated fatty acids and suggest that these mediators can be effective in promoting resolution and a return to homeostasis in both acute and chronic lung diseases.
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BACTERIAL INFECTIONS: DYNAMIC REGULATION OF PRO-RESOLVING MEDIATORS (RESOLVINS AND PROTECTINS) THAT LOWER ANTIBIOTIC REQUIREMENTS

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Underlying mechanisms for how bacterial infections contribute to active resolution of acute inflammation and infection are of interest. Here, we employed lipid mediatormetabololipidomics following *Escherichia coli* (*E. coli*) infections in mice to identify proinflammatory (prostaglandins and leukotrienes) and specialized pro-resolving mediators (SPM) as well as to establish their temporal relationship. In self-resolving *E. coli* exudates (10^5 CFU), the most abundant SPM were resolvin (Rv) D5 and protectin D1 (PD1), which at 12 h were significantly greater than levels in exudates from higher titer *E. coli* (10^7 CFU) challenged mice. In germ-free mice, endogenous RvD1 and PD1 levels were higher than in conventional mice. RvD1 and RvD5 (100 ng/mouse) each heightened host antimicrobial response, lowered bacterial titers, prevented *E. coli*-induced hypothermia and increased survival. With isolated human macrophages, RvD1 and RvD5 each enhanced phagocytosis of *E. coli* in a human G protein-coupled receptor (GPR) 32 dependent manner, and counter-regulated specific proinflammatory genes, including NF-kappaB and TNF-alpha and cyclooxygenase-2 (Chiang et al., Nature 2012). During live *E. coli* infections, a select SPM panel (RvD1, RvD5 and PD1) enhanced antibiotic ciprofloxacin's therapeutic efficacy, lowering bacterial titers and accelerating resolution. In skin infections, SPM enhanced effectiveness of vancomycin in clearing *Staphylococcus aureus*. Taken together, specific SPM are temporally and differentially regulated during infections. They are anti-phlogistic and enhance bacterial containment. Stimulation of targeted host resolution programs by SPM in combination with bacterial-directed antibiotics lowers the required antibiotic doses for bacterial clearance. These results illustrate new opportunities to address the increasing antibiotic resistance.
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REGULATION OF CANCER THROUGH CYTOCHROME P450-DERIVED EICOSANOIDS

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Arachidonic acid and its metabolites have recently stimulated great interest in cancer biology. To date, most research on eicosanoids in cancer has focused on the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. In contrast, the role of cytochrome P450-derived eicosanoids, such as epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs), in cancer has received less attention. We recently demonstrated that epoxyeicosatrienoic acids (EETs), which are cytochrome P450 (CYP) metabolites of arachidonic acid, stimulate multi-organ metastasis and escape from tumor dormancy. This eicosanoid pathway also includes 20-HETE, which is biosynthesized by CYP ω-hydroxylases (e.g. CYP4F2). The emerging role of Cytochrome P450-derived eicosanoids in cancer will be discussed.
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SOLUBLE EPOXIDE HYDROLASE ON ANGIOGEENSIS, TUMRO GRWOTH AND METASTASIS

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Epoxygenated fatty acids (EpFAs), which are lipid mediators produced by cytochrome P450 epoxygenases from polyunsaturated fatty acids, are important signaling molecules known to regulate various biological processes including inflammation, pain and angiogenesis. Pharmacological inhibitors of soluble epoxide hydrolase (sEH), which is the major enzyme to degrade EpFAs, are being evaluated for multiple human disorders. EpFAs are known to be critical regulators of inflammatory and cardiovascular diseases; however, their roles in cancer are poorly characterized. We recently discovered that EDPs, which are cytochrome P450 (CYP) epoxygenases-derived metabolites of docosahexaenoic acid (DHA, a major omega-3 fatty acid), potently suppressed tumor growth and metastasis by blocking tumor angiogenesis. In contrast, epoxyeicosatrienoic acids (EETs), which are CYP epoxygenases metabolites of arachidonic acid (ARA, an omega-6 fatty acid), are mildly pro-angiogenic to stimulate tumor progression. These findings demonstrate that the previously unappreciated CYP-metabolites (EETs and EDPs) play critical roles in mediating the opposite effects of omega-6 and omega-3 fatty acids on cancer. We also discovered that co-administration of COX-2 inhibitors and

sEH inhibitors synergistically inhibited tumor growth and metastasis. Due to the potent interactionS of COX-2 and she inhibitors, we designed and synthesized the first-in-class COX-2/sEH dual inhibitors. The dual inhibitors also potently suppressed tumor growth and metastasis in part via inhibition of tumor angiogenesis, with reduced cardiovascular toxicity compared with COX-2 inhibitors. These results suggest that dual pharmacological inhibition of COX-2 and sEH is a promising therapeutic strategy.

DELETION OF MAMMARY EPITHELIAL MPGES1 SUPPRESSES TUMOR DEVELOPMENT IN MICE: A POSSIBLE EFFECT OF SUBSTRATE RE-DIVERSION

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Microsomal prostaglandin E_2 synthase (mPGES1) is the terminal enzyme in cyclooxygenase (COX)-2-mediated prostaglandin (PG) E_2 biosynthesis. The pro-tumor contributions of COX-2 and PGE_2 are established as is the clinical efficacy of pharmacological COX-2 inhibition. However, in addition to the desired suppression of tumor PGE_2, collateral loss of vascular endothelial COX-2-derived PGI_2, an endogenous anti-platelet mediator, imposes a cardiovascular hazard that limits clinical use of COX-2 inhibitors. mPGES1 is a promising alternative target to interrupt COX-2-driven events in tumors without elevating cardiovascular risk. We engineered mice transgenic for an activated HER2/neu oncogene to lack mPGES1 in mammary epithelial cells (MEC; mPGES1 KO^{MEC}). mPGES1 KO^{MEC} mice and their wild type (WT) littermates were sacrificed at 22 weeks and mammary glands harvested. Glands were sectioned, every 10^{th} slide was H&E stained and scanned to obtain whole slide images. For each mouse, multiplicity was calculated using the slide containing the greatest number of lesions. Adjacent sections were immunostained for Ki67, caspase 3, and Factor VIII, to assess proliferation, apoptosis, and angiogenesis. Tumor multiplicity was significantly higher in WT compared to mPGES1 KO^{MEC} mice (4.17 ± 1.05 [n=6] vs 0.29 ± 0.19 [n=7], respectively; p=0.002). In addition, in a second cohort of mice followed until tumors were palpable, mPGES1 KO^{MEC} mice trended toward a longer tumor free period. Concordant with these observations, shRNA knock down (KD) of mPGES1 in mammary tumor cells dramatically suppressed their growth as orthotopic tumors in syngeneic immune competent WT hosts - 5 out of 6 non-target shRNA control tumors grew successfully while, in all cases, mPGES1 KD cells failed to grow. By immunohistochemistry, no difference was observed in proliferation or apoptosis between WT and mPGES1 KO^{MEC} spontaneous tumors. Interestingly, and in contrast to our previous study of neu-driven mammary tumors in mice lacking MEC COX-2 (COX-2 KO^{MEC}), vascularization was not different between mPEGS-1 KO^{MEC} and WT spontaneous tumors suggesting divergence in the anti-tumorigenic mechanisms in mPEGS-1 KO^{MEC} and COX-2 KO^{MEC} tumors. Multiple studies of mPGES-1 deletion report re-diversion of COX-2-derived substrate to other prostanoid synthetic pathways, in particular PGD_2. By Q-PCR, other than reduced mPGES1 mRNA, terminal prostanoid enzymes were not altered in mPGES1 KO^{MEC} tumors. Interestingly, while the expression of PGE_2 receptors EP2 and EP4 trended downwards in mPGES1 KO^{MEC} tumors, the DP2 receptor for PGD_2 was significantly increased. Taken together, these data establish mPGES1 as a promising therapeutic target in breast cancer. Further, we propose that, in addition to loss of PGE_2, re-diversion of COX-2-derived substrate to the PGD_2-DP2 pathway may be involved in delayed tumorigenesis.

ROLES OF CELL INTRINSIC EPOXYGENASE IN BREAST CANCER PROGRESSION.

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CYP3A4 is expressed in estrogen receptor positive (ER+) breast cancer and promotes cancer cell proliferation, in part, through biosynthesis of epoxyeicosatrienoic acids (EETs). CYP3A4 gene silencing blocks the engraftment of the ER+ breast cancer cell line MCF-7, in part, through inhibiting the association of CD31 and vWF+ endothelial cells with nascent tumor nodules. Because the biguanide drug metformin exhibits inhibitory effects on breast cancer growth in vitro and in vivo, albeit at high concentrations (IC50=5 mM), and because metformin has been implicated as a CYP3A4 inhibitor, we investigated whether metformin inhibits CYP3A4 epoxygenase activity in microsomes and in breast cancer cells. Metformin inhibited CYP3A4 microsomal biosynthesis of (±)-8,9-, (±)11, 12-, and (±)14,15- EET and also depleted these regioisomers in breast cancer cells. Furthermore, metformin interacted with CYP3A4 exhibiting a type II spin shift on CYP3A4 nanodiscs. CYP3A4 nanodiscs synthesized EETs in an NADPH-dependent fashion. Co-crystallization of CYP3A4 and metformin resulted in a co-crystal structure exhibiting one metformin molecule per active site, positioned above the heme plane and hydrogen bonded to residue Arg212. Addition of (±)-14,15-EET (1 uM) to MCF-7 cells inhibited by metformin, buformin or phenformin exhibited in each case partial restoration of breast cancer cell proliferation, whereas the triple negative breast cancer line MDA-MB-231 exhibited no restoration of growth. Metformin treatment inhibited tumor growth in the ER+ MCF-7 xenograft model. Together, these results suggest that metformin inhibits ER+ breast cancer, in part, through reduction of cancer cell intrinsic EET levels.

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VASCULAR BIOLOGY OF PROSTACYCLIN AND THROMBOXANE

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Prostacyclin and thromboxane mediate opposing cardiovascular effects through their receptors IP and TP respectively. When co-expressed with IP, TP shows a signaling shift from Gq-coupled inositol phosphate (InsP) to Gs-coupled cAMP production, a typical signaling for IP. Individuals heterozygous for an IP rare variant, IPR212C, displayed exaggerated loss of platelet IP responsiveness and accelerated cardiovascular disease. In this work we examined receptor dimerization, microdomain distribution within plasma membrane, and the role of rafts in receptor function. To examine receptor dimerization, we used Bioluminescence Resonance Energy Transfer (BRET), which measures energy transfer from a renilla luciferase (RLuc)-fused donor receptor to a yellow fluorescent protein (YFP)-fused acceptor receptor in transfected live cells. We demonstrated that IP and TP can associate with each other to form homo- or hetero- dimers. IPR212C displayed normal homo- and heterodimerization but reduced cAMP generation and increased endoplasmic reticulum (ER) localization. It also exerted a dominant action on the wildtype IP and TP through imerization. By measuring energy transfer from RLuc -fused receptors to fluorescently labeled rafts, we found that IP was raft associated, and TP was raft excluded but redistributed to rafts upon dimerization with IP. Disruption of rafts by cholesterol depletion of transfected cells or primary cells such as human aortic smooth muscle cells and raw macrophages impaired IP but not TP signaling further confirming IP and TP microdomain distribution. On another hand, cholesterol enrichment was used to mimic hypercholesterolemia conditions in transfected cells, primary cells, and ex-vivo in peritoneal macrophages isolated from LDLR-/- mice. Cholesterol enrichment selectively suppressed IP and IPTP function. Finally, w-3 fatty acids (DHA) reversed cholesterol inhibition of IP but not TP function in transfected cells. In summary, in this study, we showed that IPR212C likely contribute to accelerated cardiovascular disease in heterozygous individuals, through ER retention and dominant actions on IP and TP. IP and TP function within distinct microdomains, and TP signaling shift is likely explained by both its association with IP as heterodimer and its translocation to membrane rafts. We speculate that changes in IP and IPTP signaling after perturbation of membrane cholesterol, consistent with their raft localization, may contribute to cardiovascular disease associated with hypercholesterolemia. Finally, the potentially beneficial effects of w-3 fatty acids (DHA) might be mediated by reversing the inhibition of IP signaling due to elevated cholesterol levels associated with hypercholesterolemia. This could be achieved by reversing cholesterol effects on membrane fluidity.

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POSTER ABSTRACTS

Posters will be displayed in the Grand Ballroom West/DEF on the Lobby Level of the Marriott Inner Harbor Hotel, Sunday, March 9th through Tuesday evening, March 11th. Presenters are required to be by your poster for discussion during the session you are assigned to. ALL posters MUST be removed on Tuesday evening at the end of the poster session.

POSTER SESSION I
SUNDAY, MARCH 9, 2014
Grand Ballroom West/DEF (Lobby Level)
6:00-7:30 PM

CARDIOVASCULAR - RENAL

S1

INHIBITION OF PROSTAGLANDIN E2 PROMOTES HUMAN AORTIC SMOOTH MUSCLE CELL DIFFERENTIATION

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INTRODUCTION: Altered differentiation of aortic smooth muscle cells (SMCs) has been shown to be associated with the development of abdominal aortic aneurysms (AAAs). Using a mouse model of AAAs induced by chronic angiotensin II (AngII) infusion, we have previously shown that progression of AAAs is associated with reduced differentiation of SMCs in the abdominal aorta. In addition, the effectiveness of cyclooxygenase-2 (COX-2) inhibition for attenuating AngII-induced AAA progression is associated with maintenance of a differentiated SMC phenotype. However, the mechanisms responsible for COX-2-dependent aortic SMC phenotypic modulation have not been identified. Therefore, we utilized human aortic SMCs (hASMCs) to assess the role of COX-2-dependent production of prostaglandin E2 (PGE2) in contributing to reduced SMC differentiation. **METHODS:** hASMCs were cultured under conditions which promote either increased or decreased differentiation. SMCs were treated over time with varying concentrations of AngII, PGE2, or inhibitors of COX-2 or the PGE2 synthetic pathway. The effects of these treatments on the expression of SMC differentiation markers and components of the COX-2 pathway were examined. **RESULTS:** hASMCs cultured under conditions which reduce differentiation was associated with decreased expression of the SMC differentiation marker alpha-actin and increased mRNA expression of COX-2. Treatment with the COX-2 inhibitor celecoxib significantly reduced PGE2 synthesis ($p < 0.0001$; n=3; unpaired Student's t-test) and increased expression of alpha-actin, whereas treatment with 1 μM PGE2 significantly decreased expression of alpha-actin ($p < 0.05$; n=3; one-way ANOVA). Although the addition of AngII to the culture

media of differentiated hASMCs resulted in slight decrease of alpha-actin, there was no significant effect of AngII on the expression of COX-2. In contrast, AngII treatment of differentiated hASMCs did increase protein expression of microsomal PGE2 synthase (mPGES-1), an enzyme that functions down-stream of COX-2 and contributes to the synthesis of PGE2. In addition, alpha-actin expression was significantly increased by treatment with 15-deoxyPGJ2, an inhibitor of mPGES-1, and this mPGES-1 inhibitor significantly reduced PGE2 production ($p < 0.0001$ one-way ANOVA) by these cells. Furthermore, siRNA–mediated mPGES-1 knockdown in these cells significantly reduced mPGES-1 levels and PGE2 production, while significantly increasing protein levels of alpha-actin and SM22-alpha ($p < 0.05$; n=3; unpaired Student's ttest). **CONCLUSIONS:** The inhibition of COX-2 by celecoxib may increase hASMC differentiation by inhibiting production of PGE2. mPGES-1 inhibition may provide inhibition of PGE2 that is more specific than celecoxib treatment, and may serve as a potential target for attenuating AAA progression by maintaining a differentiated SMC phenotype.

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S2

OPPOSITE EFFECTS OF GENE DEFICIENCY AND PHARMACOLOGICAL INHIBITION OF SOLUBLE EPOXIDE HYDROLASE ON CARDIAC FIBROSIS

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Aims: Arachidonic acid-derived epoxyeicosatrienoic acids (EETs) are important regulators of cardiac remodeling, manipulation of their levels is a potentially useful pharmacological strategy. EETs are hydrolyzed by soluble epoxide hydrolase (sEH) to form the corresponding diols, thus altering and reducing the activity of these oxylipins. To better understand the phenotypic impact of sEH disruption, we compared the effect of *EPHX2* gene knockout (*EPHX2*-/-) and sEH inhibition in mouse models.
Methods and Results: Measurement of plasma oxylipin profiles confirmed that the ratio of EETs/DHETs was increased in *EPHX2*-/- and sEH-inhibited mice. However, the plasma concentrations of several metabolites in the lipoxygenase pathway were elevated in *EPHX2*-/- but not sEH-inhibited mice. Next, we investigated the role of this difference in cardiac dysfunction induced by Angiotensin II (AngII). Both *EPHX2* gene deletion and inhibition protected against AngII-induced cardiac hypertrophy. Interestingly, the cardiac dysfunction was attenuated by sEH inhibition rather than gene deletion. Histochemical staining revealed that compared with pharmacological inhibition, *EPHX2* deletion aggravated AngII-induced myocardial fibrosis; the mRNA levels of fibrotic-related genes were increased. Furthermore, the cardiac inflammatory response was greater in *EPHX2*-/- than sEH-inhibited mice with AngII treatment, as evidenced by increased macrophage infiltration and expression of inflammatory factors. *In vitro*, AngII-upregulated inflammatory factors were significantly attenuated by sEH inhibition but promoted by *EPHX2* deletion in cardiofibroblasts. **Conclusion:** Thus, compared with pharmacological inhibition of sEH, *EPHX2* deletion, because of the shift in arachidonic acid metabolism, led to pathological cardiac remodeling, especially cardiac fibrosis, which may have therapeutic value in treatment of heart failure.

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S3

INHIBITION OF SOLUBLE EPOXIDE HYDROLASE IMPROVES CARDIAC FUNCTION AND LIMITS MITOCHONDRIAL DAMAGE FROM ISCHEMIC INJURY

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Purpose: Cardioprotective effects of epoxyeicosatrienoic acids (EETs) toward acute myocardial ischemia-reperfusion injury have been recognized; however, the precise mechanism(s) are still largely unknown. Our study investigates the protective effects of EETs by inhibiting soluble epoxide hydrolase (sEH), the enzyme responsible for EET metabolism, following surgical occlusion of left anterior descending artery (LAD) of the heart. **Methods:** Age matched 2 month old sEH null (KO) and littermate wild-type (WT) mice were utilized in the study, as well C57Bl/6 mice which were administered an sEH inhibitor, trans-4-[4-(3-adamantan-1-y1-ureido)-cyclohexyloxy]-benzoic acid (*t*AUCB; 10mg/L) or vehicle in drinking water for 4 days prior and 7 days post-surgery. Mice from all groups were subjected to surgical occlusion of LAD and cardiac function was assessed by echocardiography prior to and 7 days post-surgery. Mice were sacrificed on day 7 and heart tissues were dissected into infarct, peri-infarct (area at risk) and non-infarct (healthy) regions to assess cellular and sub-cellular structure by electron microscopy (EM). Hearts were collected and mitochondrial respiratory enzymes in complexes I, II, III, IV and citrate synthase activities were assayed following IR injury.
Results: Hearts from *t*AUCB treated and sEH (KO) mice showed significantly improved ejection fraction ($p<0.05$) and fractional shortening ($p<0.05$) compared to WT counterparts. Echocardiogram revealed less cardiac remodeling in *t*AUCB treated and sEH KO groups evident by reduced left ventricular internal diameter ($p<0.05$) during both systole and diastole. Consistently, EM data showed

more intact cardiomyocytes with better arrangement of myofibers and mitochondria in the *t*AUCB treatment and sEH KO group. Inhibition of sEH resulted in better complex I, II and citrate synthase activities compared to control hearts. However, no significant improvements were observed in complex III or IV activities. **Conclusion:** The inhibition sEH or ablation of sEH gene provides cardiac protection against long-term ischemia, associated with preserved post-ischemic cardiac function and maintaining mitochondrial integrity and respiratory function.

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S4

INHIBITION OF EPOXIDE HYDROLASE RESTORES HYPOXIC PULMONARY VASOCONSTRICTION AND IMPROVES GAS EXCHANGE IN MURINE SEPTIC LUNG INJURY

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Recent studies report that cytochrome P450 epoxygenase-derived epoxyeicosatrienoic acids (EETs) are pulmonary vasoconstrictors. We hypothesized that inhibiting the catabolism of EETs by inhibition of soluble epoxide hydrolase (sEH) would enhance vasoconstriction in hypoxic lung regions and improve arterial oxygenation in a murine model of systemic sepsis and lung injury. Lung injury was induced in awake wild-type mice by intravenous administration of *Escherichia coli* O55:B5 lipopolysaccharide (LPS) (20 mg·kg-1). At 22 h after challenge with LPS, the mice were anesthetized, intubated, and ventilated at an inspired oxygen fraction (FIO2) of 1. Anesthetized mice received an i.v. infusion of the sEH inhibitor, AEPU, or an equal volume of vehicle 30 minutes prior to assessing hypoxic pulmonary vasoconstriction (HPV). HPV was assessed by measuring left lung pulmonary vascular resistance index (LPVRI) and systemic arterial oxygenation (PaO2) before and after left main stem bronchial occlusion (LMBO). Following thoracotomy, pulmonary artery pressure (PAP) and left lung blood flow (QLPA) were continuously measured before and during transient inferior vena cava occlusion. LPVRI was estimated from the slope of the PAP-QLPA relationship. Challenging mice with LPS markedly attenuated the increase in LPVRI in response to LMBO ($P<0.05$), consistent with impaired HPV. Intravenous administration of vehicle or AEPU to anesthetized mice at 22 hours following LPS challenge did not change baseline systemic or pulmonary hemodynamic parameters. LMBO increased LPVRI, in both AEPU- and vehicle-treated LPS-challenged mice. However, the increase in LPVRI during LMBO was greater in mice treated with AEPU than in mice treated with vehicle alone (137±14 vs. 102±7 mmHg·min·g·ml-1, respectively; $P<0.05$). PaO2 during LMBO was greater in LPS-challenged mice treated with AEPU than in LPS-challenged mice treated with vehicle (225±33 vs. 121±9 mmHg, respectively; $P<0.05$), demonstrating that inhibiting sEH augments HPV by improving matching of ventilation to perfusion (V/Q) in murine septic lung injury. Taken together, these results suggest that inhibition of sEH by AEPU restores HPV, preserves V/Q matching, and improves systemic oxygenation in murine septic lung injury. If these findings can be translated to humans, these data indicate that sEH inhibitors have great potential for the treatment of acute or chronic impairment of pulmonary gas exchange.

S5

OBESE CHILDREN AND HYPERTENSION: POSSIBLE ROLE FOR CYTOCHROME P-450 EICOSANOIDS.

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Children obesity is an increasing health emergency and is often accompanied by other metabolic and haemodynamic disorders such as hypertension. Beside other pathways, arachidonic acid (AA) can be metabolized by cytochrome P-450 (CYP450) leading to the formation of epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acids (20-HETE), vasoactive and natriuretic metabolites which can be involved in blood pressure (BP) control. EETs are further metabolized to dihydroxyeicosatrienoic acids (DHETs), compounds with little or no biological activity. The role of CYP-derived eicosanoids in BP maintenance has not been explored in children yet. Omega-3 polyunsaturated fatty acids (Ω-3 PUFA) may compete with omega-6 PUFA (Ω-6 PUFA) for CYP450 metabolism, determining a modulation in eicosanoids production. The aim of our study was to investigate the possible role of CYP450 metabolites of AA in BP control and vascular function measured by ultrasound (flow mediated dilatation; FMD, index of endothelial function) and digital photoplethysmography (Stiffness Index; SI; marker of systemic vascular stiffness) in obese children. The possible correlation with Ω-3 PUFA erythrocytes plasma membrane levels was explored. We recruited 26 obese children (BMI≥95° percentile for sex and age), aged 7-17 years, with no comorbidities including diabetes. Plasma and urinary EETs, 20-HETE and DHETs were measured by liquid chromatography/mass spectrometry (LC-ESI-MS/MS) and Ω-3 PUFA by gas chromatography and expressed as percentage of total fatty acids (Ω-3 Index). Eight children were classified as hypertensive and showed an increased urinary excretion of DHETs, compared with normotensive children (1684±766 *vs.* 1051±426 ng/24 h; $p=0.03$), without differences in the four regioisomers. Differences between normotensive and hypertensive children were found neither for Plasma EETs, DHETs and plasma and urinary 20-HETE nor for the Ω-3 Index. We did not find any significant correlation between eicosanoids and BP levels but found an inverse correlation between SI and Ω-3 index ($r=-0.39$; $p=0.05$) and between FMD and Plasma EETs levels, the latter only in males ($r=0.52$; $p<0.05$). Our preliminary study sets the stage for further studies to address the involvement of CYP pathways in BP control in children.

S6

20-HETE CONTRIBUTES TO ISCHEMIA-INDUCED COMPENSATORY NEOVASCULARIZATION

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Objective: Compensatory neovascularizaiton is an important adaptation for recovery from critical ischemia. Recent studies strongly supported that the CYP4A-20-HETE system is a novel regulator of angiogenic processes. Here, we assessed the contribution of the 20-HETE system to ischemia-mediated neovascularization and further explored its underlying molecular and cellular mechanisms. **Methods:** The mouse ischemia hindlimb angiogenesis assay was performed to evaluate the effect of systemic or local inhibition of the CYP4A-20-HETE system with the 20-HETE synthase inhibitor, DDMS or the 20-HETE antagonist, 20-HEDGE on the compensatory angiogenic responses *in vivo*. Laser Doppler Perfusion Imaging was conducted at days 0, 1, 3, 10, 17, and 21 after fermoral ligation to assess hindlimb blood flow. Blood pressure was monitored at days 1, 7, 12, 14, and 21 post-ligation with the non-invasive blood pressure monitoring system. CD31 and Tomato Lectin double staining were carried out to quantify microvessel density in hindlimb gracilis muscle. 20-HETE production in ischemic muscles was also measured by LC-MS-MS analysis. Finally, western blot was performed to explore the potential underlying signaling pathways. **Results:** Inhibition of 20-HETE synthesis or antagonizing its action either locally or systematically delayed the blood flow perfusion and microvessel formation in response to ischemia without affecting blood pressures. Importantly, ischemic hindlimb muscles showed markedly elevated 20-HETE synthesis compared to non-ischemic controls (96 ± 17 pg/mg of protein versus non-detectable amount) in ischemic hindlimb gracilis muscles 3 days post-ligation. Furthermore, the protein expression of HIF-1α, VEGF, VEGFR2, and p44/42 MAPK in day 3 post-ligation hindlimb muscles were significantly induced. These increases were negated by DDMS or 20-HEDGE. No significant difference was found in the expression of these signaling molecules at day 21 post-ligation. **Conclusions:** Early increases in 20-HETE production in ischemic muscles may regulate ischemic angiogenesis *via* the induction of the HIF-1α/VEGF and MAPK pathways. These results strengthen the notion that 20-HETE may be a key regulator of ischemia-induced compensatory neovascularization processes.

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S7

INCREASED PLACENTAL EPOXYEICOSATRIENOIC ACIDS IN PREECLAMPSIA

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Plasma levels of epoxyeicosatrienoic acids (EETs) derived from cytochrome P450 (CYP)-dependent metabolism of arachidonic acid have been shown to be increased in women with preeclampsia (PE) as compared to normal pregnancy (N), and to be even higher in fetal plasma (Herse et al. Circulation 2012, Jiang et al. Am J Hypertens 2013). We hypothesized that differences in EET synthesis or metabolism in the feto-placental unit underlies the observed differences in circulating EETs. Biopsies of placenta and umbilical cord were collected from 19 N and 10 PE at the time of surgical delivery. EETs were extracted from tissue homogenates and analyzed by LCMS. Tissue 14,15-, 11,12-, 8,9-, and 5,6 EETs and their metabolites DHETs were measured in a subgroup of tissue samples (N=10; PE=5). CYP2J2, but not CYP4A11 nor CYP2C8, and metabolizing enzyme soluble epoxide hydrolase (sEH) were detected and quantified in tissue samples by immunohistochemistry and immunoblotting. Both *cis*- and *trans*- EETs were detected in the placenta and umbilical cord in PE and N, with similar mean ratios (1.1 vs 1.0 and 1.4 vs 2.0, respectively). Concentrations of total EETs were higher in the placenta of PE compared to N (3.37±1.42 ng/mg vs 1.20±0.72 ng/mg, M±SD, $P<0.001$), with 5,6-, 8,9- and 11,12-EETs being higher, but not 14,15-EETs. A tendency towards increased ratio of total EETs to DHETs, an index of sEH activity, was observed in PE ($p=0.07$). No differences between PE and N were detected as for EETs and DHETs in umbilical cord. Staining for sEH in the placenta localized in the trophoblast was lower in PE than in N, as confirmed by western blotting analysis of placenta homogenates. Staining for sEH localized in the perivascular area of umbilical cord was similar in PE and N. Abundant CYP2J2 expression were observed in tissues of both PE and N. In conclusion, along with the enzymes implicated in their biosynthesis, significant amounts of EETs were found in the placenta and the umbilical cord. The presence of abundant *trans*-EETs suggests that non-enzymatic, peroxidative synthesis of EETs occurs in these tissues. Reduced expression of sEH in the placenta may have contributed to increased EET levels observed in PE.

S8

EETS TARGET MITOCHONDRIA TO AUGMENT SURVIVAL DURING STARVATION STRESS

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PURPOSE: Mitochondria are the primary source of energy in cardiomyocytes playing a key role in contractile function and cell survival during stress. During nutrient restriction, mitochondria fuse together into elongated networks, which delays their collapse

and enhances ATP production to sustain cell survival. Previously, we and others have reported the protective role of epoxyeicosatrienoic acids (EETs) in the heart. EETs are CYP450-depended metabolites of arachidonic acid that have robust but poorly understood anti-apoptotic and cardioprotective properties. EETs protected mitochondria against fragmentation induced by either hypoxia or oxidative stress. In the present study, we investigate the effect of EETs on cardiac mitochondria during starvation induced stress. METHODS: Rat neonatal cardiomyocytes (NCM) and HL-1 cardiac cells were treated with 14,15-EET (1μM) or UA8 (dual acting EET mimetic, 1μM) in serum free starvation buffer for 24 hours. The putative pan-EET receptor antagonist, 14,15-EEZE (10μM), was used as a negative control to confirm EET-mediated effects. Cell survival was assessed with MTT assay. Western blot analysis was used to assess alterations in mitochondrial dynamics regulators (DRP1, Fis1, and OPA1) and p62 as a marker for autophagy. Mitochondrial function was assessed by measuring changes in enzymatic activities and protein expression of key enzymes involved in the electron transport chain (ETC). Live-cell imaging was used to assess alterations in mitochondrial morphology and membrane potential with potentiometric mitochondrial dye TMRE (0.1μM). The 3D mitochondrial morphology and network structure was reconstructed and analyzed by the Filament Tracer module in Imaris software. RESULTS: Similar to our previous data, starvation caused a marked activation of autophagy as indicated by consumption of p62 levels. Starvation caused increased mitochondrial membrane potential and clear mitochondrial elongation, which correlated with significant reduction in mitochondrial fission proteins DRP1 and Fis1. UA8 treated cell showed further decrease in both DRP1 and Fis1. Interestingly, UA-8 treated cells had preserved mitochondrial cristae and increased expression the short form of OPA-1 but no starvation-induced mitochondrial elongation was observed. This coincided with UA8 enhanced cell survival and maintained mitochondrial respiration during starvation stress. CONCLUSIONS: Together, these initial data suggest that EET-mediated events preserve mitochondrial structure and minimize the loss of ETC enzymatic function, thus enhancing cell survival without the starvation induced mitochondrial elongation.

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S9

20-HYDROXYEICOSATETRAENOIC ACID (20-HETE) INDUCTION OF ANGIOTENSIN CONVERTING ENZYME (ACE) TRANSCRIPTION IS MEDIATED BY NF-kB BINDING VIA EGFR/MAPK/IKK PATHWAY IN HUMAN ENDOTHELIAL CELLS

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Increased vascular 20-hydroxyeicosatetraenoic acid (20-HETE), a cytochrome P450-arachidonic acid metabolite, promotes vascular dysfunction, injury and hypertension that is dependent, in part, on the renin angiotensin system (RAS). We showed that in cultured human microvascular endothelial cells (HMVEC), 20-HETE (5 nM) increases angiotensin converting enzyme (ACE) mRNA by 3.1-fold (± 0.16; $p<0.05$), ACE protein by 4.2-fold (±0.99; $p<0.05$) and cellular and extracellular ACE activity by 2.2-fold (±0.09; $p<0.05$) and 1.96-fold (±0.16; $p<0.05$), respectively. These effects were abrogated by co-treatment with 20-HEDE, a 20-HETE antagonist. 20-HETE's effects were distinct from known inducers of ACE as maximal ACE mRNA expression was observed at 2 hours compared to 24 hours for the PKC-dependent inducer phorbol esters (PMA). Upregulation of ACE mRNA and ACE activity by 20-HETE was abolished by EGFR-tyrosine kinase, MAPK and IKKβ inhibitors. The 11,12-epoxyeicosatrienoic acid (11-12 EET), a cytochrome P450 eicosanoid which has been shown to activate EGFR in HMVEC, did not have an effect on ACE mRNA induction illustrating the specificity of 20-HETE as an inducer of ACE. HMVEC treated with EGF (100 ng/ml) alone or with 20-HETE (10 nM) did not induce or further increase ACE mRNA levels, respectively. Moreover, pretreatment of cells with a neutralizing antibody against EGF did not prevent the 20-HETE- or 20-HETE+EGF-mediated induction in ACE mRNA indicating that the induction of ACE by 20-HETE is EGF-independent. The transcription inhibitor actinomycin negated the effects of 20-HETE on ACE mRNA indicating that 20-HETE's effect is at the level of transcription. EMSA of 20-HETE-treated cells showed increased NF-kB binding activity in nuclear extracts. Sequence analysis demonstrated the presence of two (Chromosome 17: 61554061-61554075, 61554157-61554171) and one (Chromosome 17: 61562250-61562264) putative NF-kB binding sites on the human somatic and germinal ACE promoters, respectively. In cells transfected with luciferase promoter-reporter constructs for the somatic or germinal ACE promoter regions, 20-HETE (10 nM) increased promoter activity by 4.37-fold (±0.18; $p<0.05$) and 2.53-fold (± 0.24; $p<0.05$) respectively. 20-HETE-stimulated ACE promoter activity was abrogated by 20-HEDE and by inhibitors of EGFR, MAPK, IKKβ and NF-kB activation. This is the first study to identify NF-kB as a transcriptional factor for ACE and to implicate a distinct EGFR/MAPK/IKK signaling cascade underlying 20-HETE-mediated transcriptional activation of ACE mRNA. The findings further suggest that ACE induction may be a key mechanism underlying 20-HETE's ability to promote vascular dysfunction, vascular injury and hypertension.

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S10

CYP2C44 PLAYS A CRUCIAL ROLE IN THE VASCULAR DEVELOPMENT OF THE RETINA

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Cytochrome P450 (CYP) enzymes are able to metabolize polyunsaturated fatty acids like arachidonic acid (AA), linoleic acid (LA) as well as docosahexaenoic acid (DHA) to bioactive fatty acid mediators. While the murine Cyp2c44 has already been identified as a potential source of pro-angiogenic factors, the underlying mechanisms as well as the main angiogenic mediators remain to be clarified. We investigated the role of Cyp2c44 in angiogenesis by characterizing postnatal retinal vascularization focusing on fatty acid-mediated Notch signaling. Cyp2c44 could be detected by RT-qPCR and immunohistochemistry in the adult retina and was mainly localized in astrocytes and Müller glia cells. Postnatal retinal vascularization was assessed on postnatal day 5 (P5) by quantifying branching points as well as vessel diameter in the central and the peripheral regions of the retina. Compared with retinas from wild-type mice the peripheral region of the Cyp2c44-/- retina demonstrated a significantly increased vascular density, characterized by more branching points and an enlarged vessel diameter, as well as more tip cell filopodia. This phenotype was accompanied by a decrease in the expression of *Notch1* and its target genes *Hes1* and *Hey1* in Cyp2c44-/- retinas. Lipid metabolites from plasma and P5 retina of wild-type and Cyp2c44-/- mice were quantified using LC-MS/MS and enzyme activity assays were performed with microsomes isolated from SF9 cells overexpressing Cyp2c44 and human oxidoreductase. We found that although Cyp2c44 is able to metabolize AA, DHA and LA *in vitro*, analysis of the lipid profile in plasma and retina revealed no significant differences in profile of AA-derived epoxides or diols while the LA- as well as DHA-derived epoxides and diols were significantly decreased in samples from Cyp2c44-/- mice. These results suggest that Cyp2c44 plays an important role in the development of retinal vasculature, potentially by affecting Notch signalling which can account for the observed pro-angiogenic phenotype in the Cyp2c44-/- retina.

S11

ORALLY ACTIVE EPOXYEICOSATRIENOIC ANALOG ATTENUATES DEVELOPMENT OF ANG II-DEPENDENT MALIGNANT HYPERTENSION IN RATS

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Recent studies have shown that pharmacological blockade of soluble epoxide hydrolase (sEH) increases tissue bioavailability of epoxyeicosatrienoic acids (EETs) and has considerable antihypertensive and renoprotective effects in ANG II-dependent malignant hypertension in rats. This study evaluated another approach to enhance EETs activity for treatment of hypertension, using a new orally active EET-analog (EET-A) that was administered to transgenic rats with inducible ANG II-dependent hypertension (iTGR). Malignant hypertension was induced by dietary administration of indole-3-carbinol (I3C), a natural xenobiotic that activates the mouse renin gene in Cyp1a1-Ren-2 transgenic rats. “Early treatment” and “late treatment” protocols were designed. Blood pressure (BP) was monitored by radiotelemetry. Biochemical parameters, components of renin-angiotensin system and cytochrome P-450 metabolites were assayed. Renal injury was assessed. When started simultaneously with gene induction procedure, EET-A given in drinking water at a dose of 10 mg.kg-1.day-1 attenuated the BP increase, prevented renal hypoperfusion and reduced proteinuria, renal injury and cardiac hypertrophy. This was accompanied by suppression of elevated plasma and kidney ANG II levels, ACE activity and marked natriuresis. Moreover, the treatment prevented the usual decreases in urinary excretion of nitrate/nitrite and abolished increases in urinary excretion of 8-isoprostane. Remarkably, EET-A treatment was almost ineffective when applied in the phase of established hypertension. Our results show that a new orally active EET-A attenuates the development of ANG II-dependent malignant hypertension and hypertension-associated end-organ damage. This finding should be considered in attempts to develop new antihypertensive therapies.

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S12

INHIBITION OF ACE ACTIVITY LOWERS BLOOD PRESSURE BUT NOT PREVENT 20-HETE-MEDIATED MICROVASCULAR REMODELING IN CYP4A12TG MICE

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20-hydroxyeicosatetraeonic acid (20-HETE), a product of ω-hydroxylation of arachidonic acid by enzymes of the cytochrome P450 (CYP) 4A and 4F gene families, plays an important role in the regulation of vascular tone, renal function, and blood pressure. Recent studies in our laboratory identified 20-HETE as a potent inducer of the angiotensin-converting enzyme (ACE) and demonstrated that 20-HETE-driven hypertension is mediated, in part, by induction of the ACE and activation of the renin-angiotensin system (RAS). Our studies also showed that 20-HETE-dependent hypertension is associated with remodeling of the

microvasculature that is not fully driven by the increase in blood pressure. However, the role of ACE induction in 20-HETE-mediated microvascular remodeling in hypertension is unclear. The Cyp4a12tg mice, in which the expression of the Cyp4a12-20-HETE synthase is under the control of the tetracycline (doxycycline, DOX) promoter, were used to assess the contribution of ACE/RAS to microvascular remodeling in 20-HETE-dependent hypertension. Treatment of Cyp4a12tg mice with DOX (1mg/ml in the drinking water) increased CYP4A12 protein levels by 2-fold, 20-HETE production in preglomerular microvessels (PGMV) by 2-fold and blood pressure by 33% (136±2 vs 102±1 mmHg, $p<0.05$). The increase in blood pressure was maintained as long as DOX was administered; it was prevented by co-treatment with either the 20-HETE antagonist 20-HEDGE (103±3 mmHg, $p<0.05$) or lisinopril (100±3mmHg, $p<0.05$). DOX-treated Cyp4a12tg mice (42 days) showed significant increases in PGMV remodeling determined by increases in media-to-lumen ratio and media thickness when compared to untreated Cyp4a12tg mice (M/L, 0.16±0.01 vs 0.08±0.01, $p<0.05$; media thickness, 16.1±0.9 vs. 8.2±0.5 μm2, $p<0.05$). Co-treatment of DOX-treated Cyp4a12tg mice with 20-HEDGE (10 mg/kg/day) abrogated PGMV remodeling (M/L, 0.09±0.01; media thickness, 8.8±0.6 μm2, $p<0.05$). In contrast, co-treatment of DOX-treated Cyp4a12tg mice with lisinopril (10 mg/kg/day) lessened but did not prevent PGMV remodeling (M/L, 0.13±0.01; media thickness, 12.0±0.5 μm2, $p<0.05$). The increased production of 20-HETE in PGMV in response to DOX treatment of Cyp4a12tg mice was not affected by lisinopril (1.05±0.18 vs 1.09±0.30 ng/mg). ACE expression in the PGMV was significantly higher in DOX-treated (4.0±0.8 fold; $p<0.05$) as compared to untreated Cyp4a12tg mice; administration of 20-HEDGE prevented the increase in ACE expression. Co-treatment of DOX with Lisinopril did not exhibit any significant difference in PGMV ACE expression when compared to DOX-treated mice. This study demonstrates that 20-HETE is a key determinant of microvascular remodeling in DOX-induced Cyp4a12tg hypertensive mice; 20-HETE-driving microvascular remodeling in hypertension does not fully depend on ACE activity or blood pressure elevation. Whether the increase in ACE expression brought about by the increased 20-HETE's production contributes to microvascular remodeling independent of its activity is a subject of further studies.

** Contribute equally to the study. This study was supported by NIH grants HL34300 (MLS), NHLBI Diversity Supplement to HL034300 (VG), and by the Robert A. Welch Foundation (GL625910) (JRF).*

S13

AN ORALLY ACTIVE EPOXYEICOSATRIENOIC ACID ANALOG MITIGATES EXPERIMENTAL RADIATION NEPHROPATHY

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Epoxyeicosatrienoic acids (EETs) protect kidney in different pathologies ranging from hypertension to nephrotoxic renal injury. In the current study, we investigated kidney protective role of a novel EET analog (EET-A) in experimental radiation nephropathy caused by total body irradiation (TBI) with 11Gy. We hypothesized that EET analog will mitigate radiation nephropathy. Four groups of rats (n=4-6/group) viz. normal male WAG/RijCmcr rats, TBI rats treated with vehicle (TBI-V), TBI rats treated with EET-A (TBI-EET-A, 3mg/kg/d po), and captopril treated TBI rats (TBI-Cap, 30mg/kg/d po) were studied. Kidney injury was assessed from blood urea nitrogen (BUN), albuminuria, nephrinuria, and histopathological changes 12-weeks post TBI. The TBI-V group had a 30-fold increase in BUN level compared to control (157±14 vs. 5±3 mg/dL, $P<0.05$). EET-A (84±6 mg/dL) and captopril (52±2 mg/dL) decreased the BUN level compared to TBI-V group ($P<0.05$). The albumin/creatinine ratio increased 300-fold in TBI-V group compared to control (5610±648 vs. 18±3 μg/mg, $P<0.05$), which was reduced in TBI-EET-A (1652±387 μg/mg) and TBI-Cap (113±21 μg/mg) groups ($P<0.05$). Moreover, nephrin/creatinine ratio increased 250-fold in the TBI-V compared to control group (582±67 vs. 2±1 μg/mg, $P<0.05$) and reduced by both EET-A (150±16) and captopril (8±2) ($P<0.05$). In histopathological studies, it is demonstrated that TBI-V group had tubular and glomerular injuries that were reduced by EET-A and captopril ($P<0.05$). These data demonstrate a novel EET-analog based strategy to reduce kidney injury in experimental radiation nephropathy.

S14

QUANTIFICATION OF TXB2 AND PGI2 METABOLITES IN MICE WITH HYPERTENSION INDUCED BY L-NAME

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Aim: The aim of the present work was to develop bioanalytical method for simultaneous quantification of TXA2 metabolites (TXB2, 2,3-dinor-TXB2 and 11-dehydro-TXB2), PGI2 metabolites (2,3-dinor-6-keto-PGF1α, 6-keto-PGF1α) as well as other prostanoids PGE2, PGD2, PGF2α) using LC-MS/MS to profile changes in their production in mice with NO-deficiency and hypertension induced by L-NAME. **Experiment:** 12 C57Bl/6J mice: Control group (untreated), L-NAME group (receiving L-NAME in drinking water, 100 mg/kg) were used for experiments. Blood pressure was measured by telemetry, and platelet activation by *ex vivo* dynamic TXB2 generation assay. Urine for prostanoid analysis was collected using metabolic cages. **Quantification Methods and Results:** The method of liquid ultrafast chromatograph UFLC Nexera (Shimadzu) coupled to mass spectrometer QTRAP 5500 (ABSciex) equipped with TurboV ion source was used. The limit of quantification of the method for 2,3-dinor-6-keto-PGF1α, 6-keto-PGF1α and 2,3-dinor-TXB2 was 0.25 ng/mL, 0.5 ng/mL and 1 ng/mL, respectively. PGE2, PGD2 and PGF2α concentration was below the detection limit (LOD) irrespectively to preparation techniques used (e.g. protein precipitation with and without evaporation step, solid-phase extraction). The urine concentration of 11-dehydro-TXB2 in both control and L-NAME groups was validated using ELISA. L-NAME treated mice developed sustained hypertension within 1-2 weeks after L-NAME

treatment that was associated with elevation of concentration of 2,3-dinor-TXB2 as well as 2,3-dinor-6-keto-PGF1α and 6-keto-PGF1α. However, TXB2 generation by *ex vivo* platelets was not significantly different in control and L-NAME groups. **Conclusions:** NO-deficiency induced by L-NAME treatment was associated with the higher urine excretion of TXB2 and PGI2 metabolites. However, the ratio of 2,3-dinor-6-keto-PGF1α/2,3-dinor-TXB2 was similar between control and L-NAME group throughout experimental period suggesting full compensation of NO-deficiency by PGI2, compatible with the lack of platelet activation in *ex vivo* assay.

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S15

NOVEL REGULATION OF 15-LIPOYGENASE AND 15-HETE PRODUCTION BY GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN VASCULAR SMOOTH MUSCLE

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Using immunoprecipitation combined with proteomic approach we observed that 15-lipoxygenase (15-LOX), which oxidizes arachidonic acid, forms a complex with glucose-6-phosphate dehydrogenase (Glc-6-PD), a key enzyme that regulates NADPH, in the vascular smooth muscle. To determine the physiological meaning of the complex between 15-LOX and Glc-6-PD, we studied the effects of 6-aminonicotinamide (6AN) and dehydroepiandrosterone (DHEA), known competitive and uncompetitive inhibitors of Glc-6-PD, respectively, on the 15-LOX product, 15-HETE, formation in pulmonary artery (PA). To do so, we incubated PA with 6AN and DHEA at 37ºC for 1 hr and then measured 15-HETE by LC-MS. 6AN (20.2±12.3 pg/μg protein; n=3) and DHEA (29 pg/μg protein) decreased 15-HETE by 64% (p=0.057) and 52%, respectively, of control (74±25 pg/μg protein; n=3) in the PA. 15-HETE plays a role in the pathogenesis of PA adventitial fibrosis and pulmonary hypertension by eliciting proliferation, migration and constriction of PA smooth muscle cells. Therefore, in conclusion we have a discovered a novel mechanism for regulation of 15-LOX and 15-HETE synthesis in the PA. This mechanism could be of potential therapeutic value for the treatment of pulmonary hypertension.

S16

ACUTE ADMINISTRATION HET0016 IMPROVES CEREBRAL PERFUSION AND FUNCTIONAL OUTCOME IN A PEDIATRIC ASPHYXIAL CARDIAC ARREST MODEL

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Objective: Novel therapies are needed to improve the neurological outcome after pediatric asphyxial CA. Our group developed a pediatric asphyxial CA model in immature 17 day old rats. In this model cerebral blood flow (CBF) after reperfusion is characterized by cortical hypoperfusion, which may produce secondary neuronal damage. Increased levels of the vasoconstrictor eicosanoid 20-HETE in this model could influence CBF and functional outcome. We sought to evaluate the effect of acute inhibition of 20-HETE production on CBF, cortical eicosanoid levels, neuropathological and functional outcome in our pediatric asphyxial CA model. **Methods:** Male 17 day old rats (n=6/group) were subjected to 12 min of CA followed by resuscitation. HET0016, 1 mg/kg, or vehicle was administered iv at resuscitation. Cortical CBF was measured at baseline, during CA, and continuously until 120 min post resuscitation via laser speckle photometry. Cortical CYP450 eicosanoid levels were quantified at 5 and 120 min after resuscitation using a validated UPLC-MS/MS method. In a separate cohort of rats (n=6/group), HET0016 1 mg/kg or vehicle was administered at resuscitation and every 6 h for 24 h. Short-term neurological deficits were assessed at 24 and 48 h after resuscitation using a modified neuro-deficit score (NDS) system. Neuronal degeneration at 48 h was assessed by FluoroJade-B staining. **Results:** HET0016 treatment reduced cortical 20-HETE levels (1.99±0.2 vs 8.06±1.3 pmol/g tissue, $p<0.001$ HET0016 vs. vehicle). Cortical CBF increased early after CA in rats receiving HET0016 vs. vehicle (%CBF at 5 min: 101±12.5% vs 77.8±4.2%; at 10 min: 91.6±14.2% vs 69.5±4.6%, $p<0.05$). HET0016 treatment decreased the NDS score vs. vehicle at 3h (52.2±4.8% vs 37.5±9.9%) and 24 h (10.4±4.9% vs 2.8±0.8) after resuscitation, $p<0.01$. HET0016 treatment decreased neuronal degeneration at 48 h (FluoroJade positive neurons: 22.8±6.9 vs 41.4±9.6, HET0016 vs. vehicle, $p<0.01$). **Conclusions:** Cortical hypoperfusion early after resuscitation from pediatric asphyxial CA is associated with increased levels of the vasoconstrictor eicosanoid 20-HETE. Administration of HET0016, a 20-HETE synthesis inhibitor, selectively inhibited the formation of 20-HETE, improved cortical CBF early post-resuscitation, decreased cortical neurodegeneration, and improved the short-term functional outcome.

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S17

HYPOXIA INDUCIBLE FACTOR-1α ON INFARCT SIZE-LIMITING EFFECT OF POSTCONDITIONING AFFORDED BY EPOXYEICOSATRIENOIC ACID ANALOG IN RAT HEARTS

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Epoxyeicosatrienoic acids (EETs) decrease cardiac ischemia/reperfusion injury, however, the mechanism of this protective effect remains elusive. Here we investigated the cardioprotective action of a novel EET analog in reperfusion (pharmacological postconditioning) and the role of hypoxia inducible factor-1α (HIF-1α). Adult male SD rats were subjected to 30 min left coronary artery occlusion followed by 2 h reperfusion. The endogenous EET, 14,15-EET (2.5 mg/kg, iv) or the EET analog, EET-B (2.5 mg/kg, iv) administered 5 min before reperfusion reduced infarct size expressed as a percentage of the area at risk, from 64.3±1.3 % in control to 42.6±1.9 % and 46.0±1.6 %, respectively and their co-administration did not provide stronger effect (45.9±1.4 %). On the other hand, the EET antagonist 14,15-EEZE (2.5 mg/kg, iv) inhibited infarct size limiting effect of EET-B (62.5±1.2 %). HIF-1 α inhibitors, 2-methoxyestradiol (2.5 mg/kg, iv) and acriflavine (2 mg/kg, iv) completely abolished the cardioprotective effect of EET-B (63.3±1.6 and 63.6±1.2 %, respectively). In a separate set of experiments the immunoreactivity of HIF-1α and its degrading enzyme prolyl hydroxylase 3 (PHD3) were analyzed in the ischemic area at risk and non-ischemic septa at the end of ischemia as well as after 20 min and 2 h reperfusion. HIF-1α immunogenic signal markedly increased in the area at risk compared to septum at the end of ischemia (9.3±1.1 vs 0.3±0.1 %). After 20 min and 2 h of reperfusion, HIF-1α immunoreactivity in the area at risk decreased to 2.4±0.5 % and 1.9±0.4 %, respectively in the controls. EET-B administration reduced PHD3 immunogenic signal and blunted the decrease of HIF-1α immunoreactivity in ischemized tissue following reperfusion (7.8±0.7 and 6.4±1.4 %, respectively). In conclusion, EET-B provides strong postconditioning protection against myocardial infarction in rats. We suggest that increased HIF-1α levels play an important role in this cardioprotective mechanism.

S18

ROLE OF SOLUBLE EPOXIDE HYROLASE IN AGE-RELATED VASCULAR COGNITIVE DECLINE

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Vascular cognitive impairment (VCI) is the second most common cause of dementia worldwide, and cerebrovascular disease commonly co-occurs and contributes to cognitive impairment in Alzheimer's disease (AD). The most common cause of VCI is cerebral small vessel disease, which is seen on T2-weighted MRI as white matter hyperintensities (WMH). We stratified 88 cognitively impaired individuals from the Oregon Brain Aging Study according to the presence (n=42) or absence (n=46) of vascular disease based on postmortem pathological examination, and quantified their WMH volume on premortem MRI. Individuals with vascular disease had more than 4 times the volume of WMH compared to those without vascular disease (23.21 ± 3.34 mL vs. 5.29 ± 0.68 mL, $p<0.01$). Because endothelium-derived eicosanoids play an important role in small vessel dilation, we hypothesized that cerebrovascular vasodilator eicosanoid signaling is impaired in brains of patients with VCI. We quantified eicosanoids in postmortem cortical brain tissue from patients with VCI (n=5) and aged-matched controls (n=5). We found that 14,15-dihydroxyeicosatrienoic acid (DHET), the breakdown product of 14,15-epoxyeicosatrienoic acid (EET), was significantly elevated in individuals with VCI compared to age-matched controls (19.0 ± 1.9 vs. 10.3 ± 0.9 pg/mg of brain tissue, $p<0.01$). Interestingly, levels of EETs, including 14,15-EET were not different between groups (167.9 ± 10.4 vs. 138.0 ± 10.9 pg/mg of brain tissue, $p=0.08$, n=5 per group), suggesting enhanced metabolism of EETs in VCI brains. To determine if enhanced EETs in VCI brains is linked to soluble epoxide hydrolase (sEH), we localized sEH-immunoreactivity (IR) in human brain sections, and compared sEH-IR signal intensity between VCI and control brain tissue. sEH-IR was localized in cerebral microvascular endothelium. Signal intensity increased with age and in some individuals was higher in microvessels near areas of micro-infarction. To determine whether enhanced sEH hydrolase activity in VCI brain vessels is genetically linked, we used allelic discrimination to determine the frequency of two missense polymorphisms known to affect sEH activity in VCI patients and controls. We found that K55R and R287Q polymorphisms were more frequent in VCI patients compared to control (29% vs. 11%). Our findings support a role for sEH in agerelated vascular cognitive decline, and identify a new population that may benefit from treatment with sEH inhibitors.
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S19

THE EFFECT OF A SOLUBLE EPOXIDE HYDROLASE INHIBITOR, T-TUCB, ON EET FORMATION IN THE IMMATURE RAT BRAIN

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Introduction: Cerebral blood flow (CBF) is decreased after resuscitation from pediatric cardiac arrest (CA). The level of several vasodilator eicosanoids is decreased after CA. We hypothesized that intravenous administration of the soluble epoxide hydrolase inhibitor trans-4-{4-[3-(4-Trifluoromethoxy-phenyl)-ureido]-cyclohexyloxy}-benzoic acid (t-TUCB) would increase post-resuscitation cerebral blood flow (CBF) in our pediatric rat model of CA by increasing levels of vasodilatory epoxyeicosatrienoic

acid (EET) metabolites. **Objectives:** To 1) develop an LC/MS/MS assay to quantify t-TUCB in plasma and brain of immature rats, 2) determine an optimal dose of t-TUCB in pediatric rats, and 3) determine the effect of t-TUCB on cerebral EET levels using LC/MS/MS. **Methods:** The assay for quantifying t-TUCB was validated by testing duplicate standard curves against six replicates of quality controls (QC) on days one and two and twelve replicates of QCs on day three. Postnatal day 17 rats (n=6) were subjected to asphyxia cardiac arrest (CA) using an established model. The rats were anesthetized with fentanyl and received neuromuscular blockade using vecuronium. Tracheal intubation and arterial and venous catheterization were performed. The rats were subjected to either asphyxia CA of twelve minutes or sham surgery. CBF was quantified in the somatosensory areas using a Laser Speckle Perfusion Imager (PeriCam PSI). The rats were sacrificed at 120 min after resuscitation and brains were rapidly collected on ice. We quantified the levels of t-TUCB and the concentration of eicosanoids in brain and plasma samples using LC/MS/MS. **Results:** t-TUCB measurement had inter-day and intra-day coefficients of less than 10% at each of three quality control levels (%inter-day CV 2.8, 6.71, 8.08 and intra-day CV 2.57, 6.28, 4.57, at QC15, QC75, and QC350, respectively). An IV bolus of 1 mg/kg of t-TUCB resulted in cerebral concentrations of 29.2±6 ng/mL, two times the reported IC50 for the target enzyme. Cerebral concentrations of EET did not differ in rats treated with t-TUCB vs. vehicle. No difference in CBF was observed between rats treated with t-TUCB or vehicle. **Conclusions:** We developed an assay for the quantification of t-TUCB in plasma and brain samples from pediatric rats. t-TUCB was able to penetrate the brain at concentrations two times higher than the IC50. Cerebral levels of EET and cerebral perfusion did not differ between vehicle and t-TUCB treated rats after CA. Because EET data were variable, it is difficult to conclude whether t-TUCB is not effective for CBF modulation per se or if it was sub-optimally dosed. Future studies using a higher dose of t-TUCB should be performed to assess the optimal dose that results in increased cerebral EET concentrations. Furthermore, the effect of t-TUCB administration in longer durations of CA with more decreased cerebral perfusion need to be assessed.

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S20

ANGIOTENSIN II PRIMING ENHANCES PGE2 VASOCONSTRICTOR EFFECTS

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Prostaglandins are important modulators of blood pressure and arterial tone. Prostaglandin E2 (PGE2), is a prostanoid that has vasodepressor effects; however, under certain circumstances can induce vasopressor responses as well. Recent reports in the literature demonstrated that sub-threshold concentrations of vasoconstrictors such as KCl or α-adrenergic agonists augment PGE2-mediated constriction in rat femoral arteries as assessed by *ex vivo* wire myography. We demonstrated that PGE2 had little or no effect on mouse femoral arterial rings at concentrations up to 1 μM. Pretreatment of femoral rings with a sub-threshold dose of KCl (20 mM) potentiated a PGE2-evoked constriction in a concentration dependent manner (Area Under the Curve, AUCuntreated 0.947± 0.253, AUCKCl 7.427± 0.254, P = 0.0001). Interestingly, priming of femoral arterial rings with 1 nM angiotensin II (Ang II) also potentiated PGE2-evoked constriction. Pretreatment of arterial rings with 1 μM losartan, an angiotensin receptor antagonist, blocked both Ang II constrictor effects as well as Ang II priming of PGE2 constrictor effects. Pretreatment with 1 μM DG-041, a potent EP3 antagonist, had no effect on Ang II constriction but abrogated the subsequent PGE2 constrictor response. Taken together these data are consistent with angiotensin AT1 and prostaglandin EP3 receptors mediating this synergistic vasoconstrictor response. Tempol, a superoxide dismutase mimetic, added to the wire myography bath 20 minutes before 30 nM Ang II priming of 100 nM PGE2 resulted in reduced PGE2-mediated vasoconstriction implicating reactive oxygen species as one mediator of the observed Ang II/PGE2 synergism. We are continuing to investigate the relationship between angiotensin II and PGE2 and determine the physiological relevance this may have in modulating blood pressure *in vivo*.

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S21

VASCULAR ENDOTHELIAL DELETION OF ACE ATTENUATES BUT DOES NOT PREVENT 20-HETE-MEDIATED VASCULAR REMODELING IN ANDROGEN-INDUCED HYPERTENSION

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We have previously shown that 20-HETE increases the expression and activity of angiotensin-converting enzyme (ACE) in cultured endothelial cells. In vivo, 20-HETE-mediated hypertension in rats and mice is associated with increased vascular ACE expression and circulating Angiotensin II (Ang II) levels; administration of an ACE inhibitor or an Ang II receptor (AT1R) blocker attenuates 20-HETE-mediated increases in blood pressure. We have recently shown that 20-HETE-mediated hypertension is associated with microvascular remodeling; however, the contribution of 20-HETE-induced ACE expression and activity to the 20-HETE-mediated microvascular remodeling in hypertension is unclear. To examine the role of ACE in 20-HETE-mediated vascular remodeling, Androgen (5α-dihydrotestosterone, DHT), a known inducer of 20-HETE production, was administered to ACE3/3 mice and remodeling of renal preglomerular microvessels (PGMV) was assessed. The ACE3/3 mice lack vascular endothelial ACE, exhibit attenuated ACE expression in the kidneys and display normal ACE and Ang II levels in the plasma. ACE3/3 mice and their littermate wild type (WT) mice were administered DHT or placebo by subcutaneous slow-releasing pellets (100 mg) for 21 days. The increase in the systolic blood pressure (SBP) was significantly higher in WT mice (148±4 vs. 99±2 mmHg) as compared to

ACE 3/3 mice (125±1 vs. 99±1 mmHg; p<0.05) on day 14 of the pellet placement and this difference was maintained till day 21. Administration of either losartan (10 mg/kg/day) or 20-HEDGE (10mg/kg/day) at day 14 of DHT treatment reduced blood pressure to basal levels in both WT and ACE3/3 mice. DHT treatment increased vascular ACE protein levels in WT by 2.3-fold in mesenteric and 2.6-fold in aortic arteries while ACE3/3 mice lacked vascular ACE expression which remained absent under DHT treatment. DHT treatment significantly increased PGMV-20-HETE levels, as compared to placebo, in WT (2.18±0.95 vs. 0.66±0.33 ng/mg protein, p<0.05) as well as in ACE3/3 (1.85±0.58 vs. 0.63±0.02 ng/mg protein, p<0.05) mice. DHT treatment increased PGMV remodeling in both WT and ACE3/3 mice; however, DHT-induced remodeling was attenuated in the ACE3/3 mice (media thickness, 14.54±1.24 vs. 9.80±0.56 μm; M/L, 0.17±0.01 vs. 0.10±0.01; p<0.05) as opposed to the WT mice (media thickness, 22.17±0.92 vs. 9.68±0.76 μm; M/L, 0.26±0.02 vs. 0.10±0.01; p<0.05). To the extent that DHT-induced microvascular remodeling is driven by 20-HETE (Ding et al, AJP 2013), these results suggest that vascular endothelial ACE contributes, in part, to 20-HETE-mediated microvascular remodeling in hypertension and that 20-HETE have effects on microvascular remodeling independent of its action on ACE expression in the vascular endothelium.

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P450 EICOSANOIDS AS PREDICTORS AND THERAPEUTIC TARGETS FOR SUBARACHNOID HEMORRHAGE

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Patients with aneurysmal subarachnoid hemorrhage (SAH) are at high risk for delayed cerebral ischemia (DCI). Experimental and human studies have implicated 20-hydroxyeicosatetraenoic acid (20-HETE) in the pathogenesis of DCI, which is consistent with its vasoconstrictor effect. No studies, however, have evaluated the role of vasodilator epoxyeicosatrienoic acids (EETs) in SAH. To determine if HETEs and EETs are preferentially altered in SAH patients who end up developing DCI, we collected CSF daily from 34 SAH patients for up 14 days after admission and correlated levels with the presence or absence of DCI. In control CSF, EETs and HETEs were undetectable. After SAH, the levels of 14,15-EET and 20-HETE were higher in patients who go on to experience DCI vs. those who do not (14.8 ± 10.8 vs. 4.9 ± 1.6 pg/ml, $p < 0.05$ for 14,15-EET and 170.3 ± 114.0 vs. 19.9 ± 9.0 pg/ml, $p < 0.01$ for 20-HETE). When used as an early test, peak CSF concentrations of 14,15-EET as well as 20-HETE in the first 96 hours after SAH were highly predictive for DCI development (for 14,15-EETs, ≥ 24 pg/ml, likelihood ratio 7.5, $p < 0.05$ and for 20-HETEs ≥ 148 pg/ml, likelihood ratio 11.7, $p < 0.01$). To determine the role of 14,15-EET in SAH, we subjected wild-type (WT) mice and mice with higher 14,15-EET, due to lack of soluble epoxide hydrolase (sEH knockout mice) to experimental SAH. Using a non-invasive, contrast-free in-vivo vascular imaging technique called optical microangiography, we found that sEHKO mice are protected from delayed microvascular hypoperfusion after SAH compared to WT mice. Our data suggest that early CSF eicosanoid monitoring may help predict DCI after SAH. While 20-HETE may contribute to the development of DCI, 14,15-EET may afford protection against DCI. Strategies to enhance 14,15-EET, including sEH inhibition, should be considered as part of a multi-modal approach to preventing DCI.

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S23

LIPOCALIN-TYPE BUT NOT HEMATOPOIETIC PROSTAGLANDIN D SYNTHASE DELETION CAUSES HYPERTENSION AND ACCELERATED THROMBOGENESIS IN MICE

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Lipocalin-type prostaglandin D synthase (L-PGDS) and hematopoietic prostaglandin D synthase (H-PGDS) both catalyze formation of prostaglandin D2 (PGD2). H-PGDS is a sigma class glutathione-S-transferasewhile L-PGDS is a member of the lipocalin family, the first to be recognized as an enzyme. L-PGDS is involved e in pain perception,regulation of the immune response, the sleep/wakefulness cycle and carbohydrate metabolism. L-PGDS is also the most abundant protein in cerebrospinal fluid protein. Deletion of L-PGDS depressed urinary11,15-dioxo-9alpha-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid (PGDM) by ~30% in both genders when compared to wild type littermate controls. On another hand, deletion of H-PGDS had a more pronounced effect, depressing urinary PGDM by ~90%. There was no evidence of systemic re-diversion of the PGH2 substrate as urinary PGEM, PGIM and TXM were unaltered in both mutants. . Deletion of H-PGDS, but not L-PGDS, almost completely suppressed PGD2 production by macrophages (>95%). Deletion of L-PGDS, but not H-PGDS elevated blood pressure and accelerated the thrombogenic occlusive response to a photochemical injury to the carotid artery inboth genders. A H-PGDS inhibitor (HQL-79) further depressed urinary PGDM in L-PGDS knockout mice but had no incremental effect on blood pressure or the thrombogenic response. Genes relevant to both phenomena were dysregulated in mice lacking L-PGDS. Although disruption of L-PGDS disturbs cardiovascular function in mice, this seems unrelated to its contribution to the biosynthesis of PGD2.

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CYP4A12TG MICE EXHIBIT CONCENTRIC HYPERTROPHY AND CARDIAC DYSFUNCTION

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20-Hydroxyeicosatetraenoic acid (20-HETE) is a cytochrome P450-derived eicosanoid shown to increase smooth muscle contractions and proliferation, stimulate endothelial dysfunction and activation, and promote hypertension. The Cyp4a12tg mice, in which the expression of the Cyp4a12-20-HETE synthase is under the control of the tetracycline (doxycycline, DOX) promoter, were used to assess the effects of 20-HETE-dependent hypertension on cardiac function by echocardiography. Treatment of Cyp4a12tg mice with DOX increased blood pressure (BP) within 5 days and stayed elevated during the 42-day treatment period (136±2 vs 102±1 mmHg, $p<0.05$). The BP increase was prevented by co-treatment with the 20-HETE antagonist 20-HEDGE (103±3 mmHg, $p<0.05$) and with the ACE inhibitor Lisinopril (100±3 mmHg, $p<0.05$). Renal resistance arteries from 42-day-DOX-treated Cyp4a12tg mice displayed marked increases in media-to-lumen ratio and medial cross sectional area when compared to untreated Cyp4a12 mice (M/L, 0.16±0.01 vs 0.08±0.01, $p<0.05$; mCSAx103 11.13±0.75 vs. 5.68±0.37 mm2, $p<0.05$); these changes were completely abolished by co-treatment with 20-HEDGE. Echocardiography data are depicted as the difference (Δ) between day 42 and day 0 of DOX treatment with and without 20-HEDGE (10 mg/kg/day) or lisinopril (10 mg/kg/day). DOX-treated Cyp4a12tg mice displayed significant changes in left ventricular internal diameter during systole and diastole (Untreated: Δ-1.07±0.25 vs DOX: Δ0.41±0.23mm, $p<0.05$, Untreated: Δ-0.73±0.09 vs DOX: Δ0.31±0.15mm, $p<0.05$ respectively). Left ventricular volumes were significantly increased in DOX treated mice (Δ8.14±4.97 and Δ10.01±5.32 µl, systolic and diastolic volumes, respectively) compared to water treated controls (Δ-26.99±8.36 and Δ -29.74±5.39 µl, $p<0.05$, systolic and diastolic volumes, respectively). 20-HEDGE and Lisinopril treatment prevented the increase in internal wall diameter and left ventricular volumes. Ejection fraction and fractional shortening were both significantly reduced in DOX treated mice (DOX: Δ-7.92±5.86 % and Δ-5.62±4.26 % vs Untreated: Δ28.24±6.01 % and Δ18.55±3.57 %, respectively, $p<0.05$). Stroke volume and cardiac output remained unaffected while the 42-day-DOX-treated Cyp4a12tg mice showed a marked increase in total peripheral resistance (9.33±0.57 vs 6.13±0.38 mmHg/ml/min, $p<0.05$). There were no observed changes in heart to body weight ratios throughout the treatment arms at day 42. These results suggest that the activation of Cyp4a12-20-HETE synthase causes hypertension and results in the development of concentric hypertrophy and cardiac dysfunction. However, the mechanisms underlying Cyp4a12-20-HETE-driven remodeling and cardiac dysfunction are yet to be explored.

** Contribute equally to the study. This study was supported by NIH grants HL34300 (MLS), DK38226 (JC) and NHLBI Diversity Supplement to HL034300 (VG), and by the Robert A. Welch Foundation (GL625910) (JRF)*

S25

HEMODYNAMIC EFFECTS OF IN VIVO INHIBITION OF EPOXYGENASE WITH MSPPOH IN RATS WITH LIVER CIRRHOSIS

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In liver cirrhosis, the decreased vascular reactivity to vasoconstrictors participates to mesenteric vasodilatation and increased splanchnic flow of the hyperdynamic circulatory syndrome characteristic of this pathological condition. Aim of the study was to analyze the role of the potent vasosodilators, epoxyeicosatrienoic acids (EET), in mesenteric arterial vasodilatation and in the hyperdynamic circulation of rats with experimental cirrhosis. We evaluated the effect of *in vivo* administration of an epoxygenase inhibitor, N-methanesulfonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH), on mesenteric vascular reactivity, cardiac output, and sodium excretion. The expression of genes encoding for some families of cytochrome P450 involved in the EET synthesis was also analyzed. Methods: Wistar rats (15 control and 10 with liver cirrhosis induced by CCl4 administration) were included. The two groups were further divided into treated and untreated rats. Treated rats were injected with 20mg/kg/die of MS-PPOH for three consecutive days. Cardiac output was measured by echocardiography; urinary volume, plasma and urinary sodium concentration, creatinine clearance and fractional sodium excretion were measured by standard technique. Small resistance mesenteric arteries (diameters <400mm) were mounted on micropipettes connected to a pressure servo controller in a video-monitored perfusion system. Dose-response curves to phenylephrine (PE)(10-8-10-4M) and to acetylcholine (ACh))(10-9-10-4M) were evaluated after the addition of Indomethacin (2.8 mM) and L-NAME (1mM); pEC50 and Rmax were calculated. Gene expression of CYP450 (CYP 2J3 , 2J4 , 2J10 , 2C11 , 2C12 , 2C23) in liver, kidney and mesenteric arteries of cirrhotic and control rats was analyzed by rtPCR. The results are reported as M±SE. Results: in cirrhotic rats, cardiac output was higher than in control rats (181±21 vs 92±39 ml/min; $p<0.05$). In cirrhotic rats, but not in control rats, MS-PPOH increased sodium excretion (774±227 vs 143±64µmol/24hr; $p<0.01$) and decreased cardiac output (119±26ml/min; $p<0.05$). No difference in PE sensitivity was observed among the examined groups of rats. After ACh, there was a significant difference in pEC50 between untreated cirrhotic rats and controls (-7.08±0.20 vs. -6.52±0.73, $p=0.014$), and between treated (-6.39±0.26, $p= 0.044$) and untreated cirrhotic rats. Rmax induced by ACh was decreased in treated cirrhotic rats in respect of untreated cirrhotic rats (95%±6% vs 106%±2 %, $p= 0.049$). Real-Time PCR showed that MS-PPOH decreased renal CYP2J4 mRNA level and hepatic and renal CYP2C11 mRNA only in cirrhotic rats. **Conclusions:** EETs mediate mesenteric arterial vasodilatation and hyperdynamic circulation of experimental cirrhosis. Their inhibition may represent a new therapy for cirrhotic portal hypertension.

S26

CYP2C44-DERIVED EPOXYEICOSATRIENOIC ACIDS ACT AS SECOND MESSENGERS IN INTRARENAL DOPAMINE-INDUCED NATRIURESIS

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We have recently demonstrated that intrarenal dopamine plays an important role in preventing the development of systemic hypertension. Similarly, renal cytochrome P-450 (CYP)-epoxygenasederived arachidonic acid metabolites, epoxyeicosatrienoic acids (EETs) also are antihypertensive, acting as vasodilators and inhibiting sodium reabsorption in the kidney. Because dopamine also is a vasodilator and induces natriuresis, we investigated potential interaction between renal dopamine and epoxygenase systems. Catechol-*O*-methyl-transferase (COMT)-/- mice have increased intrarenal dopamine levels due to deletion of the major dopamine metabolizing enzyme, and proximal tubule deletion of aromatic amino acid decarboxylase (ptAADC-/-) mice have renal dopamine deficiency. These mice were treated with low-salt diet or high-salt diet for 2 weeks. In addition, wild type or *Cyp2c44*-/- mice were treated with gludopa, which selectively increases renal dopamine levels. In both low-salt and high-salt treated mice, EET levels in both kidney and urine were related to renal dopamine levels, being highest in COMT-/- mice and lowest in ptAADC-/- mice. Selective increases in renal dopamine in response to gludopa administration led to marked increases in EET levels in the kidney without any changes in blood EET levels. qRT-PCR and immunoblotting indicated that gludopa increased renal Cyp2c44 mRNA and protein levels. Gludopa induced marked increases in urine volume (ml/16-h: control: 0.95 ± 0.11; gludopa: 3.77 ± 0.23, $P < 0.001$, n = 4) and urinary sodium excretion (μM/16-h: control: 99.65 ± 9.93; gludopa: 302.08 ± 23.43, $P < 0.001$, n = 4) in wild type mice. In contrast, gludopa did not induce significant increases in urine volume (ml/16-h: control: 0.67 ± 0.11; gludopa: 0.90 ± 0.30, $P > 0.05$, n = 4) or urinary sodium excretion (μM/16-h: control: 116.04 ± 7. 83; gludopa: 157.71 ± 21.72, $P > 0.05$, n = 4) in *Cyp2c44*-/- mice. In cultured mouse proximal tubule epithelial cells, dopamine caused increases in Cyp2c44 mRNA levels, which were attenuated by the selective DA1 receptor antagonist, SCH23390, but not the DA2 receptor antagonist, sulpiride. The selective DA1 receptor agonist SKF81297 stimulated Cyp2c44 mRNA levels as early as 1 hour while the DA2 receptor agonist, quinpirole had no effect. These studies demonstrate that renal Cyp2c44-derived EETs levels are maintained by intrarenal dopamine through activation of DA1 receptors, and Cyp2c44-derived EETs act as secondary messengers in intrarenal dopamine-induced natriuresis and diuresis.

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S27

VASCULAR EICOSANOID GENERATION IN RESPONSE TO EXPERIMENTAL URAEMIA

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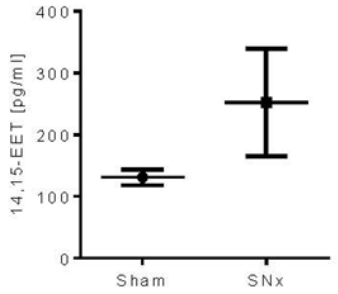

Cardiovascular disease is the leading cause of death for patients with moderate to severe chronic kidney disease. Eicosanoids and related lipids, are products of cyclooxygenase, lipoxygenase and epoxygenase enzymatic pathways, and have established and emerging roles in renal and cardiovascular physiology. Here we examined the changes in vascular eicosanoid and related fatty acid pathway production after experimental uraemia in the rat.

Male Wistar rats (n=5 per group) underwent a 2-stage subtotal nephrectomy (SNx) or a sham procedure. The left kidney was two thirds resected with right total nephrectomy. After a further 4 weeks, blood was taken for serum analysis, and aorta's were removed and placed in serum free organ culture for 30min. The resulting conditioned media was analysed by LC-MS/MS for patterns of cyclooxygenase, lipoxygenase and epoxygenase: arachidonic acid, linoleic acid, DHA and EPA metabolites.

Subtotal nephrectomy resulted in increases (P<0.05) in serum urea (sham 6.1±0.2; SNx 17.4±1.6 mmol/l), creatinine (sham 42.0±1.5; SNx 85.2±3.0 umol/l), and Ca2+ (sham 2.712±0.019; SNx 2.846±0.020 mmol/l), but not albumin (sham 32.50±0.5; SNx 32.1±0.5 g/L), Na+ (sham 140.4±0.9; SNx 140.2±0.5 mmol/l), or K+ (sham 8.1±0.3; SNx 7.9±0.3 mmol/l). Sham and SNx aorta released an almost identical pattern of eicosanoids with prostacyclin the major eicosanoid produced by both groups (Sham 65±12; SNx 58±9 ng/ml). The one exception was a significant increase in the epoxygenase arachidonic acid metabolite 14,15-EET (Figure 1). ShamSNx010020030040014,15-EET [pg/ml]

In conclusion, 14,15-EET may represent a novel biomarker and mediator of the cardiovascular response to chronic kidney disease. As 14,15-EET acts as a vasodilator and anti-inflammatory eicosanoid, drugs that elevate 14,15-EET levels, e.g. soluble epoxide hydrolase inhibitors, may help to represent a new therapy to limit the adverse cardiovascular outcomes associated with chronic kidney disease.

Funding: BHF (PG/11/39/28890), and the Intramural Research Programs of the NIH.

S28

URINARY CYP EICOSANOID EXCRETION CORRELATES WITH RENAL FUNCTION AND PROTEINURIA IN AFRICAN-AMERICANS WITH CHRONIC KIDNEY DISEASE

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Previous studies have indicated that cytochrome P450 (CYP) metabolites of arachidonic acid, ie, 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs) play an important role in the regulation of renal tubular function and vascular tone. More recent studies suggest that variants in the *CYP4A11* and *CYP4F2* genes are linked to the development of hypertension in a variety of human population studies. However, little is known about the role of 20-HETE or EETs in the pathogenesis of hypertension or diabetic induced renal disease because CYP eicosanoids have not been measured in patients with CKD. The present study profiled HETEs and the dihydroxy metabolites of EETs (DHETs) levels, from spot urines at the time of their clinic visit using LC/MS/MS in 270 African-American patients from the University of Mississippi (UMC) Chronic Kidney Disease (CKD) Clinic with various etiologies of renal disease. Informed consent was obtained for this UMMC IRB approved protocol. Significant positive correlations were found between urinary 5,6- DHETE, 8,9-DHETE, 11,12-DIHETE, 14,15-DHETE, 20-HETE and 19-, 15- and 8-HETE levels and estimated GFR (eGFR) as derived from the MDRD. The magnitude of the influence of urinary eicosanoid levels on eGFR was relatively profound since the slopes of these relationships indicated that there is a 5-10% decrement eGFR associated with each ng/ml fall in urinary eicosanoid levels. Similar significant positive associations were found between the other urinary eicosanoids and eGFR and also with 19-HETE/urine creatinine concentration and proteinuria. These results suggest that a decline in the renal formation of 20-HETE and/or EETs may contribute to the pathogenesis of CKD. At very least, urinary CYP eicosanoids may be marker for GFR.

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S29

20-HETE REGULATES THE ANGIOGENIC FUNCTIONS OF HUMAN ENDOTHELIAL PROGENITOR CELLS AND CONTRIBUTES TO ANGIOGENESIS IN VIVO

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Circulating endothelial progenitor cells (EPC) contribute to postnatal neovascularization, which is an important adaption for pathological conditions, including wound healing, ischemia and tumor development .We identified the cytochrome P450 4A/F-20-hydroxyeicosatetraenoic acid (CYP 4A/F-20-HETE) system as a novel regulator of EPC functions associated with angiogenesis *in vitro*. In the present studies, we explore cellular mechanisms by which 20-HETE regulates EPC angiogenic functions and assessed its contribution to EPC-mediated angiogenesis *in vivo*. The VEGF pathway is an important signaling mechanism of neovascularization. Our previous data suggested 20-HETE can be upstream of VEGF signaling pathway in EPC. Here, we found that both hypoxia and VEGF induces CYP4A11 gene and protein expression (the predominant 20-HETE synthases in human EPC), and this is accompanied by an increase in 20-HETE production by ~1.4- and 1.8- fold, respectively, compared to the control levels. Additional studies demonstrated that 20-HETE and VEGF have a synergistic effect on EPC proliferation, while 20-HETE antagonist, 20-HEDGE, or VEGF neutralizing antibody negated VEGF or 20-HETE-induced proliferation, respectively. These findings are consistent with the presence of a positive feedback regulation on EPC proliferation between the 20-HETE and the VEGF pathways. EPC adhesion plays a critical role in the homing processes of EPC to sites of neovascularization. We also found that 20-HETE induced EPC adhesion to fibronectin and EC monolayer by 40 ± 5.6% and 67 ± 10%, respectively, which was accompanied by a rapid induction of VLA-4 and CXCR4 mRNA and protein expression. Basal and 20-HETE-stimulated increases in adhesion were negated by the inhibition of the CYP4A-20-HETE system. Furthermore, 20-HETE productions decreased as EPC differentiate into EC, implicating that 20-HETE may be a novel regulator of the EPC to EC differentiation processes. Lastly, EPC increased angiogenesis *in vivo* by 3.6 ± 0.2- fold using the Matrigel plug angiogenesis assay, and these increases were markedly reduced by the local inhibition of the 20-HETE system. These findings provide strong evidence that 20-HETE is a key regulator of EPC functions and their contributions to postnatal neovascularization *in vivo*.

Acknowledgement: *This study was supported by the American Heart Association Scientist Development Grant 11SDG6870004 (A.M. Guo), the National Institutes of Health grants HL34300 (M.L.Schwartzman), DK38226 (J.R. Falck), and the Robert A. Welch Foundation (GL625910) (J.R. Falck), and the National Natural Science Foundation of China (30973552, 81173089) (J.Yang).*

S30

Title: PODOCYTES ARE BIOSENSORS FOR RENAL EFFECTS OF ETHANOL

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Background and Rationale: Podocytes are terminally differentiated cells that control the glomerular permeability barrier and determine protein filtration into urine. They are characterized by a unique actin cytoskeleton, interdigitating foot processes and specialized intercellular slit junctions. We have shown that the glomerular protein permeability barrier is altered prior to overt proteinuria in animal models of several glomerular diseases, and that 20-hydroxyeicosatetraenoic acid (20-HETE) protects the filtration barrier *in vitro*. Little is known about the effects of EtOH on glomerular and podocyte structure, function and metabolism. We have shown that EtOH in low concentrations (1-2µl/mL) increases podocyte expression of CYP4a isoforms, while the actin cytoskeleton is not altered. In contrast, EtOH at higher concentrations (10-20 µL/mL) suppresses CYP4a12a expression and is associated with cytoskeletal derangement. Ethanol (EtOH) is mainly metabolized by cytosolic alcohol dehydrogenase (ADH) and microsomal cytochrome P450 2E1 (CYP2E1). The role of these enzymes in podocytes during proteinuric disease is unknown**.**
Hypothesis: Cultured podocytes will be useful in delineating the effects of low and high concentrations of ethanol *in vitro*.
Methods: We examined the effect of EtOH (2-20 µl/mL) on the expression of ADH and CYP2E1 in immortalized murine podocytes using RT-qPCR and Western blotting. We examined superoxide generation using the fluorescent probe hydroethidine (HE). We tested the protective effect of 20-HETE (100 nM) in some experiments. **Results:** ADH gene and protein expression were detected in all conditions. CYP2E1 expression was undetectable in untreated podocytes. ADH gene was upregulated by 2 µl/mL (P<0.001) but not by 10 or 20 µl/mL EtOH. Expression of CYP2E1 was induced by EtOH (10 or 20 µl/mL) (P<0.001) and superoxide generation was increased as indicated by HE fluorescence (P<0.001). The increase in superoxide was prevented by 20-HETE (P<0.001).
Conclusions: Low concentrations of EtOH upregulate both CYP4a12a and ADH expression. In contrast, higher concentrations of EtOH cause oxidative stress through induction of CYP2E1 and simultaneously down regulate expression of CYP4a12a and may decrease 20-HETE generation that protects the filtration barrier. Podocytes may be a useful model system to understand the molecular effects of low and high concentrations of ethanol. **Significance:** Emerging clinical evidence suggests that moderate ethanol consumption has beneficial cardiovascular effects in contrast to its harmful effects when consumed in excess. Dose dependent molecular responses in podocytes suggest protection from oxidative injury by limited intake of EtOH that is lost with excessive intake. These results provide a novel opportunity to study the contribution of alcohol consumption to protection from or exacerbation of chronic renal disease.

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S31

CYP EPOXYGENASE AND EETS PROTECT HEART AGAINST CARDIAC DYSFUNCTION

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CYP2J2, unique 2J family epoxygenase in human, expresses dominantly in cardiomyocytes and metabolizes arachidonic acids to produce rich epoxyeicosatrienoic acids (EETs). CYP epoxygenases and EETs have diverse biological roles including anti-inflammation, endothelial protection, reduced blood pressure and anti-insulin resistance. However, their roles in heart still need more studies to be elucidated. We found, in first, that in vivo CYP2J2 overexpression attenuated TNFα-induced cardiac dysfunction via activating cardiac PPARγ and inhibiting production of inflammatory cytokines and neutrophil adhesion. In αMHC-CYP2J2 transgenic mice, CYP2J2 overexpression alleviated diabetic cardiomyopathy, increased uptake and application of glucose in heart and inhibited cardiac hypertrophy induced by STZ. 2J2 and EETs markedly inhibited endoplasmic reticulum (ER) stress and calcium overload in heart failure and improved cardiac function. These beneficial effects for heart protection involve in EETs-mediated macrophage polarization, activation of PPARγ and PPARα, as well as PI3K/Akt pathway and inhibition of cardiomyocytes-fibrogenic roles by EETs.

Grant: This work was supported by National Basic Research Program of China (973 Program) [No. 2012CB518004], National Natural Science Foundation of China Grants [Nos. 31130031 and 30930039] and Program for Changjiang Scholars and Innovative Research Team in University [PCSIRT1131].

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POSTER SESSION II
MONDAY, MARCH 10, 2014
Grand Ballroom West/DEF (Lobby Level)
6:35-8:00 PM

DIABETES - CANCER - INFLAMMATION

M1
STRUCTURAL AND TOPOLOGICAL MECHANISMS OF INFLAMMATORY PROSTAGLANDIN E2 BIOSYNTHESIS IN ER ENVIRONMENT

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In vascular inflammation, prostaglandin E2 (PGE2) is largely biosynthesized by microsomal PGE2 synthase-1 (mPGES-1) in competition with other downstream eicosanoid-synthesizing enzymes, such as prostacyclin (PGI2) synthase to isomerize the inducible cyclooxygenase (COX)-2-produced prostaglandin H2 (PGH2) in endothelial and smooth muscle cells. The molecular and cellular mechanisms involved to encourage synthesis of inflammatory PGE2 and less encourage synthesis of other eicosanoids (such as PGI2) during the inflammatory process are poorly understood. In this study, we designed experiments to test the effects of the structural and topological arrangement between COX-1/-2 and mPGES-1 or PGIS on the production of PGE2 in cell ER membranes. We found that the major product for the cells co-expressing human COX-2, mPGES-1 and PGIS, on ER membranes was PGE2. It indicated that the mPGES-1 has higher activity than PGIS in converting COX-2-produced PGH2 to PGE2 in the inflammatory cells. Based on the passive diffusion of PGH2, we predicted that the distance between COX-2 and mPGES-1 is shorter than that of COX-2 and PGIS. This hypothesis was then confirmed by the experiment, in which use of the COX-2-10aa-PGIS, which has a very short distance (approximately 10 Å) between the active sites of COX-2 and PGIS, could not reduce the efficiency of mPGES-1 to compete with PGIS—thereby high production of PGE2 remained. However, expression of COX-2-10aa-PGIS and mPGES-1 on separated ER membranes with a distance >100 Å away could reduce PGE2 production. Finally, the inhibition of mPGES-1 production of PGE2 was achieved by introducing the distance-constrained COX-1 and PGIS to compete with mPGES-1 in ER membranes. This result has led us to propose a model showing an ER-membrane mediated chain reaction for COXs coordinated with mPGES-1 and PGIS, in which mPGES-1 is closer to COX-2, and the COX-1 active site and membrane anchor could be different from COX-2 in the native ER membrane. These results provide novel structural and topological information, uncovering the molecular and cellular mechanisms of the pathogenic PGE2 production in inflammatory cells and tissues. This study also indicated that introducing our engineered COX-1-10aa-PGIS hybrid enzyme to vascular cells not only could directly increase the conversion of PGH2 to PGI2, but also could inhibit mPGES-1 to catalyze PGH2 to PGE2, which could provide dual vascular protections.

Acknowledgement: *1) RO1 HL56712 for Ke-He Ruan; 2) RO1 HL079389 for K. Ruan; 3) RC1 HL100807 for R. Dixon and K. Ruan; 4) AHA 10GRNT4470042 for K Ruan ; 5)US Army W81XWH-10-2-0125 (Y. Geng and K. Ruan)*

M2
MODULATION OF MAST CELL PROLIFERATIVE AND INFLAMMATORY RESPONSES BY LEUKOTRIENE D4 AND STEM CELL FACTOR INTERACTIONS

Nosayba Al-Azzam[1], Vinay Kondeti[1], Ernest Duah[1], Farai Gombedza[1], Charles K. Thodeti[2] and Sailaja Paruchuri[1]
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Mast cells (MC) are one of the major hematopoietic cells that are ubiquitously distributed throughout the body and involved in immediate hypersensitivity reactions and many inflammatory diseases like asthma. One of the main growth factors for MC is stem cell factor (SCF), which acts through its receptor c-Kit. MC-derived cysteinyl leukotrienes, leukotriene (LT) C4, LTD4 and LTE4, are major inflammatory mediators that can be recognized by at least two G-protein coupled receptors (GPCRs) termed CysLT1R and CysLT2R. Here, we report that LTD4 and SCF can act together to prime each other's responses leading to augmentation of mast cell growth and inflammatory signals. Stimulation of MC line, LAD2 by SCF in the presence of LTD4 enhances c-Kit phosphorylation and c-Kit-mediated proliferation. Similarly, SCF potentiates LTD4-induced calcium influx as well as c-fos expression and phosphorylation. SCF treatment also potentiates LTD4-mediated up-regulation of cyclooxygenase-2 (COX-2), TNFα and MIP1β transcripts as well as MIP1β protein secretion. Taken together these results, for the first time, suggest an integration between SCF and LTD4 signaling that may contribute to MC hyperplasia and hyper-reactivity. The fact that locally-derived LTD4 could control c-Kit responses, enhancing proliferation and SCF potentiating LTD4-mediated inflammatory responses is very intriguing and can carry substantial pathogenic and therapeutic implications for asthma and allergic diseases.

This work is supported by University of Akron start-up funds and James Foght Assistant Professor support.

M3

THE ELECTROPHILIC FATTY ACID SPECIES, NITRO-OLEIC AND NITROLINOLEIC ACID, SPECIFICALLY INHIBIT 5-LIPOXYGENASE *IN VITRO* AND ATTENUATE SEPSIS-INDUCED PULMONARY INFLAMMATION *IN VIVO*.

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Nitric oxide and nitrite-derived species readily react with polyunsaturated fatty acids to yield nitro–fatty acids (NO2-FA), like nitro-oleic (NO2-OA) and nitro-linoleic acid (NO2-LA). The latter have been attributed anti-inflammatory properties via covalent electrophilic adduction to nucleophilic residues within target proteins. Given that the 5-lipoxygenase (5-LO) possesses critical nucleophilic amino acids, that are potentially sensitive to electrophilic modifications, we determined the consequences of NO2-FA on 5-LO activity *in vitro* and 5-LO-mediated inflammation *in vivo*. Incubation of human polymorphonuclear leukocytes (PMNL) with either NO2-OA or NO2-LA resulted in a concentration-dependent attenuation of the calcium ionophoreinduced generation of the 5-LO products; 5-hydroxyeicosatetraenoic acid (5-HETE) and leukotriene B4. The inhibition of 5-LO inhibition was stimuli- and phospholipase A2-independent and was equally effective versus the stimulation of PMNL with NaCl, granulocyte macrophage colony stimulating factor and N-formyl-methionine-leucinephenylalanine, exposure to sodium arsenite or the supplementation with arachidonic acid. Similar effects were observed using the recombinant human protein, indicating that NO2-FAs directly target the enzyme. Neither of the NO2-FAs affected the activity of 12-LO or 15-LO enzymes in intact cells or recombinant systems. At the molecular level, the posttranslational modification of susceptible nucleophilic amino acid residues in 5-LO by NO2-OA was assessed using a combination of mutagenesis and proteomic studies. Incubation of human recombinant 5-LO with NO2-OA resulted in the adduction of histidine residues His125, 360, 362, 367, 372 and 432 and the cysteine residues Cys 416 and 418. The mutation of His367 and 372 were previously shown to release the nonheme iron from the catalytic domain of 5-LO. Correspondingly, nitroalkylation of these residues resulted in a 30% reduction of the 5-LO iron content. Both the NO2-FAmediated inhibition of the 5-LO as well as iron release were abolished by mutation of Cys418 to serine. To determine whether the NO2-OA-mediated inhibition of 5-LO is pathophysiologically relevant, the effect of NO2-OA on lipopolysaccharide (LPS)-induced 5-LO activation and neutrophil infiltration were studied in the mouse lung. Systemic administration of NO2-OA to wild type mice, but not 5-LO knockout mice, decreased LPS-induced neutrophil as well as monocyte mobilization and attenuated lung damage. These effects were paralleled by a decrease in the pulmonary 5-HETE and leukotriene B4 levels and were comparable to the effects of the 5-LO inhibitor, zileuton. In summary, NO2-FAs directly and irreversibly inhibit the 5-LO by the nitroalkylation of functionally-critical histidine and cysteine residues and attenuate acute inflammation.

M4

REDUCED INCIDENCE OF TYPE 1 DIABETES FOLLOWING INHIBITION OF THE CA2+-INDEPENDENT PHOSPHOLIPASE A2β BY A NOVEL FLUOROKETONE-BASED COMPOUND

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Type 1 Diabetes (T1D) is an autoimmune disease that leads to ablation of insulin-producing pancreatic islet β-cells, but the mechanism(s) involved are not fully understood. Our findings that the group VIA Ca2+-independent phospholipase A2 beta (iPLA2β) derived signals contributed to β-cell apoptosis & that $iPLA_2\beta$ expression is increased in islets of diabetes-prone non-obese diabetic (NOD) mice prompted us to assess whether $iPLA_2\beta$ activation contributes to the development of diabetes in this model of autoimmune T1D. FKGK18, a recently available fluoroketone-based reversible inhibitor of $iPLA_2\beta$ was administered (20 mg/kg, i.p.) tri-weekly to female NOD mice. As expected, 80% of vehicle-treated mice developed diabetes by 25 weeks of age. In contrast, with FKGK18 treatment 10 of 11 mice remained diabetes free at 25 weeks. The reduced incidence of diabetes was associated with higher circulating insulin levels, improved glucose tolerance, and preservation of β-cell mass. Intriguingly, islet infiltration, a hallmark of T1D, was dramatically reduced with treatment, as reflected by reduced abundance of CD4+ T-cells and B-cells in the islets of FKGK18-treated mice. The β-cell subcellular membranes are enriched in arachidonate-containing phospholipid substrates and activation of $iPLA_2\beta$ in β-cells promotes hydrolysis of arachidonic acid, which can be metabolized via cyclo- and lipo-oxygenases to eicosanoids, some of which can serve as chemoattractants and/or promote immune responses. The reversible nature of FKGK18 inhibition prompted us to measure PGE2 metabolites (PGEM) in urine to assess the *in vivo* inhibitory ability of the drug. We find that the PGEM were decreased ~25%, suggesting decreased generation of PGE2 due to inhibition of arachidonic acid hydrolysis in the presence of FKGK18. PGE2 has been reported to exacerbate immune response by virtue of inhibiting debris clearance. Thus decreased production of PGE2 might be expected to relieve such inhibition and restore a cleaner environment around the islet. We further find that generation of cytokines by inflammatory cells is significantly reduced by FKGK18, suggesting a direct effect of $iPLA_2\beta$-derived lipid signals on immune cell function. Taken together, our findings suggest that generation of $iPLA_2\beta$-derived lipid signals play critical roles in the pathogenesis of autoimmune-mediated type 1 diabetes.

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M5

CONTROL OF TUMOR LYMPHANGIOGENESIS BY EPOXYEICOSANOIDS

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Background: Epoxyeicosatrienoic acids (EETs) are lipid autacoids biosynthesized by cytochrome P450 epoxygenases and metabolized by soluble epoxide hydrolase (sEH) to less active dihydroxyeicosatrienoic acids (DHETs). EETs are autocrine and paracrine mediators of arachidonic acid-induced vasorelaxation in the cardiovascular and renal systems. Thus, inhibitors of sEH, which raise endogenous EET levels, are being considered for long-term use in hypertension, diabetes, stroke and other medical conditions. 20-hydroxeicosatetraenoic acid (20-HETE) is a small molecule biosynthesized by cytochrome P450 ω-hydroxylases. We recently demonstrated that EETs stimulate multi-organ and lymph node metastasis, a major cause of disease dissemination and death. However, the mechanism of EETs in lymph node metastasis and tumor lymphangiogenesis is unknown. Thus, we hypothesize that EETs stimulate lymph node metastasis via tumor lymphangiogenesis. Methods and Results: Using two murine tumor lines transfected with VEGFC, B16F10-VEGFC melanoma and T241-VEGFC fibrosarcoma, we demonstrate that EETs stimulate primary tumor growth via tumor lymphangiogenesis. Systemic administration of 14,15-EET and/or 20-HETE by osmotic minipump accelerated primary B16F10-VEGFC melanoma and T241-VEGFC fibrosarcoma tumor growth. Inhibitors of soluble epoxide hydrolase (sEH) elevated endogenous EET levels and also promoted B16F10-VEGFC tumor growth. Conversely, systemic administration of a 20-HETE antagonist (HET0016) and/or EET antagonist (14,15-EEZE) regressed established primary B16F10-VEGFC tumors. 14,15-EET and 11,12-EET stimulated lymphatic endothelial cell proliferation, viability and migration. To determine whether tumor lymphangiogenesis contributes to the increase in B16F10-VEGFC tumor growth in 14,15-EET treated mice, we analyzed tumors for the specific lymphangiogenesis markers podoplanin and LYVE-1. Immunohistochemistry studies revealed an increase in podoplanin-positive and LYVE-1-positive vessels in B16F10-VEGFC tumors treated with 14,15-EET compared to vehicle treatment. 14,15-EET, 11,12-EET, and 20-HETE increased the production of VEGFC by B16F10-VEGFC and T241 fibrosarcoma tumor cells. Using a well-established model in which resection of a primary tumor (Lewis lung carcinoma) reproducibly stimulates the development of distant metastases 14-17 days after resection, we investigated whether EET antagonists inhibit spontaneous metastatic growth. The EET antagonist 14,15-EEZE and/or siRNA to VEGFR3 potently inhibited lung metastasis and the combination showed additive inhibition. Conclusion: EETs and 20-HETE stimulate tumor lymphangogenesis, offering a mechanistic rationale for using EET and 20-HETE antagonists as novel anti-cancer therapeutics to inhibit lymph node metastasis and tumor lymphangiogenesis.

M6

THE ROLE OF THE PROSTAGLANDIN E2 EP3 RECEPTOR IN GLYCEMIC CONTROL

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Prostaglandin E2 (PGE2) impairs glucose stimulated insulin secretion (GSIS) through its cognate G-protein coupled E-Prostanoid receptors. Targeted disruption of the EP3 receptor gene *Ptger3,* was investigated as a mechanism for improving glycemic control. $EP3^{-/-}$ mice fed standard chow (13.5% calories from fat) showed improved glucose tolerance at 40 weeks of age. Wild-type (WT) or $EP3^{-/-}$ mice fed a high-fat diet (HFD; 45% calories from fat) was used to further investigate the effects of EP3 action on glucose homeostasis. In contrast to chow fed mice, $EP3^{-/-}$ HFD fed animals gained more weight than did WT animals, while no difference in weight was observed with mice fed a micronutrient matched control diet (10% calories from fat). The response to exogenous insulin in $EP3^{-/-}$ mice was decreased as compared to WT mice at 4 and 16 weeks whether fed control or HFD. Homeostatic model assessment of insulin resistance (HOMA-IR) showed that EP3 loss in combination with 16-weeks of HFD feeding caused a significant worsening of insulin sensitivity. $EP3^{-/-}$ mice fed HFD also developed elevated hepatic triglyceride levels and more severe hepatic steatosis. These results suggest that global EP3 deletion improves GSIS in chow fed mice; this effect is overwhelmed by obesity, hepatic lipidosis, insulin resistance, and glucose intolerance, which are significantly enhanced in mice fed HFD.

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M7

OVEREXPRESSION OF CYP2J2 AND ITS METABOLITE ATTENUATE ADVENTITIAL REMODELING AND OXIDATIVESTRESS

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Background: Vascular adventitial remodeling is an important pathologic process in multiple vascular diseases, characterized by increased thickness of adventitia, activation of adventitial fibroblasts (AFs) and deposition of extracellular matrix (ECM). Reactive oxygen species (ROS) is thought to be an important inducer in this process. Previous studies demonstrated that cytochrome P450 2J2 (CYP2J2) and its arachidonic acid metabolites had anti-inflammation, anti-atherosclerosis and elicit protective effects on vascular system. Herein, we assumed that CYP2J2 and its metabolite epoxyeicosatrienoic acids could prevent the adventitial remodeling via anti-oxidative stress in angiotensin II (Ang II) treated mice. **Methods and results**: C57BL/6J mice were treated with two-week Ang II infusion by mini-pump accompany with rAAV-mediated CYP2J2. Results showed that Ang II treatment induced vascular adventitial remodeling, while CYP2J2 overexpression significantly reduced adventitial hyperplasia, collagen accumulation, and phenotype transformation of adventitial cells. Moreover, Ang II induced increase of ROS level and gp91phox expression and decrease of catalase expression in aorta were also reversed by rAAV-mediated CYP2J2. In vitro, both overexpression of CYP2J2 and exogenous addition of 11,12- epoxyeicosatrienoic acids (11,12-EETs) prevented AFs remodeling caused by Ang II (0.1μM) via their anti-oxidative effects. In addition, activation of mitogen-activated protein kinases (MAPK) in Ang II-treated AFs, especially p38MAPK, was reduced by 11,12-EETs administration. **Conclusions:** These data indicated that overexpression of CYP2J2 attenuated vascular adventitial remodeling via oxidative stress in AFs. Exploitation of these beneficial effects may contribute to a potential therapy for remodeling in multiple kinds of vascular diseases.
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M8

PROSTAGLANDIN TRANSPORTER PGT MODULATES ENERGY HOMEOSTASIS BY REGULATION OF THERMOGENESIS

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Metabolic disorders, including diabetes and liver steatosis, are currently epidemic, driven by the increased prevalence of obesity. Prostaglandins (PGs) regulate both thermogenesis by brown adipose tissue (BAT) and the "browning" of white adipose tissue (WAT), as well as the conversion of pre-adipocytes to adipocytes, i.e. adipogenesis. The amount of PGs is determined both by the rate of synthesis (e.g. cyclooxygenases) and by the rate of catabolism, for which the rate-limiting step is the prostaglandin transporter PGT. Here we tested the hypothesis that PGT regulates thermogenesis and adipogenesis. We previously generated PGT knockout (KO) mice and developed a potent PGT inhibitor, PV-02076. Both genetic deletion (KO) and pharmacological inhibition (PV-02076) of PGT result in higher circulating levels of PGE2 compared to controls. In the high fat diet (HFD) mouse model, both methods of PGT inhibition significantly reduced body weight gain. PV-02076 had no further effect on PGT KO mice. Whereas neither PGT KO nor PV-02076 mice on HFD exhibited increased physical activity compared to controls, both showed significantly increased body temperature under ambient conditions. Although deletion or inhibition of PGT did not significantly affect the abundance of BAT, both genetic and pharmacological inhibition of PGT increased WAT mRNA levels for uncoupling protein (UCP)1 and UCP3, suggesting that inhibiting PG metabolism at the PGT step modulates the browning process of WAT and induces energy release in form of heat. In growing mice that were first allowed weight gain by being fed a HFD, adding PV-02076 to the drinking water stopped any further weight gain, suggesting that PGT may also modulate adipogenesis. In summary, inhibition of PG clearance by pharmacological blockade of the PG uptake carrier PGT offers a potential new therapeutic target for obesity.
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M9

LEUKOTRIENE D4 MODULATE HUMAN ENDOTHELIAL AND MACROPHAGES FUNCTION LEADING TO ATHEROSCLEROSIS

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Cysteinyl leukotrienes (cys-LTs) comprising of leukotriene (LT), C_4 LTD_4 and LTE_4 are potent inflammatory mediators derived from arachidonic acid and induce their action through two receptors, CysLT1R and CysLT2R. Although cys-LTs have been implicated in atherosclerosis, the mechanism of action is not well understood. We investigated the role of cys-LTs on two important aspects of atherosclerosis 1) endothelial dysfunction 2) ability of macrophages to uptake oxidized LDL (ox-LDL) and form foam cells. We recently demonstrated that LTD4 induces endothelial dysfunction as evidenced by EC monolayer disruption, increase in TNFα-induced VCAM-1 expression and leukocyte recruitment to ECs through $CysLT_2R$ but not $CysLT_1R$ in human endothelial cells. In the present study, we found that THP-1 derived macrophages expressed functional CysLTR and exhibited robust calcium influx in response to LTD4. Further, LTD_4 induced significant phosphorylation of ERK1/2 and JNK in these cells. Importantly, we found that LTD_4 treatment up-regulated the ox-LDL receptors CD36 and OLR1 in macrophages. Finally, LTD_4 induced significant

uptake of ox-LDL by macrophages compared to untreated cells. Taken together, our results demonstrate that the inflammatory mediator LTD_4 can promote endothelial and macrophage dysfunction which could play critical role in the etiology of cardiovascular diseases such as atherosclerosis and myocardial infarction.

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M10

INVOLVEMENT OF RENAL CYTOCHROME P450 AND ARACHIDONIC ACID METABOLITES IN DIABETIC NEPHROPATHY

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Background and aims: Diabetic nephropathy (DN), a major complication of type 1 and type 2 diabetes, is characterized by hypertrophy, extracellular matrix accumulation, fibrosis and proteinuria leading to loss of renal function. Hypertrophy is a major factor inducing proximal tubular epithelial cells (PT) injury. However, the mechanisms leading to tubular injury is not well defined. We hypothesize that levels of cytochromes P450 (CYP), particularly those involved in arachidonic acid (AA) metabolism, 20-HETE and EETs are altered by hyperglycemia during the onset and development of diabetes and are involved in the pathophysiology of DN. ***Results:*** In our study, we show that exposure of PT cells to high glucose (HG) resulted in increased extracellular matrix accumulation, hypertrophy and apoptosis. HG treatment increased ROS production and was associated with alteration in CYPs 4A, 4F, 2C, and 2J expression concomitant with alteration in 20-HETE and EETs formation. HG-induced tubular injury were blocked by HET0016, an inhibitor of CYPs 4A and 4F. In contrast, inhibition of EETs promoted the effects of HG on cultured PT cells. Our results also show that alteration in CYPs and their metabolites regulates the activation of the AMPK/mTOR/p70S6Kinase pathway, known to play a major role in the development of DN. In addition to alteration of AA metabolism, uncoupled redox cycling by these CYPs contribute to the increase in reactive oxygen species (ROS) and to an increase in TGF-β production, proposed to be major contributing factor to damage the diabetic kidneys. ***Conclusion:*** In conclusion, we show that hyperglycemia in diabetes has a significant effect on the expression of Arachidonic Acid (AA)-metabolizing CYPs, and might thus alter kidney function through alteration of type and amount of AA metabolites.

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M11

DHA-DERIVED SPECIALIZED PRORESOLVING MEDIATORS INCREASE B CELL ANTIBODY PRODUCTION

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The resolution of inflammation is regulated by specialized proresolving mediators (SPM). SPM are derived from essential polyunsaturated fatty acids, such as docosahexaenoic acid (DHA). B lymphocytes, which circulate between peripheral blood and secondary lymphoid organs, are essential during the adaptive immune response. The actions of SPM on B lymphocytes are not known. Our study showed that the DHA-derived SPM are present in the spleen, a secondary lymphoid organ. Furthermore, resolvin D1 (RvD1), protectin D1 (PD1) and 17-hydroxy-docosahexaenoic acid (17-HDHA) increased antibody production on human B cells in part due to an increased B cell differentiation towards an antibody-secreting cell phenotype. Increased antibody production and differentiation is suggestive of an important role for SPM during the resolution of inflammation and antigen clearance. Therefore, immuno-stimulatory SPM have the potential to be use as natural adjuvants by enhancing adaptive immune protection.

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M12

HYPERTENSION IS A MAJOR CONTRIBUTOR OF 20-HYDROXYEICOSATETRAENOIC ACID-MEDIATED KIDNEY INJURY IN DIABETIC *CYP4A14*-NULL MALE MICE

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In the kidney, 20-hydroxyeicosatetraenoic acid (20-HETE) is a primary cytochrome P450 (Cyp4)-derived eicosanoid that enhances vasoconstriction of renal vessels and induces hypertension, renal tubular cell hypertrophy and podocyte apoptosis. Hypertension and podocyte injury contribute to diabetic nephropathy and are strong predictors of disease progression. In this study we defined the mechanisms whereby 20-HETE affects the progression of diabetic nephropathy. We used Cyp4a14(-/-) male mice which exhibit androgen-sensitive hypertension due to increased Cyp4a12-mediated 20-HETE production. We show that upon induction of diabetes type 1 via streptozotocin injection, Cyp4a14(-/-) male mice developed worse renal disease than streptozotocin-treated wild type mice characterized by increased albuminuria, mesangial expansion, glomerular matrix deposition and thickness of the glomerular

basement membranes. Castration blunted androgen-mediated Cyp4a12 synthesis and 20-HETE production, normalized blood pressure, and ameliorated renal damage in diabetic Cyp4a14(-/-) mice. Importantly, treatment with agents that normalized blood pressure without affecting Cyp4a12 expression and 20-HETE biosynthesis also ameliorated diabetes-mediated renal damage and albuminuria in Cyp4a14(-/-) male mice. Taken together, these results suggest that hypertension is the major contributor of 20-HETE-driven diabetes-mediated kidney injury.

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M13

SOLUBLE EPOXIDE HYDROLASE (SEH) BIOLOGY IN HEALTHY AND LAMINITIC HORSES

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This study determined the levels of epoxy-fatty acid (EpFA) and corresponding diols and soluble epoxide hydrolase (sEH) activity in digital venous blood (DVB), laminar tissue (LT), cerebrospinal fluid (CSF) and/or liver of healthy (n=7) and laminitic (n=11) horses. The potency of sEH inhibitors for the equine sEH was eevaluated *in vitro*, as well as the pain relief from treatment with one sEH inhibitor in laminitic horses (n=5). Heart rate, blood pressure, visual analog pain scores (VAS), complete blood cell counts and serum biochemistry were evaluated to assess pain relief and adverse events in laminitic horses. Data (mean±SEM or median and range), were analyzed with one-way ANOVA or t-tests and Bonferroni post-tests as appropriate. $P<0.05$ was considered statistically significant. EpFA from linoleic and linolenic acid were similar whereas the respective diols was significantly higher in DVB from laminitic compared to unaffected horses. Arachidonic acid-derived EpFA and corresponding diols were increased in LT but not in DVB and CSF of laminitic compared to unaffected horses. Preliminarily, sEH activity in LT was not significantly different between laminitic and unaffected horses (0.1±0.01 and 0.16±0.04 nmol/mg of protein/min, respectively). Sixteen sEH inhibitors were tested and potencies (IC_{50}) ranged between 1±0.2 and 10,000±1,000 nM. Laminitic horses treated with t-TUCB, a potent sEH inhibitor, had lower VAS scores (4.3; 3-6.3) compared to baseline (8.7; 7-9.5) with no adverse effects detected. There is increased degradation of EpFAs in horses with chronic laminitis with no significant changes in sEH activity. Inhibition of sEH may improve pain relief in horses with laminitis.

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M14

20-HETE TRIGGERS RAS ACTIVATION, PANCREATIC CANCER GROWTH AND METASTASIS THROUGH INFLAMMATORY MACROPHAGE SIGNALING

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Prolonged, unresolving inflammation is increasingly implicated in the pathogenesis of pancreatic cancer, but the critical inflammatory mediators remain largely unknown. Most efforts to elucidate these mediators have focused on protein cytokines, rather than lipid autacoids. Lipid autacoids are metabolites of arachidonic acid and other related fatty acids that regulate inflammation and tumor growth. Among these, the metabolites of the cytochrome P450 (CYP) epoxygenases and ω-hydroxylases, including EETs and 20-HETE, respectively, have been largely neglected in cancer. Recently, we showed that epoxyeicosatrienoic acids (EETs) stimulate multi-organ metastasis and tumor dormancy escape. We report for the first time, that elevated levels of 20-hydroxyeicosatetraenoic acid (20-HETE), a product of the ω-hydroxylases CYP4A/F, stimulates primary pancreatic tumor growth and metastasis. Using genetic and pharmacological manipulation of endogenous 20-HETE, we demonstrate that systemic administration of 20-HETE promotes primary human and murine pancreatic cancer in subcutaneous and orthotopic models. A transgenic murine model of endogenously high vascular 20-HETE level (Tie2-CYP4F2 Tr), exhibited spontaneous liver and mediastinal lymph node metastasis after resection of subcutaneous primary pancreatic tumors, in contrast to wild-type mice. We show that 20-HETE activated Ras in human pancreatic tumor cell lines and murine macrophages, while HET0016, a selective inhibitor of 20-HETE synthesizing enzymes, decreased Ras activity. The p65 subunit of NF-κB also increased in a 20-HETE-dependent manner in these tumor cell lines. 20-HETE induced migration of human macrophages in vitro, and tumor-associated macrophages expressed the 20-HETE generating enzyme CYP4F2. Depletion of macrophages with clodronate liposomes impaired CYP-metabolite-stimulated tumor growth. Moreover, inhibitors of soluble epoxide hydrolase, the enzyme that metabolizes EETs, elevated EET levels and promoted pancreatic tumor growth. Either the 20-HETE inhibitor HET0016, or the EET antagonist 14,15-EEZE alone significantly inhibited primary tumor growth without toxicity. Remarkably, systemic administration of both HET0016 and 14,15-EEZE together resulted in sustained regression of established pancreatic tumors, and inhibited tumor cell proliferation in vitro. Combining the 20-HETE inhibitor and EET antagonist suppressed ascites formation and prolonged survival in an aggressive orthotopic pancreatic cancer model. Thus, 20-HETE and EET production by macrophages in the tumor microenvironment critically

regulate Ras activation, pancreatic tumor growth and metastasis. Our studies offer a mechanistic rationale for combining 20-HETE inhibitors and EET antagonists as a novel approach in pancreatic cancer therapy.

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M15

ATF3 NEGATIVELY REGULATES COX-2 DURING ACUTE INFLAMMATION IN MICE.

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By generating prostaglandins, cyclooxygenase-2 (Cox-2/Ptgs2) plays a critical role in regulating inflammatory responses. While *Ptgs2* is induced by several inflammatory stimuli, less is known about how its transcription is terminated. Here, we show that stimulation of macrophages with TLR2 agonist, zymosan, causes transient expression of *Ptgs2* and an increase in the transcriptional repressor, Activating transcription factor-3 (*Atf3*). Expression of *Ptgs2* was significantly higher in macrophages isolated from *Atf3*$^{-/-}$ mice, which was associated with increased prostaglandin production. In activated macrophages, Atf3 accumulated in the nucleus and chromatinimmunoprecipitation analysis showed that Atf3 binds the *Ptgs2* promoter. In acute peritonitis and cutaneous wounds, *Atf3*$^{-/-}$ mice had increased leukocyte accumulation and their inflammatory exudates had higher levels of prostaglandins and downstream mediator, IL-17, than WT mice. Collectively, these results demonstrate that during acute inflammation, Atf3 negatively regulates *Ptgs2*, and therefore dysregulation of this axis could contribute to aberrant COX-2 expression in chronic inflammatory diseases.

M16

EOSINOPHILS AND EICOSANOIDS IN ASPIRIN EXACERBATED RESPIRATORY DISEASE (AERD): PGD SYNTHASE AND LTE4 SYNTHASE GENE EXPRESSION INCREASES IN EOSINOPHILS OF AERD PATIENTS AFTER ORAL GRADED ASPIRIN CHALLENGE

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Rationale: 10% of adult asthmatics suffer from AERD, characterized by adult onset of moderate to severe asthma and nasal polyps. AERD patients typically manifest a hypersensitivity reaction (bronchospasm, rhinorrhea, and/or conjunctivitis) in response to cyclooxygenase-1 (COX-1) inhibitors and have eosinophilia in peripheral blood, nasal polyps, and lung tissue. However, the relationship between the abnormal COX-1 inhibitor response in AERD and the eosinophilic inflammation is not clear. **Objective:** The goal of this pilot study was to determine the pattern of eosinophil eicosanoid expression in AERD. We hypothesized that an eicosanoid abnormality in AERD is mediated through a distinct eosinophil gene expression profile. **Methods:** We studied two groups of asthmatics: 8 AERD patients and 5 ASA-tolerant asthmatics, age >21 years. We targeted five arachidonic acid pathway-relevant genes: arachidonate 5-lipoxygenase-activating protein (ALOX5AP), leukotriene C4 synthase (LTC4S), leukotriene B4 receptor (LTB4R), prostaglandin D (PGD)-synthase (PGDS), and prostaglandin E synthase (PGES). We measured gene expression levels in eosinophils at baseline and 2 h after graded oral aspirin challenge. We also measured corresponding urine and plasma levels of three eicosanoid metabolites: LTE4, tetranor PGDM, and PGE metabolite. Eosinophils were isolated to 95-99% purity by depletion of non-eosinophils by magnetic labeling and conjugation to MicroBeads. RNA was extracted from eosinophils and RNA expression level was measured by RNA-specific RT-qPCR method. **Results:** After an aspirin-induced hypersensitivity reaction, eosinophil PGDS gene expression increased two-fold from baseline and LTC4S gene expression increased 1.5-fold in AERD patients. In contrast, in ASA-tolerant asthmatics, there was no change in PGDS or LTC4S gene expression after the ASA challenge ($p<0.01$ comparing the two groups). The increase in gene expression in AERD positively correlated with increased levels of corresponding urine eicosanoids. PGDM and LTE4 levels after aspirin challenge were significantly greater in AERD patients than in aspirin-tolerant asthmatics, $p<0.01$ and $p=0.04$, respectively. There was no significant change from baseline in ALOX5AP or LTB4R expression in either group, and PGES was not expressed in eosinophils. **Conclusion:** Peripheral blood eosinophils differentially express PGDS and LTC4S gene in response to aspirin challenges in AERD patients as compared to ASA-tolerant asthmatics. Prior studies suggested that PGD is produced exclusively by mast cells in AERD patients. However, our results indicate that eosinophils also produce PGD. It remains to be established whether eosinophils are causative or effector cells in AERD.

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M17

SPECIALIZED PRORESOLVING LIPID MEDIATORS (SPMS) DECREASE HUMAN B CELL IGE PRODUCTION

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Resolution of inflammation is a biologically active process regulated by newly identified specialized proresolving lipid mediators (SPMs). These endogenous lipid-derived mediators promote the resolution of inflammation and maintain homeostasis by regulating the immune system. Little is known about the functions of SPMs on adaptive immune cells, such as B cells. Our laboratory has discovered that certain omega-3 fatty acid derived SPMs promote B cell IgM and IgG antibody production. Human B cells also

produce IgE antibodies, which play an essential role in the onset of inflammatory diseases, such as seasonal allergies and asthma. Here, we show that certain SPMs decrease human B cell IgE production *in vitro*. Omega-3-derived SPMs, 17-HDHA (17-hydoxydocosahexaenoic acid) and RvD1 (resolvin D1), strongly decreased the IgE production in B cells, as well as the number of IgE-secreting cells in peripheral blood of healthy and asthmatic human donors. In addition, 17-HDHA and RvD1 also reduced IgE production by the spontaneously IgE-secreting multiple myeloma B cell line, U266. These results show that SPMs are non-immunosuppressive endogenous mediators that could specifically dampen IgE production and have potential as therapeutics for asthma. Moreover, SPMs might also be effective on other IgE-mediated inflammatory diseases such as atopic dermatitis.
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M18

MULTIPLE DRUG RESISTANCE-ASSOCIATED PROTEIN 4 (MRP4) MAY CONTRIBUTE TO BREAST CANCER METASTASIS BY EXPORTING THE COX-2 PRODUCT, PGE2

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Cancer is becoming the most prevalent disease in Western countries and breast cancer is the most frequently diagnosed cancer among women. Breast cancer accounts for 23% of total cancer diagnoses and 14% of cancer-related deaths. Metastatic disease accounts for the majority of cancer mortality, and therefore, elucidating new targets in the metastatic process can prevent cancer-related death. As with many solid tumors, cyclooxygenase-2 (**COX-2**) and its enzymatic product, prostaglandin E2 (**PGE2**), are elevated in breast cancer, and are associated with a poor prognosis and increased metastatic potential. PGE2 initiates various signaling pathways upon binding to each of four cognate EP receptors. We have previously shown that PGE2 signaling through the **EP4** receptor increases the metastatic potential of breast cancer cells and supports the survival of breast cancer stem cells. Multiple drug resistance-associated protein 4 (**MRP4**) is responsible for the active export of PGE2 from cells, while the prostaglandin transporter (**PGT**) imports PGE2 for 15-hydroxyprostaglandin dehydrogenase (**15-PGDH**)-mediated degradation. The role of neither MRP4 nor PGT has been investigated in breast cancer progression. We hypothesize that elevated expression of MRP4 would cause increased PGE2 signaling and, therefore, enhance metastatic potential and progression of breast cancer and may also support breast cancer stem cells. We used MCF10A (immortalized normal breast epithelium), MCF7 (luminal), T47D (luminal), MDA-MB-231 (basal B), MDA-MB-436 (basal A), and SKBR3 (HER2-enriched) cell lines. These cell lines span not only a range of molecular subtypes, but also a range of metastatic potential. MRP4 mRNA and protein expression is increased in tumor cell lines with high metastatic potential (MDA-MB-231, MDA-MB-436, SKBR3) while expression of PGT mRNA and protein is decreased in these cells when compared to cells with lower metastatic potential. This inverse relationship between MRP4 and PGT should lead to higher concentrations of extracellular PGE2 in the tumor microenvironment, and this hypothesis is being tested through pharmacologic and genetic approaches. We show that pharmacologic inhibition of MRP4 through antagonism with MK571 (MRP-family inhibitor) or competition with 6-mercaptopurine (6-MP) results in decreased efflux of PGE2 and cyclic-AMP (cAMP), two substrates of MRP4. Likewise, genetic suppression by MRP4 RNA-interference (RNAi) results in lower levels of PGE2 exported from cells. These cells with suppressed MRP4 expression also show decreased migration when compared to control. These data support the hypothesis that MRP4 is a critical member of the PGE2 signaling pathway that leads to high extracellular PGE2, increased PGE2 signaling, and increased metastatic potential, implicating MRP4 as a possible therapeutic target.
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M19

THROMBOXANE (TXA2) ATTENUATED LUNG INFLAMMATORY RESPONSES AND TH9 DIFFERENTIATION DURING ALLERGIC LUNG INFLAMMATION

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Thromboxane (TXA2) is a potent bronchial smooth muscle spasmogen *in vitro* and is implicated in airway inflammation/bronchial hyperresponsiveness in asthma. Through thromboxane receptor (TP) signaling, TXA2 negatively regulates the interaction of dendritic cells (DC) and T cells and regulates acquired immunity. T helper type 9 (Th9) cells, a subpopulation of CD4+ T cells, play an important role in pathogenesis of asthma; however, it is unknown whether TXA_2 regulates Th9 cells during allergic lung inflammation. We used an *in vivo* ovalbumin (OVA)-induced allergic inflammation model to study the role of TXA2 in Th9 cell differentiation during allergic lung inflammation. Flow cytometry, cytokine analysis, confocal microscopy, and RT-PCR were used to assess the role of TXA2 in Th9 cell differentiation. In addition, the interaction of TXA_2 and its receptor was examined using a synthetic TP agonist (carboxocyclic thromboxane A2; CTA), selective TP antagonist (iodophenyl sulfonyl amino pinane TXA_2; I-SAP), TP-deficient mice ($TP^{-/-}$) and siRNA knockdown. Our results indicated that the percentage of IL-$9^{+}CD4^{+}$ T cells was significantly decreased in lungs of mice that were implanted with mini-pumps containing CTA during OVA exposure vs. mice implanted with I-SAP or vehicle containing mini-pumps (CTA: 3.9 ± 1.9% vs. I-SAP: 9.3 ± 2.5% or vehicle: 9.7 ± 5.5%). Similar results were obtained with BALF (CTA: 9.12 ± 3.4% vs I-SAP: 12.4 ± 3.5% or vehicle: 15.2 ± 5.4%), lymph nodes (CTA: 4.9 ± 3.1% vs I-SAP: 7.6 ± 3.1% or vehicle: 10.4 ± 3.2%), and blood (CTA: 4.9 ± 3.1% vs I-SAP: 7.6 ± 3.1% or vehicle: 10.44 ± 3.2%). $TP^{-/-}$ mice have significantly impaired Th9 cell differentiation in lung after OVA exposure as compared to WT mice (5.6 ± 1.3% vs

8.8 ± 2.1%). Similar results were obtained in BALF (9.8 ± 2.4% vs 12.86 ± 2.8%), blood (5.5 ± 1.8% vs 7.6 ± 2.4%), lymph nodes (6.2 ± 2.6% vs 9.8 ± 2.8%) and spleen (4.23 ± 1.5% vs 5.3 ± 1.4%). *In vitro* experiments determined that IL-9 and IL-10 expression during Th9 cell differentiation were significantly inhibited by TXA_2 in both murine and human naive $CD4^+$ T cells. TXA_2 also inhibited Th9 cell differentiation from OVA-sensitized $CD4^+$ T cells. Interestingly, lung $CD11b^+F4/80^+$ macrophages had higher TXA2 secretion than $CD11b^+F4/80^-$ macrophages after LPS stimulation. We also examined the role of $CD11b^+F4/80^+$ and $CD11b^+F4/80^-$ cells in Th9 cell differentiation. Co-culture of $CD11b^+F4/80^+$ macrophages with naïve $CD4^+$ T cells significantly impaired Th9 cell differentiation compared to CD11b+F4/80- macrophages. $TP^{-/-}$ naïve $CD4^+$ T cells showed decreased Th9 cell differentiation compared with WT naïve $CD4^+$ T cells. In summary, TXA2 significantly suppressed Th9 cell differentiation of murine and human naïve $CD4^+$ T cells and murine OVA-sensitized $CD4^+$ T cells by inhibiting TP signaling during allergic lung inflammation**.**

M20

15-LIPOXYGENASE PROMOTES CHRONIC HYPOXIA-INDUCED PULMONARY ARTERY INFAMMATION VIA POSITIVE INTERACTION WITH NUCLEAR FACTOR-KB

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Objective-Our laboratory has previously demonstrated that 15-lipoxygenase (15-LO)/15-hydroxyeicosatetraenoic acid (15-HETE) is involved in hypoxic pulmonary arterial hypertension. Chronic hypoxia-induced vascular inflammation has been considered as an important stage in the development of pulmonary arterial hypertension. Here, we determined the contribution of 15-HETE in the hypoxia-induced pulmonary vascular inflammation. Approach and Results-Chronic hypoxia-induced monocyte/macrophage infiltration and the expressions of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 were analyzed in hypoxic rat model and cultured pulmonary arterial endothelium cells using immunochemistry methods. We found that monocyte/macrophage infiltration and the expressions of intercellular adhesion molecules under hypoxia were markedly inhibited by 15-HETE inhibitors or 15-LO1/2 small interfering RNA. In addition, exogenous 15-HETE enhanced the expression of both adhesion molecules in pulmonary arterial endothelium cells in a time-dependent manner. Hypoxia-induced 15-LO1/2 expression in rat pulmonary arterial endothelium cells was significantly abolished by nuclear factor-κB inhibitors. Meanwhile, nuclear factor-κB activity was enhanced prominently by the 15-LO1/2 product, 15-HETE, suggesting a positive feedback mechanism. Conclusion-Taken together, our results suggest that chronic hypoxia promotes monocyte infiltration into the vasculature and adhesion molecules upregulation in pulmonary arterial endothelium cells via a positive interaction between 15-LO/15-HETE and nuclear factor-κB. Our study revealed a novel mechanism underlying hypoxia-induced pulmonary arterial inflammation and suggested new therapeutic strategies targeting 15-LO/15-HETE and nuclear factor-κB in the treatment of pulmonary arterial hypertension.

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M21

INCREASED 12/15-LIPOXYGENASE AND CYCLOOXYGENASE RESPONSE PATHWAYS IN AFFECTED AND NON-AFFECTED SKIN OF HUMAN ATOPIC DERMATITIS PATIENTS

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Lipoxygenases (LOX) and cyclooxygenases (COX) are the main enzymes for PUFA metabolism to highly bioactive prostaglandins, leukotrienes, thromboxanes, lipoxins, resolvins and protectins. LOX and COX pathways are important for the regulation of pro- and anti-inflammatory active metabolite synthesis and metabolism in various inflammatory diseases like atopic dermatitis (AD). In this study, we determined PUFAs and various PUFA-metabolites in serum as well as affected and non-affected skin samples from AD-patients and the expression of various enzymes, binding proteins and receptors involved in these LOX and COX pathways just in the skin. Decreased EPA and DHA levels in serum and reduced EPA level in affected and nonaffected skin were found. In addition, n3-/n6-PUFA ratios like EPA/AA and DHA/AA were lower in affected and non-affected skin as well as in serum. Mono-hydroxylated PUFA metabolites of AA (HETEs), EPA (HEPEs), DHA (HDHAs) and the sum of AA, EPA and DHA metabolites were increased in affected and non-affected skin. COX-1 and ALOX12B expression and in addition to COX and 12/15-LOX metabolites were increased both in non-affected and affected AD-skin. Expression of COX1, COX metabolites and ALOX12B-metabolites were even higher in non-affected AD-skin. To conclude, 12/15-LOX and COX pathways were mainly upregulated in AD skin and serum and n3-/n6-PUFA ratios were lower in skin and serum of AD-patients.

M22

MARESIN 1: A NOVEL MACROPHAGE-DERIVED ENDOGENOUS PRORESOLVING INHIBITOR OF TUMOR GROWTH AND METASTASIS

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Background: Current clinical approaches to cancer therapy including chemotherapy, radiation, and targeted therapy, treat inoperable or incompletely resected tumors by killing tumor cells. However, this is a *doubled-edged sword*. The debris of dead cancer cells acts as a source of tumor-stimulation on the few surviving cells contributing to tumor relapse. In tumors, apoptotic (dying) cells release mediators (e.g. prostaglandin E2) that attract inflammatory infiltration, a recent hallmark of cancer. Furthermore, chemotherapeutic agents stimulate pro-inflammatory and pro-tumorigenic cytokine production by macrophages in the tumor microenvironment. Maresin 1 (MaR1), a key mediator of inflammation resolution, is biosynthesized by human macrophages from endogenous docosahexaenoic acid. We *hypothesize* that maresins represent a novel modality in cancer treatment by pharmacologically promoting the clearance (resolution) of tumor cell debris via macrophage phagocytosis of apoptotic tumor cells, thereby depriving the surviving tumor cells of inflammatory stimuli. **Methods**: Apoptotic tumor cell debris was generated by treatment of tumor cells with chemotherapeutic agents. To study the pro-resolving efficacy of maresin 1, human macrophages were incubated with Mar1 or the epimer 7(S)-MaR1. We focused on a subset of cytokines whose abundance was altered by maresins via human cytokine array and confirmed by ELISA. **Results**: Flow cytometry confirmed apoptotic tumor cell debris. We demonstrate that maresin 1 enhances macrophage phagocytosis of apoptotic tumor cells (e.g. prostate and melanoma) by 32% to 206%. Maresin 1 also stimulates macrophage phagocytosis of chemotherapyinduced cell debris (e.g. melanoma and oral carcinoma) by 50% to 117%. The maresin 1 epimer, 7(S)-MaR1 also enhances macrophage phagocytosis, with enhanced phagocytosis of chemotherapy-induced tumor cell debris (melanoma and oral carcinoma) by 58% to 121%. MaR1 counter-regulated pro-tumorigenic and pro-inflammatory cytokines/chemokines secreted by macrophages, including: IL-6, IL-8, CXCL1, CCL1, CCL2, CCL5, and Serpin E1. Systemic administration of maresin 1 inhibits primary tumor growth (human melanoma-A375-SM) and spontaneous lung metastases (post primary Lewis lung carcinoma removal) by promoting the resolution of inflammation without overt toxicity. **Conclusions**: We demonstrate for the first time that maresins inhibit primary tumor growth and metastasis via the macrophage stimulation of the phagocytosis of chemotherapy-induced tumor cell debris. Furthermore, maresin 1 reduces tumor inflammation by counter-regulating pro-inflammatory cytokines secreted by macrophages. Thus, the maresin 1 pathway or the enhancement of endogenous resolution processes, offers an entirely novel approach for clearing tumor cells debris induced by conventional cancer therapy.
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M23

DGLA DIFFERENTIALLY REGULATES PLATELETS REACTIVITY IN TYPE 2 DIABETES MELLITUS

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Over 380 million people live with diabetes worldwide and suffer a 65% mortality rate due to a thrombotic event. To prevent unwanted vessel blockage, fatty acid supplements, containing DHA/EPA and GLA (DGLA), are often recommended for individuals being treated for type 2 diabetes mellitus (T2DM); however, the underlying mechanism of protection afforded by these lipid products remains unclear. Our previous studies have shown that the ω-6 fatty acid, DGLA, its platelet 12-lipoxygenase (12-LOX) peroxidized product, 12(S)-HPETrE, and its reduced form, 12(S)-HETrE, significantly inhibit platelet reactivity (Ikei et al., 2012). Therefore, we decided to investigate how these three components affect platelet activation in healthy subjects and subjects diagnosed with T2DM. Washed human platelets from both experimental groups were treated with DGLA, 12(S)-HETrE or 12(S)-HPETrE and platelet activation was measured following stimulation with multiple agonists including thrombin, PAR1-activating peptide (PAR1-AP), PAR4-AP and convulxin. Three biochemical endpoints were assessed in these subjects: 1) aggregation, 2) integrin αIIbβ3 activity and 3) granule secretion. Our results suggest DGLA, 12(S)-HETrE, 12(S)-HPETrE potently inhibit platelet aggregation in healthy subjects stimulated with thrombin, PAR1-AP and PAR4-AP. Platelets from subjects with T2DM are more resistant to DGLA inhibition and required higher concentration of lipid products to exert a similar inhibitory effect. Convulxin-initiated aggregation, which uses a different pathway from other agonists, required higher concentrations of DGLA and its metabolites in healthy subjects, and only partially inhibited platelet activation in subjects with T2DM. All three tested bioactive lipid components significantly inhibited integrin activation and granule secretion. The fact that DGLA is able to inhibit these critical endpoints at much lower concentration than the 12-LOX metabolites, 12(S)-HPETrE and 12(S)-HETrE, suggests that while 12-LOX activity is an important regulator of platelet function in the DGLA pathway, the COX-1 pathway may also play a significant role in suppressing integrin activation and granule secretion. Current results give us good bases to further investigate which are the pathways DGLA modifies leading to lowered platelet activity and why patients with T2DM may need higher levels of fatty acid supplementation to achieve the same protection from thrombosis and stroke.
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M24

PROSTAGLANDIN E2 LEADING TO EXAGGERATED HUMAN MAST CELL INFLAMMATORY RESPONSES

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Cysteinyl leukotrienes (cys-LTs) are potent bronchoconstrictors, powerful inducers of vascular leakage, potentiators of airway hyperresponsiveness and play an essential role in asthma. They act through two receptors, CysLT1R and CysLT2R. On the other hand, Prostaglandin E^2 (PGE_2), was shown to be both pro- and anti-inflammatory depending on the receptors ($EP_{1\text{-}4}$), through which it exerts its effect in a cell. In the present study, we investigated the cross talk between cys-LTs and PGE_2, the receptors involved and the impact of this cross talk on mast cell (MC) inflammatory responses. In human MCs, PGE_2 by itself does not activate calcium and MIP1β while cys-LT priming prior to PGE_2 stimulation synergistically enhanced calcium flux, c-fos phosphorylation and MIP-1β generation. Supporting this, combined treatment of LTD4 and PGE_2 significantly enhanced TNFα, COX-2 and IL-8 transcripts than either of the agonists alone. Interestingly, exaggerated c-fos expression and MIP1β generation by both the agonists were completely abolished by the combined treatment of CysLT1R and EP3 receptor antagonists. Further, this synergism is completely abolished by Protein Kinase A (PKA) inhibitor, H7. Importantly, in an in vivo ear inflammation assay, combined injection of PGE_2 and LTD_4, but not alone, caused significant ear swelling in mice. Our results, thus suggest that Cys-LTs prime PGE_2 pro-inflammatory function such as increased c-fos expression, secretion of chemokines and cytokines through the modulation of protein kinase A pathway. The fact that cys-LTs prominently regulate MC development in mucosal inflammation suggests that locally-derived cys-LTs could control PGE_2 responses and alter MC function which could carry substantial pathogenic and therapeutic implications for asthma and allergic diseases in particular.

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M25

Arg287Gln VARIANT OF *EPHX2* AND EPOXYEICOSATRIENOIC ACIDS ARE ASSOCIATED WITH INCREASED INSULIN SENSITIVITY IN HUMANS

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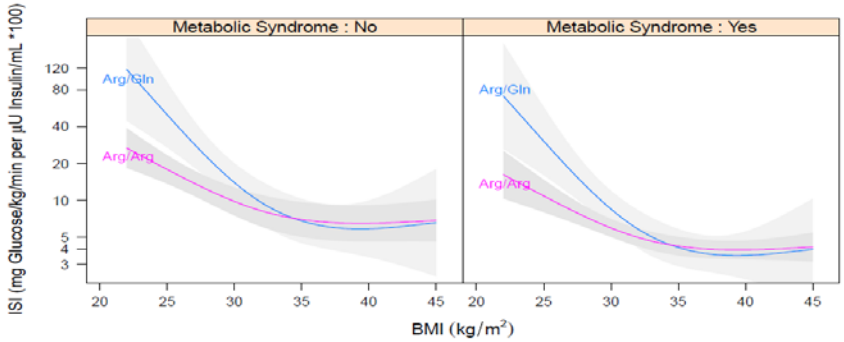
Studies in rodents suggest that epoxyeicosatrienoic acids protect against the development of insulin resistance. Epoxyeicosatrienoic acids are rapidly hydrolyzed to their less biologically active diols by the enzyme soluble epoxide hydrolase, encoded for by *EPHX2*. The Arg287Gln variant of *EPHX2* results in a missense mutation that encodes for an enzyme with decreased epoxide hydrolase activity compared with the wild-type enzyme. This study tested the hypothesis that the Arg287Gln variant of *EPHX2* is associated with enhanced insulin sensitivity or secretion in humans. Subjects participating in metabolic phenotyping studies were genotyped for the *EPHX2* Arg287Gln polymorphism. Eighty-two subjects underwent hyperglycemic clamps (61 with the metabolic syndrome, and 21 without metabolic syndrome). The *EPHX2* 287Gln variant was associated with higher insulin sensitivity index (p=0.019 after controlling for body mass index and metabolic syndrome). There was an interactive effect of *EPHX2* Arg287Gln genotype and body mass index on insulin sensitivity index (p=0.029). Thus, for subjects with a body mass index lower than $30kg/M^2$ the insulin sensitivity index was significantly higher in carriers of the *EPHX2* 287Gln allele compared with Arg/Arg homozygotes. The enhanced insulin sensitivity index in 287Gln allele carriers decreased in a hyperbolic fashion with increased BMI (Figure). Although there was no relationship between *EPHX2* Arg287Gln genotype and acute glucose-stimulated insulin response or late phase glucose-stimulated insulin secretion, the disposition index was higher in 287Gln carriers compared with Arg/Arg homozygotes (p=0.043). Plasma epoxyeicosatrienoic acids correlated with the insulin sensitivity index (r=0.64, p=0.015 for total EETs) and were significantly decreased in subjects with the metabolic syndrome (0.83±0.38 ng/mL versus 1.55±1.40 ng/mL, p=0.026). A loss-of-function variant of *EPHX2*is associated with enhanced insulin sensitivity in humans. The finding that epoxyeicosatrienoic acids correlate with insulin sensitivity suggests that enhanced glucose homeostasis in carriers of this variant could be mediated through a decreased degradation of epoxyeicosatrienoic acids.



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M26

LIPOXIN A4 A SPECIALIZED PRORESOLVING MEDIATOR MODULATES HUMAN B CELL FUNCTIONS

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Lipoxins, resolvins, maresins and protectins are members of the specialized proresolving mediators (SPM) family. SPM are bioactive lipid mediators critical for the resolution of inflammation. SPM have important immunological functions including the increase of monocyte recruitment and macrophage efferocytosis. The effects of lipoxins on B cells are not known. Here, we report on the ability of LXA_4 to decrease human B cell IgM and IgG production through an ALX/FPR2-dependent mechanism. Specifically, LXA_4 decreased memory B cell antibody production and proliferation but it did not affect the naïve B cell population. In addition, LXA4 decreased antigen-specific antibody production *in vivo*. These results show the different effects of LXA_4 on separate B cell populations, which provide a link between the resolution programme and the adaptive immune response. An uncontrolled humoral response can prolong inflammation and lead to disease, such as autoimmunity. Therefore, lipoxins and other SPM have great potential to be used as therapeutics for inflammatory and autoimmune disorders.

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M27

MECHANISTIC STUDIES OF THE METASTASIS SUPPRESSOR PROSTAGLANDIN E2 RECEPTOR EP1 IN BREAST CANCER

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Prostaglandin E2 (PGE2), the chief cyclooxygenase-2 enzyme (COX-2) product in tumors, is the predominant protumorigenic prostanoid and mediates biological effects by binding to each of four EP receptors (EP1-4). Each receptor is coupled to different intracellular signaling pathways with EP1 coupled to calcium mobilization and PKC. EP4, expressed on malignant breast cells, promotes metastasis, but a role for EP1 in metastasis has not been investigated. Our published studies indicate that EP1 was detected in the cytoplasm and nucleus of benign ducts and malignant cells in invasive ductal carcinomas, and overall survival for women with tumors negative for nuclear EP1 was significantly worse than for women with EP1 expression. Pharmacologic antagonism and reduction of EP1 expression increased metastatic capacity in our murine model of metastatic breast cancer. These data support our hypothesis that EP1 functions as a metastasis suppressor. We now report that murine metastatic mammary tumor cell lines 410.4 and 66.1 have decreased EP1 mRNA expression compared to the non-metastatic cell line 410. We have also identified the presence of a variant EP1 (EP1v) transcript. EP1v has been identified previously in murine mast cell line MC/9 and rat uterus but not in malignant cells. EP1v has a pattern of mRNA expression different than full-length EP1. Compared to the cell line 410, EP1v expression is slightly increased in 410.4 and decreased in 66.1 cell lines. Previously we determined that reduction of EP1 expression lead to increased metastatic capacity; therefore, we investigated the effect of EP1 and EP1v overexpression on metastasis in 410.4 and 66.1 cells via tail vein injection in a murine model of breast cancer. In 410.4 cells, EP1 overexpression lead to a 57%-97% decrease in metastasis; likewise, EP1v overexpression reduced metastasis by 80%. Overexpression of EP1 in 66.1 cells resulted in a 10% - 38% decrease in metastatic lung weight compared to vector control. EP1-v overexpression decreased lung weight by 12%. The inverse correlation between EP1 expression and metastatic capacity supports our hypothesis that EP1 functions as a metastasis suppressor. In the quest to identify a clinically relevant strategy to increase expression of this protective receptor, we performed bioinformatic analysis of the EP1 gene and identified several CpG islands. DNA methylation analysis revealed hypermethylation of the CpG island nearest to the promoter in normal, non-metastatic and metastatic murine mammary tumor cell lines. Treatment with 5-azacytidine, a clinically approved demethylating agent, on 410.4 and 66.1 cells resulted in an increase in EP1 expression. These findings suggest that EP1 has the potential to be a new therapeutic target in reducing breast cancer metastasis and increasing overall cancer survival.

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M28

IN HEALTHY ADULTS SUPPLEMENTED WITH MARINE OMEGA-3 FATTY ACIDS TOTAL ISOPROSTANE RESPONSE TO LOW-DOSE ENDOTOXIN CHALLENGE IS ATTENUATED DESPITE INCREASED F3-ISOPROSTANE PRODUCTION

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Increased eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake is associated with prevention of chronic diseases involving inflammation and oxidative stress. However, the mechanisms responsible for the anti-inflammatory effects of EPA + DHA and their role in the development or attenuation of oxidative stress remain incompletely understood. Increased intake of EPA and DHA decreases production of F_2-isoprostanes and increases formation of F_3-isoprostanes derived from EPA and DHA peroxidation. Compared to F_2-isoprostanes formed from arachadonic acid peroxidation, F3-isoprostanes are less biologically active and do not

exert the same potent vasoconstrictive effects or changes in platelet shape and aggregation. We evaluated whether levels of EPA + DHA intake were predictive of acute oxidative stress responses as assessed by urine concentrations of 15-F_{3t}-isoprostane (F3-IsoP), 15-F_{2t}-isoprostane (F_2-IsoP), and its metabolite 2,3-dinor-5,6-dihydro-15-F2t-isoprostane (F2-Iso-M), in a human model of induced inflammation (intravenous 0.6 ng/kg purified lipopolysaccharide [LPS], or endotoxin challenge). Participants were healthy adults (n = 19, age 20-44, BMI 20-30 kg/m2) enrolled in a fish oil supplementation study and received 0, 300, 600, 900, or 1800 mg/d EPA + DHA for approximately 5 months. Following this supplementation period, participants underwent an IV endotoxin challenge, during which urine was collected for 8 h post injection. Due to sample size limitations, analyses of total IsoP (F_2-IsoP + F_2-Iso-M + F_3-IsoP) were performed using a dichotomous variable of low intake (0 and 300 mg/d, n = 8) and high intake (600, 900, and 1800 mg/d, n = 11). In a univariate model, each 1 g/d increase in EPA + DHA resulted in a 0.4 ng/mg creatinine increase in urinary F3-IsoP concentrations ($p < 0.001$). Despite the increase in F3-IsoP production, higher intake of EPA + DHA remained associated with an attenuated oxidative stress response, as measured by total IsoP production (F_3-IsoP + F_2-IsoP + F_2-Iso-M), in a multivariate model including gender and BMI. Higher intake of EPA + DHA significantly ($p = 0.03$) decreased total IsoP by 0.7 ng/mg; male gender and higher BMI were also associated with decreased total IsoP (model $p = 0.002$, model $R^2 = 62\%$). Our study demonstrates that in a small sample of healthy individuals, despite producing elevated levels of F_3-isoprostranes, marine omega-3 fatty acid supplementation remained predictive of attenuated oxidative stress (in terms of total isoprostane production) during acute inflammation, which may have important implications for the prevention of chronic diseases.
Support: USDA, CSREES, grant #2009-65200-05973. ClinicalTrials.gov #: NCT01078909

M29

SOLUBLE EPOXIDE INHIBITION AND EXOGENOUS EPOXYEICOSATRENOIC ACIDS INCREASE PRESENCE OF CD206-POSITIVE MICROGLIAL

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As stroke still remains one of the leading causes for long-term disability in adults, novel interventions to reduce morbidity beyond the acute ischemic phase are needed. Epoxyeicosatrenoic acids (EETs) are anti-apoptotic, angiogenic, anti-inflammatory, and anti-nociceptive lipids synthesized in astrocytes by CYP 2C11, a hypoxia inducible enzyme, and are released upon activation of glutamate receptors. Rapid degradation of EETs by the soluble epoxide hydrolase enzyme (SEH) limits their sustained activity. This can be overcome with SEH inhibitors (SEHi). Here we investigated the ability of SEHi and/or exogenous EETs to the promote M2 phenotype in microglia, which is a phenotype thought to promote neural repair. To assess potential effects SEHi on microglial cells further, organotypic slices that were obtained from P4-P8 Sprague Dawley rats were treated with lipopolysaccharide (LPS) 1ug/mL or exposed to oxygen-glucose deprivation (OGD) (1 hour for cortical slice, 30 minutes for hippocampal slices). Slices were treated with vehicle or SEHi N-[1-(1-oxopropyl)-4-piperidinyl]-N'-[4-(trifluoromethoxy)phenyl)-urea (TPPU) at 100uM and in conjunction with 14,15-EET 1 uM for 24 hours after the addition of LPS or at the time of reoxygenation of the OGD-exposed slices. Iba-1 staining was used to assess for microglial activation. The CD-206 microglial marker was used to stain microglia of M2 phenotype. Lastly, BV-2 cells were treated with SEHi TPPU 100um and 14,15 EET 1uM and co-cultured for 24 hours with neurons exposed to 1 hour of OGD. Treating the slices with TPPU and exogenous EETs in conjunction with LPS dampened Iba-1 up-regulation. Also, the predominance of CD206-positive cells increased with TPPU treatment in LPS-treated slices in the cortical slices. Cell death was reduced in slices subjected to OGD, as detected by TUNEL and propidium iodide staining. Moreover, the CD206-positive cells were increased with TPPU and exogenous EET treatment. BV-2 cells treated with TPPU and EET and co-cultured with neurons exposed to OGD had a higher predominance of CD-206 staining in comparison to vehicle treated group. Neuronal cell death from OGD was also reduced with the TPPU and exogenous EET treatment. These data suggest that SEHi may influence microglial phenotype by promoting M2 polarization. Given that M2 phenotype microglia have been proposed to possibly assist with neural repair, SEHi are potential therapeutic agents for use in treatment of recovery.
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M30

CANCER PROGRESSION: THE FAILURE TO RESOLVE?

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Background: Failure to resolve inflammation contributes to disease pathogenesis. Inflammation in the tumor microenvironment is now recognized as a hallmark of cancer. Anti-inflammatory therapies have focused on suppressing pro-inflammatory mediators, cytokines and eicosanoids. However, a new direction has emerged with the discovery of a novel genus of endogenous anti-inflammatory and pro-resolving lipid-autacoid mediators derived from omega-3 polyunsaturated fatty acids, specifically resolvins (Rvs). Resolvins have potent novel inflammation clearing (proresolution) activity without being immunosuppressive and are anti-angiogenic, anti-infective and anti-fibrotic. We hypothesize the failure of the resolution of inflammation leads to cancer progression

and resolvins inhibit primary tumor growth/metastasis. **Results:** Resolvins potently inhibited primary tumor growth in subcutaneous, orthotopic and genetically engineered models of cancer without toxicity. After primary tumor resection, resolvins inhibited spontaneous lung metastasis. Resolvins prevent apoptosis-stimulated tumor growth (Révész effect) more potently than hemotherapy at nanogram levels (at doses over 10,000 fold less than aspirin or omega-3 fatty acids). Resolvins (RvD1, RvD2, and RvE1) activated human macrophage phagocytosis of apoptotic human tumor cells (including prostate and cisplatin-resistant ovarian), which was reversed with a lipoxygenase inhibitor (baicalein). Similarly, RvD1 stimulated murine macrophage phagocytosis of chemotherapy-induced apoptotic murine tumor cells (including ovarian), which was reversed by the ALX/FPR-2 selective antagonist BOC-1. Resolvins also counter-regulated chemotherapy-induced pro-inflammatory cytokines/chemokines including
TNFα, IL-6, IL-8, CCL4 and CCL5. Furthermore, resolvins reduced leukocyte infiltration and VEGF-dependent tumor angiogenesis. Resolvins induce a new phenotype of tumor-associated macrophages that are M2-like (increased CD206+/F4/80+), but possess phagocytic activity (CD11bhigh/F4/80+). Electron microscopy confirmed phagocytosis of tumor cells in macrophages in RvD2-treated tumors. Depletion of macrophages promoted tumor growth in RvD2-treated mice. Resolvins display synergistic anti-tumor activity with the chemotherapeutic agent cisplatin to regress established primary tumors. The expression of the RvD1 receptor, ALX/FPR-2, decreased with spontaneous tumor (TRAMP) progression. Finally, cancer progression resulted in the loss of the endogenous pro-resolving lipid mediator RvD1. **Conclusions:** Resolvins inhibit primary tumor growth and metastasis via the resolution of inflammation. The resolvin pathway, or the enhancement of endogenous resolution processes, therefore offers an entirely novel approach for controlling chronic inflammation as a modality of cancer treatment.
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M31

DOWNREGULATION OF COX-2/PGE2 SIGNALING BY ISOQUIRITIGENIN IMPROVES ANTITUMOR EFFICACY OF TEMOZOLOMIDE THROUGH NORMALIZING VASCULATURE IN C6 RAT GLIOMA ORTHOTOPIC MODELS

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Cyclooxygenases-2 (COX-2) is expressed highly in glioma and implicated in tumor progression. COX-2 inhibition can enhance the effect of standard therapy for pancreatic cancer through promoting vascular normalization. Isoquiritigenin (ISL), a flavonoid from licrorice, has potent antitumor and antiangiogenic activity in several solid tumors. However, its mechanisms of action have not been fully defined. Here, we demonstrate that ISL increase the antitumor efficacy of temozolomide through inducing vascular normalization in C6 rat glioma. After stereotactic injection of C6 rat glioma cell into the Sprague Dawley rat brain, the rats were randomly assigned to four treatment groups [control, ISL (25 mg/kg/d, i.p.×7 consecutive days) alone, temozolomide (5 mg/kg i.p. either as a single dose to determine the levels of temozolomide in tumor tissues or once daily for 5 consecutive days) alone, and a combination of ISL and temozolomide]. There was a significant reduction in tumor volume and an increase in the life span with the elevated levels of temozolomide in tumor tissues in combination group when compared with ISL or temozolomide alone. In contrast, ISL had no direct effects on the growth and survival of tumor cells. Importantly, ISL resulted in a dose-dependent increase of pericyte coverage (the ratio of $CD31^+NG2^+$ vessels/$CD31^+$ vessels), and a moderate but significant decrease in $CD31^+$ microvessel density. In addition, ISL improved the functions of these vessels, evidenced by an approximately 2.1-fold increase in perfusion and a 2.7-fold decreases in hypoxia and vessel leakiness in tumor tissues. Notably, ISL inhibited the expression of COX-2 and vascular endothelial growth factor (VEGF), and decreased production of prostaglandin E2 (PGE2) and VEGF *in vivo* and *in vitro*. Furthermore, ISL inhibited angiopoietin 1 expression in pericytes, and activated tumor suppressor phosphatase and tensin homolog in endothelial cells. In contrast, exogenous addition of PGE2, EP3-or EP4-specific agonists reversed ISL-induced downregulation in expression of VEGF and angiopoietin 1, whereas, neither EP1 nor EP2 agonists did. Taken together, these data suggest that downregulation of COX-2/PGE2-EP3/EP4 signaling by isoquiritigenin augments standard therapy for C6 rat glioma through promoting vascular normalization. The study highlights the importance of the microvascular effects of COX-2 inhibition by plant flavonoids and needs to take those changes into account in the design of clinical trials many of which use combinations of chemotherapeutic agents. **Keywords**：Vascular normalization; COX-2; VEGF; Glioma; Isoquiritigenin
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M32

SOLUBLE EPOXIDE HYDROLASE ON ANGIOGEENSIS, TUMRO GRWOTH AND METASTASIS

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Epoxygenated fatty acids (EpFAs), which are lipid mediators produced by cytochrome P450 epoxygenases from polyunsaturated fatty acids, are important signaling molecules known to regulate various biological processes including inflammation, pain and angiogenesis. Pharmacological inhibitors of soluble epoxide hydrolase (sEH), which is the major enzyme to degrade EpFAs, are being evaluated for multiple human disorders. EpFAs are known to be critical regulators of inflammatory and cardiovascular diseases; however, their roles in cancer are poorly characterized. We recently discovered that EDPs, which are cytochrome P450 (CYP) epoxygenases-derived metabolites of docosahexaenoic acid (DHA, a major omega-3 fatty acid), potently suppressed tumor growth and metastasis by blocking tumor angiogenesis. In contrast, epoxyeicosatrienoic acids (EETs), which are CYP epoxygenases

metabolites of arachidonic acid (ARA, an omega-6 fatty acid), are mildly pro-angiogenic to stimulate tumor progression. These findings demonstrate that the previously unappreciated CYP-metabolites (EETs and EDPs) play critical roles in mediating the opposite effects of omega-6 and omega-3 fatty acids on cancer. We also discovered that co-administration of COX-2 inhibitors and sEH inhibitors synergistically inhibited tumor growth and metastasis. Due to the potent interactionS of COX-2 and she inhibitors, we designed and synthesized the first-in-class COX-2/sEH dual inhibitors. The dual inhibitors also potently suppressed tumor growth and metastasis in part via inhibition of tumor angiogenesis, with reduced cardiovascular toxicity compared with COX-2 inhibitors. These results suggest that dual pharmacological inhibition of COX-2 and sEH is a promising therapeutic strategy.

M33

12/15-LIPOXYGENASE DERIVED EICOSANOIDS ACTIVATE HUMAN RETINAL ENDOTHELIAL CELLS AND INDUCE DIABETIC RETINOPATHY PHENOTYPE

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Background: Diabetic retinopathy (DR) is characterized by early inflammatory response (leukostasis and hyperpermeability) followed by retinal neovascularization. Our previous studies established 12/15-lipoxygenase (12/15-LOX)-derived eicosanoids (12- and 15- hydroxyeicosatetraenoic acids or HETEs) as proangiogenic mediators during DR via disrupting the retinal levels of vascular endothelial growth factor and pigment epithelium derived factor. Here, we tested the hypothesis that NADPH oxidase and endoplasmic reticulum (ER) stress contribute to the inflammatory and angiogenic effects of the 12/15LOX-derived eicosanoids
Methods: Fluorescein Angiogram (FA) was used to characterize the effect of intravitreal injection of 12- HETE (1 μl containing 0.1μM) on retinal vascular function in living mice. Optical Coherence tomogram (OCT) was also used to evaluate retinal changes after HETE injection. Retina, were then collected for further assessment of glial cell activation by GFAP staining, and oxidative and inflammatory markers. Human retinal endothelial cells (HRECs) were used to examine the effect of 12 and 15-HETEs on leukocyte adhesion and angiogenesis *in vitro*. Effects of 12- or 15-HETE (0.1 μM) on HREC were tested in cells transfected with NOX2 siRNA or in the presence or absence of the inhibitors of NADPH oxidase or ER stress, apocynin, 30μM and 4-phenylbutyric acid (PBA, 5μM) respectively. Human leukocytes (PMNs) were labeled with lipophilic fluorescent probe for leukostasis assay. Tube formation and migration assays were performed to evaluate the angiogenic properties of 12- and 15-HETEs. Migration assay was performed using the Electrical Cell Impedance Sensor (ECIS). The levels of ER stress markers (BIP, PERK and IRE1) were detected with Western blotting. We also tested the effect of 12- and 15-HETEs on cytokines production by Multiplex assay system.
Results: FA and OCT showed marked increase in the inflammatory and angiogenic response to the intravitreal injection of 12-HETE as evidenced by marked vascular leakage and pre-retinal new vessel formation. These vascular changes were also associated with remarkable ganglion cell loss and glial cell activation as well as upregulation of the NADPH oxidase catalytic subunit (NOX2). 12 and 15-HETE increased endothelial cell migration, tube formation, leukostasis and ER stress ($P<0.05$). These effects were inhibited by the concomitant use of apocynin or PBA and by knocking down the NOX2. Furthermore, 12- and 15-HETEs significantly increased IL6, IL8, IL17 and MCP1 production in HRECs ($P<0.003$).
Conclusion: 12/15-LOX –derived eicosanoids 12 and 15-HETEs promote inflammatory and angiogenic response in HRECs via NADPH oxidase dependent mechanism which involves enhanced ER stress and cytokine production. Targeting 12/15LOX-NADPH-ER stress signaling system represents an attractive therapeutic strategy to treat DR.
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M34

ARG287GLN VARIANT OF *EPHX2* IS ASSOCIATED WITH FASTING TRIGLYCERIDES IN THE FRAMINGHM HEART STUDY

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Studies in rodents suggest that epoxyeicosatrienoic acids (EETs) protect against insulin resistance. EETs are hydrolyzed to less active diols by soluble epoxide hydrolase (*EPHX2*, human chromosome 8). An Arg287Gln (rs751141) variant of *EPHX2* encodes for an enzyme with decreased hydrolase activity. We have found that carriers of the 287Gln allele demonstrate increased insulin sensitivity during hyperglycemic clamps compared to Arg/Arg. To validate these findings in a general population, we examined the relationship between rs751141 genotype (MAF~10%) and three measures of insulin sensitivity in the Framingham Heart Study – fasting plasma glucose (FPG), high density lipoprotein cholesterol (HDL-C), and triglycerides (TRG) using linear mixed-effects models. SNP rs751141 was imputed (imputation $R^2=0.9$) from 550K Affymetrix variants (call rate >97%, MAF >1%, Hardy Weinberg Equilibrium $p >10^{-6}$ and Mendelian errors ≤1000) to the Integrated 1000G Phase I, Version 3 reference panel using miniMAC v 2012-08-15. Models were adjusted for age, age^2, body mass index (BMI), cigarette use, alcohol use, sex (or menopausal status and estrogen status), and principle components for population substructure. We also stratified analyses by gender or by the presence or absence of metabolic syndrome, defined using NCEP criteria. In the last model, we tested for an interaction between BMI and rs751141 genotype. Participants numbered 7172 (53% female) from the Offspring and Third Generation Cohorts. Measurements were taken from the first examination. Mean age was 38.0±9.7 years, BMI 26.2±5.1 kg/M^2, SBP/DBP 118.5±14.7/76.6±10.1 mmHg, HDL-C 52.8±15.6 mg/dL, TRG 202.8±200.8 mg/dL and FPG 97.8±15.5 mg/dL. In all subjects

combined, rs751141 287Gln allele was significantly associated with decreased log TRG in an unadjusted model (p=0.04), after adjustment for age and sex (p=0.014), and after full adjustment (p=0.007). In sex-stratified analyses, the relationship between rs751141 and log TRG was significant in women (p=0.021 in the fully adjusted model), but not men (p=0.145). Among individuals with the metabolic syndrome, there was no association between *EPHX2* rs751141 genotype and HDL-C, log TRG, or FPG. In those without metabolic syndrome, the loss-of-function variant 287Gln was associated with lower log TRG (p=0.0099) in those with normal BMI and there was an interactive effect of BMI and rs751141 genotype on log TRG (p=0.025). There was no association of rs751141 genotype and HDL-C or FPG. After adjustment for age and sex, the association between rs751141 genotype and log TRG and the BMI x genotype interaction remained significant in those without metabolic syndrome; these relationships were not significant in the fully-adjusted model. A loss-of-function variant of *EPHX2* is associated with decreased fasting TRG concentrations, consistent with enhanced insulin sensitivity, in the Framingham study.

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M35

CYP2C-DERIVED EETS CONTRIBUTE TO INSULIN SENSITIVITY IN MICE AND IN HUMANS

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Background: The P450 arachidonic acid epoxygenases (CYP2C, CYP2J) modulate blood pressure via renal and vascular effects, and interventions which prevent degradation of their epoxyeicosatrienoic acid products (EETs) via epoxide hydrolase inhibition may favorably alter glucose homeostasis. **Methods:** We tested the hypothesis that endogenous CYP2C-derived epoxyeicosatrienoic acids alter insulin sensitivity in vivo using *Cyp2c44*(-/-) mice during 16 weeks of high fat or regular chow diet. We assessed whole animal insulin sensitivity using hyperinsulinemic clamps and tissue insulin sensitivity using 2-deoxyglucose uptake. To examine this association in humans, we assessed insulin sensitivity and free plasma EETs during standardized dietary sodium intake in human subjects. **Results:** Despite similar body weight and adiposity, insulin sensitivity decreased in *Cyp2c44(-/-)* mice compared to wild-type (WT) controls (40.4±2.7 vs 57.8±3.8 mg/kg/min; p=0.003). High fat-fed WT and *Cyp2c44(-/-)* mice were both significantly insulin resistant compared to WT mice, but were similar between genotype. Hepatic glucose production during regular chow diet was similar at baseline, but was incompletely suppressed in *Cyp2c44(-/-)* mice during insulin-glucose infusion (-1.2±1.8 vs 6.4±0.6; p<0.05). Muscle and adipose tissue 14C 2-deoxyglucose uptake *in vivo* was diminished in *Cyp2c44(-/-)* mice. However, insulin-stimulated 2-deoxyglucose uptake was similar in isolated muscle *ex vivo*, suggesting that tissue perfusion may contribute to the defect. Vascular KATP-induced relaxation was impaired in isolated *Cyp2c44*(-/-) vessels *ex vivo*, suggesting that impaired vascular reactivity impairs insulin sensitivity *in vivo*. In human subjects, plasma EETs were positively associated with insulin sensitivity but not insulin secretion assessed during intravenous glucose tolerance tests (R^2=0.62, $p<10^{-4}$). **Conclusions:** These studies demonstrate that CYP2C-derived EETs contribute to insulin sensitivity in mice and in humans. Interventions which increase circulating EETs in humans could provide a novel approach to improve insulin sensitivity and treat hypertension.

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M36

EXPRESSION OF THE PROSTACYCLIN RECEPTOR (IPR) IS INVERSELY CORRELATED WITH HEMOGLOBIN A1c LEVELS IN PLATELETS OF HUMANS WITH TYPE 2 DIABETES

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Platelet aggregation is a necessary mechanism for hemostasis. However, excessive or inappropriate platelet aggregation can lead to the occlusion of blood vessels resulting in tissue ischemia. Platelets of humans with type 2 diabetes (DM2) synthesize augmented thromboxane and exhibit increased agonist (ADP, thrombin or collagen)-induced aggregation (hyperactivity) when compared to platelets of healthy humans. Activation of the platelet prostacyclin receptor (IPR) results in increases in platelet cAMP accumulation and inhibition of platelet aggregation. We hypothesized that platelets from humans with DM2 exhibit decreased IPR expression when compared to platelets of healthy humans, resulting in decreased IPR agonist-induced cAMP accumulation. Using ^{3}H-iloprost (a chemically stable prostacyclin analog) in IPR ligand binding studies, we identified a decreased number of IPR binding sites (Bmax) and decreased iloprost-induced cAMP accumulation in platelets of humans with DM2 when compared to platelets from healthy humans. These data differ from previously published studies in which no difference was reported for agonist binding to the IPR receptors of platelets obtained from healthy humans and humans with DM2. However, those studies assessed IPR ligand binding using the chemically unstable ^{3}H-prostacyclin as the agonist. To support the findings of the current IPR binding studies, Western blot analysis was used to identify IPR expression in platelets. In these studies we observed an inverse correlation between platelet IPR expression and the level of glycosylated hemoglobin (HbA1c), an index of glycemic control. These results suggest that

as glycemic controls worsens in humans with DM2 (increased HbA1c), platelet IPR expression decreases. Taken together, these findings suggest that reduced IPR expression in DM2 platelets may contribute to platelet hyperactivity.
(Supported by ADA and NIH grants)

M37

COMBINED THERAPY WITH COX-2 AND 20-HETE INHIBITOR REDUCES COLON TUMOR GROWTH AND THE ADVERSE STROKE EVENTS ASSOCIATED WITH COX-2 INHIBITION

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Background: Cyclooxygenase-2 (COX-2) selective inhibitors, coxibs, are potent anti-inflammatory agents. Although the reduction of cancer risk in colon polyp recurrence trials by coxibs is substantial, the increased risk of stroke and cardiovascular diseases by coxibs has led to the withdrawal of coxibs from the market. Therefore, the harmful side effects of coxibs -especially stroke- continue to be a barrier to their therapeutic usefulness. 20-Hydroxyeicosatetraenoic acid (20-HETE), CYP4A-derived eicosanoid, is a lipid mediator that promotes tumor growth as well as causing detrimental effects in cerebral circulation. **Purpose:** In this study, we determined whether the combination of cyclooxygenase-2 (COX-2) and 20-HETE inhibition affects colon tumor growth and ischemic stroke outcomes. **Research Design:** The expression of CYP4A and COX-2 and production of 20-HETE and PGE_2 were determined in murine colon carcinoma (MC38) cells. We then examined the effects of combined treatment with rofecoxib, a potent COX-2 inhibitor, and HET0016, a potent CYP4A inhibitor, on the growth and proliferation of MC38 cells. Subsequently, we determined the effects of HET0016 + rofecoxib in colon tumor and ischemic stroke models. **Results:** CYP4A and COX-2 are highly expressed in MC38 cells. Respectively, HET0016 and rofecoxib inhibited 20-HETE and PGE_2 formation in MC38 cells. Moreover, rofecoxib + HET0016 treatment had greater inhibitory effects on the growth and proliferation of MC38 cells than did rofecoxib alone. Importantly, rofecoxib + HET0016 treatment provided greater inhibition on tumor growth than did rofecoxib alone in MC38 tumor-bearing mice. In MC38 tumor mice, prolonged rofecoxib treatment (50 mg/L in drinking water for 3 weeks) selectively increased circulating 20-HETE levels by 50% without affecting the levels of other eicosanoids including EETs/DHETs, 5-HETE, 8-HETE, 11-HETE, and 15-HETE. Furthermore, therapy with rofecoxib (50 mg/L) + HET0016 (5 mg/kg/day, ip) attenuated 20-HETE levels. In MC38 mice, rofecoxib increased vascular injury (bleeding and edema) in thromboembolic stroke and HET0016 attenuated the side effects induced by rofecoxib in ischemic stroke. **Conclusion:** These results demonstrate that combination therapy with rofecoxib and HET0016 provides a new treatment of colon cancer, which can not only enhance the anti-cancer efficacy of rofecoxib, but also reduce the adverse stroke events of rofecoxib.
(Grant support: AHA Grant-in-Aid (AHASE0054)).

M38

INHIBITION OF SOLUBLE EPOXIDE HYDROLASE ATTENUATES FIBROSIS AND ENDOPLASMIC RETICULUM STRESS IN A CARBON TETRACHLORIDE INDUCED MODEL OF LIVER DAMAGE

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Liver fibrosis is a pathological condition in which chronic inflammation and changes to the extracellular matrix lead to alterations in hepatic tissue architecture and functional degradation of the liver. Inhibitors of the enzyme soluble epoxide hydrolase (sEH) reduce fibrosis in the heart, pancreas and kidney in several disease models. In this study, we assess the effect of sEH inhibition on the development of fibrosis in a carbon tetrachloride (CCl4)-induced mouse model by monitoring changes in the inflammatory response, matrix remolding and endoplasmic reticulum stress. The sEH inhibitor 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU) was administered in drinking water. Collagen deposition in the liver was increased five-fold in the CCl_4 treated group, and this was returned to control levels by TPPU treatment. Hepatic expression of Col1a2 and 3a1mRNA was increased over fifteen-fold in the CCl_4-only group relative to the control group, and this increase was reduced by 50% by TPPU treatment. Endoplasmic reticulum stress observed in the livers of CCl_4-treated animals was attenuated by TPPU treatment. Taken together, these data indicate that the sEH inhibition may represent a promising tool to study the effect of systemic modulation of oxylipids on some of the pathological states associated with liver fibrosis.
Funding: This work was supported by US National Institute of Environmental Health Sciences (NIEHS) Grant R01 ES002710 (to B.D.H.), NIEHS Superfund Basic Research Program P42 ES004699, NIH Grant T32 HL86350 (to T.R.H.). B.D.H. is a George and Judy Marcus Senior Fellow of the American Asthma Foundation.

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POSTER SESSION III
TUESDAY, MARCH 11, 2014
Grand Ballroom West/DEF (Lobby Level)
5:15-6:45 PM

MECHANISM-STRUCTURE-GENETICS

T1

ADVANCED INHIBITORS OF THE PROSTAGLANDIN (PG) TRANSPORTER PGT AND THEIR POTENTIAL THERAPEUTIC APPLICATION

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Metabolic inactivation of prostaglandin E2 (PGE2) occurs by the two-step sequential process of carrier-mediated uptake across the plasma membrane, which is mediated by the PG transporter PGT, followed by oxidative inactivation at the 15-hydroxyl position by the cytoplasmic enzyme 15-HPGD. PGT-mediated uptake is rate limiting for PGE2 metabolic inactivation. Mice that are null at the PGT locus, once rescued through the neonatal period, are normal (Chang et al, *Circulation*, 2010) and human PGT nulls have only mild skin and skeletal disorders (Diggle et al, *Hum Mutat*, 2012). Because PGs are useful therapeutically, we have focused on PGT as a therapeutic drug target. We have previously reported on two rounds of PGT inhibitors. The initial round of library screening yielded a lead compound, TGBz T34, which is a competitive PGT inhibitor with a Ki of 3.7 μM (Chi et al, *JPET*, 2006). Our second round, structure-activity-relationship (SAR) study built upon T34 and yielded a more potent inhibitor, T26A, with a Ki of 378 nM (Chi et al, *JPET*, 2011). T26A has a triazine core connected to three side chains, R1, R2 and R3. R1 contains an azide group. Because the azide group is medicinally suboptimal, and because the potency of T26A is still only moderate, we decided to further improve the potency of PGT inhibitor and its applicability as a potential therapeutic agent. We rationally designed derivatives of T26A and measured their inhibitory activities. Out of 151 compounds that we synthesized and tested, 26 compounds have an IC50 lower than 100 nM. One of the most potent inhibitors is PV02076, a Na+ salt with an IC50 of 44.8 nM. Pharmacokinetics analysis indicates that single intravenous bolus PV-02076 has a t1/2 of 0.410 ± 0.10 (SD) hrs, whereas subcutaneous PV-02076 has a t1/2 of 6.97 ± 1.42 (SD) hrs. Indeed, in a rat model of pulmonary hypertension (monocrotaline injection), twice-daily subcutaneous PV-02076 significantly ameliorated the increase in right ventricular pressures. We conclude that PV-02076 has significant potential as a medicinal prototype in conditions such as pulmonary hypertension, glaucoma, vascular insufficiency, and others.
NIH RO1-DK-49688, American Soc of Nephrology, American Heart Association 0735066N and American Diabetes Association 1-11-JF-06

T2

ELUCIDATING THE MOLECULAR INTERACTIONS OF DIETARY LIPIDS AND ENDOCANNABINOIDS WITH CYP 2J2 EPOXYGENASE-NANODISCS

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CYP2J2 is the predominant epoxygenase expressed in the human heart. It synthesizes four regioisomers of epoxyeicosatrienoic acids (EET) from arachidonic acid that are implicated in inflammation, cardiovascular disease and cancer. The interactions of CYP2J2 with lipids and drugs are quite complex and can have a significant effect on the observed kinetics of lipid metabolism thereby influences disease states. In the present work, we investigate the binding interactions and metabolism of arachidonic acid (AA) and two AA derived endocannabinoids - anandamide (AEA) and 2-arachidonoylglycerol (2-AG) by CYP2J2. We use Nanodiscs (nanoscale lipid bilayers) to solubilize and stabilize membrane bound CYP2J2 *in vitro* for these studies. We elucidate the nuances of ligand-protein interactions using spectral titrations, small molecule ligand egress with stopped-flow spectroscopy, molecular modeling and LC-MS. The binding study shows that simply by changing the residue at the carboxylic acid end of AA to ester (in 2-AG) or amide (in AEA) changes the binding interaction of these lipids with CYP2J2 active site. While AA and AEA binding to CYP2J2 produced a type II binding spectrum (red shift in the Soret), binding of 2-AG produces type I binding spectrum (blue shift in the Soret). Furthermore molecular modeling using MOE confirmed that this carboxylic acid site indeed plays an important role in docking of the substrate molecule to the active site. Additionally, we show that the presence of AA, AEA or 2-AG in the CYP2J2 active site can induce conformational change in the protein that blocks the small molecule ligand egress. While the binding of the substrate is an indication of interaction but that does not imply that the transition state is conducive to forming desired products. Therefore we further studied the metabolism and kinetics of product formation using LC-MS. Reactions of CYP2J2 with AEA formed four AEA-epoxide products (EET-EA) where the predominant product was 14(15)-EET-EA. Interestingly, incubations of 2-AG with CYP2J2 yielded detectable levels of only two 2-AG epoxides (EET-G) and considerable amount of free AA, glycerol and EETs in solution, that are products of oxidative ester hydrolysis of 2-AG. This shows that CYP2J2 not only metabolizes 2-AG but also provides an alternative pathway for its hydrolysis. In summary, we successfully identified a cardiovascular CYP that binds and metabolizes both AEA and 2-AG as well as oxidatively cleaves the 2-AG ester, effectively attenuating 2-AG activity. Given the

mounting evidence that the cannabinoids and PUFA play important physiological roles in the cardiovascular system, our investigation of the interactions of the lipids with heart CYP2J2 suggests potential cross talk between the endocannabinoind metabolism and lipid metabolism pathways in the heart.

T3

PREEXISTENT ASYMMETRY IN THE HUMAN CYCLOOXYGENASE-2 SEQUENCE HOMODIMER

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Prostaglandin endoperoxide H synthase-2 (PGHS-2) also known as cyclooxygenase-2 (COX-2) is a sequence homodimer that functions as a conformational heterodimer having E_{allo} and E_{cat} subunits. E_{cat} but not E_{allo} binds heme with high affinity. Here we describe studies to delineate interactions unique to E_{allo} vs. E_{cat} that underlie allosteric regulation of PGHS-2 by fatty acids (FAs) and COX inhibitors. A Y385F/Native human (hu) PGHS-2 heterodimer was developed as a platform for these studies. Monomers with the Tyr385Phe mutation cannot catalyze the COX reaction and can only function as E_{allo} subunits. Native/Y385 huPGHS-2 when tested with AA has similar COX kinetic properties and forms the same products as the Native/Native huPGHS-2 homodimer. For this to occur, Native/Y385 huPGHS-2 must become lodged in a form in which the Y385F monomer is E_{allo} and the Native monomer is E_{cat}. In studying mutants of Y385F/Native huPGHS-2, we established that FAs (including AA) interact differently with the active sites Arg-120 of E_{allo} and E_{cat}. Binding of AA to Arg-120 of E_{cat} involves an ionic interaction. FA interactions with Arg-120 of E_{allo} affect the V_{MAX} and not the K_M for AA. Both allosteric modulation and half-sites activity depend on FA binding to Arg-120 of E_{allo}. We also envisioned that ligand-induced stabilization enables such heterodimers to become lodged in a catalytically competent [E_{allo}-Mutant-FA/E_{cat}-Native-heme] form. Specifically, we hypothesized that the A and B monomers comprising a PGHS-2 dimer normally flux between two E_{allo}/E_{cat} forms (i.e. [E_{allo}-Native-A/E_{cat}-Native-B] <-> [E_{cat}-Native-A/E_{allo}-Native-B]), and that heme and/or FAs that bind E_{allo} and/or E_{cat} stabilize the dimer and slow or prevent the flux. To test this hypothesis, we characterized a number of recombinant heterodimers. Studies of aspirin acetylation with one particular variant—S530A/Native huPGHS-2—led us to the conclusion that apo-PGHS-2 actually assumes a stable conformational heterodimeric form relatively early in their lifetimes—during their folding and processing—and do not undergo ligand induced fluxes between E_{allo} and E_{cat} forms.

This work was supported in part by grants from the National Institutes of Health GM68848, CA130810 and HL117798

T4

PGE2 INHIBITS PULMONARY FIBROBLAST DIFFERENTIATION VIA MODULATION OF A MECHANO-SENSITIVE ION CHANNEL, TRPV4

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Pulmonary fibrosis is a progressive interstitial lung disease characterized by fibroblast proliferation and differentiation of fibroblasts to myofibroblasts as determined by expression of α-SMA in stress fibers, increased synthesis of collagen and extracellular matrix proteins. Prostaglandin E_2 (PGE_2), a derivative of arachidonic acid is known to attenuate TGFβ-mediated differentiation of fibroblasts and reverse fibrosis in the lung tissue. However, the molecular mechanism by which PGE_2 exerts this protective effect is not clear. In the present study, we investigated if PGE_2 mediates anti-fibrotic effects through the modulation of a mechanosensitive TRPV4 channel recently implicated in cardiac fibroblast differentiation to myofibroblasts. We found that primary normal human lung fibroblasts (NHLF) express all PGE_2 receptors, $EP_{1\text{-}4}$ with EP_2 as the predominant receptor. Importantly, PGE_2 treatment reversed TGFβ-induced differentiation of NHLF through EP_2 receptor. Interestingly, we found that an antagonist of TRPV4, AB159908 (AB1), significantly inhibited TGF-β-induced differentiation of NHLF to myofibroblasts, similar to PGE_2. Further, PGE_2 or TRPV4 inhibition reduced the TGF-β-induced expression of trans-differentiation markers like sm22, Collagen1A1 and MRTF1 transcripts. Surprisingly, however, combined treatment of PGE_2 together with TRPV4 inhibitor had no additive effect on TGFβ-induced fibroblast differentiation. Taken together these results suggest that PGE_2 mediates its anti-fibrotic effects through the modulation of TRPV4 signaling and unravel a novel eicosanoid dependent regulation of mechanical signaling. This could offer potential therapeutic strategies for pulmonary fibrosis.

This work is supported by University of Akron start-up funds and James Foght Assistant Professor support.

T5

GENERATION AND INITIAL CHARACTERIZATION OF EPOXIDE HYDROLASE 3 (EPHX3)-DEFICIENT MICE

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Epoxide hydrolases are a family of enzymes that hydrolyze epoxyeicosanoids such as epoxyeicosatrienoic acids (EETs) to their corresponding diols, the dihydroxyeicosatrienoic acids (DHETs). Soluble epoxide hydrolase (EPHX2) is a well-characterized member of this family. EETs are products of cytochrome P450 epoxygenase metabolism of arachidonic acid, which control blood pressure, post-ischemic heart function, angiogenesis, and inflammation. Another epoxide hydrolase, EPHX3, was recently identified

by sequence homology. Previous *in vitro* studies have shown that Ephx3 also exhibits hydrolase activity with a high efficiency for 9,10-EpOME, followed by 8,9-EET, 11,12-EET and 14,15-EET. Quantitative RT-PCR indicated that *Ephx3* is highly expressed in the skin, lung, stomach, esophagus, and tongue; however, the endogenous functions of EPHX3 remain unknown. Therefore, we developed global EPHX3 knockout (KO) mice to analyze the impact of genetic disruption of *Ephx3* in the mouse. EPHX3 KO mice were generated by deleting the promoter and first four exons of the *Ephx3* gene using standard methodology. Analysis of the lung, skin, stomach, esophagus and tongue mRNAs by quantitative RT-PCR indicated that there was > 96% reduction in expression of *Ephx3* in the KO mice relative to wild type (WT) controls. Quantitative RT-PCR also showed that disruption of *Ephx3* did not alter expression of other epoxide hydrolases (*Ephx1*, *Ephx2*, and *Ephx4*) or cytochrome 450 epoxygenases in these tissues. Initial characterization showed that body weight:organ weight ratios were similar between EPHX3 KO mice and WT controls. Moreover, reproductive capacity was normal and there were no overt abnormalities in the EPHX3 KO mice. Since prior studies showed that EPHX3 is capable of fatty acid epoxide hydrolysis *in vitro*, we determined if EPHX3 KO mice exhibited altered endogenous fatty acid epoxide or diol levels. LC/MS/MS analysis of EPHX3 KO heart, lung, and skin lysates revealed no differences in endogenous epoxide:diol ratios compared to WT. Plasma levels of fatty acid epoxides and diols were also comparable between EPHX3 KO and WT mice. Finally, incubations of stomach, lung, and skin lysates with synthetic 8,9-EET, 11,12-EET, and 9,10-EpOME revealed no significant differences in rates of fatty acid diol formation between the genotypes. We conclude that loss of EPHX3 does not produce an overt phenotype and has no significant effects on the metabolism of fatty acid epoxides *in vitro* or *in vivo*.

T6

EPOXY FATTY ACIDS AND INHIBITION OF THE SOLUBLE EPOXIDE HYDROLASE SELECTIVELY MODULATE GABA MEDIATED NEUROTRANSMISSION TO DELAY ONSET OF SEIZURES

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In the brain, seizures lead to release of large amounts of polyunsaturated fatty acids including arachidonic acid (ARA). ARA is a substrate for three major enzymatic routes of metabolism by cyclooxygenase, lipoxygenase and cytochrome P450 enzymes converting PUFAs to potent lipid mediators including prostanoids, leukotrienes and epoxyeicosatrienoic acids (EETs). The prostanoids and leukotrienes are largely pro-inflammatory molecules that sensitize neurons whereas EETs are anti-inflammatory and reduce the excitability of neurons. Recent evidence suggests a GABA-related mode of action potentially mediated by neurosteroids. Here we tested this hypothesis using models of chemically induced seizures. The level of EETs in the brain was modulated by a) inhibiting the soluble epoxide hydrolase (sEH), the major enzyme that metabolizes EETs to inactive molecules, b) by genetic deletion of sEH and c) by direct administration of EETs into the brain. All these approaches delayed onset of seizures and prevented lethality instigated by GABA antagonists but not seizures through other mechanisms. Inhibition of neurosteroid synthesis by finasteride partially blocked the anticonvulsant effects of sEH inhibitors while the efficacy of an inactive dose of neurosteroid allopregnanolone was enhanced by sEH inhibition. Consistent with earlier findings, levels of prostanoids in the brain were elevated following seizures. In contrast EET levels were decreased. Overall these results demonstrate that EETs are natural molecules which suppress the tonic component of seizure related excitability through modulating the GABA activity and that exploration of the EET mediated signaling in the brain could yield alternative approaches to treat convulsive disorders.

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T7

DIETARY OMEGA-3 FATTY ACIDS MODULATE THE EICOSANOID PROFILE IN MAN PRIMARILY BY ENHANCING EPA-DERIVED EPOXYGENASE METABOLITES

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Cytochrome P450 (CYP) enzymes initiate the third branch of the arachidonic acid (AA 20:4 n-6) cascade and their metabolites contribute to the regulation of cardiovascular function. Various CYP isoforms also accept eicosapentaenoic acid (EPA 20:5 n-3)) and docosahexaenoic acid (DHA 22:6 n-3) to yield more potent vasodilatory and potentially antiarrhythmic metabolites, suggesting that the endogenous CYP-eicosanoid profile can be favorably shifted by omega-3 fatty acid-rich diets. To test this hypothesis, 20 healthy volunteers were treated with an EPA/DHA-supplement and analyzed for concomitant changes in the circulatory and urinary levels of AA-, EPA-, and DHA-derived metabolites produced by the cyclooxygenase (COX)-, lipoxygenase (LOX)- and CYP-dependent pathways (EudraCT: 2009-013458-33). The participants ingested 1 Omacor® capsule (480 mg EPA + 360 mg DHA) daily for the first 4 weeks, and two capsules daily in the subsequent 4 weeks, followed by 8 weeks of wash-out. Raising the Omega-3 Index (EPA + DHA as percentage of total fatty acids in red blood cells) from about 4 at baseline to 8 after maximal EPA/DHA supplementation primarily resulted in a large increase of EPA-derived CYP-epoxygenase metabolites followed by increases of EPA- and DHA-derived hydroxy-metabolites including the precursors of resolvin E (18-HEPE) and D families (17-HDHA). The obtained metabolite/precursor fatty acid ratios indicated that EPA was metabolized via the CYPepoxygenase pathway with an 8.6-fold, and DHA with a 2.2-fold higher efficiency than AA. In contrast, effects on leukotriene, prostaglandin E as well as prostacyclin and thromboxane formation remained rather weak or were not expressed at all in response to the applied relatively low, but recommended, cardioprotective dose of the EPA/DHA-supplement. In conclusion, our data show that the CYP-epoxygenase pathway was most responsive to dietary EPA/DHA-supplementation compared to the COX- and LOX-initiated pathways of

eicosanoid formation. We propose that CYP-dependent epoxy-metabolites of EPA and DHA may function as mediators of the vasodilatory and cardioprotective effects of omega-3 fatty acids and could serve as suitable biomarkers in clinical studies investigating the cardiovascular effects of omega-3 fatty acid supplementation.

T8

EFFECT OF INTRAVENOUS ADMINISTRATION OF A SOLUBLE EPOXIDE HYDROLASE INHIBITOR T-TUCB ON P450 DEPENDENT EICOSANOID CONCENTRATIONS IN PND 17 RATS BRIAN

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Introduction Hypoxic ischemic encephalopathy (HIE) occurs after resuscitation from pediatric cardiac arrest (CA) in 50-76% of patients. We have shown that cerebral blood flow is significantly reduced post-CA potentially leading to secondary neuronal damage. Epoxyeicosatrienoic acids (EETs) play a critical role in vasodilation thus regulating the cerebral blood flow. Recently our group validated a model of HIE in the immature post-natal day (PND) 17 rats, and observed decreased EET levels post-CA in this model. Our goal is to explore the role of EETs in the pathogenesis of HIE. The optimal dose of soluble epoxide hydrolase inhibitors necessary to increase EET production in the immature brain is unknown. **Objective** The purpose of this study was to determine the dose of t-TUCB needed to alter cerebral EETs and DiHETEs levels in PND17 rats. **Methods** t-TUCB was formulated with 10% PEG 400 and 20% Ethanol, dosed at 5mg/kg to PND 17 rats intravenously. Rats were sacrificed at 2, 6 and 24 hours after iv infusion (n=4/time point). Plasma and brain were collected to detect t-TUCB concentration using UPLC/MS/MS. EETs and DiHETE concentrations in the brain were also determined by an established UPLC/MS/MS assay. All results were compared with vehicle treated rats. **Results** t-TUCB concentration at 2, 6, 24 hours were 2465.42±370.39, 3256.50±235.66, 685.19±63.54 ng/mL (mean±SD) in the plasma and 872.36± 174.80, 815.44± 341.12, 82.52± 24.77 ng/mL in the brain. At 2 and 6 hours, concentration of t-TUCB in the brain was 20 times higher than IC50 value. Cerebral 11, 12-EET concentrations at 2 and 24 hours were higher (6.5±3.3 and 7.0±1.7 pmol/g tissue) than vehicle group (2.1±0.8 pmol/g tissue), $p<0.05$. Cerebral 14, 15-EET concentration at 2 hours was also higher (6.8±2.3 vs. 2.7±1.4 pmol/g tissue in vehicle group, $p<0.05$). 14, 15-DiHETE in all three treatment groups were significant lower than vehicle group (0.8±0.2, 0.9±0.4, 1.0±0.3 vs. 2.1±0.6 pmol/g tissue, $p<0.01$). Additionally, the ratios of 11, 12-EET/11, 12-DiHETE increased 4-5 times at all three time points, and 14, 15-EET/ 14, 15-DiHETE increased 4-6 times at all three time points ($p<0.05$). **Conclusion** In this in study, intravenous administration of t-TUCB increased EETs level while decreased DiHETEs level. Future investigation is necessary in optimization of the formulation and application to relevant injured animal models.

Support: PRISE, American Heart Association (VN), NIH R01HD075760 (MDM), University of Pittsburgh CMRF (MDM).

T9

UNBIASED RNA-SEQ ANALYSIS OF GENES REGULATED BY THE PROSTAGLANDIN TRANSPORTER PGT

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Metabolic inactivation of prostaglandin E2 (PGE2) occurs by the two-step sequential process of carrier-mediated uptake across the plasma membrane, which is mediated by the PG transporter PGT, followed by oxidative inactivation at the 15-hydroxyl position by the cytoplasmic enzyme 15-HPGD. PGT-mediated uptake is rate limiting for PGE2 metabolic inactivation. Mice rendered null (knockout, KO) at either the 15-PGDH locus or the PGT locus, once rescued through the neonatal period, are normal (Coggins et al, *Nature Med*, 2002; Chang et al, *Circulation*, 2010). In contrast, human males (but not females) who are null at either the 15-HPGD locus or the PGT locus exhibit a condition variably known as "primary hypertrophic osteoarthropathy", "hypertrophic osteoarthropathy with clubbing", or "pachydermoperiostosis". This condition is characterized by thickened skin of the tips of the fingers and toes ("clubbing"), thickened forehead and scalp, and calcification of the periosteum (the fibrous sheath encasing long bones) (Uppal et al, *Nature Genet*, 2008; Tariq et al, *J Med Genet*, 2009; Yuksel-Konuk et al, *Rheum Int*, 2009; Diggle et al, *Rheumatol*, 2010; Bergmann et al, *Exp Derm*, 2011; Diggle et al, *Hum Mut*, 2012; Busch et al, *J Invest Derm*, 2012; Sasaki et al, *J Derm Sci*, 2012; Seifert et al, *Hum Mut*, 2012; Zhang et al, *Am J Hum Genet*, 2012). Because the phenotypic changes in humans null for 15-HPGD or PGT are in mesenchymal/mesodermal pathways, and because of the striking sex-dependence in humans, we have returned to the mouse to investigate further. We harvested ~13 day embryos, and generated mouse embryonic fibroblasts (MEFs), from male and female mice that were wild type or PGT KO. Total RNA was extracted and treated with DNase-I and ribosomal RNA was removed; then RNAseq was performed, i.e. mRNA was fragmented, converted to cDNA, primed, sequenced, and analyzed. Fold changes of genes was analyzed using the “R” program. GeneVenn and Ingenuity pathway analysis ("IPA") were used in tertiary data analysis. A list of genes that have two-fold changes (wt vs PGT KO) was generated. These numbered 3,027 in male and 1,013 in female MEFs; GeneVenn analysis showed that only 350 of these PGT-regulated genes were common to males and females. Preliminary confirmation has been carried out using qPCR. Genes involved in bone development exhibited significant gender dependence: 177 genes in males, 20 in female, and 11 in both were involved in osteoblast, osteocyte, and osteoclast development. We conclude that PGT regulates mesenchymal/mesodermal genes in mice, and does so differently in males and females. Further pursuit of these genes in the mouse model, and of the involved mechanisms, will likely lead to insights into the bone and soft tissue phenotypes in male humans who carry disruptions in the genes for PG inactivation.

NIH RO1-DK-49688, American Soc of Nephrology

T10

STEREOSELECTIVITY OF ARACHIDONATE OXYGENATION: THE ROLE OF PHE-205 IN ASPIRIN-ACETYLATED CYCLOOXYGENASE-2

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Non-steroidal anti-inflammatory drugs (NSAIDs) are inhibitors of the cyclooxygenases (COX-1 and -2), which generate prostaglandins (PGs) from arachidonic acid (AA). Despite high sequence and structural similarity between the isozymes, research has shown that the classically "nonselective" inhibitor aspirin (ASA) exerts a differential effect. Ser-530 acetylation following ASA treatment leads to complete inhibition of COX-1; however, acetylation of COX-2 leads to a shift of the product profile from generation of primarily 15*S*-prostaglandin H2 (PGH2) to that of 15*R*-hydroxyeicosatetraenoic acid (15*R*-HETE). 15*R*-HETE, in turn, is a precursor in the production of 15-epi-lipoxins, which are inflammation-resolving lipid mediators. Molecular dynamics studies suggest that the product shift in stereochemistry and relative abundance may be the result of the steric environment within the active site. The covalently modified Ser-530 is proposed to force AA to adopt an altered conformation and enhance the shielding on the side of AA opposite the catalytic Tyr-385 (i.e. antarafacial face). The altered AA conformation and local shielding from the acetyl group is such that the native trajectory of the initial oxygen attack is disrupted, allowing an alternate attack on the suprafacial face at carbon 15 to occur. The molecular dynamics studies further demonstrate that this attack is likely to occur from a small pocket bordered by Phe-205, Thr-206, and the catalytic Tyr-385. In order to assess this steric shielding hypothesis, we have generated a set of Phe-205 mutant COX-2 proteins that will alter the nature of the proposed secondary oxygen binding pocket. The mutants have been subjected to kinetic analysis and treatment with ASA and our initial findings indicate that there is a trend between increased polarity at position 205 and decreased sensitivity of oxygenation rate to aspirin treatment. A report of additional progress to date is presented.

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T11

CYP-13A12 OF THE NEMATODE *C. ELEGANS* IS A PUFA-EPOXYGENASE AND INVOLVED IN THE BEHAVIORAL RESPONSE TO REOXYGENATION

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Oxygen deprivation followed by reoxygenation causes pathological responses in many disorders, including ischemic stroke, heart attacks, and reperfusion injury. Studies of the nematode *C. elegans* led to the discovery of an evolutionarily conserved family of O2-dependent enzymes (EGL-9 in *C. elegans* and EGLN2 in mammals) that hydroxylate the HIF transcription factor and link hypoxia to HIF-mediated physiological responses. EGL-9 inactivation blocks *C. elegans*' behavioral response to reoxygenation (after anoxia), characterized by a rapidly increased locomotion speed, called the "O2-ON response". Moreover, C20-PUFA-deficient, Δ-12 and Δ-6 fatty acid desaturase mutants (*fat-2* and *fat-3*, respectively) exhibited a complete lack of the O2-ON response. A suppressor screen for mutations that can restore the defective O2-ON response in *egl-9* mutants identified a gain-of-function allele of the *cyp-13A12* gene. The *cyp-13A2* gene encodes a cytochrome P450 protein closely related to human CYP3A4. In the present study, we show that the defective O2-ON response of *fat-2* mutants can be restored by feeding the nematodes AA or EPA, but not ETYA, a non-metabolizable AA-analog. Long-term treatment with AA, but not ETYA, also rescued the impaired locomotion of the *fat-3* strain as detectable already under normoxic conditions. These results indicated an essential role of PUFA derived metabolites for the O2-ON response and the locomotion behavior of *C. elegans*. Co-expression of CYP-13A12 with the *C. elegans* CPR gene (*emb-8*) in insect cells resulted in the reconstitution of an active microsomal monooxygenase system that metabolized EPA (and also other PUFAs) to a specific set of regioisomeric epoxy- and hydroxy-derivatives. The main product was 17,18-epoxyeicosatetraenoic acid (EEQ). Already short-term incubation with **17,18-EEQ** (20 min) was sufficient to rescue the impaired locomotion of the *fat-3* strain. LC-MS/MS analysis revealed that wild-type nematodes produce 17,18-EEQ as the major CYP-eicosanoid under normoxic conditions. The endogenous 17,18-EEQ levels declined during anoxia and were rapidly restored in response to reoxygenation. Based on these results, we suggest that CYP-dependent eicosanoids such as 17,18-EEQ function as signaling molecules in the regulation of the O2-ON response of *C. elegans*. Moreover, these signaling molecules are also required under normoxic conditions as indicated by the impaired locomotion of the *fat-2* and *fat-3* mutant strains that completely lack these metabolites.

T12

FINDING A NON-TOXIC SUBSTITUTE FOR VALPROIC ACID FOR TREATING BIPOLAR DISORDER

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Background: FDA-approved mood stabilizers, lithium, carbamazepine and valproate (VPA), chronically given to rats at therapeutically doses relevant to bipolar disorder (BD), downregulate arachidonic (AA, 20:4n-6) but not docosahexaenoic (DHA,

22:6-n-3) or palmitic acid (16:0) turnover in brain phospholipids, cyclooxygenase (COX)-2 activity and prostaglandin E2 (PGE2) concentration. Lithium and carbamazepine but not VPA also reduce brain calcium-dependent cytosolic phospholipase A2 (cPLA2), which selectively releases AA from membrane phospholipid. VPA is teratogenic, and is not recommended for women of childbearing age or pregnant. VPA uncompetitively inhibits *in vitro* recombinant acyl-CoA synthetase (Acls)-4 activity, which selectively activates AA to AA-CoA in AA recycling within brain. **Hypothesis.** Acls-4 inhibition may account for VPA's reduction of rat brain AA turnover *in vivo*, and VPA's efficacy against BD. **Methods.** We used Michaelis-Menten kinetics to test whether nonteratogenic VPA structural analogues could inhibit recombinant Acls-4 in vitro. We studied propylisopropylacetic acid (PIA), propylisopropylacetamide (PID), *N*-methyl-2,2,3,3-tetramethylcyclopropanecarboxamide (MTMCD), and 2-Ethyl-3-methylpentanamidevalnoctamide (valnoctamide, VCD), in addition to VPA. Recombinant rat Acsl4-flag protein was expressed in *E. coli*. **Results**: Acsl-4-mediated conversion of AA to AA-CoA was inhibited uncompetitively by PIA and VCD with a Ki of 11.4 and 6.38 mM respectively, compared to a Ki of 25 mM for VPA, while PID, MTMCD and sodium butyrate had no inhibitory effect. VPA uncompetitively inhibited Acsl-4 activation of DHA less effectively than of AA; palmitate was not activated. **Conclusions:** PIA and VCD but not PID, MTMCD or sodium butyrate selectively inhibited AA conversion to AA-CoA by recombinant Acsl-4, with K_i's less than for VPA. If VCD like VPA can inhibit AA turnover in brain phospholipids of unanesthetized rats, as well as COX-2 activity and PGE2 concentration, VCD might be considered further as a potential nonteratogenic mood stabilizer for treating BD.

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T13

IDENTIFICATION OF INITIAL LEAD COMPOUND TARGETING INFLAMMATORY PGE2 RECEPTOR BY INTEGRATED VIRTUAL AND HIGH THROUGHPUT SCREENING.

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Prostaglandin E2 (PGE2) synthesized by inducible cyclooxygenase-2 (COX-2) and microsomal PGE2 synthase-1 (mPGES-1) acts as a ligand and exerts its pro-inflammatory and carcinogenic action by binding on a family of four subtype PGE2 receptors, named EP1, EP2, EP3 and EP4. Recently, our study has showed that EP1 is likely one of the major EPs mediating PGE2's the pathological processes. Targeting EP1 could be a novel approach to develop specific drugs against inflammation and cancers. However, no therapeutic drugs have been discovered to inhibit EP1 yet. In this study, virtual and high throughput screening (HTS) were integrated to search for novel lead compounds inhibiting EP1 activity. 3D-structural model of human EP1 was created by homology modelling using X-ray structure of beta2 (β2) receptor as a template. Virtual screenings against selected 300,000 phenyl compounds were performed as an initial step. Secondary, a HEK293 cell line stably expressing recombinant human EP1 was successfully created, maintained and used as a target for cell based HTS. Finally, the four hundreds of the screened compounds showing top-binding possibility from the virtual approach were subjected to the cell-based HTS using highly reliable and sensitive isotope-labelled ligand, [^{3}H]-PGE2. The radioisotope screening assay confirmed that 32 of the four hundreds compounds were able to reduce the [^{3}H]-PGE2 binding to the EP1 stable cell line. Dose response curves showed that the top three of the 32 compounds have a range of the IC50 value of approximately 2-3 microM. These initial findings revealed that the three compounds with better binding affinities for EP1 could be used as potential candidates for developing antagonist to block the PGE2's pathological activity. Further inhibition and signalling assay will be required for their use as lead compound to be designed and modified into novel drugs for anti-inflammatory and cancer therapy.

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T14

A NOVEL ROLE OF MICROSOMAL EPOXIDE HYDROLASE IN THE CONTROL OF EICOSANOIDS

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The CYP450 epoxigenase CYP2J5 is an ER resident, heme-containing monoxygenase that is mainly known to turn over arachidonic acid (AA) to epoxyeicosatrienoic acids (EETs). The product is a mixture of four *cis*-regioisomers: 5,6-, 8,9-, 11,12- and 14,15 EET. The microsomal epoxide hydrolase (mEH) is also an ER-resident enzyme mainly recognized as an enzyme involved in the detoxification of genotoxic epoxides produced during the metabolism of xenobiotics, such as 1,3-butadiene, styrene oxide and BaP-7,8-epoxide. However, it has been shown that it can turn over EETs to dehydroeicosatrienoic acids (DHETs). The mEH is expressed in extrahepathic tissue. In the brain it was been shown to be expressed in a cell-specific manner, mainly brain vascular cells, choroid plexus and hippocampus. All four EET regioisomers are substrates for the enzyme. A hypotheis of EET modulation is the rapid conversion to DHETs by the mEH. The potential mechanism by which mEH and CYP transfer epoxides is unknown. The possible interaction between these enzymes was studied using intermolecular FRET, which consists in measuring the energy transfer from the donor cyan fluorescent protein (CFP) to the acceptor yellow FP (YFP) when they are in close proximity (<10nm) due to the interaction of proteins labeled with these FPs. Mouse mEH and CYP2J5 were labeled with CFP and YFP, respectively, via the N-terminal ER membrane anchor and cloned into a polycistronic construct in which the expression of the second cistron (mEH-CFP) is mediated by a viral P2A sequence. The positive and negative FRET controls were also polycistronic systems, the first made up of Cytochrome P450 reductase (CPR)-CFP and CYP2J5-YFP and the latter consisting of an ER anchored-CFP (ANC-CFP) and

CYP2J5-YFP. Constructs expressing single labeled mEH-CFP and CYP2J5-YFP proteins were used as controls for bleed through correction. Images of HEK293 cells transfected with the FRET constructs showed that the fluorescent fusion-proteins were localized in the ER. The expression analysis by western blot confirmed the identity of the fluorescent chimeras. FACS-FRET measurements showed FRET between the CYP2J5 and mEH comparable to the signal obtained from the positive control CPR-CFP CYP2J5-YFP pair. A direct interaction between these enzymes would suggest that the exchange of EETs occurs when the enzymes are in close proximity. This has potential implications in the regulation mechanism of free EETs and DHETs in the cell, and therefore on their downstream effects in signaling pathways.

T15

INVESTIGATING CYCLOOXYGENASE-2 FUNCTION IN DETERGENT-FREE NANOSCALE LIPID BILAYER DISCS

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The vast majority of investigations into Cyclooxygenase (COX) structure and function have been conducted with enzyme stabilized in a detergent solubilized state. However, alterations to protein structure, dynamics, and function caused by detergent mediated extraction from the lipid membrane may often go unrecognized. Detergent molecules are resolved in crystal structures of COX-2, and the influence of these molecules on COX-2 structure and function are largely unknown. Previous studies have demonstrated that certain detergents can influence kinetic analyses of COX-2 due to sequestration of hydrophobic COX ligands into detergent micelles. In order to investigate these possible limitations associated with the use of detergent, and to explore the role of the lipid bilayer in COX-2 catalysis, we have incorporated COX-2 into lipid bilayer nanodiscs of controlled size and lipid composition. A dual affinity purification approach was developed to ensure isolation of COX-2:nanodisc complexes with high purity and monodispersity. Size exclusion chromatography and negative-stain EM confirmed that COX-2:nanodisc complexes had been properly formed and purified. Compared to detergent solubilized enzyme, nanodisc incorporated COX-2 had similar kinetic characteristics and product profiles with various fatty acid substrates and COX inhibitors. The nanodisc incorporated COX-2 was also allosterically activated by palmitic acid in a similar fashion to detergent solubilized enzyme. We analyzed COX-2:nanodiscs formed with POPC, DOPC, POPS, or DOPS lipids and determined that lipid composition caused no significant alterations to COX catalysis or inhibition. Thus, detergent and nanodiscs maintain COX-2 in a similar catalytically active state. However, functional reconstitution of COX-2 and other fatty acid oxygenase family members into lipid bilayer nanodiscs will serve as a future utility in investigating the structure, dynamics and function of these important enzymes in the lipid bilayer bound state. The controlled size, monodispersity, and lack of detergent micelles make nanodisc incorporated COX-2 an attractive candidate for future studies including SAXS, DLS, and SDSL-EPR which are difficult or impossible in detergent micelle or liposome solutions.

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T16

Not Attending

T17

MODULATION OF FREE AND ESTERIFIED PLASMA OXYLIPIN LEVELS IN RESPONSE TO LONG-CHAIN OMEGA-3 FATTY ACID SUPPLEMENTATION

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It is believed that many of the beneficial effects of long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) are mediated by their oxidized metabolites, the oxylipins. While from arachidonic acid (AA) derived prostanoids and leukotrienes have been intensively studied in the past, the formation and biological role of many cytochrome P450 and lipoxygenase derived hydroxy, epoxy and dihydroxy fatty acids from LC n-3 PUFAs remain unclear. We recently found that the concentration of circulating free oxylipins derived from eicosapentaenoic acid (EPA) strongly correlates with the nutritional status with their precursor fatty acid [1]. In line with this observation we could show, that the concentration of free hydroxy, epoxy and dihydroxy EPA can be selectively elevated by supplementation of docosahexaenoic acid (DHA) and EPA [2]. Similar observations were made by several groups monitoring the total amount of oxylipins in plasma by release of the esterified fraction via saponification [3]. However, due to different study protocols and strong interindividual differences in the oxylipin concentrations it is difficult to compare the results. Thus it remains unclear, if LC n-3 PUFA supplementation affects free and esterified oxylipins differently. Therefore, we compared the impact of a three month LC n-3 PUFA supplementation on both the patterns of free and total oxylipins. 10 subjects (5 male; 5 female) were supplemented with a daily dose of 1008 mg EPA and 672 mg DHA. Blood samples were drawn before and after 12 weeks of treatment. Oxylipins in plasma were analyzed by LC-MS directly for free oxylipins and after saponification to obtain total (sum of free and esterified) levels. As expected, LC n-3 PUFA treatment let to a slight significant increase in the relative amounts

EPA and DHA in erythrocyte membranes determined by GC-FID. Both total and free plasma EPA derived oxylipins were highly increased (70 to 150%), while total AA derived oxylipins were decreased on average by 30%. There was no effect on DHA-metabolites. Concentrations of total hydroxy and epoxy FAs in plasma were considerably higher compared to free hydroxy and epoxy FAs (up to 350 times), while levels of most free dihydroxy FAs were in a similar range to total dihydroxy FAs. However, the individual ratios between total and free plasma concentration for each oxylipins remained almost unchanged after LC n-3 PUFA treatment. Both free plasma oxylipins as well oxylipins esterified in plasma lipids can only be regarded as a proxy reflecting the (effective) concentration of these lipid mediators in various tissues. Thus, it is unclear which biomarker is biologically more relevant for the effects of oxylipins. However, the unchanged ratio of free and esterified oxylipins in plasma in response to LC n-3 PUFA treatment indicates that both analytical parameters are valuable biomarkers for assessing the individual status of LC n-3 PUFA derived oxylipins.

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T18

A COMPREHENSIVE ANALYSIS OF THE EFFECTS OF POLYPHENOLS ON COX-2 ACTIVITY

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Several *in vitro* and *in vivo* studies demonstrate that plant derived polyphenols possess anti-inflammatory properties. One described mode of action is the downregulation and/or inhibition of cyclooxygenase-2 (COX-2). Surprisingly, the described potency of few polyphenols towards COX-2 is comparable to steroidal anti-inflammatory drugs (SAIDs) and non-SAIDs (NSAIDs) used to pharmacologically modulate COX activity, e.g. for resveratrol [1, 2]. However, comparing the results of different studies is difficult and particularly hampered by the use of different assays. The aim of this study was to comprehensively evaluate the effects of food derived polyphenols on the COX-2 activity, confirming earlier results about their potency and identifying the most potent compounds. For this purpose the effect of a small library of stilbene and flavanoid polylphenols on COX-2 catalyzed PGE_2 formation and COX-2 expression was investigated in the colon carcinoma derived cell line HCA-7 – which constitutively expresses the enzyme - and in primary monocytes after lipopolysaccharide (LPS) stimulation. PGE_2 levels in cell culture media were rapidly quantified by a sensitive LC-MS method with an automated solid-phase-extraction (SPE) as online sample preparation. This newly developed method proved to be a powerful tool for the quantitative analysis of PGE_2, PGD_2, and thromboxane B_2 (TXB_2), with a short analysis time of 7 minutes per sample, low limits of detection (TXB_2: 0.13 nM; PGE_2/PGD_2: 0.25 nM) and good accuracy (89-113%) and precision (variation < 10%) for protein containing cell culture media. In the HCA-7 cells, most polyphenols showed at a concentration of 10 µM no inhibitory effects on COX-2 activity or an inhibition below 50 %. Only resveratrol let to a potent dose dependent inhibition with an IC50 value of 6.4 µM. In the LPS stimulated monocytes the effects of all compounds were more pronounced. As an example the metoxylated flavonoid Nobiletin, which showed no inhibitory effect in the HCA-7 cell line, inhibited PGE2 formation with an IC50 of 18.6 µM in the monocyte based assay. In comparison to the selective COX-2 inhibitor celecoxib and the SAID dexamethasone the obtained IC50 values were even for the most potent polyphenols about two orders of magnitude higher. Combining the observed moderate to low activity with the low bioavailability of the polyphenols, a biological relevant inhibition of COX-2 activity by these food ingredients seems unlikely and is negligible if compared to that of pharmaceuticals.

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T19

APPLICATION OF SEQUENCING, FATTY ACID PROFILING, AND METABOLOMICS INVESTIGATION TO EXPLORE PATHOGENESIS AND TREATMENT STRATEGY FOR ANOREXIA NERVOSA

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Individuals with anorexia nervosa (AN) restrict eating and become emaciated. They tend to have an aversion to foods rich in fat. We have identified a novel AN susceptibility gene, Epoxide Hydrolase 2 (EPHX2), through a series of complementary genetic study designs (GWAS, exon-based sequencing, single-locus association and replication studies) in 1205 AN and 1948 controls (p=0.0004 to 0.00000016). To assess the mechanisms by which EPHX2 influences AN risk, here we applied a multi-disciplinary approach using lipidomics, metabolomics, and ex vivo techniques to evaluate the biological functions of EPHX2 in AN. EPHX2 codes for soluble epoxide hydrolase (sEH) which converts epoxides to the corresponding diols; thereby it plays a major role in the metabolism

of endogenous lipid epoxides, such as the epoxyeicosatrienoic acids (EETs), a derivatives of arachidonic acid (ARA). We measured polyunsaturated fatty acids (PUFAs) and eicosanoids/oxylipins (bioactive lipid mediators that are derived from the metabolism of PUFAs) in 30 ill AN cases, 30 weight-recovered AN cases, and 36 age-, gender- and race-matched controls using the GC/MS and LC/MS/MS systems. Eicosanoid/oxylipin ratios (e.g. DiHETrE-to-EpETrE ratios) were calculated as proxy markers of in vivo sEH activity. The free form of n-6 PUFAs (DGLA, ARA, and Osbond acid) and n-3 (ALA, SDA, EPA, and DHA) PUFAs were significantly elevated in ANs compared to controls (p=0.0003 to 0.00004), whereas matrix bound fatty acid levels (total PUFAs: ARA, and osbond acid of n-6, and ALA and DPA of n-3) were significant different in ANs and controls. A number of PUFA biomarkers significantly differed between ill AN (BMI<17.5) and recovered AN(BMI>=17.5), but these biomarkers only correlated with BMI in ANs but not in healthy controls, suggesting the presence of biomarker-to-disease interactions. A number of oxylipin markers from precursor ARA, LA, ALA, and DHA PUFAs were associated with AN risk, and the diol:epoxide ratios suggest the sEH activity to be significantly different between AN and controls. Our data demonstrate that both PUFAs and sEH activity/action may contribute to the pathogenesis of anorexia nervosa. This study suggests that EPHX2 influences AN risk through biological interaction with the PUFA pathway. It demonstrates that rare variant study is a useful approach to identify otherwise unsuspected risk genes in AN; that joint investigations of genetic mechanisms with their biological non-genetic partners (e.g. diet, stress) may lead to improved understanding of pathophysiology and new treatment strategies for AN.
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T20

ATP ALLOSTERICALLY ACTIVATES THE MOLECULAR MECHANISM OF HUMAN 5- LIPOXYGENASE

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5-Lipoxygenase (5-LOX) and ATP have both been implicated in inflammatory diseases, such as chronic obstructive pulmonary disease (COPD) and emphysema. Since ATP is a known activator of 5-LOX, we investigated this relationship in greater detail. In the current work, we describe how ATP activates both hydroperoxidation and epoxidation by 5-LOX of multiple fatty acids, arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). For AA, a specific step in its molecular mechanism is changed, the dependency of the rate-limiting step on hydrogen-bond rearrangement. This change is the same for both hydroperoxidation and epoxidation, further supporting the hypothesis that both catalytic processes follow the same reaction trajectory. ATP activation also changes the product profile of the fatty acids mixture (AA, EPA and DHA). The products of these fatty acids, leukotrienes and resolvins, promote and inhibit inflammation, respectively, and thus changes in their relative concentrations in the cell could have implications in the regulation of inflammation. Additionally, the extensive mutations used to stabilize 5-LOX for crystallization also remove the ability of ATP to activate 5-LOX, raising the question whether the instability of 5-LOX is the structural cost of ATP activation.
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T21

THE SOLUBLE EPOXIDE HYDROLASE IS A TARGET FOR NEUROPATHIC PAIN

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Epoxy-fatty acids (EpFAs), such as epoxyeicosatrienoic acids from arachidonic acid, are endogenous lipid mediators created by cytochrome P450 enzymes and subsequently degraded by the enzyme soluble epoxide hydrolase (sEH) in a branch of the arachidonic acid cascade. Recently the importance of lipids as signaling mediators and their functional significance in nociception has received attention. Inhibitors of sEH have been demonstrated to elevate EpFA levels *in vivo* and are antihyperalgesic in several animal pain models. Direct administration of exogenous epoxides of arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid are also significantly antihyperalgesic. The EpFAs formed from these parent fatty acids are all demonstrated substrates of the sEH as well, therefore inhibiting the sEH enzyme is an attractive strategy for eliciting antihyperalgesia. The EpFAs have multiple modes of analgesic action including acting in presence of elevated cAMP which occurs with inflammation and pain. However, the EpFAs are also active against a model of type I diabetic neuropathic pain where the role of inflammation is minimal. Here we describe the antihyperalgesic efficacy of sEH inhibitors against neuropathic pain using traditional withdrawal threshold assays but also the conditioned place preference (CPP) assay. The CPP assay was used to measure the negative reinforcement indicative of pain relief but was also used to demonstrate the absence of positive reinforcement or rewarding effects of the small molecule sEH inhibitors. There is still a need to better understand the pathophysiology of chronic pain as well as develop new therapeutic approaches for these conditions. The sEH inhibitors have shown efficacy in chronic pain models as well as clinical equine laminitis. While they have not yet been tested in humans, they are a promising approach to meeting this need.
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T22

NEWLY DESIGNED AND IMPROVED HUMAN SOLUBLE EPOXIDE HYDROLASE INHIBITORS.

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Soluble epoxide hydrolase (sEH, EC 3.3.2.10) is responsible for the metabolism of endogenously derived fatty acid oxiranes, epoxyeicosatrienoic acids (EETs) to the corresponding diols, dihydroxyepoxyeicosatrienoic acids (DHETs). EETs are the products of arachidonic acid by cytochromes P450 epoxygenases and are key modulators involved in inflammation, blood pressure and pain. Inhibition of sEH that results in an increase in endogenous EETs, which have a significant effect on resolving inflammation and high blood pressure in rodent models. Therefore, the sEH has emerged as a pharmaceutical target for hypertension, inflammation and organ-protection. Recently, we have demonstrated that elevation of epoxy-fatty acids through sEH inhibition is able to resolve chronic pain in rodents. Over the last decade, a series of N,N'-disubstituted ureas has been developed to study the physiological effects of sEH inhibition and their beneficial effects in several murine and rodent disease models have been demonstrated. To better study the effect of epoxy-fatty acids on health a set of new compounds with dramatically increased potency and high target occupancy as well as better solubility and improved pharmacokinetic profiles has been synthesized. The PK-ADME of these compounds and their efficacy on modeled diabetic neuropathic pain will be examined and discussed here.

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T23

SYNTHESIS AND BIOLOGICAL EVALUATION OF Ω-HYDROXY FATTY ACIDS FROM Ω-6 AND Ω-3 POLYUNSATURATED FATTY ACIDS.

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Polyunsaturated fatty acids such as arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are converted by cytochrome P450 ω-hydroxylases to bioactive metabolites. Most notably, the CYP 4A and 4F ω-hydroxylases produce 20-HETE, 20-HEPE, and 22-HDoHE products from ARA, EPA, and DHA respectively. 20-HETE has been known to be an important modulator of vascular, kidney, gastrointestinal and bronchial cell reactivity, angiogenesis, and inflammation. A recent study also demonstrated that 20-HETE is an endogenous ligand for the transient receptor potential ion channel, TRPV1, which suggests that these fatty acid metabolites may have a role in modulating nociception. However, the biological roles of both 20-HEPE and 22-HDoHE have not been well studied so far. This may be due in part to their limited supply as no commercial source is available. Therefore, to explore the biology of these metabolites we have developed effective and scalable synthetic methodology for the hydroxy-fatty acids including 20-HETE. We have examined their interactions and metabolism with cyclooxygenase enzymes and their effect on nociception which we present here.

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T24

A CYCLOOXYGENASE-2 DEPENDENT PGE2 BIOSYNTHETIC SYSTEM IN THE GOLGI APPARATUS

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Cyclooxygenases (COXs) catalyze the committed step in prostaglandin (PG) biosynthesis. COX-1 and COX-2 are found on the luminal surfaces of the ER and inner membrane of the nuclear envelope. COX-1 is constitutively expressed and stable, while COX-2 is a short-lived inducible enzyme. Mature COX-2 can be degraded via ER-associated degradation (ERAD) involving post-translational glycosylation of Asn-594 in a 27-aa degradation motif (degron) near its C-terminus. Immunohistochemical analyses indicate that a significant proportion of both COX-2 and microsomal prostaglandin E synthase-1 (mPGES-1), but not COX-1, are present in the Golgi apparatus. Pharmacologic inhibitors and a dominant negative Sar1 which slow trafficking between the ER and the Golgi, retard COX-2 degradation. Substitution of the normal STEL sequence at the C-terminus of COX-2 with a robust ER retention signal (KDEL) causes KDEL COX-2 to become concentrated in the ER where, although glycosylated on Asn594, the KDEL COX-2 variant is not degraded. Finally, we observe that the amount of PGE_2 produced from endogenous arachidonic acid is six times higher in HEK293 cells co-expressing COX-2 and mPGES-1 than in HEK293 cells co-expressing COX-1 and mPGES-1. We conclude that glycosylation of Asn-594 of COX-2 occurs in the ER leading to its anterograde movement to the Golgi where the Asn-594-linked glycan is trimmed by ER mannosidase-I in advance of the retrograde transport of COX-2 to the ER for ERAD. Apparently, having a weak ER retention signal (STEL) slows Golgi to ER transport of COX-2 leading to a distinct residence period for COX-2 in the Golgi. Cytosolic PLA_2, which mobilizes AA for PG synthesis is known to be preferentially translocated to the

Golgi in response to physiological Ca^{2+} mobilization. We suggest that cytosolic PLA_2, COX-2 and mPGES-1 team in the Golgi apparatus to provide a unique COX-2 dependent PGE2 biosynthetic system. This compartmentalization may explain how COX-2 can operate independently of COX-1 when the two isoforms are co-expressed in the same cell.
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T25

THE CONSTRUCTION OF EICOSANOIDS METABOLOMIC METHOD AND ITS APPLICATION IN FISH OIL-FED MICE

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Eicosanoids are a kind of bioactive lipid molecules metabolized from polyunsaturated fatty acids (PUFA). Common omega6 and omega3 PUFA such as arachidonic acid (AA), linoleic acid (LA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) can be metabolized to hundreds of eicosanoids by cyclooxygenases (COX), lipoxygenases (LOX), cytochrome P450s (CYP450) enzymes and non-enzymatic auto-oxidation pathways. These eicosanoids take part in important biological processes such as reproduction, development, maintenance of body temperature and blood pressure, and play an important role in the development of a series of critical diseases, for instance, atherosclerosis, cardiomyopathies, hypertension, cancer and diabetes. Assessment of these eicosanoids levels as much as possible is important for understanding their homeostatic and pathophysiological roles. In this work, we constructed a LC-MS/MS based method to monitor these derivatives (covering various PGs, LTs, HETEs, EETs, LXs, HEPEs, HDoHEs, EEQs, EDPs, Resolvins, Protectins, etc.). 32 ARA and 39 omega-3 PUFA metabolites were quantified in 18 min. The limits of detection (LOD) were between 0.0625 and 1 pg. The calibration curves for most metabolites were linear over the range of 0.25–2000 pg (on column, R>0.998). The intraday precision and accuracy were within a coefficient of variation of 5% and 10%. This eicosanoids metabolomic method has “4S” advantages. It can detect a spectrum of eicosanoids in one experiment with high sensitivity and specificity, and its speed also affords high-throughput analysis. It was reported that EPA and DHA have cardiovascular protective effect, but whether it is related to their eicosanoids derivatives and how omega3 PUFA uptake influenced the eicosanoids network were unknown. We quantified the basal level of dozens of eicosanoids in mice plasma, heart and aorta and discovered the change of them after feeding with fish oil added diet using the eicosanoids metabolomic method. Many omega3 PUFA derivative eicosanoids were up-regulated significantly including EEQs, EDPs, HDoHEs, etc. The change of eicosanoids profile displayed tissue specificity. The results suggested the change of eicosanoids profile could be a factor of omega3 PUFA’s biological activity, and proved eicosanoids metabolomic method could be a powerful approach for discovering important eicosanoids molecules in pathophysiological processes.
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T26

CYP EICOSANOIDS CONTRIBUTE TO THE REGULATION OF PHARYNGEAL PUMPING ACTIVITY IN *C. ELEGANS*

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Cytochrome P450 (CYP)-dependent eicosanoids are epoxygenated and/or hydroxylated metabolites of long-chain polyunsaturated fatty acids (LC-PUFAs). As known from mammalian systems, CYP-eicosanoids are involved as signaling molecules in the regulation of various physiological and pathophysiological processes. Recent studies revealed that the nematode *Caenorhabditis elegans* also produces a broad spectrum of CYP eicosanoids, in particular, epoxy- and hydroxy-metabolites derived from EPA and AA. Here we tested the hypothesis that these molecules serve as regulators of the pharyngeal muscle activity in the nematode. The pharynx of *C. elegans* is a rhythmically active pump that sucks nutrients (bacteria) and passes them to the intestine of the animal. The pumping frequency is under neuronal control and regulated in response to satiation and food supply. LC-PUFA-deficient, Δ-12 and Δ-6 fatty acid desaturase mutants (*fat-2* and *fat-3*, respectively) of *C. elegans* exhibited multiple impairments of muscle cell activity, including a strongly reduced pharyngeal pumping frequency. Supplementation of EPA and AA for several days clearly rescued these impairments, whereas ETYA, a non-metabolizable analogue of AA had no effect suggesting an essential role of PUFA derived metabolites for the pharynx activity. Already short-term treatment (40 min) with 17,18-EEQ, the most abundant CYP-derived eicosanoid in *C. elegans*, mimicked the stimulating effect of long-term EPA feeding. In contrast, 19-HETE and 20-HETE caused the opposite effect and decreased the pumping frequency. In *C. elegans*, a decrease of pumping activity normally occurs in response to satiation, which can be simulated in the laboratory by adding neurotransmitters such as glutamate or octopamine to the medium. Indeed, eicosanoid-deficient strains, as *fat-2* or *emb-8* (encoding the *C. elegans* NADPH-CYP reductase) failed to respond to these neurotransmitters in the presence of abundant food. This behavioral impairment was rescued by adding 19- or 20-HETE. Moreover, the determination of the *in vivo* pattern of free eicosanoids confirmed an increased production of 19- and 20-HETE in response to octopamine treatment. These results provide first evidence for an important role of CYP eicosanoids as second messengers of neurotransmitters that regulate pharyngeal pumping activity of *C. elegans* in response to environmental cues.

T27

PPARδ SIGNALING MEDIATES CYTOTOXICITY OF DHA IN H9C2 CELLS

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Dietary polyunsaturated fatty acids (PUFA) have been well established for many years as important mediators in regulating cellular function. Docosahexaenoic acid (22:6n3, DHA) is a n-3 PUFA that is known to evoke differing effects on primary cells compared to immortal cell lines. Treatment with DHA induces cytotoxic effects in immortalized cell lines, while inducing either negligible, or protective effects in primary cells. The molecular mechanisms underlying this exclusive cytotoxicity toward immortalized cell lines remains largely unknown. The aim of this study was to examine the involvement of PPARδ in the cytotoxicity of DHA on the cardiac H9c2 cell line. PPARδ is abundantly expressed in H9C2 cells as analyzed with western blot. Treatment with DHA (100μM) resulted in a significant decline in cell viability and cellular metabolic activity. Furthermore, treatment with DHA robustly increased total proteasome activities, LDH release and an inflammatory response. While no significant changes to ROS production or lipid peroxidation were observed following DHA treatment, evidence for an apoptotic cell death was inferred by decreased phosphorylation of Akt and activation of caspase-3 activity. Importantly, DHA robustly enhanced PPARδ DNA binding activity in H9c2 cells suggesting that cytotoxic effect of DHA might be mediated via PPARδ signaling. Indeed, co-treatment with GSK 3787, 1uM (specific PPARδ antagonist) abolished the cytotoxic effects of DHA on H9c2 cells. Intriguingly, DHA-mediated cytotoxicity was also greatly reduced by addition of myriocin, a specific inhibitor of ceramide synthesis. Taken together, these data suggest the involvement of PPARδ signaling in DHA-induced cytotoxicity, which potentially involves formation of ceramide as an integral part of this mechanism.

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T28

FLUCTUATIONS IN LIPOXYGENASE HELIX 2 ARE RELATED TO FUNCTION

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Dynamics and solution structure of helix 2 in a lipoxygenase are examined by cysteinescanning mutagenesis and spin-label EPR (electron paramagnetic resonance) spectroscopy. Most of the residues in helix 2 of soybean lipoxygenase-1 (SBL1) are substituted with spin labeled cysteine, with high retention of enzyme activity. Side chain dynamics at the optimum pH for enzyme activity, and at ambient temperature, are examined for the spin labeled side chains, residues 255-275. The SBL1 isoform has an activity optimum at pH 8-9, and little activity at pH 6; a crystal structure for comparison was determined at pH 5.6 (). The crystal structure shows a bend in direction of helix 2 at I265. Spin label results show that helix 2 may be divided roughly into three segments with regard to dynamics at ambient temperature. Residues 255-260, the N-terminal end of the helix, are relatively immobile and insensitive to changes from pH 7 to 9 and to binding of a lysolipid substrate analog, except for I257, a surface-exposed hydrophobic. In contrast, residues 263-268 undergo large changes in pH-dependent dynamics, and this middle of helix 2 undergoes faster ns fluctuations, correlated over several residues, at the higher pH. The residue at the bend changes from very mobile at pH 7 to less mobile at pH 9, suggesting that the average structure at the bend becomes more restricted by neighboring parts of the structure at the higher pH, and that the bend has functional significance. The C-terminal segment, which is largely solvent exposed in crystal structures, exhibits fast side-chain motion, as expected. To ask how observed changes in helix 2 dynamics are related to changes in structure in the middle of helix 2, distances between the spin labels and catalytic iron have been examined by EPR power saturation, in frozen solutions. These power saturation experiments show that residues 257-270 remain helical at elevated pH and that the mid part of helix 2 (residues 259-267) undergoes structural changes in the presence of a substrate analog. These results suggest that nanosecond fluctuations in helix 2 provide the opportunity for an acyl chain to enter the substrate-binding site by sliding in between helices 2 and 11, rather than by entering through small rearrangements of selected side chains (for instance, T259 and L541) that block access to the active site.

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T29

AUGMENTATION OF ASTROCYTE RELEASE OF VEGF AND NEURONAL RECOVERY AFTER OXYGEN-GLUCOSE DEPRIVATION BY INHIBITORS OF SOLUBLE EPOXIDE HYDROLASE

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Objective: Epoxyeicosatrienoic acids (EETs) are synthesized from arachidonic acid by cytochrome P450s with epoxygenase activity. In brain, astrocytes are a major source of EETs. Soluble epoxide hydrolase (sEH) metabolizes EETs into diols, reducing EETs biological activity. Male sEH null mice have reduced injury from ischemic stroke, and pretreatment with sEH inhibitors reduces infarct volume. In various model systems, EETs may be pro-angiogenic, anti-apoptotic, and anti-inflammatory. Increasing EETs bioavailability after stroke by inhibiting sEH may facilitate recovery. We tested this hypothesis in vitro by determining 1) if sEH inhibitors increase secretion of growth factors, such as VEGF, by astrocytes after oxygen-glucose deprivation (OGD), and 2) if this effect on astrocytes subsequently protects neurons during reoxygenation after OGD. **Materials and Methods:** In the first experiment, primary rat astrocytes were allocated into the following groups: normoxia, normoxia plus sEH inhibitor, ODG plus DMSO vehicle, and OGD with 1 of 6 doses of 2 different sEH inhibitors (TPPU or t-AUCB). Astrocyte OGD was induced for 6 h

and was followed by 48 h of reoxygenation/glucose restoration (OGD/R). Vehicle, TPPU or t-AUCB were added to the media for this 48 h period. In the second experiment, primary rat neuronal cultures were exposed to 1 h of OGD. At reoxygenation, wells of neurons were exposed through transwell membranes to media from astrocytes previously exposed to 6 h of OGD, 48 h reoxygenation with vehicle, TPPU or t-AUCB, followed by an additional 24 h in neurobasal media. TUNEL staining of neurons was assessed at 24 h of reoxygenation with or without exposure to OGD/R-conditioned astrocyte media. **Results:** In the first experiment, astrocyte OGD/R with vehicle led to a significant 25% increase of VEGF in the media from primary astrocytes compared with normoxia. Administration of TPPU or t-AUCB after OGD/R dose-dependently promoted much higher astrocyte VEGF secretion. For example, 1 μM TPPU increased VEGF in the media by 50%, whereas 100 μM increased VEGF by 300%. This increase was blocked by the EETs antagonist 14,15-EEZE. In the second experiment, OGD/R in neurons without astrocyte media exposure increased TUNEL-positive neurons from 1% to 63%. Exposure to OGD/R-conditioned media from astrocytes with vehicle reduced TUNEL-positive neurons to 47%, whereas exposure to OGD/R-conditioned astrocyte media with TPPU or t-AUCB significantly further reduced TUNEL-positive neurons to 33% and 38%, respectively, in association with increased VEGF. **Conclusions:** Our findings indicate that post-OGD treatment with sEH inhibitors can increase astrocyte VEGF secretion and that this augmented the protective effect of astrocyte media on neuronal recovery after reoxygenation. Inhibition of sEH may represent a therapeutic approach for attenuating reperfusion injury and promoting brain repair.

T30

SYNERGISM BETWEEN PHOSPHODIESTERASE 5 (PDE5) INHIBITORS AND UT-15C (TREPROSTINIL) IN STIMULATING INCREASES IN CAMP AND ATP RELEASE FROM ERYTHROCYTES OF HUMANS WITH PULMONARY ARTERIAL HYPERTENSION (PAH): THERAPEUTIC IMPLICATIONS

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PAH is characterized by sustained increases in pulmonary vascular resistance leading to right ventricular failure and death. The disease has a poor prognosis in the absence of pharmacological intervention. Drugs used to treat PAH include both PGI_2 analogs and PDE5 inhibitors. Although PGI_2 analogs clearly can dilate blood vessels in the absence of erythrocytes, we have shown an alternative mechanism of vasodilation when these drugs are administered in vivo. We have shown that; 1) human erythrocytes express PGI2 receptors (IPRs) and 2) PGI_2 analogs activate an IPR-mediated signaling pathway in these cells that requires involves increases in cAMP and culminates in the release of ATP. In this pathway, levels of the critical second messenger, cAMP, are regulated by PDE3. Importantly, PDE3 is inhibited by cGMP which is, in turn, regulated by PDE5. We have shown that both PDE3 and PDE5 are present in healthy human (HH) erythrocytes and that PDE5 inhibitors augment UT-15C-induced ATP release from these cells. Here, we investigated if PDE5 inhibitors also potentiate UT-15C-mediated increases in cAMP and ATP release from PAH erythrocytes. Blood was obtained from HH (n = 22, 9 male, 13 female, age = 24 to 65 years) and PAH patients (n = 14, 7 male, 7 female, age = 18 to 67 years, WHO group 1). Isolated erythrocytes were incubated with UT-15C (1 μM) or its vehicle (saline) in the absence or presence of PDE5 inhibitors (zaprinast, ZAP or tadalafil, TAD; 10 μM) or their vehicle (DMF). ATP and cAMP were measured by luminometry and ELISA assay, respectively. UT-15C stimulated increases in cAMP and ATP release from both HH (n=9) and PAH (n=8) erythrocytes, but the response in PAH patients was significantly greater in both cases ($P<0.01$). Neither ZAP nor TAD alone stimulated ATP release. However, both PDE5 inhibitors augmented UT-15C-induced increases in cAMP and ATP release, again with greater increases in PAH erythrocytes (n=8, $P<0.01$). We also determined that a concentration of UT-15C (100 nM) that alone did not stimulate ATP release was effective in the presence of TAD (n=6, $P<0.01$). To determine if the effects of TAD are mediated via cGMP, we determined that the sGC inhibitor, ODQ (10 μM), prevented the augmentation of UT-15C-induced ATP release produced by TAD (n=5, $P<0.05$). These findings demonstrate that UT-15C stimulates cAMP accumulation and ATP release from erythrocytes and that PDE5 inhibitors potentiate these responses, likely via increasing cGMP. Synergism between PDE5 inhibitors and PGI_2 analogs in stimulating release of the potent vasodilator, ATP, from PAH erythrocytes suggests a new rationale for the use of these drugs in combination in these patients. Moreover, these results suggest that the erythrocyte is a novel target for drug development for treatment of PAH.

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