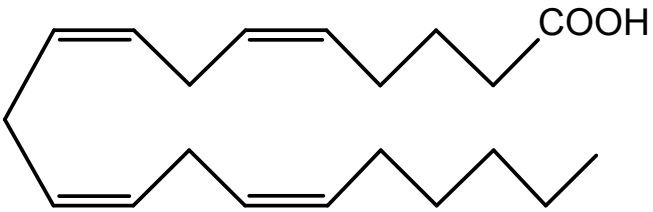
THE 19TH INTERNATIONAL WINTER EICOSANOID CONFERENCE

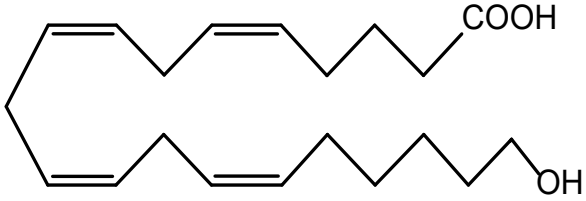
October 15-17, 2023

Sheraton Inner Harbor Hotel

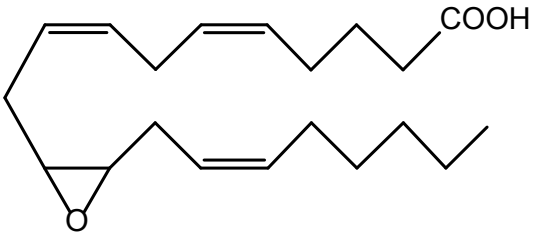
300 South Charles Street
Baltimore, Maryland



Arachidonic Acid



20-HETE



11,12 EET

COMMITTEES

Organizing Committee

William B. Campbell, Ph.D.
Medical College of Wisconsin

Kenneth V. Honn, Ph.D. *(In Memoriam)*
Wayne State University

John D. Imig, Ph.D.
University of Arkansas Medical School

Dipak Panigrahy, M.D.
Beth Israel Deaconess Medical Center/ Harvard

Michal L. Schwartzman, Ph.D.
New York Medical College

Darryl C. Zeldin, M.D.
NIEHS/NIH

Symposium Organizers

Nabil Alkayed, M.D., Ph.D.,
Knight Cardiovascular Institute
Oregon Health & Science University

Sean Davies, Ph.D.
Vanderbilt University

Ingrid Fleming, Ph.D.
Vascular Research Centre
Goethe Universitat

Victor Garcia, Ph.D.
New York Medical College

Michael Holinstat, Ph.D.
University of Michigan

John Imig, Ph.D.
University of Arkansas for Medical Sciences

Annet Kirabo, Ph.D.
Vanderbilt University Medical Center

Krishnarao Maddipati, Ph.D.
Wayne State University

Dipak Panigrahy, M.D.
Beth Israel Deaconess Medical Center
Harvard Medical School

Stokes Peebles, Ph.D.
Vanderbilt University Medical Center

Charles N. Serhan, Ph.D.
Brigham & Womens' Hospital
Harvard Medical School

Scientific Advisors

Alan R. Brash, Ph.D.
Vanderbilt University

Nancy Brown, Ph.D.
Yale University

John R. (Camille) Falck, Ph.D.
University of Texas Southwestern Med. Ctr.

Ingrid Fleming, Ph.D.
Goethe University

Bruce D. Hammock, Ph.D.
University of California, Davis

Craig R. Lee, PharmD, Ph.D.
University of North Carolina at Chapel Hill

Jerry L. Nadler, M.D.
VA Northern California Healthcare System

Paola Patrignani, Ph.D.
Universita degli Studi "G d'Annunzio"

Ann Skulas-Ray, Ph.D.
University of Arizona

John Seubert, MSc, Ph.D.
University of Alberta

Wolf-Hagen Schunck, Ph.D.
Max Delbrück Center for Molecular Medicine

Charles T. Stier, Ph.D.
New York Medical College

Dao Wen Wang, M.D., Ph.D.
Huazhong University of Science & Technology

Kenneth K. Wu, M.D., Ph.D. (Co-Founder)
University of Texas Health Science Center

Awards Committee

Claus Schneider, Ph.D., Chairman
Vanderbilt University

Ingrid Fleming, Ph.D.
Vascular Research Centre, Goethe Universitat

Austin Guo, Ph.D.
New York Medical College

Gabrielle Fredman, Ph.D.
Albany Medical College

Jason Hellmann, Ph.D.
University of Louisville

Poster Committee

John Seubert, Ph.D., Co-Chair
University of Alberta

Charles Stier, Ph.D., Co-Chair
New York Medical College

Poster Judges

Alan R. Brash, Ph.D.
Vanderbilt University

Annet Kirabo, Ph.D.
Vanderbilt University Medical Center

Aditi Das, Ph.D.
Georgia Tech

James M. Luther, Ph.D.
Vanderbilt University Medical Center

Sean Davies, Ph.D.
Vanderbilt University

Brendon Naicker, M.S.
New York Medical College

Matthew Edin, Ph.D.
NIEHS/NIH

Paola Patrignani, Ph.D.
Universita degli Studi "G d'Annunzio"

Fan Fan, M.D.
Augusta University

Nils Helge Schebb
University of Wuppertal

Austin Guo, Ph.D.
New York Medical College

Pei-an Betty Shih, Ph.D.
University of California-San Diego

Conference Staff

Gail D. Anderson, Conference Coordinator
Jennifer Brown
Lori Dean

Valerie Hurt
Caryn Volpe

Audiovisual – NIH Events Management

The 19th International Winter Eicosanoid Conference
October 15-17, 2023
Sheraton Inner Harbor Hotel

Registration:	**Sunday, October 15th**	**12:00 pm - 8:00 pm**	**Ballroom Foyer**
	Mon & Tues, October 16th-17th	**7:15 am - 5:00 pm**	**Ballroom Foyer**
Welcome Reception	**Sunday, October 15th**	**7:00 pm – 9:00 pm**	**Ballroom Foyer**
Continental Breakfast:	**Monday & Tuesday**	**7:00 am – 8:30 am**	**Ballroom Foyer**

ORAL PRESENTATIONS
SUNDAY - TUESDAY: CHESAPEAKE II & III (2ND FLOOR)

POSTER SESSIONS
SUNDAY - MONDAY: CHESAPEAK I (2ND FLOOR)
(POSTERS WILL BE DISPLAYED THROUGH TUESDAY)

Poster Session I
Sunday, October 15, 2023
7:00-8:30 pm

Poster Session II
Monday, October 16, 2023
7:00-8:30 pm

EXHIBITORS
SUNDAY - TUESDAY: CHESAPEAK GALLERY (2ND Floor)

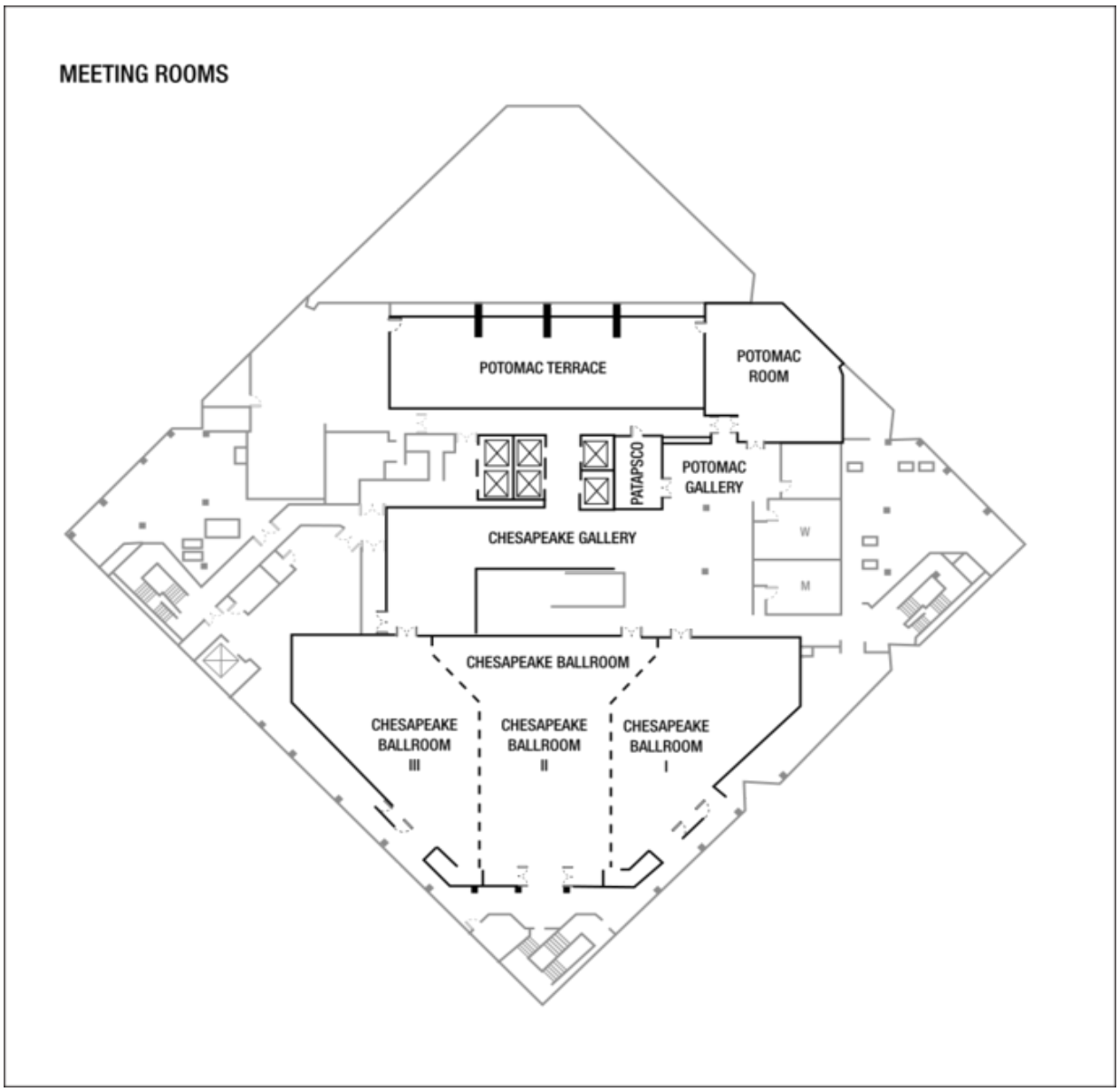
TRAINEE ROUNDTABLES
Potomac Room (2nd Floor)

Monday, October 16th
7:15-8:15 am

Tuesday, October 17th
7:15-8:15 am

CONFERENCE DINNER AND AWARDS
Tuesday, October 17th
8:00 pm
Ruth's Chris Steakhouse
Pier V
711 Eastern Avenue

Sheraton Inner Harbor Hotel
Floor Plan



..

<u>Conference takes place on the 2nd Floor</u>

Registration & Exhibitors	**Chesapeake Gallery**
Oral Presentations	**Chesapeake II & III**
Poster Presentations	**Chesapeake I**
Trainee Sessions	**Potomac**

AWARDS RECIPIENTS

JOHN C. McGIFF MEMORIAL LECTURE AWARD

Kenneth V. Honn, Ph.D.
Wayne State University
(In Memoriam)

POLM YOUNG INVESTIGATOR AWARDS

Veronica Andre
University of Sydney

Genesee Martinez
University of Kentucky

Katherine Quinlivan
Beth Israel Deaconess Medical Center

Alexandra Wolf
New York Medical College

NIEHS/NIH TRAVEL AWARDS

Yanis Afir
Goethe University

Thierno Madjou Bah
Oregon Health & Science University

Rachel Bayer
Beth Israel Deaconess Medical Center

Danielle Diegisser
New York Medical College

Melinee D'Silva
New York Medical College

Liye Fang
University of Alberta

Krista Goerger
University of Michigan

Zhijun Guo
University of Minnesota

Zachary Kipp
University of Kentucky

Jianxun Lei
University of Minnesota

Abhishek Mishra
University of Arkansas

Zumer Naeem
Goethe University

Nhien Nguyen
University of California-San Diego

Saori Noguchi
Vanderbilt University

Abdulmusawwir Alli Oluwafuyi
Vanderbilt University

Andrew Rickenberg
University of Michigan

Dominic Siler
Oregon Health & Science University

Amy Stark
Vanderbilt University

Kimberly Vazquez
Beth Israel Deaconess Medical Center

Ala Yousef
University of Alberta

NUTRIENTS TRAVEL AWARD

Timo Frömel
Goethe University

SUNDAY, OCTOBER 15, 2023

Location: CHESAPEAKE II & III (2ND Floor)

3:00 pm **Opening Remarks**
Darryl C. Zeldin, MD, NIH/NIEHS, Research Triangle Park, NC

3:05 pm **JOHN C. McGIFF MEMORIAL LECTURE AWARD**
Chair: Michal L. Schwartzman, PhD, New York Medical College

KENNETH V. HONN, Ph.D., Wayne State University
Posthumous Recipient – Award to be received by Caryn Volpe (wife)

Tribute to Kenneth V. Honn, Ph.D.
Speaker: Gabor J. Tigyi, Ph.D., The University of Tennessee Health Science Center, Memphis, TN

SESSION I: PrOLM YOUNG INVESTIGATORS SESSION
(SPONSORED BY ELSEVIER/*PROSTAGLANDINS AND OTHER LIPID MEDIATORS*)
Chair: Matthew L. Edin, Ph.D., National Institute of Environmental Health Sciences, Research Triangle, Park, NC

3:30 pm ***Introduction of Young Investigator Session***

3:35 pm Veronica Andre, M.D., University of Sydney, St. Leonards, Australia
Characterization of an Eicosanoid Driven, Clinically Relevant Mouse Model of Peripartum Cardiomyopathy

3:50 pm Genesee Martinez, University of Kentucky College of Medicine, Lexington, KY
Glucocorticoid Resistance as an Inducer of Adiposity and Hepatic Lipid Accumulation

4:05 pm Katherine Quinlivan, Beth Israel Deaconess Medical/Harvard Medical School, Boston, MA
Resolvin E1-mediated Stromal Reprogramming Via Adaptive Immunity Enhances Targeted Cancer Therapy

4:20 pm Alexandra Wolf, New York Medical College, Valhalla, NY
Deconstructing High Fat Diet-Induced Cardiometabolic Disease: The Role of 20-HETE Receptor (GPR75)

4:35 pm **Coffee Break (20 min)**

SESSION II: EICOSANOIDS AND THE LUNG

Co-Chairs: Stokes Peebles, Ph.D., Vanderbilt University, Nashville, TN
Tanya M. Laidlaw, M.D., Brigham & Women's Hospital, Boston, MA

4:55 pm Tanya M. Laidlaw, M.D., Brigham & Women's Hospital, Boston, MA
Role for TP Receptor Signaling in Eicosanoid Homeostasis in the Respiratory Tract

5:20 pm Daniel Menendez, Ph.D., NIEHS/NIH, Research Triangle Park, NC
Regulation of Eicosanoid Signaling by COX-2 during Allergic Lung Inflammation in Mice

5:45 pm Allison E. Norlander, Ph.D., Indiana University School of Medicine, Indianapolis, IN
Regulation of Pulmonary T Regulatory Cell Function by PGI_2

6:10 pm Katherine Niederer Cahill, M.D., Vanderbilt University Medical Center, Nashville, TN
The GLP-1 Receptor as a Novel Regulator of Thromboxane-induced Human Platelet Activation Relevant in Asthma

6:35 pm Weisong Zhou, Ph.D., Vanderbilt University, Nashville, TN
PGI_2 Regulation of Allergen-induced ILC_2 Memory Response

7:00 pm End of Sessions

7:00-8:30 pm Poster Session I - Chesapeake I

7:00-9:00 Welcome Reception/Meet the Exhibitors
Chesapeake Gallery (2nd Floor)

****

MONDAY, OCTOBER 16, 2023

7:15-8:15 am **Trainee Roundtable: Potomac Room (2nd Floor)**
Ways to leverage Artificial Intelligence (AI) Tools (if available, bring laptop)

ORAL PRESENTATIONS
Location: Chesapeake II & III (2nd Floor)

SESSION III: ADVANCES IN CARDIOVASCULAR EICOSANOIDS

Co-Chairs: Annet Kirabo, Ph.D., Vanderbilt University, Nashville, TN
Ingrid Fleming, Ph.D., Goethe University, Frankfurt, Germany

8:30 am Kafait U Malik, D.Sc., Ph.D., University of Tennessee Health Science Center, Memphis, TN
CYP1B1-Testosterone and Estrogen-Generated Metabolites Reciprocally Modulating Activity of Group IV cPLA2a/Arachidonic Acid System Contribute to Sex Differences in Angiotensin II-Induced Hypertension and Pathogenesis

8:55 am Ingrid Fleming, Ph.D., Goethe University, Frankfurt, Germany
Roles of Epoxides and Diols in the Diabetic Heart and in Lymphangiogenesis

9:20 am Flávia Rezende, Ph.D., Goethe-Universität, Frankfurt, Germany
Loss of Endothelial Cytochrome P450 Reductase Induces Vascular Dysfunction and Promotes Angiogenesis

9:45 am Michal L. Schwartzman, Ph.D., New York Medical College, Valhalla, NY
20-HETE-GPR75 Pairing in Cardiometabolic Disease

10:10 am Annet Kirabo, Ph.D., Vanderbilt University, Nashville, TN
Eicosanoid-regulated Myeloid ENaC and Isolevuglandin Formation in Human Salt-sensitive Hypertension

10:35 am **Coffee Break (20 min)**

SESSION IV: EICOSANOID SIGNALING IN CEREBROVASCULAR AND NEUROLOGICAL DISEASE
Co-chairs: Nabil Alkayed, M.D., Ph.D., Knight Cardiovascular Institute, Oregon Health & Science University, Portland, OR
Lauren H. Sansing, M.D., M.S., Yale School of Medicine, New Haven, CT

10:55 am Lauren H. Sansing, M.D., M.S., Yale School of Medicine, New Haven, Ct
Prostaglandin E2 in Intracerebral Hemorrhage

11:20 am David Hasan, M.D., MSc, Duke University, Durham, NC
15-PGDH as a Better Therapeutic Target than Aspirin in Decreasing Risk of Intracranial Aneurysm Rupture in Men and Women Equally

11:45 am Jin Wang, Ph.D., Baylor College of Medicine, Houston, TX
Novel Soluble Epoxide Hydrolase Inhibitors for CNS Disorders

12:10 pm Jieru Wan, PhD, Johns Hopkins University School of Medicine, Baltimore, MD
Neuroprotective Effects of Soluble Epoxide Hydrolase Inhibition in Traumatic Brain Injury

12:35 pm Dominic A. Siler, MD, PhD, Oregon Health & Science University, Portland, OR
GPR39 as a Dual Eicosanoid Receptor in Subarachnoid Hemorrhage

1:00 pm Lunch Break (1 hr, 30 min)

SESSION V: LIPIDOMICS
Co-Chairs: Krishna Rao Maddipati, Ph.D., Wayne State University, Detroit, MI
Ginger Milne, Ph.D., Vanderbilt University Medical Center, Nashville, TN

2:30 pm Ginger L. Milne, Ph.D., Vanderbilt University Medical Center, Nashville, TN
Identification and Characterization of Novel F2-Isoprostane Metabolites

2:55 pm Matthew B. Lawrenz, Ph.D., University of Louisville School of Medicine, Louisville, KY
Eicosanoids and the Black Death: Yersinia pestis Actively Inhibits the Synthesis of Leukotriene B4 During Infection

3:20 pm Anna N. Bukiya, Ph.D., The University of Tennessee Health Science Center, Memphis, TN
Role of Dietary Lipids in Shaping the Effect of Alcohol on Brain Arteries

3:45 pm Krishna Rao Maddipati, Ph.D., Wayne State University, Detroit, MI
Chronic Inflammation: Failure of Unalamation, Not Just a Delay of Acute Inflammation

4:10 pm Matthew L. Edin, Ph.D., NIEHS/NIH, Research Triangle Park, NC
Inhibition of the COVID Eicosanoid Storm by Soluble Epoxide Hydrolase Inhibitors

4:35 pm **Coffee Break (20 min)**

SESSION VI: RESOLUTIONS AND INFLAMMATION
Co-Chairs: Matthew Spite, Ph.D., Harvard Medical School, Boston, MA
Ann Skulas-Ray, Ph.D., Arizona State University, Tucson, AZ

4:55 pm Matthew Spite, Ph.D., Brigham and Women's Hospital, Harvard Medical School, Boston, MA
Regulation and Function of the Resolvin D2 Receptor in Myeloid Cells

5:20 pm Ganesh V. Halade, Ph.D., University of South Florida, Tampa, FL
Inflammation-Resolution Signaling in Cardiac Repair

5:35 pm Thomas Van Dyke, Ph.D., Forsyth Institute and Harvard University Faculty of Medicine, Cambridge, MA
Applied Resolution Pharmacology in Periodontal Disease Treatment

6:00 pm Imad Shureiqi, M.D., University of Michigan Cancer Center-Michigan, Ann Arbor, MI
ALOX15 Modulation of Colonic Resolvin Production to Suppress Colorectal Carcinogenesis

6:25 pm Ann Skulas-Ray, Ph.D., University of Arizona, Tucson, AZ
Resolution Markers in the Blood: Clinical Translation

6:50 pm End of Monday Sessions

7:00-8:30 pm **Poster Session II - Chesapeake I**
Meet Exhibitors - Chesapeake Gallery

****

TUESDAY, OCTOBER 17, 2023

7:15-8:15 am **Trainee Roundtable: Potomac Room (2nd Floor)**
Tools to Improve Presentations and Generate Better Figures for Manuscripts
(if available, bring laptop)

ORAL PRESENTATIONS
Location: Chesapeake II & III (2nd Floor)

SESSION VII: EICOSANOIDS AND CANCER

Co-Chairs: Michael Holinstat, Ph.D., University of Michigan, Ann Arbor, MI
Paola Patrignani, Ph.D., "G. d'Annunzio" University, Chieti, Italy

8:30 am Paola Patrignani, Ph.D., "G. d'Annunzio" University, Chieti, Italy
Platelet and extracellular vesicle crosstalk with cancer cells PhD promote tumorigenesis through eicosanoid biosynthesis

8:55 am Guodong Zhang, Ph.D., University of California Davis, Davis, CA
CYP Eicosanoid Pathway Mediates Colon Cancer-Promoting Effects of Dietary Linoleic Acid

9:20 am Anthony W. Ashton, Ph.D., Lankenau Institute for Medical Research, Wynnewood, PA
Thromboxane A2-Mediated Heterotypic Intercellular Communication in Tumor Pathogenesis

9:45 am David Potter, MD, PhD, Professor, University of Minnesota
Modulating the Immune Microenvironment of ER+HER2 Breast Cancer

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

SESSION VIII: sEH INHIBITORS

Co-Chairs: Cindy McReynolds, Ph.D., University of California-Davis, Davis, CA
John Imig, Ph.D., University of Arkansas for Medical Sciences, Little Rock, AR

10:30 am John D. Imig, Ph.D., University of Arkansas for Medical Sciences, Little Rock, AR
Soluble Epoxide Hydralase Inhibition as a Foundation for Multi-target Drugs to Treat Kidney Diseases

10:55 am Walter Swardfager, Ph.D., University of Toronto, Toronto, Ontario, Canada
Dementia, It's Risk Factors, and What the Blood Can Tell Us

11:20 am Jeffrey M. Saffitz, M.D., PhD, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA
Targeting Pro-Inflammatory Eicosanoids and Soluble Epoxide Hydrolase in Arrhythmogenic Cardiomyopathy

11:45 am Cindy Brown McReynolds, Ph.D., EicOsis, Davis, CA
Advancing Soluble Epoxide Hydrolase Inhibitors into Proof of Concept Clinical Trials for Pain and Alzheimer's Disease

12:10 pm John M. Seubert, M.Sc., Ph.D., University of Alberta, Edmonton
PUFA Metabolites and Soluble Epoxide Hydrolase in the Aged Heart

12:35 pm **Lunch Break (1 hr, 25 min)**

SESSION IX: EICOSANOIDS AND THE METABOLIC SYNDROME

Co-Chairs: Victor Garcia, Ph.D., New York Medical College, Valhalla, NY
Hebe Agustina Mena, PhD, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

2:00 pm Hebe Agustina Mena, PhD, Brigham and Women's Hospital, Harvard Medical School
Maresin 2 is Produced by Brown Adipose Tissue and Resolves Liver Inflammation in Obesity

2:25 pm Terry D Hinds, Jr., Pharmacology and Nutritional Sciences, University of Kentucky
Bilirubin Functioning as a Hormone Prompts Hepatic Fat Utilization and Ketosis

2:50 pm Victor Garcia, PhD, Department of Pharmacology, New York Medical College
The Impact of 20-HETE and its Receptor on the development of NAFLD, Diet-induced Obesity and Cardiometabolic Disease

3:15 pm James P. Hardwick, M.S., Ph.D., Northeast Ohio Medical University, Rootstown, OH
The Paradox of Fatty Acid Omega Hydroxylase P450s (CYP4) in Metabolic Associated Fatty Liver Disease (MAFLD)

3:40 pm James Matt Luther, M.D., MSCI, Vanderbilt University Medical Center, Nashville, TN
Epoxyeicosatrienoic Acis in CardioMetabolic Disease

4:05 pm Coffee Break (20 min)

SESSION X: REGULATION OF EFFEROCYTOSIS BY LIPID MEDIATORS

Co-Chairs: Sean S. Davies, Ph.D., Vanderbilt University, Nashville, TN
Stephania Libreros, Ph.D., Yale School of Medicine, New Haven, CT

4:25 pm Edward B. Thorp, Ph.D., Northwestern University, Chicago, IL
Thromboxane, Macrophages, and Cardiac Regeneration

4:50 pm Azuah L. Gonzalez, PhD Candidate., Vanderbilt University School of Medicine, Nashville, TN
Innate Immune Memory and Efferocytosis

5:15 pm Sean S. Davies, Ph.D., Vanderbilt University, Nashville, TN
N-acyl-phosphatidylethanolamine Hydrolyzing Phospholipase D Regulates Efferocytosis by Macrophages

5:40 pm Stephania Libreros, Ph.D., Yale School of Medicine, New Haven, CT
Role of Resolvins in Regulating Efferocytosis and Deployment

6:05 pm End of Tuesday Sessions

****

8:00 pm Conference Dinner and Awards Presentations
Ruth's Chris Steakhouse
Pier V
711 Eastern Avenue

POSTER ABSTRACTS

Posters will be displayed in the Chesapeake I Room on second floor of the Sheraton Inner Harbor Hotel, Sunday, October 15th through Tuesday evening, October 17th. Presenters are required to stand by their posters for discussion during the session to which they are assigned. ALL posters MUST be removed on Tuesday evening by 6:00 pm as the boards will be dismantled. The room is not available after this time.

Poster Session I
Sunday, October 15th
7:00-8:30 pm
Chesapeake I Room

CAN-1

PPAR-A ANTAGONIST ENHANCES ANTI-ANGIOGENIC THERAPY AND IMMUOTHERAPY TO INHIBIT THE GROWTH OF MOUSE RENAL CANCER

Michael Gillespie, Isabella Howard, Dara Burdette, Keira Smith, Thomas W. Dubensky, Dipak Panigrahy

Beth Israel Deaconess Medical Center, Center for Vascular Biology Research, Harvard Medical School

Peroxisome proliferator activated receptor alpha (PPAR-α) is a transcriptional regulator that can have wide ranging effects in different cancer types through its action on the NF-κB signaling pathway and regulation of fatty acid oxidation. In the absence of PPAR-α, infiltrating lymphocytes regain effector function and inhibit tumor growth and proliferation. PPAR-α may also inhibit tumor growth by modulating stromal processes, such as angiogenesis. PPAR-α knockout mice are resistant to tumorigenesis induced by PPAR-α agonists. Renal cell carcinoma (RCC) is a highly angiogenic cancer and is the most common type of kidney cancer in adults. Advanced RCC patients have a poor prognosis and express high levels of PPAR-α. RCC is characterized as having one of the highest amounts of immune infiltration among solid tumors. Current frontline treatments for RCC include chemotherapies, anti-angiogenic (e.g. cabozantinib) and immunotherapies (e.g. anti-PD1) are limited by the immune invasion of the tumor microenvironment. We have developed a first-in-class PPAR-α antagonist for the treatment of human cancer patients (TPST-1120). In a murine RENCA renal cell carcinoma, systemic therapy with PPAR-α antagonist TPST-1120 at 30 mg/kg/day suppressed tumor growth up to 52% as monotherapy ($p<.0001$). Importantly, TPST-1120 potently enhanced the anti-tumor activity of frontline anti-angiogenic therapy and immunotherapy. Combination treatment with TPST-1120 and anti-PD1 (200 μg Q3D) or cabozantinib (15 mg/kg QD) inhibited RENCA renal cell carcinoma by 74% and 82%, respectively ($p<.0001$). These combination treatments studies exhibited no overt toxicity. The PPAR-α antagonist TPST-1120 suppressed tumor angiogenesis as measured by microvessel density (as quantified by CD31, an endothelial cell marker) as well as reducing the amount of Ki-67-stained proliferating cancer cells by 33% ($p<.05$). Further immunohistochemical analysis showed modulation of CD8+ T lymphocytes as well as the class switching of M2 macrophages to the less immunosuppressive M1 macrophages in the tumor microenvironment (TME). Importantly, we demonstrate that TPST-1120 polarizes an immunosuppressive class of immune cells to promote anti-tumor immunity and anti-angiogenesis in the TME. Thus, our results demonstrate that PP

CAN-2

HEXL-(CUBAN-1YL-METHYL)-BIGUANIDE (HCB) SUPPRESSES ±14,15-EET-DEPENDENT REGULATORY T CELLS AND MODULATES THE TUMOR MICROENVIRONMENT BY INCREASING INTRATUMORAL CD8+ T CELLS IN THE OVARIAN-DEPENDENT ER+HER2-SSM2UCD MAMMARY CANCER ALLOGRAFT MODEL

Zhijun Guo[1], Jianxun Lei[1], Hrishi Venkatesh[1], David Owen[1], Adam Bass[2], Christine Cannon[3], Joshua McCarra[1], Brenda Koniar[1], Craig M. Flory[1], Beverly Norris[1], Robert J. Schumacher[1], Swaathi Jayaraman[4],

John Hawse[4], Robert D. Cardiff[5], John R. Falck[6], Elizabeth A. Ambrose[1], Gunda I. Georg[1], Kaylee L. Schwertfeger[1], Michael A. Farrar[1], Matthew P. Goetz[4], and David A. Potter[1].

[1]University of Minnesota, Minneapolis, MN; [2]Macalester College, St Paul, MN; [3]Pomona College, Claremont, CA; [4]Mayo Clinic, Rochester, MN; [5]University of California, Davis, CA; [6]University of Texas Southwestern Medical Center, Dallas, TX

Introduction: Immune checkpoint blockade (ICB) has utility in triple negative breast cancer (TNBC) but is less effective in the ER+HER2- signature, where tumor microenvironment (TME) is cold and regulatory T cells (Tregs) may suppress effector T cells. The biguanides hexyl-benzyl-biguanide (HBB) and its bioisosteres hexyl-(cuban-1-yl-methyl)-biguanide (HCB), 6,6,6-trifluoro-hexyl-(cuban-1-yl-methyl)-biguanide (C6F3-HCB), and 5,5-difluoro-hexyl-(cuban-1-yl-methyl)-biguanide (C5F2-HCB) are candidate agents to activate the TME because they potently inhibit biosynthesis of immunosuppressive epoxyeicosatrienoic acids (EETs) and EET-driven oxidative phosphorylation (OXPHOS). We hypothesized that reversal of hypoxia by biguanides in the ovarian dependent ER+HER2- STAT1 KO SSM2ucd mouse mammary carcinoma (MC) model would suppress Tregs and promote effector T cells in the tumor TME. We hypothesized that by inhibiting OXPHOS HBB and HCBs may promote efficacy of ICB. We chose the ovarian dependent SSM2ucd model to test the impact of HCB on the TME.
Results: C5F2-HCB and C6F3-HCB inhibited CYP3A4-mediated (±)14,15-EET biosynthesis more potently than HBB and HCB. For C57BL/6 mouse splenocytes under conditions of CD3 and CD28 stimulation, (±)14,15-EET co-treatment increased Tregs (1.22-fold; P=0.02) and suppressed the CD8+:Treg ratio (0.79; P=0.02); while concomitant HBB, HCB, C5F2-HCB, and C6F3-HCB decreased Tregs (P<0.05); increased CD4+ T-cells (P<0.05); promoted the CD4+:Treg ratio (P<0.05); increased CD8+ T-cells (P<0.05); and promoted the CD8+:Treg ratio (P<0.05). While HCB (12mg/kg daily) did not inhibit tumor growth in the SSM2ucd mammary allograft model, it reduced intratumoral hypoxia by 20% (P=0.01), increased CD8+ TIL 4.4-fold (P=0.04), decreased the Treg:CD4+ TIL ratio by 76% (P=0.02), and decreased the Treg:CD8+ TIL ratio by 88% (P=0.01) relative to control.
Conclusion: HCB, an inhibitor of OXPHOS and (±)14,15-EET biosynthesis, reduced intratumoral hypoxia, and while failing to inhibit tumor growth, increased CD8+ TIL and reduced the Treg:CD4+ and Treg:CD8+ ratios, transforming an ER+HER2- TME from cold to hot.

CAN-3

B7-H3 AND B7-H4 IMMUNE CHECKPOINT PROTEINS ARE REGULATED, IN PART, BY (±)14,15-EET MEDIATED N-GLYCOSYLATION IN ER+HER2- MAMMARY CANCER, WHERE THEIR EXPRESSION CORRELATES WITH ESCAPE FROM DORMANCY IN MICE AND CYP3A4 ARACHIDONIC ACID EPOXYGENASE CO-EXPRESSION IN HUMAN BREAST CANCER

Jianxun Lei[1], Zhijun Guo[1], Adam Bass[2], Christine Cannon[3], Joshua McCarra[1], Brenda Koniar[1], Craig M. Flory[1], Beverly Norris[1], Robert J. Schumacher[1], Swaathi Jayaraman[4], John Hawse[4], Emmanuel S. Antonarakis[1], Emanuel F. Petricoin[5], Julia Wulfkuhle[5], Robert D. Cardiff[6], Victor Garcia[7], John R. Falck[8], Dafydd G. Thomas[9], Elizabeth A. Ambrose[1], Gunda I. Georg[1], Kaylee L. Schwertfeger[1], Michael A. Farrar[1], Matthew P. Goetz[4], and David A. Potter[1].

[1]University of Minnesota, Minneapolis, MN; [2]Macalester College, St Paul, MN; [3]Pomona College, Claremont, CA; [4]Mayo Clinic, Rochester, MN; [5]George Mason University, Fairfax, VA; [6]University of California, Davis, CA; [7]New York Medical College, New York, NY, [8]University of Texas Southwestern Medical Center, Dallas, TX, [9]University of Michigan, Ann Arbor, MI

INTRODUCTION: Immune checkpoint blockade is less effective in the ER+HER2- breast cancer correlating with a cold immune tumor microenvironment. Cytochrome P450 (CYP) mediated biosynthesis of epoxyeicosatrienoic acids (EETs) promotes tumor growth. A potent metformin derivative, HBB, suppressed ER+HER2- tumor growth, in part, by inhibiting CYP-mediated EET biosynthesis, thus serving as chemical probes for EET-dependent tumor growth. In the ovarian dependent SSM2ucd murine allograft mammary cancer model tumors expressed the immune checkpoint proteins B7-H3 and B7-H4. N-glycosylation of B7-H3 and B7-H4 is known to be important for their immunosuppressive function, but the mechanisms regulating N-glycosylation of B7-H3 and B7-H4 are unknown.

RESULTS: (±)14,15-EET promoted glycosylation of B7-H3 in human breast cancer cell lines MCF-7, T47D, and ZR75 (all P<0.05) as well as in the murine mammary carcinoma cell line SSM2ucd (P=0.02). (±)14,15-EET also increased glycosylated B7-H4 in MCF-7, T47D, and SSM2ucd cells (all P<0.01). The N-glycosylation inhibitor NGI-1 blocked (±)14,15-EET-induced N-glycosylation of B7-H3 and B7-H4. The biguanides metformin and more potent derivatives HBB and HCB did not alter the amount of B7-H3 or B7-H4 protein but reduced their N-glycosylation in MCF-7, T47D, and SSM2ucd cells (all P<0.05). In the SSM2ucd allograft model, SSM2ucd tumor reimplantation shortened tumor latency by more than half, to 20 days. In reimplanted tumors, higher levels of B7-H3 (P<0.01) and B7-H4 (P=0.04) correlated with shorter latency. In human breast cancer, assayed by tissue microarray, CYP3A4 expression correlated with higher expression of B7-H3 (r=0.97, P<0.0001) and B7-H4 (r=0.92, P<0.0001).
CONCLUSION: In ER+HER2- breast cancer, CYP arachidonic acid epoxygenase promotes N-glycosylation of B7-H3 and B7-H4 and is co-expressed with these proteins. Biguanides inhibited (±)14,15-EET biosynthesis and reduced N-glycosylation of B7-H3 and B7-H4, suggesting that biguanides may be used as chemical probes for EET dependent tumor growth. Higher B7-H3 and B7-H4 expression in the SSM2ucd model correlated with shorter escape of mammary tumors from dormancy suggesting that biguanide-mediated inhibition of B7-H3 and B7-H4 N-glycosylation may be a target for breast cancer therapeutics.

LIP-1

THE MOLECULAR CROSSTALK BETWEEN AGING, POLYUNSATURATED FATTY ACID OXIDATIVE METABOLISM, AND NEUROGENERATION IN C. ELEGANS

Jennifer Hinman[1,2], Morteza Sarparast[1], Elham Pourmand[1], Jamie Alan[3], Kin Sing Stephen Lee[1,2,3]

[1]Department of Chemistry; [2]Institute of Integrative Toxicology; [3]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI, 48824

Cardiorenal syndrome encompasses a spectrum of disorders where acute or chronic dysfunction of one organ can induce dysfunction in the other organ, reflecting the interdependent relationship between the heart and kidney. CYP450 enzymes metabolize N-3 and N-6 polyunsaturated fatty acids (PUFAs) into various bioactive lipid mediators, with cardioprotective properties. These lipids are further metabolized into less active or toxic diols by soluble epoxide hydrolase (sEH). Inhibition of sEH significantly attenuates the adverse effects of myocardial infarction but its role in protecting the kidney following cardiac MI is unknown. The objective of this study was to investigate cardiorenal protective effects of the global sEH genetic deletion in both aged male and female mice following MI. In this study, male and female WT and sEH null mice (15-18 mo) were subjected to permanent left anterior descending artery ligation to induce myocardial infarction. Echocardiography was used to assess the cardiac function at baseline and 7 days post MI. Frailty index was collected as a marker for aging/body condition. Tissues were collected for molecular and biochemical analyses. Echocardiography analyses demonstrated a reduced systolic and diastolic cardiac function after LAD surgery in all mice. However, female sEH null mice showed significantly better cardiac function compared to all other groups. Overall markers such as frailty index and plasma glucose levels (acute hyperglycemia) were attenuated in sEH null mice post MI. Activation of NLRP3 was observed in only WT mice post MI suggesting genotype differences in the innate immune response. Sex-dependent differences in mitochondrial antioxidant gene expression demonstrated female mice had significantly lower renal GPX1 gene expression. While aged sEH null female mice exhibited preserved markers of cell injury, ICAM-1 and KIM-1, compared to WT kidneys. Interestingly, all groups had significantly higher levels of renal mitochondrial DNA transcription factor Tfam post MI but increased mitochondrial DNA content was only observed in sEH null mice, suggesting better mitochondrial quality control in sEH null mice. Our data highlight both sex- and genotype-differences in renal injury following myocardial infarction in aged mice. Importantly, these preliminary data demonstrated aged female sEH null mice had better cardiac and renal protection.

Support acknowledgment: This study was partially supported by NIA R03 AG075465.

LIP-2

DOCOSAHEXAENOIC AND ARACHIDONIC ACID-DERIVED OXYLIPINS DEMONSTRATE REVERSED ASSICIATIONS WITH PSYCHOPATHOLOGY IN ANOREXIA NERVOSA

Nhien Nguyen[1], Jun Yang2, Christophe Morisseau[2], Dongyang Li[2], Eileen Lam[3], D. Blake Woodside[3], Bruce D. Hammock[2], and Pei-an Betty Shih[1]

[1]University of California, San Diego; [2]University of California, Davis; [3]University of Toronto, Canada

Oxylipins are bioactive lipid mediators derived from n-3 and n-6 polyunsaturated fatty acids (PUFA) via CYP450, LOX, COX, or non-enzymatic pathways. Anorexia Nervosa (AN) is a life-threatening psychiatric disorder with unknown etiology. This study clarified the role of oxylipins in AN risk and psychopathology using 45 women with AN (age: 29 ± 8) and 45 age-matched control women (age: 29 ± 8). 59 total oxylipins analyzed were derived from CYP450 (n=32), LOX (n=17), and COX (n=6), and non-enzymatic pathways (n=4). In addition, soluble epoxide hydrolase (sEH) activity and expression were analyzed.

Forty-seven% (n=9 epoxides and 6 diols) derived from n-3 docosahexaenoic acid (DHA) and n-6 arachidonic acid (ARA) of the CYP450 pathway were 22% to 45% lower in AN (age- and BMI- and FDR-adjusted $p<0.05$). Three ARA-derived HETEs (17% of LOX oxylipins) were 47% to 60% lower in AN (adjusted $p<0.05$), while COX oxylipin levels did not differ significantly between groups. Of the AN-associated oxylipins, four DHA-derived diols were linked to lower psychopathology severity scores (Eating Disorder Inventory [EDI]-3 and Eating Disorder Examination Questionnaire [EDE-Q]) with correlations ranging from -0.30 to -0.52 (all $p<0.05$ in Pearson correlation or covariate-adjusted regression tests). By contrast, ARA-derived diols were linked to higher severity scores on EDI-3 scales and composites ($r = 0.30$ to 0.40; all $p<0.05$), while none of the CYP450 epoxides were associated with scores. Because sEH is responsible for the conversion of epoxides to diols, sEH protein expression and enzyme activity were assessed and both showed higher levels in AN by 22% and 23% (adjusted $p=0.043$ and 0.024), respectively.

Our data reveal that oxylipins derived from DHA and ARA, the two most abundant PUFAs in the brain, significantly associate with AN psychopathology in the opposite direction, even after adjusting for PUFA levels. The opposite findings between DHA and ARA oxylipins with eating disorder severity may be due to the differential inflammatory properties known in n-3 versus n-6 PUFAs or their respective diol metabolites, sEH binding differences to epoxides, or other factors. Inflammation-modulating properties of oxylipins hold promise in future clinical applications if results are replicated and further research is warranted.

LIP-3

ASSOCIATIONS OF OXYLIPINS FROM CYTOCHROME P450 AND CYCLOOXYGENASE PATHWAYS WITH BRAIN BASED PHENOTYPES

Nhien Nguyen[1], Jun Yang[2], Christophe Morisseau[2], Dongyang Li[2], Eileen Lam[3], D. Blake Woodside[3], Bruce D. Hammock[2], and Pei-an Betty Shih1

[1]University of California, San Diego, [2]University of California, Davis, [3]University of Toronto, Canada

Oxylipins, bioactive lipid metabolites derived from n-3 and n-6 polyunsaturated fatty acids via enzymatic pathways (CYP450, LOX, COX) or auto-oxidation, are involved in many biological processes including inflammation. Oxylipin dysregulation has been implicated in psychiatric disorders, yet little is known about how they impact mental health traits in the healthy population. This study analyzed the associations of 59 oxylipins from CYP450 (n=32), LOX (n=17), COX (n=6), and non-enzymatic pathways (n=4) with brain-based phenotypes: depression, anxiety, and tolerance for high-fat food. In 45 healthy women (age: 29 ± 8), those with higher pro-inflammatory COX pathway prostaglandin from arachidonic acid (ARA), 6-keto-PGF1a, and CYP450 pathway diol oxylipin from α-linolenic acid, 15,16-DiHODE, had higher tolerance for high-fat food ($r= -0.34$ and -0.31, $p=0.02$ and 0.04, respectively). Pro-inflammatory 9-HODE from linoleic acid via auto-oxidation was linked to lower anxiety ($r= -0.31$; $p=0.04$), while LOX pathway oxylipins were not associated with brain phenotypes. In 45 women with anorexia nervosa (AN, age: 29 ± 8), those with higher levels of proinflammatory oxylipins from ARA (5 LOX pathway HETEs and 1 COX pathway PGF2) and the

anti-inflammatory 12- HEPE from eicosapentaenoic acid (EPA) had higher tolerance for high-fat food (r= -0.33 to -0.43, p=0.01 to 0.05). By contrast, AN women with higher 15,16-DiHODE level had lower tolerance for high-fat food (r= 0.33, p=0.04). Anxiety and depression are prevalent (32%-55%) in women with AN. Higher levels of ARA-derived 6-keto-PGF1a and 15- deoxy-PGJ2 (COX pathway) and docosahexaenoic acid (DHA)-derived 13,14-DiHDPE (CYP450 pathway) were linked to lower anxiety (r= -0.29 to -0.31, all p=0.05) in AN but not in healthy women. DHA-derived 4,5-DiHDPE and 7,8- DiHDPE were linked to lower depression (r= -0.35 and -0.39, p=0.02 and 0.01) in AN only. This study shows that CYP450- (diols) and COX- (prostaglandins) pathway oxylipins may be associated with brainbased phenotypes in healthy women and women with AN. Many COX pathway prostaglandins are known to be proinflammatory, whereas CYP450 pathway diols are considered more inflammatory than their epoxide precursors, reinforcing the likely link between inflammation, brain health, and behavioral phenotypes.

LIP-4

STRUCTURAL BASIS FOR THE COVALENT BINDING OF ACYLCERAMIDES IN FORMATION OF THE SKIN PERMEABILITY BARRIER

Saori Noguchi, William E. Boeglin and Alan R. Brash

Department of Pharmacology, Vanderbilt University, Nashville, TN 37232

Enzymes of the 12R-lipoxygenase pathway play a crucial role in forming the skin permeability barrier. The gene deficiencies are neonatal lethal in the mouse and in humans result in ichthyosis, a scaly skin disease. The 12R-LOX pathway enzymes oxidize linoleate esterified in skin-specific acylceramides to a 9,10- trans-epoxy-11trans-13-keto-octadecenoate. We hypothesize that the chemical reactivity of the oxidized linoleate facilitates covalent binding of the acylceramides to epidermal protein, forming the corneocyte lipid envelope, a critical structure of the barrier. The 11trans-13-ketone moiety of the oxidized 9,10-epoxy-linoleate, also in its 9,10-dihydroxy hydrolysis product, has potential reactivity via Michael addition to histidine or cysteine, or Schiff base with lysine, and here we addressed the amino acid target of the covalent binding. The oxidized lipids were synthesized from 13-HODE via DDQ oxidation, I2 isomerization to the t,t13-ketone, epoxidation with mCPBA to the 9,10-trans-epoxy-13-ketone, and sEH hydrolysis to the 9,10- dihydroxy-13-ketone. Rates of reaction with amino acids were assayed as decreases in UV absorbance (240 nm) of the epoxy-ketone and dihydroxy-ketone. The oxidized linoleates (100 μM) reacted in seconds with 1 mM cysteine, with rates of 4.9±0.76 AU/min with the epoxy-ketone and about ten-fold lower, 0.4±0.03 AU/min, with the dihydroxy-ketone, while there was no observable reaction with histidine or lysine. The cysteine conjugates were identified by LC-MS (glutathione adducts, m/z 616 and m/z 634, for epoxy-ketone and dihydroxy-ketone adducts) and 1H-NMR. Significantly, two unusual cyclic linoleates formed by KOH treatment of skin barrier proteins (C18 hydroxy-cyclohexenones, JBC 2023) were detected only from alkali treatment of the dihydroxy-ketone cysteine adduct, which strongly implies occurrence of this conjugate in the skin barrier. Taken together, our results point to the oxidized acylceramide of the 12R-LOX epidermal pathway reacting rapidly via Michael addition with cysteine residues, and being at least partly transformed to the dihydroxy-ketone conjugate, together forming the covalently-bonded corneocyte lipid envelope. The epidermal barrier contains cysteine-rich proteins such as loricrin and cysteine-rich envelope protein (CREP), and these are potential targets for the covalent binding of the oxidized-acylceramides.

Supported by NIH grant GM-134548 and by CABRI.

LIP-5

THE EFFECTS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) ON UNSATURATED LIPIDS UPTAKES AND THEIR OXIDATIVE METABOLISM IN THE WESTERN POPULATION

Elham Pourmand, Wenjuan Ma, Jenifer I Fenton, Angel Edwards, Faith Strickland, Tracy Fuller, Suzu Thompson, Emily C Somers, James Pestka, Kin Sing Stephen Lee

Department of Pharmacology and Toxicology, Department of Chemistry, and Department of Food Science and Human Nutrition. Michigan State University, East Lansing, MI, USA; Schools of Medicine and Public Health, University of Michigan, Ann Arbor, MI, USA

Omega-3 (ω-3) fatty acid levels have been associated with benefits in systemic lupus erythematosus (SLE). However, the impact of dietary unsaturated fatty acids (UFAs) on SLE pathogenesis is unclear, and the corresponding mechanisms are understudied. Oxylipins, potent lipid mediators, are UFA metabolites influencing inflammation. A Chinese study identified 26 altered oxylipins in serum of persons with SLE compared to controls. Thus, we hypothesize that SLE modulates UFA oxidative metabolism, affecting patient outcomes. This research delves into how 1) UFA levels, 2) SLE severity, 3) age, and 4) race affect oxylipin metabolism in adults with lupus from the US. Serum samples and questionnaires from a sociodemographically diverse study population of 372 persons with SLE and 158 general population controls. Oxylipin analysis utilized solid phase extraction, and HPLC coupled with tandem mass spectrometry was performed,. and serum fatty acids were analyzed using GC-MS spectrometry.
Our analysis revealed that arachidonic acid (AA) metabolite concentration, 14,15-DiHETrE, exhibits significant changes among sex, race, and age groups. Controls in all three study groups showed a significantly higher concentration of 14,15-DiHETrE than persons with lupus. However, a reverse trend was observed for cytochrome P450 (CYP) metabolites of AA; the level of 14,15-EpETrE was lower in the control compared to the lupus group. Regarding the ω-3 PUFA CYP metabolites, the concentration of 19,20-DiHDPE in female controls is higher than SLE. Both of these metabolites are substrates or products of soluble epoxide hydrolase (sEH) suggesting that sEH metabolism is significantly affected by SLE. sEH is an enzyme that converts largely anti-inflammatory epoxy fatty acids (EpFAs) to less active or proinflammatory dihydroxyFAs. The ratio of EpFAs to dihydroxyFAs indicates the balance between anti-inflammatory and pro-inflammatory processes. Persons with SLE consistently exhibited higher ratios of several specific EpFAs to dihydroxyFAs, indicating a decreased sEH activity or expression in the SLE group. These findings offer insights into SLE-related shifts in enzymatic pathways and inflammatory processes.
All in all, our results show that the sEH metabolites could be potential biomarkers to monitor lupus. Given the bioactivity of these metabolites, they could be key lipid mediators involved in modulating lupus activity, and thus potential novel therapeutic targets for treating or preventing SLE.

MET-1

PROTECTIVE EFFECTS OF GPR39 AGONISTS AGAINST VASCULAR COGNITIVE IMPAIRMENT AND DEMENTIA

Thierno M. Bah[1], Ph.D.; Elyse M. Allen[1], B.Sc.; Wenri H. Zhang[1], M.D.; Ph.D.; Catherine M. Davis[1], Ph.D.; Wenbin Zhu[1], MD, Ph.D.; Destine Krenik[1], B.Sc.; Jacob Raber[2], Ph.D.; Nabil J. Alkayed, M.D., Ph.D.[1,3]

[1]Department of Anesthesiology and Perioperative Medicine, Oregon Health & Science University, Portland, Oregon, USA; [2]Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, Oregon, USA; [3]Knight Cardiovascular Institute, Oregon Health & Science University, Portland, Oregon, USA

Vascular cognitive impairment (VCI) is the second most common cause of dementia, and rates are increasing due to population aging and increasing obesity rates. There is currently no disease modifying therapy for VCI. We previously observed upregulation of the enzyme soluble epoxide hydrolase (sEH) in cerebrovascular endothelium of postmortem human brain tissue with a history of VCI, and in cerebral microvessels from mice with high-fat diet (HFD)-induced cognitive impairment. sEH is responsible for the breakdown of anti-inflammatory and vasodilator eicosanoid called 14,15-epoxyeicosatrienoate (14,15-EET). We have recently identified GPR39 as a receptor for 14,15-EET. This study was designed to characterize the plasma pharmacokinetics (PK), blood-brain barrier penetration and effects of five publicly available GPR39 agonists on cognitive dysfunction due to metabolic syndrome in mice induced by HFD. Twelve-week-old mice were randomly assigned to receive one of five GPR39 agonists or vehicle by oral gavage. Blood samples and brain tissues were collected at six designated time points over 24 hours after oral administration. Another separate cohort of six-week-old mice were assigned to either HFD or standard chow for six months. Mice were then treated with GPR39 agonists GSK2636771 or TM-N1324 or vehicle for four weeks. Cognitive performance was evaluated using the Novel Object Recognition Test (NORT) and the Morris Water Maze (MWM). Two compounds reached plasma concentrations above their EC50 and were detected in the brain: GSK2636771 and TM-N1324. Mice treated with these compounds exhibited improved learning in the MWM compared to vehicle-treated mice. HFD caused an increase in fasting glucose, with no treatment effect for either drug. HFD decreased exploratory movement in NORT, but there was no treatment effect. Neither treatment nor diet influenced time spent in the anxietyevoking center of the open

field containing the objects. We conclude that GPR39 stimulation may serve as a therapeutic target in cognitive dysfunction due to metabolic syndrome. Results support previous findings that GPR39 endogenous agonist 14,15-EET is deficient in vascular cognitive impairment and dementia (VCID) in humans.

Support acknowledgement: This work was supported by the Harrington-Vinney Discovery grant to NJ. Alkayed.

MET-2

PREVENTION AND REVERSAL OF DIET-INDUCED OBESITY BY THE 20-HETE RECEPTOR BLOCKER, AAA, AND ITS IMPACT ON GLUCOSE HANDLING

Danielle Diegisser[1], Alexandra Wolf[1], Anna Varunok[1], Zixuan Wang[1], Adeniyi M. Adebesin[2], John R. Falck[2], Michal L. Schwartzman[1], Victor Garcia[1]

[1]Department of Pharmacology, New York Medical College, Valhalla, NY; [2]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX

20-HETE (20-Hydroxyeicosatetraenoic acid) has long been known as a mediator of inflammation and a driver of various pathological processes including hypertension, vascular remodeling, and most recently, disruption of insulin receptor signaling, which plays a critical role in glucose handling and body weight management. Recent studies have identified the 20-HETE receptor, GPR75, as a novel and effective target for ameliorating obesity and obesity-driven complications. In mice, Gpr75 global knockouts exhibit protection from obesity when fed a high-fat diet (HFD), and significantly improved associated complications of obesity including inflammation, glucose handling, and insulin sensitivity. In humans, it has been shown that individuals with loss-of-function GPR75 mutations exhibit lower BMI and lower incidence of diabetes. In light of these recent discoveries, we developed receptor blockers targeting GPR75 as potential therapeutics against diet-induced obesity. The current study utilizes the water-soluble 20-HETE receptor blocker AAA (N-disodium succinate-20-hydroxyeicosa-6(Z),15(Z)-diencarboxamide) and examines its ability to prevent and reverse body weight gain in a murine (B6, Jackson Lab) model of diet-induced obesity. In experiments studying the prevention of body weight gain of mice on HFD, our results show that AAA (50 mg/kg/day, i.p.) was able to prevent 76% of the weight gain observed in saline treated mice for three weeks (delta body weight gain @ 3 weeks: 9.2g vs 2.7g in saline and AAA-treated mice, respectively). Additionally, AAA-treated mice for 3 weeks exhibited improved glucose handling compared to saline-treated mice. Body composition analyzed by microCT showed a prevention of 61% of total adipose tissue volume gain. Interestingly, when AAA (50 mg/kg of AAA for two weeks, 3 injections per week) was administered after 16 weeks of HFD feeding, mice exhibited a reduction of 8% body weight and the severe impairment in glucose handling previously observed at 16 weeks was reversed. Further studies are necessary to dissect the array of molecular mechanisms altered and disrupted that provide this level of therapeutic benefit across our animal studies. Nevertheless, these preliminary data illustrate AAA's ability as a potential therapeutic for the prevention and treatment of obesity and obesity-driven complications.

MET-3

12-LOX PLAYS A KEY ROLE IN ISLET INFLAMMATION AND DYSFUNCTION

Melinee D'silva[1], Ercument Dirice[1], Hyunki Kim[2], Rohit N. Kulkarni[2] Raghavendra G. Mirmira[3] and Jerry Nadler[1,4]

[1]Department of Pharmacology, New York Medical College, New York, Valhalla, NY; [2]Joslin Diabetes Center; Department of Medicine, BIDMC; Harvard Stem Cell Institute; Harvard Medical School, Boston, MA.; [3]Department of Medicine, University of Chicago, IL; [4]VA Research Service, Mather, CA.

Inflammation in islets leads to pancreatic β-cell dysfunction and loss of mass leading to Type 2 diabetes (T2D). The lipoxygenases (LOXs) are a family of enzymes that catalyze the oxygenation of cellular poly-unsaturated fatty acids to form lipid inflammatory mediators. 12-lipoxygenase (12-LOX) converts arachidonic acid to 12-hydroperoxyeicasatetranoic acid (12-HPETE) which is then reduced to a more stable 12-hydroxyeicosatetraenoic acid (12-HETE) by glutathione peroxidase. In the islet, increased expression or activity of 12-LOX catalyzes the production of 12-HETE which accelerates inflammation and promotes

oxidative stress. 12-LOX has been identified in both rodent and human endocrine pancreas and in both are upregulated in the setting of pre-diabetes and T2D. 12-LOX in the mouse (unlike in humans) can be generated by an Alox15 or Alox12 gene while in humans only Alox12 leads to expression of 12-LOX. Selective 12-LOX inhibition prevents islet inflammation and β-cell damage in human islets treated with cytokines linked to T2D development. There have been no reported mouse models of T2D that demonstrate increased Alox12 expression in the islet, thus limiting the testing of inhibitors useful for human disease. The Kulkarni Lab has developed a β-cell insulin receptor knockout mouse (βIRKO) that has a local β-cell signaling defect and develops age-related T2D. We have identified that at the time of developing diabetes (9-11weeks age), the βIRKO mouse showed a selective increase of 12-LOX in their islets. We examined different tissues in these mice which included liver and inguinal white adipose tissue and compared expression to control mice at similar ages. We observed increases in Alox12 mRNA and 12-LOX protein expression by PCR and western blotting respectively. This increase of 12-LOX and inflammatory lipids may underlie or exacerbate the endoplasmic reticulum stress and dysfunction of β-cells in the setting of insulin resistance. These data suggest that the βIRKO mouse is a useful model to directly test the role of increased 12-LOX in the pathogenesis of T2D and examine the effect of selective 12-LOX inhibitors during development of the disease.

MET-4

THE ROLE OF SEH DELETION ON HEPATIC LIPID METABOLISM AND LIPID DROPLET DYNAMICS AND ITS EFFECT ON CARDIOVASCULAR FUNCTION UNDER A FAT AND CARBOHYDRATE RICH DIET

Timo Frömel,[1] Laila R. B. Dos Santos,[1] Nicole Mangels,[1] Zumer Naeem,[1] Sven Zukunft,[1] Johannes Graumann,[2] Rüdiger Popp,[1] Bruce D. Hammock,[3] Ingrid Fleming,[1,4]

[1]Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe University, Frankfurt, Germany. [2]Max Planck Institute for Heart and Lung Research. [3]Department of Entomology and UCD Comprehensive Cancer Center, University of California, Davis, USA. [4]German Centre for Cardiovascular Research (DZHK) partner site Rhein-Main, Germany.

Therapeutic approaches to lower lipid accumulation in steatohepatitis by increasing lipolysis in the liver can result in toxic effects caused by free fatty acids and subsequent systemic inflammatory responses. Lipophagy, the autophagic removal of excessive lipids would be an alternative approach to lower the hepatic lipid load. Given that soluble epoxide hydrolase deficiency is associated with a decrease in cholesterol synthesis, the downregulation of key lipid-metabolizing enzymes, such as HMG-CoA reductase, fatty acid synthase, and the lowdensity lipoprotein receptor under normal diet in hepatocytes, the following study investigated the effects of soluble epoxide hydrolase (sEH) deletion on lipid metabolism and cardiovascular function. We could show that sEH-deficient mice displayed improved glucose handling, insulin sensitivity, and reduced blood pressure when fed a high fat and high carbohydrate diet in comparison to the respective control animals. Furthermore, the study revealed that sEH deletion led to alterations in hepatic cholesterol and triglyceride levels, with decreased hepatic triglyceride content and altered lipid droplet size. This effect could be linked to changes in lipid metabolism related proteins, including Perilipin 2 and lysosome-associated proteins. The study suggested that sEH deletion could impact lipid droplet formation by altering the interactions with the endosomes or autophagosome as well as the mitochondria, leading to a modified lipid metabolism. These findings proposed sEH as a potential target for addressing metabolic diseases protecting animals against hyperglycaemia, insulin insensitivity, hypertension as well endothelial dysfunction associated with over nutrition and abnormal lipid accumulation in the liver.

MET-5

ROSIGLITAZONE AND DIET REGULATES THE PPARγ COREGULATOR INTERACTOME TO CONTROL FAT PAD SIZE

Zachary A. Kipp[1], Evelyn A. Bates[1], Sally Pauss[1], Genesee J. Martinez[1], Wang-Hsin Lee[1], Mei Xu[1], Terry D. Hinds Jr.[1,2,3]

[1]Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY. [2]Barnstable

Brown Diabetes Center, University of Kentucky, Lexington, KY. [3]Markey Cancer Center, University of Kentucky, Lexington, KY

Nuclear receptors are transcription factors that control the expression of their target genes once activated by a ligand. Nuclear receptors can be regulated through protein-protein interactions with coregulators that can either increase transcription activity (coactivator) or decrease transcriptional activity (corepressor). Peroxisome proliferatoractivated receptor gamma (PPARγ) is a nuclear receptor that has an important function in regulating adipogenesis and is the pharmacological target of the insulin-sensitizing drug Rosiglitazone (Rosi). Rosi has been previously shown to change the distribution of adipose tissue in humans. However, it's unknown how Rosi changes the coregulator interactome and if it's affected by a high-fat or a low-fat calorie matched diets in in vivo adipose depots. To determine this, we utilized the PamGene PamStation Nuclear Hormone Receptor (NHR) analysis, which uses the Microarray Assay for Realtime Coregulator-Nuclear Receptor Interaction (MARCoNI) technology. Mice were fed a normal chow diet (NCD) or a high-fat diet (HFD) for 16 weeks, followed by an administration of Rosi (15 mg/kg) or vehicle every other day for 4 weeks. On NCD and HFD there was no difference in total body weight, but Rosi significantly increased fat mass on the HFD measured via echoMRI. Rosi significantly reduced plasma glucose levels on a HFD. On a NCD there were no differences in the adipose depots. On a HFD, Rosi significantly decreased the mass of the retroperitoneal white adipose tissue (rWAT) depot and liver mass while increasing the brown adipose tissue (BAT) mass. We then determined the coregulator interactome in the tissues that were significantly changed using the PamGene PamStation NHR analysis. We determined that Rosi differentially regulated the PPARγ coregulator interactome compared to the vehicle-treated mice in the NCD and HFD-fed groups in the rWAT, liver, and BAT. Rosi had similar effects on the number of coregulators associating and disassociating with PPARγ in the rWAT and BAT. However, in the liver, there were significantly fewer coregulators disassociating from PPARγ with Rosi on a HFD than the NCD. We then characterized the top 25 coregulators that were associating and disassociating with the Rosi treatment in the rWAT, BAT, and liver in the NCD and HFD groups. To determine how the Rosi-induced coregulator interactome effects PPARγ activity we measured the expression of its target genes by Real-Time PCR. In the HFD group, Rosi induced the expression of the PPARγ target gene, Pdk4, in the rWAT and BAT measured. Rosi and diet alter the coregulator interactions with PPARγ which may be a factor that regulates PPARγ activity leading to changes in adipose tissue distribution. Support acknowledgement:
This work was supported by the National Institutes of Health F31HL170972 (Z.A.K.) and R01DK121797 (T.D.H.J.) and R01DA058933 (T.D.H.J

MET-6

PROSTAGLANDINS INTERGRATE ENTEROCYTE-HEPATOCYTE FASTING RESPONSES

Run Lu[1], Matthew Edin[2], Fred B. Lih[2], Darryl Zeldin[2], and Victor L. Schuster[1,3]

[1]Medicine and 3Biochemistry, Albert Einstein College of Medicine, Bronx, NY; [2]Environmental Cardiopulmonary Disease Group, NIEHS, Durham, NC

The intestine and liver must coordinate their responses to fasting and feeding. Prior studies indicate that exogenous prostaglandins (PGs) applied experimentally to hepatocytes suppress nutrient-induced de novo lipogenesis (DNL). Here we asked whether endogenous intestinal and liver PGs might coordinately control liver DNL. To approach this question, we probed published circadian gene/protein expression databases for PG-related enzymes, transporters, and receptors; this revealed that many intestinal and liver PG genes exhibit circadian rhythmicity, suggesting a role in modulating response to nutrient ingestion. When we fed chow + sucrose drinking water to fasted wild type (WT) mice, jejunal Cox1 mRNA and portal vein plasma PGE2, PGF2α, and PGD2 concentrations fell. Concurrently, mRNA for liver PGT, the rate-limiting in PG inactivation, increased. Together, these intestinal and liver changes would limit hepatocyte exposure to intestinal PGs after eating, thus avoiding unwanted suppression of DNL. To test this hypothesis further, we globally deleted PGT (GKO). In contrast to WT mice, upon eating, GKO mice displayed markedly increased portal vein plasma PGE2 and PGF2α concentrations. Because liver PGT is absent in GKO mice, these high portal PGs were passed on to hepatocytes, resulting in marked blunting of the normal liver response to carbohydrate ingestion. Indeed, in GKO there was no post-prandial rise in hepatocyte carbohydrate response element binding protein (ChREBP) or fatty acid synthase (Fasn). When we combined paired data

from three feeding conditions (fasting, fructose gavage, and chow/sucrose feeding) and two genotypes (WT and GKO), high portal PGF2α stood out as significantly associated with lower liver ChREBPβ mRNA (r2 = 0.53, p = .00009). When we gave the PGF2α inhibitor AL8810 to GKO mice, the suppression of DNL genes was reversed. We conclude that, during fasting, high intestinal-portal PGF2α, and low liver PGT, combine to (appropriately) suppress liver ChREBP, Fasn, and DNL. During feeding, intestinal-liver PGs are reduced, allowing DNL. These data thus reveal a previously unappreciated metabolic role for enterocyte-hepatocyte PG signaling. ***Support acknowledgement***: NIH 2 R01 DK110063-07

sEH-1

DELETION OF THE SOLUBLE EPOXIDE HYDROLASE IMPAIRS INTESTINAL ANGIOGENESIS AND LACTEAL DEVELOPMENT

Yanis Afir, Rüdiger Popp, Timo Frömel, Zumer Naeem, Ingrid Fleming

Institute for Vascular Signalling, Goethe University, Frankfurt am Main.

Blood and lymphatic capillaries of the small intestine are characterized by their key role in nutrient absorption as well as their unique dynamic regenerative state. Despite their importance, the molecular mechanisms governing their generation and homeostasis are insufficiently understood. Epoxides of polyunsaturated fatty acids (PUFAs), generated by cytochrome P450 enzymes, and the diols generated by the subsequent activity of the soluble epoxide hydrolase (sEH), have been shown to play an important role in angiogenesis and lymphangiogenesis in the retina and in cancer. Here, we assessed the impact of the sEH on intestinal angiogenesis and lymphangiogenesis.

Mapping of the expression of the sEH revealed its high abundance in enterocytes, predominantly in the proximal small intestine. Expression of the protein was also higher at the top of the villi. Developing lacteals also expressed the sEH (P7 and P21), as did the nascent capillaries but sEH expression was not detectable in lacteals and blood capillaries from adult mice.

Deletion of the sEH had a clear impact on blood vessel and lymphatic development at P7 and P21, with a significant decrease in the lacteal/villi ratio but with a higher number of lacteal sprouts and a higher expression of Prox1. In sEH-/- mice we also observed a decrease in blood capillary density within the villi.

Moreover, we found evidence suggesting that sEH inhibition modulates lipid uptake in the gut, with a bigger size of lipid droplets inside the enterocytes of the top villi.

Besides, sEH deletion reshapes the immune cells landscape in the proximal intestine, with, in particular, a massive upregulation of myenteric resident macrophages.

Overall, our findings suggest an important role for sEH-related lipid mediators in the intestinal angiogenesis and lymphangiogenesis in a developmental model, as well as regulatory effect of lipid absorption. Experiments are ongoing to determine the mechanisms by which PUFA epoxides and diols mediate these effects.

sEH-2

DUAL COX-2/sEH INHIBITION ENHANCES IMMUNOTHERAPY AND CHEMOTHERAPY TO INDUCE BLADDER CANCER REGRESSION

Kimberly Vazquez[1,2], Eva Rothenberger[1,2], Weicang Wang[3], Sung Hee Hwang[3], Diane R. Beilenberg[4], Bruce D. Hammock[3], Dipak Panigrahy[1,2]

1Dept. of Pathology and 2Center for Vascular Biology Research, BIDMC, Harvard Medical School, Boston, MA; 3Dept. of Entomology/Comprehensive Cancer Center, UC Davis, CA; 4Vascular Biology Program, Boston Children's Hospital, Boston, MA

Unresolved inflammation plays a critical role in bladder cancer initiation and progression. Chemotherapy (e.g. gemcitabine and cisplatin), the standard of care for advanced bladder cancer, disrupts inflammation resolution and is only partially effective in preventing tumor recurrence after treatment. Immunotherapy has emerged as a potential treatment option for bladder cancer patients, however it is ineffective in 80% of patients and can induce a pro-inflammatory cytokine storm. Therefore, there is a critical unmet medical need to improve chemotherapy and immunotherapy in bladder cancer. To address this, we have developed dual COX-2/sEH inhibitors (e.g. PTUPB). This lead compound is representative of a newly patented class of "dual acting" molecules that target two important enzymes in the arachidonic acid cascade with nanomolar

binding affinity: cyclooxygenase-2 (COX-2) and soluble epoxide hydrolase (sEH). Oral dosing of PTUPB is well-tolerated in animal models. Since chemotherapy and immunotherapy both induce tumor-promoting inflammation via an eicosanoid/cytokine storm, we hypothesized that dual COX-2/sEH inhibition would enhance immunotherapy in experimental bladder cancer via anti-inflammatory mechanisms. When syngeneic (MB49) bladder tumors reached ~200 mm3 in immunocompetent mice, treatment was initiated with PTUPB, anti-CTLA-4, anti-PD1, gemcitabine & cisplatin, or various combinations thereof. Here we demonstrate that chemotherapy and/or immunotherapy stimulated sEH and COX-2 expression in tumor tissue which was counter-regulated by PTUPB. While monotherapy treatment with PTUPB, gemcitabine & cisplatin, anti-PD1 or anti-CTLA-4 suppressed tumor growth compared to control, the tumors escaped single treatments by the end of the study. Remarkably, dual COX-2/sEH inhibition in combination with chemotherapy (gemcitabine and cisplatin) and/or immune checkpoint blockade (anti-CTLA-4 or anti-PD1) induced sustained tumor regression via synergistic anti-tumor activities. Chemotherapy and/or immunotherapy induced the expression of ER stress response genes (e.g. BiP and CHOP) and angiogenic factors (e.g. EGF and VEGF-C) in bladder cancer tissue, which were repressed by PTUPB. PTUPB also prevented chemotherapy-induced toxicity and prolonged survival in an orthotopic MB49 tumor model. Taken together, our results demonstrate dual COX-2/sEH inhibition as a novel therapeutic approach to enhance immunotherapy in bladder cancer without overt toxicity.

sEH-3

INHIBITION OF SOLUBLE EPOXIDE HYDROLASE, A POTENTIAL NOVEL AD/ADRD TREATMENT?

Jane J. Border,[1] Chengyun Tang[1,2], Huawei Zhang[1], Xing Fang[1], Yedan Liu[1], Sung Hee Hwang[3], Bruce D. Hammock[3], Richard J. Roman[1], and Fan Fan[1,2]

[1]University of Mississippi Medical Center, Jackson, MS; [2]Medical College of Georgia, Augusta University, Augusta, GA; [3]University of California, Davis, Davis, CA

Recent human studies have provided substantial genetic evidence linking soluble epoxide hydrolase (sEH) to Alzheimer's Disease and Alzheimer's Disease-Related Dementias (AD/ADRD). Inhibition of sEH has been documented to enhance cognition in AD mice due to its anti-inflammatory and neuroprotective effects. Our previous work confirmed that inhibiting soluble epoxide hydrolase mitigates cognitive impairments in rat models of AD/ADRD, potentially by ameliorating impairments in cerebral hemodynamics. In this study, we aimed to investigate whether the cerebral vascular protective effects associated with sEH inhibition extend to other aspects of AD/ADRD pathology. We administered the sEH inhibitor TPPU (1 mg/kg/day in drinking water for 9 weeks) to 18-month-old diabetes (DM)-related ADRD rats and 6-month-old TgF344-AD rats. Notably, TPPU treatment did not alter body weight, plasma glucose, or HbA1C levels. Using an 8-arm water maze, we assessed learning, short-term, and long-term memory dysfunction in both DM and AD rats. The results demonstrated that TPPU treatment effectively rescued these cognitive deficits in AD/ADRD. Moreover, we observed that inhibiting sEH in AD/ADRD rats normalized cerebral hemodynamics, including the myogenic response of the middle cerebral artery (MCA) and parenchymal arteriole (PA), as well as the autoregulation of cerebral blood flow. In isolated vascular smooth muscle cells (VSMCs) from AD MCAs, contractility was markedly reduced (3.56 ± 0.33%) compared to controls (8.32 ± 0.42%). Interestingly, treatment of control cells with Aβ (1-42) led to a dose-dependent decrease in cell contractility. Moreover, diminished VSMC contractility was rescued by TPPU treatment in AD cells (7.14 ± 0.59). Furthermore, TPPU-treated AD brains exhibited significantly reduced amyloid plaques in the cortex and hippocampus. Neuronal counts in the hippocampus increased in both TPPU-treated AD (86.91 ± 3.71 vs. 64.57 ± 3.92) and DM-ADRD (109.81 ± 3.60 vs. 86.42 ± 3.44) rats. In conclusion, our study underscores the effectiveness of chronic sEH inhibition in reversing cerebrovascular dysfunction, neurodegeneration, and reducing amyloid plaques. Moreover, our findings demonstrate that this intervention successfully mitigates cognitive impairments in animal models of AD/ADRD. These collective results strongly advocate for the potential of sEH inhibition as an innovative and promising therapeutic avenue for addressing AD/ADRD.

Support acknowledgment:
This study was supported by grants AG050049 (FF), AG057842, AG079336 (FF, RJR), HL138685 (RJR), and P20GM104357 (FF, RJR) from the National Institutes of Health.

****

Poster Session II
Monday, October 16th
7:00-8:30 pm
Chesapeake I Room

BRN-1

SERUM SOLUBLE EPOXIDE HYDROLASE DERIVED LINOLEIC ACID SPECIES AND THE COGNITIVE BENEFITS OF EXERCISE IN TYPE-2 DIABETES

Natasha Z. Anita[a,b,c], Felicia Kwana[b,c], Si Won Ryoo[a,b,c], Chelsi Major-Orfao[b,c], William Z. Lin[a,b,c], Shiropa Noor[a,b,c], Sean Nestor[b,d], Myuri T. Ruthirakuhan[a,b], Bradley J Macintosh[b,e], Maged Goubran[b,d],Krista L. Lanctôt[a,b,c,d], Nathan Herrmann[b,d,f], Jeremy Gilbert[f], Ameer Y. Taha[g], Walter Swardfager[a,b,c *]

[a]Department of Pharmacology & Toxicology – University of Toronto, Canada; [b]Sunnybrook Research Institute, Toronto, Ontario, Canada; [c]KITE Research Institute, Toronto Rehabilitation InstituteUniversity Health Network, Canada; [d]Department of Psychiatry - University of Toronto, Canada; [e]Department of Medical Biophysics – University of Toronto, Canada; [f]Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada; [g]Department of Food Science and Technology, College of Agriculture and Environmental Sciences, University of California, Davis; West Coast Metabolomics Center, Genome Center, University of California - Davis; Center for Neuroscience, One Shields Avenue, University of California - Davis, CA, USA.

Type 2 diabetes mellitus (T2DM) increases the risk of cognitive decline and dementia. While exercise improves cognition in healthy individuals, there has been little evidence of benefit in T2DM. One theory to explain this is the disruptions in oxidative and metabolism processes; specifically, we investigate the cytochrome P450-soluble epoxide hydrolase (CYP450-sEH) pathway. Polyunsaturated fatty acids can be converted by CYP450s into proresolving epoxides; however, they are quickly metabolized by sEH into inactive or cytotoxic diols. Circulating linoleic acid (LA)-derived diols can be elevated by exercise in healthy adults, but their association with poor cognitive performance in T2DM suggests that these species may limit the brain benefit of exercise in this population. Here, we examine the association between these oxylipins and cognition in T2DM participants (glycosylated hemoglobin [HbA1c] > 6.4%, impaired fasting glucose/glucose intolerance) beginning an exercise program and in non-exercising T2DM. Participants completed baseline and 3-months visits where they provided fasting blood for serum measures of four LA-derived oxylipins via ultra-high-performance liquid chromatography tandem mass spectrometry. Executive function was assessed using a composite z-score from the Stroop Colour-Word Interference, FAS Verbal Fluency, Digit Symbol Substitution, and Trails Making Test Part B. Verbal memory was assessed using the California Verbal Learning Test, 2nd edition. Repeated measures tests were used to determine differences in oxylipin concentrations and cognitive performance over 3 months. To test interaction effects between oxylipins and exercise on cognition, analyses of covariance models controlled for the following terms: oxylipin, age, exercise status, baseline cognitive scores and an exercise×oxylipin interaction. Across 24 exercisers and 27 controls with T2DM (mean age 62±10, 43% female, HbA1c 7±1%), there were no significant differences across demographic characteristics, cognitive scores and oxylipin concentrations at baseline. After 3 months, no differences in oxylipins were observed across groups. Verbal memory increased from baseline in exercisers compared to controls (time×exercise interaction $F_{1,45}=7.789$, $p=0.008$), whereas executive function did not differ between groups over time. There was a significant interaction between higher baseline 9,10-dihydroxyoctadecenoic acid (9,10-DiHOME) concentrations predicting poorer executive function at 3 months, which was stronger in exercisers (exercise×9,10-DiHOME interaction $F_{1,45}=5.429$, $p=0.024$). Findings were consistent when controlling for education. The results suggest that LA-derived diols might predict exercise-induced executive function changes in T2DM. Combined administration of an sEH inhibitor may boost exercise benefits to address executive dysfunction in T2DM.

Support acknowledgement: Canadian Institutes of Health Research; Banting & Best Diabetes Centre; National Institute of Diabetes and Digestive and Kidney Diseases

BRN-2

SOLUBLE EPOXIDE HYDROLASE INHIBITION REVERSES COGNITIVE DYSFUNCTION IN A MOUSE MODEL OF METABOLIC SYNDROME BY MODULATING INFLAMMATION

Thierno M. Bah[1], Ph.D.; Catherine M. Davis[1], Ph.D.; Elyse Allen[1], B.Sc.; Rohan N. Borkar[1], B.Sc.; Ruby Perez[2], B.Sc; Marjorie R. Grafe[3], MD, Ph.D.; Jacob Raber[2,4], Ph.D.; Martin M. Pike[5], Ph.D.; Nabil J. Alkayed, M.D., Ph.D.[1,6]

[1]Department of Anesthesiology and Perioperative Medicine, Oregon Health & Science University, Portland, Oregon, USA; [2]Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, Oregon, USA; [3]Department of Pathology, Oregon Health & Science University, Portland, Oregon, USA; [4]Departments of Neurology and Radiation Medicine, Division of Neuroscience, ONPRC, Oregon Health & Science University, Portland, Oregon, USA; [5]Advanced Imaging Research Center, Oregon Health & Science University, Portland, Oregon, USA; [6]Knight Cardiovascular Institute, Oregon Health & Science University, Portland, Oregon, USA

Midlife metabolic syndrome (MetS) is associated with cognitive impairment in late life. The mechanism of delayed MetS-related cognitive dysfunction (MetSCD) is not clear, but it has been linked to systemic inflammation and chronic cerebral microangiopathy. Other than early risk factor modification, there is currently no treatment for late life MetSCD. We investigated the effect of soluble epoxide hydrolase (sEH) inhibitor 4-[[trans-4-[[(tricyclo[3.3.1.13,7]dec-1-ylamino)carbonyl]amino]cyclohexyl]oxy]-benzoic acid (tAUCB) on cognitive performance, cerebral blood flow (CBF) and central and peripheral inflammation in the high fat diet (HFD) model of MetS. At 6 weeks of age, mice were randomly assigned to receive either HFD or standard chow (STD) for 6 months. Mice received either t-AUCB or vehicle for 4 weeks. Cognitive performance was evaluated, followed by CBF measurement using magnetic resonance imaging (MRI). At the end of the study, blood was collected for measurement of eicosanoids and inflammatory cytokines, and brains were analyzed by immunohistochemistry for glial activation markers. HFD caused a significant impairment in novel object recognition. Treatment with t-AUCB increased plasma levels of 14,15-EET, prevented this cognitive impairment and modified hippocampal glial activation and plasma cytokine levels, without affecting CBF in mice on HFD. sEH inhibition for four weeks prevents cognitive deficit in mice on chronic HFD, by modulating inflammatory processes, without affecting CBF.
Support acknowledgement: This work was supported by the National Institutes of Health grant AG058273-01 to NJ. Alkayed. Eicosanoid Research Association, Inc. The 19th International Winter Eicosanoid Conference Wint

BRN-3

SOLUBLE EPOXIDE HYDROLASE INHIBITORS AND PET IMAGING IN CLINICAL TRIALS

Dominic Siler MD PhD[1], Ross Martini MD[2], Ines Koerner MD PhD[2], Holly Hinson MD[3], Miriam Treggiari MD PhD[4], Nabil Alkayed MD,PhD[1,2]

[1]OHSU Dept. of Neurosurgery; [2]OHSU Dept. of Anesthesiology and Perioperative Medicine; [3]UCSF Dept. of Neurology; [4]Duke University Dept of Anesthesiology

Despite half a century of active research, delayed cerebral ischemia (DCI) remains a common and devastating complication of subarachnoid hemorrhage (SAH) that is difficult to predict and even more difficult to prevent. Novel therapeutic and diagnostic tools are needed to improve the outcomes in this patient population where morbidity and mortality remain high. We have identified a novel mechanism by which derangements in brain epoxyeicosatrienoate (EET) metabolism is associated with the onset of DCI and have identified interventions targeting soluble epoxide hydrolase (sEH), a key enzyme that controls the bioavailability of EETs, that may prove beneficial in preventing DCI and improving outcomes in SAH patients. In an ongoing clinical trial, we are testing clinical use of a novel radiolabeled sEH inhibitor 18F-fluoronicotinamide (18F-FNDP) for positron emission tomography (PET) quantification of sEH activity in critically ill SAH patients at risk for developing DCI. In this study, we evaluate safety and efficacy of 18F-FNDP to quantify brain sEH activity in critically ill patients and test the hypothesis that sEH brain activity is altered in SAH and associated with the risk of DCI. To test this, enrolled patients with aneurysmal SAH as well as patients with elective clipping/coiling of unruptured cerebral aneurysms admitted to the neuroscience intensive care unit, undergo PET-CT quantification of 18F-FNDP uptake within 72 hours of admission.

Single nucleotide polymorphisms in the gene encoding sEH, EPHX2, that are known to alter sEH activity are quantified by qPCR in all patients. Biomarkers of EETs metabolism, including plasma EETs concentrations are obtained in all participants. Cerebrospinal fluid EETs concentrations are obtained when clinically available. Clinical outcomes include incidence of DCI, angiographic vasospasm, elevated trans-cranial Doppler values, mortality, and GOS at discharge. Regional brain 18F-FNDP uptake will be compared between groups, and correlated with plasma/CSF concentrations, EPHX2 status and clinical outcome. Here we summarize our ongoing work characterizing the role of sEH and EETs in the development of DCI and clinical trials of pharmacological sEH inhibitors to prevent DCI and improve outcomes in SAH patients. Finally, we share early results of our clinical study using 18F-FNDP PET to quantify changes in brain sEH activity in critically ill SAH patients at risk for developing DCI.
Support acknowledgement: Brain Aneurysm Foundation, Medical Research Foundation, GSK (compounds only), Foundation for Anesthesia Education and Research

BRN-4

APPLICATION OF TARGETED BIOACTIVE LIPIDS AND PHOSPHOLIPIDS TO ATTENUATE EPIGENETIC INSULT IN DEMENTIA AND ALZHEIMER'S

Kane PC[1], Pouria S[2], Speight MO[3], Matzinger CA[4]

[1]Cellular Health Foundation, Chesterfield, Virginia, United States, drpckane@protonmail.com [2]Kings College, Private Practice, London, United Kingdom [3]Private Practice, Charlotte, North Carolina, United States 4Private Practice, Henderson, Nevada, United States

Our clinical findings over the past 25 years have revealed that neuroinflammation, dementia and Alzheimer's may be hallmarked by an increase in very long chain fatty acids (VLCFAs) forming ceramides, which create aberrations in cell/organelle membranes interrupting membrane integrity, endoplasmic reticulum, organelle and neurometabolic function. Epigenetic insult results in disruption of organelle interplay reflected in both aberrant lipids (ceramides, lipid rafts, VLCFAs, sphingomyelin, oxidized lipids & cholesterol) and aberrant proteins (misfolded, aggregated, unfolded) throughout the cellular components (ER, mitochondrion, peroxisome, cytosol, nuclear envelope, membranes). Epigenetic insult may involve either/both nuclear and mitochondrial DNA adducts that can compromise gene expression in normal individuals, but may also explain the variations in disease progression in patients with the same diagnosis, including those with inherited disease. Altered gene expression following epigenetic insult results in impaired peroxisomal and mitochondrial respiration, derangement of neural, cellular and organelle membranes, neuroinflammation, aberrant phospholipid architecture, ceramide formation and endoplasmic reticulum / ER stress (both smooth and rough ER are involved) with the unfolded protein response via mitochondria-associated membranes. Disease progression in neurological disease may be identified with a sharp increase in sphingomyelin in the outer membrane cellular leaflet with concomitant decline in unsaturated phosphatidylcholine levels. Derangement of neural, cellular and organelle membranes, along with deficits of the phospholipids, serve as primary therapeutic targets, using phospholipids to clear debris from epigenetic insult appearing as nuclear and mitochondrial DNA adducts and to optimize membrane function. Therapeutic modalities include application of targeted bioactive lipids/phospholipids,SR3 4:1 oil, linoleic acid, caviar, glutathione, stimulation of resolvins, protectins, maresins with chaperones butyrate, phenylbutyrate, TUDCA, and a membrane stabilizing diet. To optimize membrane architecture, we clinically address appropriate balance, fluidity and content of phospholipids with bioactive lipids, which is crucial toward optimizing neurometabolic function. Our lipid therapeutic approach has yielded marked clinical improvement in subjects following 3 to 6 months of a targeted regimen corresponding with normalization in red cell lipids. Stabilization of the membranes of organelles, cell leaflets, cardiolipin along with identification and clearance of DNA adducts may be new therapeutic targets to address epigenetic-induced neuroinflammatory dementia.

BRN-5

CYTOCHROME P450-EPOXIDE HYDROLASE METABOLISM OF POLYUNSATURATED FATTY ACIDS ON NEURODEGENERATION

Kin Sing Stephen Lee, Morteza Sarparast, Elham Pourmand, Jennifer Hinman, Jamie Alan

Departments of Pharmacology & Toxicology and Chemistry, Michigan State University, East Lansing, MI

Dementia significantly contributes to disability in elderly patients and is ranked as the seventh global cause of death. Amidst all forms of dementia, Alzheimer's disease and related dementia (ADRD) is the most common dementia, affecting six million individuals in the US alone, casting immense economic burdens on families and societies. The number of ADRD cases is expected to triple by 2050 due to global aging, underscoring a pressing crisis. Addressing this challenge hinges on understanding the complicated mechanisms driving ADRD pathogenesis to drive the development of innovative interventions. Recent insights highlight cytochrome P450-epoxide hydrolases (CYP-EH) metabolism of polyunsaturated fatty acids (PUFAs) as critical pathways in ADRD pathogenesis. Inhibition of this pathway, through pharmacological intervention or genetic manipulation, alleviates tauopathy, amyloidosis, and neuroinflammation across diverse ADRD animal models. Nonetheless, the mechanism of how CYP-EH metabolites influence ADRD pathogenesis is understudied due to the complexity of CYP-EH metabolism and the pathogenesis of ADRD. This challenge is exacerbated by the intricate nature of aging-related disease research. To tackle these challenges, our team has turned to Caenorhabditis elegans (C. elegans), a versatile model organism widely used for aging studies. C. elegans offers numerous advantages including ease of handling, cost-effectiveness, genetic malleability, and compatibility with high-throughput screening. These advantages make this organism an ideal tool to study the unknown mechanisms underlying the beneficial effects of CYP-EH PUFA metabolites in ADRD and neurodegeneration. This presentation explores C. elegans as a novel model organism to delineate the complex molecular crosstalk between CYP-EH PUFA metabolism and neurodegeneration. Our findings confirm the conservation of mammalian CYP-EH metabolism in C. elegans and validate the applicability of insights to rodent models. Notably, we identified dihydroxyeicosadienoic acid as a critical lipid mediator that drives ferroptosis-induced neurodegeneration in C. elegans. In essence, our study positions C. elegans as an innovative animal model, shedding light on the complicated interplay between CYP-EH PUFA metabolism and neurodegeneration. The implications extend beyond ADRD, beckoning exploration into the broader landscape of diseases associated with PUFA metabolism.

Support acknowledgment**:** This project is partially supported by the National Institute of Aging (R03 AG076465) and the National Institute of General Medical Sciences (R35 GM146983). The abstract was partly edited by ChatGPT 3.5.

CDV-1

RENAL PROTECTIVE EFFECTS OBSERVED IN AGED SOLUBLE EPOXIDE HYDROLASE NULL MICE FOLLOWING MYOCARDIAL INFARCTION

Liye Fang[1], Ala Yousef[2], Faqi Wang[3], Jia You[1], Zamaneh Kassiri[3], John M. Seubert[1,2*]

Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB T6G 2H7, Canada Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB T6G 2H7, Canada Department of Physiology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB T6G 2H7, Canada

Cardiorenal syndrome encompasses a spectrum of disorders where acute or chronic dysfunction of one organ can induce dysfunction in the other organ, reflecting the interdependent relationship between the heart and kidney. CYP450 enzymes metabolize N-3 and N-6 polyunsaturated fatty acids (PUFAs) into various bioactive lipid mediators, with cardioprotective properties. These lipids are further metabolized into less active or toxic diols by soluble epoxide hydrolase (sEH). Inhibition of sEH significantly attenuates the adverse effects of myocardial infarction but its role in protecting the kidney following cardiac MI is unknown. The objective of this study was to investigate cardiorenal protective effects of the global sEH genetic deletion in both aged male and female mice following MI. In this study, male and female WT and sEH null mice (15-18 mo) were subjected to permanent left anterior descending artery ligation to induce myocardial infarction. Echocardiography was used to assess the cardiac function at baseline and 7 days post MI. Frailty

index was collected as a marker for aging/body condition. Tissues were collected for molecular and biochemical analyses. Echocardiography analyses demonstrated a reduced systolic and diastolic cardiac function after LAD surgery in all mice. However, female sEH null mice showed significantly better cardiac function compared to all other groups. Overall markers such as frailty index and plasma glucose levels (acute hyperglycemia) were attenuated in sEH null mice post MI. Activation of NLRP3 was observed in only WT mice post MI suggesting genotype differences in the innate immune response. Sex-dependent differences in mitochondrial antioxidant gene expression demonstrated female mice had significantly lower renal GPX1 gene expression. While aged sEH null female mice exhibited preserved markers of cell injury, ICAM-1 and KIM-1, compared to WT kidneys. Interestingly, all groups had significantly higher levels of renal mitochondrial DNA transcription factor Tfam post MI but increased mitochondrial DNA content was only observed in sEH null mice, suggesting better mitochondrial quality control in sEH null mice. Our data highlight both sex- and genotype-differences in renal injury following myocardial infarction in aged mice. Importantly, these preliminary data demonstrated aged female sEH null mice had better cardiac and renal protection.

CDV-2

EPA, AN OMEGA-3 FATTY ACID, INHIBITS PLATELET ACTIVATION THROUGH ITS 12-LOX METABOLITE 12(S)-HEPE

Krista Goerger[1], Taekyu Lee[2], Michelle Tran[3], Theodore R. Holman[3], Michael Holinstat[1]

[1]University of Michigan Medical School, Ann Arbor, MI; [2]Emory University, Atlanta, GA; [3]University of California Santa Cruz, Santa Cruz, CA

Arterial thrombosis is the underlying cause of several cardiovascular-related events. Platelets play a key role in the hemostatic response following vascular injury, but platelet hyperreactivity in pathophysiological conditions can lead to thrombosis and vessel occlusion. Dietary supplementation with eicosapentaenoic acid (EPA), an omega-3 polyunsaturated fatty acid (PUFA), has long been used to slow the progression of cardiovascular disease. However, the mechanisms underlying the cardioprotective effects of EPA and its ability to regulate platelet function are controversial. In the platelet, PUFAs are metabolized into oxidized lipids by oxygenase enzymes, including lipoxygenases. Our group has previously shown that the antiplatelet effects of several PUFAs require their metabolism to bioactive lipid through 12-lipoxygenase (12-LOX). The goal of this study is to determine whether omega-3 EPA, and its 12-LOX derived oxylipin, 12(S)-hydroxyeicosapentaenoic acid (12(S)-HEPE), play a role in the regulation of platelet activity. Washed platelets or platelet rich plasma (PRP) from healthy human donors were treated with EPA and 12(S)-HEPE to assess their ability to inhibit platelet activation. EPA and 12(S)-HEPE attenuate collagen and thrombin-induced platelet aggregation. Furthermore, 12(S)-HEPE more potently inhibits surface expression of platelet activation markers, integrin αIIbβ3 and P-selectin, in comparison to EPA. PRP treated with EPA and 12(S)-HEPE delayed thrombin-induced clot retraction. Additionally, treatment with EPA or 12(S)-HEPE does not result in phosphorylation of vasodilator-stimulated phosphoprotein (VASP) suggesting they do not signal through the activation of the eicosanoid Gαs-GPCRs. Here, we show for the first time that EPA directly inhibits platelet activation through its 12-LOX metabolite, 12(S)-HEPE. These findings support the beneficial effects of EPA as a therapeutic intervention in atherothrombotic diseases and provides further insight into the mechanisms by which EPA is cardioprotective. A better understanding of the current PUFA supplementations can inform treatment and recommendations for optimal prevention of the development of cardiovascular disease leading to occlusive thrombotic events, myocardial infarction, and stroke. Support acknowledgement: This research is partially funded by the National Institutes of Health grants R35 GM131835 (M.H. and T.R.H.) and T32 TR004371 (K.G.).

CDV-3

POST-ISCHEMIC INTERVENTION WITH A SYNTHETIC 19,20-EDP ANALOG SA-22 AMELIORATES CARDIAC INJURY IN A SIRT DEPENDENT MANNER

Joshua Kranrod, Ahmed Darwesh, John Falck, John Seubert

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada Cardiovascular Research Institute, University of Alberta, Edmonton, AB, Canada Department of

Pharmacology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada Division of Chemistry, Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX

Purpose: Evidence suggests that CYP epoxygenase metabolites of docosahexaenoic acid (DHA), called epoxydocosapentaenoic acids (EDPs), limit mitochondrial damage following cardiac injury. In particular, the 19,20-EDP isomer has demonstrated potent cardioprotective action. We investigated our novel synthetic 19,20-EDP analog compound SA-22 for protection against cardiac ischemia-reperfusion (IR) injury. Methods: Isolated C57BL/6J mouse hearts were perfused via Langendorff apparatus for 20 minutes to obtain baseline function followed by 30 minutes of global ischemia. Hearts were then treated with either vehicle, 19,20-EDP (1 μM), SA-22 (1 μM), or SA-22 with the pan-sirtuin inhibitor nicotinamide (NAM) (30 μM), or the SIRT3-selective inhibitor 3-(1H-1,2,3-triazol-4-yl) pyridine (3-TYP) (50 μM) at the start of 40 minutes reperfusion. We continuously assessed IR injury-induced changes in recovery of myocardial function, using left ventricular developed pressure, systolic and diastolic pressure change. Tissues were assessed for electron transport chain function, SIRT-1 and -3 activity, optic atrophy type-1, manganese superoxide dismutase (MnSOD) acetylation, caspase-1, IL-1B, and Gasdermin D cleavage. H9c2 cells were used in an in vitro model of hypoxia/reoxygenation injury. Results: Perfusion with SA-22 significantly enhanced postischemic functional recovery, preserved ETC function, SIRT activity and reduced activation of pyroptosis. Interestingly, while NAM (SIRT3 inhibitor) co-treatment worsened functional outcomes, cell survival, and attenuated sirtuin activity, it only partially attenuated SA-22-induced protection against pyroptosis, suggesting involvement of other protective mechanisms. Conclusions: The data demonstrate cardioprotective effects of our novel synthetic 19,20-EDP analog against IR injury. Treatment resulted in improved postischemic cardiac function correlated with evidence of limited mitochondrial degradation and enhanced SIRT3 activity. This pilot data provides the framework for the development of superior therapeutic agents.

Support: FRN 156393, Canadian Institutes for Health Research, Ottawa, Canada. I-0011, Robert A. Welch Foundation, Houston, USA. DK126452, U.S. Public Health Service National Institutes of Health, Bethesda, USA.

CDV-4

DEVELOPMENT OF 8,9-EET ANALOGS FOR THE MITIGATION OF VEGF-KINASE INHIBITOR LINKED NEPHROTOXICITY

Abhishek Mishra[1], Ashraf El-Meanawy[2], Jawad Belayet[3], Weicang Wang[4], Yuxin Wang[4], Bruce Hammock[4], Marcus Bourg[5], Rawand Mohammad[5], Anders Vik[5], John D. Imig[1]

[1]University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA; [2]Medical College of Wisconsin, Milwaukee, Wisconsin, USA; [3]University of Wisconsin Milwaukee, Milwaukee, Wisconsin, USA; [4]University of California, Davis, Davis, California, USA; [5]University of Oslo, Oslo, Norway

Background: The use of VEGF-tyrosine kinase inhibitors (VEGF-TKIs) has greatly increased cancer survival rates but worries remain about their possible side effects on the kidneys. Nephrotoxicity is caused by the interaction between VEGF-TKIs and normal kidney function, which can result in hypertension and glomerular injury that may have a negative impact on mortality even if therapy is suspended. Glomerular injury is often irreversible, as there is damage to mesangial cells. The epoxyeicosatrienoic acid, 8,9-EET, has been demonstrated to protect the glomerular filtration barrier. This has pushed researchers to investigate 8,9-EET analogs as preventative treatments to protect renal filtration from damage due to VGF-TKI therapies. The aim of this study is to develop such protection.
Hypothesis: 8,9-EET analogs reduce the nephrotoxic effects of the VEGF-TKI, sorafenib, on HRMCs.
Methodology: Human renal mesangial cells (HRMCs) were grown at 37˚C in RPMI 1640 medium with 10% FBS. Live cell imaging was conducted to analyze the apoptotic activity of the HRMCs every two hours for 48 hours via an Incucyte system. To evaluate the effect of sorafenib and 8,9-EET analogs, four conditions were tested: treatment with sorafenib only (10 μM) and with 1, 3, and 10 μM 8,9-EET analogs. The sEH inhibitory potency of thirteen 8,9-EET analogs was done on the human sEH recombinant protein. RNA sequencing study was performed at the UAMS Genomics Core.
Results: Our current study's live cell imaging showed sorafenib caused HRMC cell death, as seen in elevated caspase 3/7 activity. Four of the thirteen 8,9-EET analogs tested during screening decreased sorafenib-linked HRMC cell death and caspase 3/7 activity. Substituting an oxamide at the 8,9-position was

protective against sorafenib-induced cell death, while not having sEH-inhibitory action. RM-84 and MBD-32 lowered caspase 3/7 activity by 20 to 40%, while MDB52-I and MDB52-II successfully reduced it in a dose dependent manner up to 90%.We evaluated the effect of sorafenib and 8,9-EET analog at the pathway level by carrying out a differential RNA sequencing study on MDB-52-I. Comparing group A (no treatment) with group B (10μM sorafenib), 1244 genes were found to be significantly expressed. HRMCs treated with the 8,9-EET analog, group E, had significant expression of ANGPTL4 and RP11-566K11.4. This is relevant because ANGPTL4 has been known to inhibit lipoprotein lipase activity, which plays an important role in metabolism and vascular homeostasis. There was an interesting difference between groups B and D (sorafenib, B or 10 μM MDB-52 I and sorafenib, D); RP11-178L8.4 displayed more expression in group D than B; this indicates that 8,9 EET analogs activate glomerular protective molecular machineries within HRMC cells.
Conclusion: 8,9-EET Analog demonstrate promise in preventing nephrotoxicity caused by sorafenib.
Disclose Support/Funding: - Arkansas Research Alliance.

CDV-5
ROLE OF LIPID MEDIATORS IN LYMPHANGIOGENESIS AND LYMPHATIC MATURATION

Zumer Naeem, Timo Frömel, Andreas Weigert, Ingrid Fleming

Institute for Vascular Signalling, centre for Molecular Medicine, Goethe-University, Frankfurt am Main

Lymphatic vessels are essential for tissue homeostasis in physiological conditions as we all as in diverse pathologies, including cancer metastasis, lymphedema, and organ graft rejection. In our study we set out to investigate the role of cytochrome P450 (CYP)-derived epoxides and the rrespective soluble epoxide hydrolase-derived diols in lymphangiogenesis and lymphatic maturation during development. In a spheroid-based assay using embryonic stem cells (mESC) and tumour cells, we observed that PUFA epoxide/diol pairs elicited differential effects on mESC differentiation into either blood endothelial cells (BECs) or lymphatic endothelial cells (LECs). Indeed, while 12,13-EpOME and 11,12-EET increased the expression of genes implicated in angiogenesis e.g. Vegfr1 and Ephb4, the corresponding diols; 12,13-DiHOME and 11,12-DHET promoted the expression of Vegfr3, Coup-tf, Sox18 and Prox1 i.e., genes linked to lymphangiogenesis. Based on this information, we studied lymphangiogenesis in the ears of young (P21) wild-type, and sEH-/- pups and discovered profound effects. Deletion of sEH (to increase epoxide and decrease diol levels) resulted in a less dense lymphatic network with fewer branching points. Although valve morphology seemed normal in these mice, the lymphatic collecting vessels showed a higher coverage of smooth muscle cells. Moreover, sEH-/- pups showed severely reduced lymphatic drainage as assessed by lymphangiography. Of the mediators tested, 12,13-DiHOME, which is a PUFA diol generated by the sEH, had the biggest impact on LEC development. 12,13-DiHOME is also a lipokine which can increase fatty acid uptake and fatty acid oxidation. Therefore, we also assessed the impact of CYP/sEH derived PUFA-lipid mediators on fatty acid oxidation in human dermal lymphatic cells. Lipid mediators including 11,12-DHET and 12,13-DiHOME which promoted lymphatic differentiation also increased the fatty acid oxidation and mitochondrial ROS production. We also checked Cyp2c44-/- (to decrease epoxide and diol formation), an upstream enzyme of sEH, and showed that Cyp2c44 deletion attenuated lymphatic vessel maturation and severely affected valve formation, without having any major effect on lymphatic vessel density or branching. In conclusion, we could show that lipid mediators generated by the actions of Cyp2c44 and sEH play an important role in endothelial cell specification, lymphatic development, lymphatic vessel maturation, and valve formation.

CDV-6
PLATELET-DERIVED 12(S)-HETrE REGULATES ENDOTHELIAL CONTRACTILITY AND PERMEABILITY

Andrew Rickenberg[1], Adriana Yamaguchi[1], Simon Hogan[2], and Michael Holinstat[1]

[1]Department of Pharmacology, Michigan Medicine, University of Michigan, Ann Arbor, MI, USA. [2]Mary H Weiser Food Allergy Center, Department of Pathology, Michigan Medicine, University of Michigan, Ann Arbor, MI, USA.

Cardiovascular disease is the leading cause of death worldwide, accounting for more than 32% of deaths globally. The integrity of the endothelium, which lines blood vessels, is essential for maintaining hemostasis.

Under physiological conditions, endothelial cells form tight junctions that create a semipermeable barrier, which selectively regulates the movement of macromolecules between the blood compartment and surrounding tissues. However, increased endothelial contractility and permeability, leading to endothelial dysfunction, is known to be involved in the pathogenesis of vascular diseases, such as pulmonary edema and hypertension. Currently, prostacyclin (PGI2) analogs are FDA approved to treat pulmonary arterial hypertension due to their vasodilatory effects. However, due to their short half-life and limited selectivity, PGI2 analogs are not well tolerated, and patients often suffer significant side-effects. Our group recently identified that platelet-type 12(S)-lipoxygenase (12(S)-LOX) oxidizes the omega-6 fatty acid, dihomo-γ-linolenic acid (DGLA) to form the eicosanoid, 12(S)-hydroxyeicosatrienoic acid (12(S)- HETrE), in human platelets. We have shown that platelet-derived 12(S)-HETrE selectively binds to the prostacyclin (IP) receptor and effectively attenuates platelet reactivity (Tourdot et al., Blood Adv. 2017). However, it is unknown if platelet-derived 12(S)-HETrE regulates other cells in the vessel, such as the endothelium. In this study, we used human umbilical vein endothelial cells (HUVEC) to assess the ability of 12(S)-HETrE to inhibit thrombin-induced endothelial barrier dysfunction. Through immunofluorescence, we demonstrate that 12(S)-HETrE prevents thrombin-induced endothelial gap formation and cytoskeletal rearrangement. Additionally, using transwell permeability assays and Electric Cell-substrate Impedance Sensing (ECIS), we show the ability of 12(S)-HETrE to prevent thrombin-induced increases in inter-endothelial permeability. Our findings suggest that 12(S)-HETrE may represent a new class of inhibitors for the prevention of a wide range of diseases in the vessel and blood, including ARDS, pulmonary edema, and hypertension. A better understanding of the mechanism of 12(S)-HETrE on endothelial contractility and permeability has significant implications in advancing our understanding of how transcellular regulation by this eicosanoid could be exploited to develop novel therapeutic approaches to treat endothelial dysfunction. Key Words: cardiovascular disease, polyunsaturated fatty acids, lipoxygenases, endothelial cells, endothelial dysfunction 5) Acknowledgement of grants: This study was partially funded by National Institutes of Health grant R35 GM131835 (M.H. and T.R.H.).

CDV-7

RATIONALE FOR A TRIAL IN TYPE 2 DIABETES AND/OR CORONARY ARTERY DISEASE: COMBINED INTERVENTION WITH EXERCISE AND A SOLUBLE EPOXIDE HYDROLASE INHIBITOR

Myuri Ruthirakuhan[a,b], Natasha Z. Anita[a,b,c] , Felicia Kwan[a,b,c], Si Won Ryoo[a,b,c], Chelsi Major-Orfao[b,c], William Z. Lin[a,b,c], Shiropa Noor[a,b,c], Jennifer S. Rabin[b,d,e,f], Joel Ramirez[b], Susan Marzolini[c,d,g], Sean Nestor[b,h], Bradley J Macintosh[b,i], Maged Gourbran[b,i], Nathan Herrmann[b,h], Paul I. Oh[c,e], Baiju R. Shah[b,e], Jeremy Gilbert[e], Angela Assal[e], Ilana J. Halperine, Ameer Y. Taha[i], Sandra E Black[b,c,d,e], Fuqiang Gao[b], Heather Edgell[k], Vicki Shienfield, Carmela Tartaglia[l], Ana Andreazza[a], Hugo Cogo-Moreira[m], Jodi Edwards[n], Damien Gallagher[b,h], Benjamin Goldstein[a,h], Mario Masellis[b,e], Mark Rapoport[b,h], Rick Swartz[b,e], Pearl Yang[o,p] , Krista L. Lanctôt[a,b,c,h], Walter Swardfager[a,b,c]

[a]Department of Pharmacology & Toxicology – University of Toronto, Canada; [b]Hurvitz Brain Sciences Research Program, Sunnybrook Research Institute, University of Toronto, Ontario, Canada; [c]KITE Research Institute, Toronto Rehabilitation Institute-University Health Network, Canada; [d]Rehabilitation Sciences Institute, Temerty Faculty of Medicine, University of Toronto, Canada; [e]Department of Medicine (Neurology) Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada; [f]Harqual Centre for Neuromodulation, Sunnybrook Research Institute, Toronto, Ontario, Canada [g]Department of Exercise Sciences, Faculty of Kinesiology and Physical Education, University of Toronto, Canada; [h]Department of Psychiatry, Sunnybrook Health Sciences Centre, University of Toronto, Canada; [i]Department of Medical Biophysics – University of Toronto, Canada; [j]Department of Food Science and Technology, College of Agriculture and Environmental Sciences, University of California, Davis; West Coast Metabolomics Center, Genome Center, University of California - Davis; Center for Neuroscience, One Shields Avenue, University of California - Davis, CA, USA; [k]School of Kinesiology and Health Sciences - York University, Canada; [l]Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Canada; [m]Department of Education, ICT and Learning, Østfold University College – Østfold, Norway; [n]School of Epidemiology and Public Health-University of Ottawa, Canada; [o]Primary Care Research Unit, Sunnybrook Health Sciences Centre, University of Toronto, Canada; [p]Department of Family Medicine – University of Toronto, Canada

Background: Vascular inflammatory conditions such as coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM) are risk factors for vascular cognitive impairment (VCI) and dementia. Recent consensus recommendations strongly support lifestyle interventions, such as physical activity to combat cognitive decline. However, not everyone benefits as people with CAD or T2DM show variable cognitive responses. Identifying new targets and treatments to enhance the cognitive benefits of exercise in T2DM and/or CAD is a clinical priority.
Methods: We rationalize the need for a clinical trial to improve cognition in T2DM and/or CAD by: 1) Investigating potential target populations for intervention, based on the results of studies quantifying the effects of exercise-based rehabilitation on cognition. 2) Identifying evidence supporting a detrimental effect of cerebral small vessel disease (SVD) on cognition based on neuroimaging markers of SVD. 3) Identifying blood markers of SVD that implicate a specific targetable lipid pathway in multiple clinical populations. 4) Investigating relationships between targetable lipid pathway markers and cognition over the course of a 6-month exercise intervention for people with T2DM and/or CAD in our Pro-Resolving Inflammatory Mediators in Neurovascular Gains in Aerobic Training study (PRIMiNG-AT).
Results: 1) Though physical exercise is one of the most potent stimuli known to combat cognitive decline, individuals with CAD (Swardfager 2010; Saleem 2013) and T2DM (Fiocco 2013) have demonstrated variability in cognitive response following the completion of a structured exercise-based rehabilitation program. 2) In CAD, cognitive non-response to exercise was linked to white matter hyperintensities (WMH), a neuroimaging marker of SVD (Santiago 2017). This finding implicates SVD as a barrier to benefitting cognitively from exercise. 3) In stroke, neuroimaging markers of SVD were related to blood markers of the soluble epoxide hydrolase (sEH) pathway (WMH: $\beta_{1,79}$=.364, p<.001, MRI-visible perivascular spaces: $\beta_{1,79}$=0.302, p=0.011) (Yu 2023). In transient ischemic attack (Yu 2019), stroke (Yu 2023), and T2DM (Anita 2023), sEH markers were associated with poorer VCI scores. These findings implicate the sEH pathway as a treatment target for SVD. 4) In PRIMiNG-AT study, higher sEH markers predicted poorer cognitive outcomes with exercise ($F_{1,45}$=5.4, p=.03). These findings specify a link between sEH markers and cognitive non-response to exercise.
Conclusions: We implicate sEH in SVD, and in limiting the cognitive benefits of exercise in T2DM and/or CAD. These findings present new opportunities to boost the brain benefits of exercise by combining it with the use of an sEH inhibitor.

CDV-8

$PGF_{2\alpha}$ IS ELEVATED IN CONDITIONS OF DIABETES AND PROMOTES RETINAL LEUKOSTASIS IN VITRO

Amy K. Stark[1], Warda Amin[2], Stephanie Sanchez[2], Ginger L. Milne[1,2], John S. Penn[1,3]

[1]Department of Pharmacology, Vanderbilt University, Nashville, TN; [2]Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, TN; [3]Department of Ophthalmology and Visual Sciences, Vanderbilt University, Nashville, TN

Diabetic retinopathy (DR), the leading cause of irreversible blindness in working-age Americans, involves early retinal inflammation driven by systemic conditions of diabetes mellitus. As all available treatments for DR target late stages of disease once damaging neovascularization has already occurred, targeting the inflammatory components of DR could address the disease at its earliest stages before damage begins. We found that primary human retinal microvascular endothelial cells consistently produced elevated levels of proinflammatory prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) when cultured in conditions modeling diabetic hyperglycemia (2.50-fold, p<0.001), dyslipidemia (4.67-fold, p<0.001), or chronic inflammation (2.89-fold, p<0.001) compared to controls. Subsequently, we analyzed the effects of $PGF_{2\alpha}$ in endothelial cell experiments of retinal leukostasis—a hallmark of early DR marked by adhesion molecule expression and leukocyte adhesion to retinal vessels. *SELE* (E-selectin) gene expression was elevated 2.84-fold by 1μM PGF2α (p=0.047) and 4.83-fold by 10μM $PGF_{2\alpha}$ (p<0.001); *ICAM1* and *VCAM1* were also dose-dependently elevated. Further, ICAM-1 and VCAM-1 protein expression was elevated 1.86-fold (p=0.018) and 3.19-fold (p<0.001), respectively, by $PGF_{2\alpha}$. To model leukostasis *in vitro*, we performed static adhesion assays. Endothelial cells were treated with $PGF_{2\alpha}$ before adding human peripheral blood mononuclear cells (PBMCs) to the monolayers and washing to retain only adhered PBMCs. Increased PBMC adhesion was observed with 1μM (1.52-fold, p=0.004) and 10μM (1.92-fold, p<0.001) PGF2α treatment. To determine if

the effects of $PGF_{2\alpha}$ were mediated by its cognate receptor, the FP receptor, we used the selective antagonist AL8810 (Ki=2.6μM). Pretreatment with 1μM AL8810 fully prevented induction of *SELE* by 10μM $PGF_{2\alpha}$ (p=0.476), likewise observed for *ICAM1* and *VCAM1* genes. Western blots indicated ICAM-1 and VCAM-1 levels were not different from vehicle with 1μM AL8810 pretreatment + PGF2α (p=0.888; p=0.672). In static adhesion assays, 10μM AL8810 decreased $PGF_{2\alpha}$-induced PBMC adhesion to vehicle levels (p=0.218). Together, these results indicate a pathogenic role of $PGF_{2\alpha}$ produced by retinal endothelial cells in response to systemic diabetes, promoting retinal leukostasis in early DR. These effects are mediated selectively by the FP receptor of $PGF_{2\alpha}$. Therapeutic targeting of $PGF_{2\alpha}$-FP signaling may provide a novel strategy to slow DR progression at its earliest stages.
Support: NIH F31EY034386 (AS), NIH R01EY023397 (JP), Vanderbilt Institute for Clinical and Translational Research Grants (AS)

CDV-9

AGE-SPECIFIC RESPONSE TO LPS-INDUCED ENDOTOXEMIA IN SEH NULL FEMALE MICE

Ala` Yousef, Deanna Sosnowski, Jacob Korodimas, John M Seubert

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada. Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

Purpose: Older individuals become more susceptible to external stressors due to a decline in their biological systems. Exposure to environmental toxins are well known to cause adverse effects which are exacerbated in older individuals, for example, lipopolysaccharide (LPS)-induced endotoxemia can result in a multiorgan inflammatory response leading to cardiac dysfunction worsening with age. The metabolism of PUFAs by CYP450 enzymes produces numerous bioactive lipid mediators that can be further metabolized by soluble epoxide hydrolase (sEH) into diol metabolites, often with reduced biological effects. Previous research has demonstrated genetic deletion of sEH is cardioprotective and limits LPS-induced inflammation in young male mice. However, the cardioprotective effect of sEH deletion in young and aged female mice has not been investigated. Methods: Young (2-5mo) and aged (18-25mo) female wild type (WT) and sEH null mice were administered either saline (control), 1mg/kg or 10 mg/kg LPS via i.p. injection. Echocardiography was used to assess cardiac function at baseline and 24 hours after injections. Cardiac inflammatory markers IL-6, MCP-1, NLRP3, and Il-1β and senescence markers p21, p16, and senescence-associated β-galactosidase were determined using qPCR. Oxidative DNA damage was assessed using an 8-OHdG assay and GDF15 concentrations were determined. Results: Both WT and sEH null young female mice demonstrated tolerability toward LPS exposure without significant changes in overall health status or cardiac function. However, sEH deletion improved overall survival, cardiac function and significantly reduced inflammatory and senescent markers in aged female mice. In addition, oxidative DNA damage and GDF15 levels were significantly lower in aged sEH null mice. Conclusion: These data highlight age-dependent differences in female response to acute LPS exposure. And targeting sEH in older females may be an effective strategy for alleviating age-related susceptibility to endotoxemic-induced cardiac dysfunction.

CDV-10

DECONSTRUCTING HIGH FAT DIET-INDUCED CARDIOMETABOLIC DISEASE: THE ROLE OF THE 20-HETE RECEPTOR (GPR75)

Alexandra Wolf[1], Jonathan V. Pascale[1], Danielle Diegisser[1], Danait Yemane[1], Anna Varunok[1], Shan Daniel[2], Zixuan Wang[1], Melissa-Maria Kulaprathazhe[3], Artiom Gruzdev[4], Darryl C Zeldin[4], Michal L. Schwartzman[1], Victor Garcia[1]

[1]Department of Pharmacology, New York Medical College, Valhalla, NY; [2]Case Western Reserve University, Cleveland, Ohio; [3]Hunter College, New York, New York; [4]Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina

Elevations in 20-Hydroxyeicosatetraenoic acid (20-HETE) are intimately linked to the pathogenesis of various cardiovascular pathologies including endothelial dysfunction, hypertension, atherosclerosis, and

myocardial infarction. Additionally, clinical studies demonstrate that plasma levels of 20-HETE correlate with body-mass index (BMI) and are elevated in subjects who are overweight, are diabetic, or exhibit metabolic syndrome. Recently, our lab has identified GPR75, a G-protein coupled receptor (GPCR), as a high-affinity receptor for 20-HETE. Studies in humans also identified several loss-of-function mutations of GPR75 that are associated with lower BMI. With this in mind, we look to better understand the ligand-receptor relationships associated with the 20-HETE-GPR75 pairing and how systemic elevations in 20-HETE contribute to the pathogenesis and severity of cardiometabolic disease. To explore this, our lab has developed a genetically altered mouse line that is deficient in GPR75 (Gpr75-/- global knockout (KO) mice) and in which the dominant 20-HETE synthase, Cyp4a12, can be induced to overproduce 20-HETE globally via the administration of doxycycline (DOX) in the drinking water (Cyp4a12-Gpr75-/-). As expected, the administration of DOX to male Cyp4a12-Gpr75+/+ (WT) mice elevated blood pressure when compared to male Cyp4a12-Gpr75-/- (KO) mice (133 ± 3 vs 104 ± 2 mmHg @ 42 days DOX-treatment, WT vs KO, respectively). Cyp4a12-Gpr75-/- mice also exhibited protection from diet-induced obesity when challenged with high-fat diet for 28-weeks in the presence and absence of DOX (Body Weight Gain: 24g vs 11g, @ 28 weeks of DOX+HFD-treatment, WT vs KO, respectively). Moreover, KO mice were protected from 20-HETE- and GPR75-dependent changes in vascular remodeling, more specifically, changes in media thickness, media:lumen ratio (M:L) and cross sectional area (CSA) which were significantly elevated in WT mice exposed to HFD for 28 weeks (Media Thickness: 31μm vs 15μm, M:L: 0.36 vs 0.14, CSA: 1.96 x 104 vs 1.07 104 (@28 weeks of DOX+HFD, WT vs KO, respectively). These changes correlated with improved glucose handling and fasting blood sugar values in KO mice when compared to DOX+HFD-treated WT mice (FBS: 125 mg/dL vs 105 mg/dL @ 28 weeks of DOX+HFD, WT vs KO, respectively). Taken together, these data illustrate the cardiometabolic consequences associated with the 20-HETE-GPR75 pairing and highlight the potential therapeutic applications of receptor blockers.

CDV-11

EXPOSURE TO A DIET RICH IN LINOLEIC ACID PROMOTES NOCICEPTIVE HYPERSENSITIVITY AND ELEVATED SYSTEMIC BLOOD PRESSURE IN BOTH SPINAL-INTACT AND SPINALIZED RATS

Christian A. Reynolds, Toni Azar, Zeljka Minic, Zeljka Minic

Department of Emergency Medicine, Wayne State University School of Medicine, Detroit, Michigin

Pain is associated with the development of cardiovascular disease and nociceptive hypersensitivity may contribute to increased systemic blood pressure. Various fatty acyl lipid mediators (e.g. oxylipins) are derived from dietary polyunsaturated fatty acids (PUFAs) and modulate nociception. The modern diet is rich in the omega-6 PUFA, linoleic acid (LA), which may present a risk factor for developing pain conditions and associated risk for development of cardiovascular disease. In this study rats were randomized, at the time of weaning, to receive one of two modified AIN-76A diets each containing 5.1% fat. The standard corn oil was replaced with a custom triglyceride blend rich in either LA or oleic acid (OA; 18:1n-9), a monounsaturated fatty acid that is not metabolized to form oxylipin lipid mediators. The average body weight at 9 weeks of age in rats fed the LA-rich diet was not different than that of rats fed the OA-rich diet. In general, rats maintained on the LA-rich diet displayed greater plasma accumulation of pro-nociceptive oxylipin lipid mediators when compared to rats maintained on the OA-rich diet. The accumulation of pro-nociceptive oxylipin lipid mediators was associated with a significant increase in thermal nociceptive hypersensitivity. Using an unanesthetized, decerebrate preparation splanchnic sympathetic nerve activity (sSNA), arterial blood pressure and heart rate were measured at baseline and in response to ganglionic blockade. Rats maintained on the LA-rich diet displayed higher baseline mean arterial pressure (MAP) compared to littermates maintained on the OA-rich diet (94±16 vs 78±14mmHg; $p<0.02$), while baseline heart rate was not influenced by diet. Ganglionic blockade with hexamethonium (20 mg/kg, i.v.) produced a larger fall in baseline in MAP in rats maintained on the LA-rich diet compared to littermates maintained on the OA-rich diet (-54±15 vs -41±12 mmHg, respectively; $P<0.05$). Moreover, the effects of diet on nociceptive hypersensitivity and baseline MAP were preserved in chronic spinalized (T2 transection) rats. These findings illustrate the potential of intraspinal circuits to modulate systemic blood pressure and support the notion that elevated systemic blood pressure associated with chronic pain may involve intraspinal circuitry.

CDV-12

CCL5-20-HETE-GPR75 RECEPTOR ON CEREBRAL VASCULAR TONE

Jane J. Border[1], Huawei Zhang[1], Xing Fang[1], Reece F. Crumpler[1], Yendan Liu[1], Ezekiel Gonzalez-Fernandez[1], Fan Fan[1,2], Richard J. Roman[1]

[1]Department of Pharmacology, University of Mississippi Medical Center, Jackson, MS
[2]Department of Physiology, Medical College of Georgia, Augusta University, Augusta, GA

We have reported that loss of function variants in CYP4A/F that produce 20-HETE are linked with dementia in elderly patients and that a homologous deficiency in the formation of 20-HETE is associated with cerebral vascular and cognitive dysfunction in Dahl S rats. GPR75 has been identified as the 20-HETE receptor that activates Gq signaling in HUVEC and aortic VSM cells. CCL5 also binds to, but does not activate, GPR75. We have reported that CYP4A and GPR75 are expressed in VSM, endothelial cells, and capillary pericytes in the cerebral microcirculation. However, little is known about the interactions of 20-HETE, CCL5 and the GPR75 receptor in controlling vascular tone. In the present study, the 20-HETE inhibitor, HET0016, dilated the parenchymal arteriole (PA) by 30±4, 24±5, and 15±7% in SD, CYP4A transgenic and 20-HETE-deplete SS rats, respectively. It completely blocked the myogenic response of PAs to increases in pressure in SD rats. Surface and deep CBF rose by 22±4 and 17±2% in SD rats treated with HET0016. A 20-HETE agonist, 20- hydroxy-5,14-dienoic acid (20-5,14-HEDE) (0.1-10 uM) constricted the MCA and PA by 10±2 and 23±4% in SD rats. Administration of new water-soluble 20-HETE agonists, 20-5,14-AAA and 20-5,14-SOLA (10 uM), had similar effects and reduced PA diameter by 18±4 and 12±3%, respectively. CCL5 (0.1-10 nM) was a 1000X more potent constrictor than 20-5,14 HEDE. It reduced PA diameter by 24±3, 29±2, and 12±3%, respectively in SD, CYP4A and SS rats. The vasoconstrictor response to 20-5,14-HEDE was blocked in PAs preconstricted with 10 nM CCL5. The response to CCL5 was not altered by the 20-HETE antagonist, 20-6,15-HEDE, suggesting that GPR75 does not mediate this response, but it may be due to activation of CCR1/3/5 receptors. Surprisingly, the vasoconstrictor response to CCL5 was blocked by HET0016, suggesting it is dependent on the production or release of 20-HETE. These studies indicate that 20-HETE and CCL5 play a critical role in controlling cerebral capillary perfusion. Elevations in 20-HETE and CCL5 may contribute to cerebral vasospasm and the no-reflow phenomena following TBI or stroke. On the other hand, decreases in 20-HETE and GPR75 receptor activation that impair cerebral autoregulation may lead to capillary damage, BBB leakage, edema, inflammation, neurodegeneration and loss of cognitive function in hypertensive, elderly, diabetic, and AD patients.

Support acknowledgment: This study was supported by grants HL138685 (RJR), P20GM104357 (FF, RJR), AG079336 (FF, RJR), and AG066245-01 (EGF, RJR) from the National Institutes of Health.

EFF-1

UNRAVELING THE ROLE OF NAPE-PLD IN MACROPHAGE FUNCTION

Abdulmusawwir Alli Oluwafuyi[1], Cristina Youwakim[2], Amanda C. Doran[3], Danielle Michell[4], Kasey Vickers[4], Stetler Tanner[5], Sean Davies[1]

[1]Department of Pharmacology, Vanderbilt University; [2]Department of Medicine, Division of Cardiology, Vanderbilt University Medical Center, Nashville, TN; [3]Molecular Physiology and ,Vanderbilt University; [5]Brigham Young University

Macrophages play a critical role in maintaining vascular health by carrying out homeostatic functions such as reverse cholesterol transport and phagocytosis of apoptotic cells (efferocytosis). Impaired efferocytosis is observed in advanced atherosclerosis and contributes to the formation of rupture-prone atherosclerotic plaques. N-acyl phosphatidylethanolamine hydrolyzing phospholipase D (NAPE-PLD) enzyme generates bioactive N-acylethanolamines (NAEs) including palmitoylethanolamine which has been shown to increase efferocytosis capacity of bone marrow derived macrophages (BMDMs). However, diminished NAPE-PLD expression has been reported in human atherosclerotic lesions and in mice fed atherogenic diets, leading us to hypothesize that reduced macrophage NAPE-PLD activity contributes to the progression of atherosclerosis.

To test this hypothesis, BMDMs isolated from wild-type (WT) and NAPE-PLD-/- mice were used in an

efferocytosis assay measuring uptake of UV-induced fluorescent-labelled Jurkat T cells. The apoptotic index was calculated from flow cytometric analysis of F4/80 macrophages colocalized with Jurkat cells. Compared to WT BMDM, 50% less NAPE-PLD-/- BMDM took up apoptotic cells ($p < 0.0001$) supporting an essential role for NAPE-PLD in efferocytosis. RNA sequencing identified 30 genes with significant differential expression (greater than a 2-fold change, $p \leq 0.05$). Based on literature, 5 genes with probable efferocytosis role were selected for validation using quantitative PCR. Lpin1 was reduced to 0.07-fold relative to control ($P < 0.001$). Similarly, Adgrg1, Odc1, Hmox1 and Acp1 were all reduced 0.4-, 0.5-, 0.5-, 0.7-fold respectively relative to control (all $P < 0.05$). Importantly, treatment of NAPE-PLD-/- BMDMs with 10 μM PEA for 6 h restored expression of Acp1, Adgrg1, Hmox1 and Lpin1 but not Odc1. Together, our results support a critical role of NAPE-PLD in regulating efferocytosis, potentially through maintaining expression of signaling pathways not previously recognized to be essential for efferocytosis.

LNG-1

BIFUNCTIONAL SEH/COX-2 INHIBITOR, PTUPB, AS ALTERNATIVE THERAPY TO ALLEVIATE BRONCHOCONSTRICTION IN MURINE PRECISION CUT LUNG SLICE MODEL

Jacqueline Capuano, Niccole Schaible, Mehran Moghaddam, Reyes, Gregory, Ramaswamy Krishnan[1]

[1]Dept. of Pathology and [2]Center for Vascular Biology Research, BIDMC, Harvard Medical School, Boston, MA; [3]Center for Vascular Biology Research, Department of Emergency Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA; [4]Orox Biosciences, Inc.; [5]Orox Pharmaceuticals, Inc.

The necessity of effectively treating asthma is indisputable. Affecting nearly 262 million people world-wide, this prevalent disorder is characterized by airway constriction and inflammation that, when left untreated, may lead to death. Currently, the standard treatment of asthma includes treatment with long-acting beta-agonists coupled with high-dose oral or inhaled corticosteroids. While this remains an effective treatment for some patients, approximately 55% of those affected by asthma continue to experience episodes of acute airway bronchospasm. In response to this unmet need, alternative therapeutics such as antibodies targeting IgE, IL-4, IL-5/IL-5 receptors have been developed. However, these alternative therapies have proven costly and have a multitude of adverse side-effects. Further, it has been discovered that patients with severe asthma experience airway smooth muscle (ASM) enhancement following hyper-contraction during an asthma attack. This muscle enhancement has been found to persist past the resolution of initial inflammation of the asthma attack. There has been noteworthy excitement in inflammatory research regarding bifunctional sEH/COX-2 inhibitors and their potential to alleviate inflammation and oxidative stress in acute and chronic pathologies. We hypothesized bifunctional sEH/COX-2 inhibition can serve as a novel therapeutic strategy to inhibit methacholine (MCh)-induced airway constriction and ASM contraction. Specifically, we predict that by alleviating bronchoconstriction, bifunctional sEH/COX-2 inhibition can inhibit the two hallmarks of asthma: ASM airway hyper-constriction and airway inflammation. To test this hypothesis, we utilized murine precision cut lung slices (mPCLS), MCh to induce airway constriction, and the bifunctional sEH/COX-2 inhibitor OX-001 (PTUPB). We found pre-treatment, 24 hours prior to stimulation, of mPCLS samples with PTUPB inhibited MeCh-induced airway constriction as shown through decreased luminal area, IC50=0.6μM. Further, we found PTUPB pre-treatment, 24 hours prior to stimulation with MCh, inhibited constriction for 30 minutes in human ASM cells. Lastly, pre-treatment with PTUPB, 48 hours prior to treatment with isoproterenol, enhances β agonist relaxation and even partially overcomes the desensitization of the isoproterenol response. Further studies need to be conducted to elucidate the mechanism behind these results and larger sample sizes will be used to verify these promising results.

LNG-2

ROLE OF PROSTAGLANDINS IN REGULATING GAMMADELTA T CELLS FUNCTION IN ALLERGIC LUNG INFLAMMATION

Chiguang Feng, Hong Li, Matt Edin, Daniel Menendez, Shreyas Kanwar, Darryl C. Zeldin

Immunity, Inflammation, and Disease Laboratory, National Institute of Environmental Health Sciences, NIH

Background: Cyclooxygenase-2 (COX-2)-derived prostaglandins regulate the differentiation of αß T helper

cells to Th2, Th9, and Th17 subsets during allergic lung inflammation. IL-17, the signature cytokine of Th17 cells, is a critical regulator of allergic immunopathology. The majority of studies on IL-17 have focused on Th17 cells, even though γδ T cells are the primary IL-17-producing lymphocyte subset and most lung-infiltrating γδ T cells express IL-17.
Unlike αß T cells and B cells, γδ T cells preferentially colonize non-lymphoid tissues, such as the intestines and the epithelial/mucosal tissue of the airways. In addition, lung γδ T cells regulate airway function independently of αß T cells and can maintain and protect normal airway function. Interestingly, peripheral blood γδ T cells are decreased in asthmatic patients. However, it is unknown if COX-2-derived prostaglandins regulate γδ T cells during allergic lung inflammation.
Methods: In this study, we examine how COX-2 derived prostaglandins regulate γδ T cells during allergic lung inflammation using in vivo and in vitro assays with Cox2-/- and Cox2+/+ mice and isolated γδ T cells.
Results: Our results show that OVA-induced allergic lung inflammation activates γδ T cells to produce a significant amount of IL-17 upon restimulation ex vivo. γδ T cells from Cox2-/- mice produced less IL-17 than their wildtype littermates. PGE2 promoted the IL-17 production of γδ T cells when stimulated by IL-1ß and IL-23. This suggests that PGE2 promotes IL-17 production of γδ T cells in vivo after OVA challenge, likely through enhancing the stimulation from IL-1ß and IL-23, which is likely produced by DCs or other myeloid cells in vivo. RT-qPCR detected PGE2 receptors EP2 and EP4 from γδ T cells. Further, EP2 and EP4 antagonists suppress the PGE2 stimulant effect, suggesting that PGE2 promotes IL-17 production via both EP2 and EP4 receptors. In contrast, PGE2 attenuated IL-17 productions when γδ T cells were restimulated with anti-CD3/CD28 antibodies. EP2 but not EP4 antagonists suppress the PGE2 effect, most likely via the EP2 receptor.
Conclusions: Our results indicate that PGE2 has dual roles in IL-17 production of γδ T cells, depending on the activation status of the cells.
Support acknowledgment: This work was supported by the Intramural Research Program of the NIH, NIEHS (Z01 ES025034, to DCZ)

RES-1
PRO-RESOLVING LIPID MEDIATORS PREVENT CANCER CACHEXIA

Rachel Bayer, Victoria Haak, Eva Rothenberger, Haixia Yang, Steven D. Freedman, Charles N. Serhan, Dipak Panigrahy

Department of Pathology, Center for Vascular Biology Research, Division of Gastroenterology and Pancreas Center, Beth Israel Deaconess Medical Center and Harvard Medical School; Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Most patients with advanced cancer suffer from cachexia, which results in the death of 20% of patients. There are no approved therapies for cancer cachexia as the underlying mechanisms remain poorly characterized. The hallmark of cachexia is unresolved hyperinflammation resulting in a devastating muscle wasting syndrome. Cachexia-induced apoptotic cell death occurs in many tissues by pro-inflammatory cytokines. A paradigm shift is emerging in understanding the resolution of inflammation as an active biochemical process with the discovery of novel specialized pro-resolving mediators (SPMs). SPMs stimulate clearance of debris, promote muscle regeneration, and counter-regulate cytokines. We hypothesized that cachexia results from disrupted resolution of inflammation. We profiled lipid mediators in cachexia models via state-of-the-art targeted metabololipidomics. Here, we've identified dysregulated SPMs in six cancer cachexia models. SPMs (e.g., RvD2 and MaR1) were markedly reduced in colon cancer (CT26)-induced cachectic mice on day 35 post-tumor cell injection vs. non-tumor bearing mice (NTB). SPMs (RvD1, RvD2, LXA4, and MaR1) were also dramatically dysregulated in Lewis lung carcinoma (LLC)-induced cachectic mice, including the gastrocnemius and tibialis anterior muscles, heart, liver, and spleen, on day 20 post-tumor cell injection. Cachexia induced a pro-inflammatory eicosanoid storm in plasma from CT26-induced cachectic mice. Chemotherapy also induced cachexia via loss of SPMs in a lymphoma (EL4) and ovarian cancer (ID8) mouse model. At 10 days post-LLC tumor resection, the RvD1 receptor (ALX/FPR2) KO and RvE1 receptor (ChemR23/ERV) KO mice exhibited 20-23% loss in body weight compared to WT mice. Thus, cancer cachexia is resolvin receptor-dependent. RvD2 and PCTR2 prevented LLC- and B16F10 melanoma-induced cachexia at 15 nanograms/day compared to control without

immunosuppression. RvD2 prevented pancreatic cancer (KPC)-induced loss of grip strength and prolonged survival compared to control. In contrast to celecoxib, SPMs prevented the cachexia-induced cytokine storm, including inhibition of TNF-α, CCL2, CCL3, CCL4, CXCL2, G-CSF, and PAI-1. SPMs were sharply reduced by up to 85% in the plasma of pancreatitis patients at risk for cachexia compared to healthy individuals. Thus, our studies shall provide the basis for the clinical translation of SPM-directed treatments in humans as a new direction to potentially prevent and/or reverse cancer cachexia.

RES-2

EX-VIVO SUPPLEMENTATION OF PRIMARY HUMAN MACROPHAGES WITH N-3 PUFA

Rebecca Kirchhoff[1], Carina Rothweiler[1], Nadine Rohwer[2,3,4], Karsten-Henrich Weylandt[2,3] and Nils Helge Schebb[1]

[1]Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal, Gaussstrasse 20, 42119 Wuppertal, Germany; [2]Division of Medicine, Department of Gastroenterology, Metabolism and Oncology, University Hospital Ruppin-Brandenburg, Brandenburg Medical School, Neuruppin, Germany; [3]Faculty of Health Sciences, Joint Faculty of the Brandenburg University of Technology, Brandenburg Medical School and University of Potsdam, Potsdam, Germany; [4]Department of Molecular Toxicology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany

Increased dietary intake of long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) has been associated with beneficial health effects such as anti-inflammatory effects. Inside the human body n-3 PUFA undergo enzymatic and non-enzymatic oxidation giving rise to a large number of oxylipins. Several of those are potent bioactive lipid mediators involved in the regulation of biological processes such as pain and inflammation. It is believed that these oxylipins are part of the anti-inflammatory mode of action of n-3 PUFA. However, the underlying mechanisms of actions are not yet fully understood.
For a detailed investigation of the effects of n-3 PUFA and arising oxylipins on human immune cells, an ex-vivo supplementation strategy was developed in human macrophages. Primary human macrophages derived from blood monocytes were supplemented with different concentrations of n-3 PUFA for different time periods. For investigation of potential antiinflammatory effects of the n-3 PUFA, macrophages were stimulated with bacterial lipopolysaccharide (LPS) and analyzed for changes in the oxylipin, protein and mRNA levels.
After careful optimization of all steps of the experimental strategy, a reliable and reproducible supplementation with n-3 PUFA was achieved: We show, how the cellular FA pattern of monocytes derived from human subjects following a typical Western diet was changed to macrophages with a FA pattern comparable to subjects having a high n-3 PUFA intake.
On poster, we present the developed ex-vivo n-3 PUFA supplementation strategy in primary human macrophages as well as the effects of the n-3 PUFA supplementation based on the response of the cells to LPS treatment. The supplementation strategy developed is a helpful tool for mechanistic investigation of n-3 PUFA effects on human immune cells under strictly controlled conditions without the need of carrying out intervention studies in humans.
Support acknowledgment: This work is supported by a PhD fellowship of the Friedrich-EbertStiftung e.V. to RK.

RES-3

ASSOCIATIONS BETWEEN NLRP3 INFLAMMASOME ACTIVATION SENSITIVITY AND CYTOCHROME P450-SOLUBLE EPOXIDE HYDROLASE OXYLIPINS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Felicia Kwan[a,b,c], Natasha Z. Anita[a,b,c], Si Won Ryoo[a,b,c], Chelsi Major-Orfao[b,c], Shiropa Noor[a,b,c], William Z. Lin[a,b,c], Ana C. Andreazza[a,d], Krista L. Lanctôt[a,b,c,d], Nathan Herrmann[b,d,e], Jeremy Gilbert[e], Ameer Y. Taha[f], Walter Swardfager[a,b,c]

[a]Department of Pharmacology & Toxicology – University of Toronto, Canada; [b]Sunnybrook Research Institute, Toronto, Ontario, Canada; [c]KITE Research Institute, Toronto Rehabilitation Institute-University Health Network, Canada; [d]Department of Psychiatry - University of Toronto, Canada; [e]Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada; [f]Department of Food Science and Technology, College of

Agriculture and Environmental Sciences, University of California, Davis; West Coast Metabolomics Center, Genome Center, University of California - Davis; Center for Neuroscience, One Shields Avenue, University of California - Davis, CA, USA.

Type 2 diabetes mellitus (T2DM) is a chronic inflammatory disease. Epoxide cytochrome p450 (CYP450) metabolites of fatty acids (e.g. linoleic acid [LA], arachidonic acid [AA], and eicosapentaenoic acid [EPA]) can mediate inflammation or its resolution, but their activities are limited by their conversion into diols by soluble epoxide hydrolase (sEH), a possible contributor in T2DM complications. Inflammation in T2DM may also arise from increased activation of the nucleotide binding domain (NOD)-, leucine-rich repeat (LRR)-, and pyrin domain (PYD)- containing protein 3 (NLRP3) inflammasome. This multiprotein complex (NLRP3, apoptosis-associated speck-like protein containing a CARD [ASC], and procaspase-1) regulates innate immunity and increases pro-inflammatory interleukin-1β (IL-1β). Cell and animal studies suggest that sEH inhibitors impede the inflammasome, but findings need corroboration in humans. To address this, the present study sought to investigate the association between epoxides and diols ("oxylipins") and inflammasome activation sensitivity in people with pre-diabetes (glycosylated hemoglobin [HbA1C]: 6-6.4%) or T2DM (HbA1C > 6.4%, impaired fasting glucose and/or impaired glucose tolerance). Fasting blood samples were collected from participants enrolled in the Sunnybrook Type 2 Diabetes Study (NCT04455867). White blood cells extracted using a Ficoll gradient were treated with lipopolysaccharide and nigericin, and inflammasome activation sensitivity (ΔASC Specks, ΔCaspase-1, ΔIL-1β) was quantified using fluorescence microscopy and ELISA tests. Serum oxylipins were measured using ultra-high-performance liquid chromatography tandem mass spectrometry, and samples under the detection limit were imputed using the imputeLCMD package in R. Non-parametric partial correlations were run in SPSS, adjusting for age, sex, BMI, and total cholesterol. Among 83 people (mean age 63.6+11.5, 48.2% female), ΔASC Specks were positively correlated with ΔCaspase, but ΔIL-1β was not significantly correlated with other inflammasome components. For ΔASC Specks, negative associations were found with AA pro-resolving epoxides. Positive associations were found between ΔASC Specks and potentially pro-inflammatory diols of AA, LA, and EPA, and with ratios of omega-6 diols to epoxides. For ΔCaspase, positive associations were seen with the 11,12 AA diol, 14,15 EPA diol, and diol/epoxide ratios of 11,12 AA and 8,9 AA species. ΔCaspase and the 8(9) AA epoxide were negatively correlated. ΔIL-1β was associated negatively with the 11,12 AA diol/epoxide ratio, and positively with 11(12) AA epoxide. October 15-17, 2023 Sheraton Inner Harbor Hotel Baltimore, Maryland In conclusion, individuals with higher serum sEH products or lower sEH substrates have greater inflammasome activation sensitivity in their white blood cells. Future studies should explore inconsistencies with IL-1β, and clinical manifestations related to the findings. Support acknowledgement: Canadian Institutes of Health Research; Banting & Best Diabetes Centre

RES-4

PRO RESOLVING LIPID MEDIATORS STIMULATE THE RESOLUTION OF BREAST CANCER IN MICE

Sarina A. Virani, Michael Gillespie, Haixia Yang, Diane R. Bielenberg, Sui Huang, Charles N. Serhan, Dipak Panigrahy

Department of Pathology and Center for Vascular Biology Research, Beth Israel Deaconess Medical Center and Harvard Medical School; Vascular Biology Program, Boston Children's Hospital, Boston, MA; Institute of Systems Biology, Seattle, WA Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Tumor recurrence is the principal cause of mortality in breast cancer, yet the underlying mechanisms are not well understood. Anti-estrogen therapy, including tamoxifen, induces apoptotic cell death that may stimulate tumor growth by disrupting inflammation resolution. Compared to estrogen receptor (ER)-positive breast cancer, ER-negative behaves aggressively and patients have a poor prognosis due to the lack of treatment options. Here, we demonstrate that breast tumor cells killed by cytotoxic anti-estrogen therapy or chemotherapy ("tumor cell debris") stimulate primary tumor growth when co-injected with a subthreshold (nontumorigenic) inoculum of tumor cells by triggering a macrophage-derived pro-inflammatory and pro-angiogenic "cytokine storm." Thus, tumor cell debris is a critical risk factor for aggressive breast cancer growth. To assess whether stimulating the clearance of debris would impact breast cancer progression in vivo, we utilized maresin 1 (MaR1), maresin conjugates in tissue regeneration (MCTR1, MCTR2) and protectin conjugates in tissue regeneration (PCTRs), which are specialized pro-resolving lipid autacoid

mediators (SPMs). We treated established models of ER-positive (EO771) and negative (4T1) breast cancer. Each maresin sharply reduced tumor growth in debris-stimulated and spontaneous (e.g. MMTV-PyMT) breast cancer models at nanogram concentrations without toxicity. Notably, PCTR1 inhibited orthotopic triple-negative (4T1) growth and proliferation compared to chemotherapy (paclitaxel) or immunotherapy (anti-cytotoxic T-lymphocyte-associated protein 4, CTLA4). Similarly, PCTR1 alone or in combination with chemotherapy (paclitaxel) reduced gene expression and protein levels of pro-angiogenic factor CXCL12/SDF1 in the ER-negative (4T1) tumor microenvironment. In ER-positive (EO771), a triple combination of PCTR1, chemotherapy (paclitaxel), and immunotherapy (anti-CTLA4) treatment potentiated the immune checkpoint blockade. Maresins also stimulated macrophage phagocytosis of therapy-generated breast tumor cell debris, inhibited tumor angiogenesis, and dampened a therapy-induced cytokine storm, including TNF-α, MIP-2/CXCL2, CCL2/MCP-1, IL- 1ra/IL-1F3, and G-CSF. Expression of SPM receptors (e.g. resolvin D2 (RvD2) receptor GPR 18) was specifically identified in breast cancer tissue. Altogether, the maresin and protectin pathway mediators may represent a new therapeutic approach to stimulate the resolution of inflammation in breast cancer.

****

SPEAKER ABSTRACTS

ROLE OF EPOXIDES AND DIOLS IN THE DIABETIC HEART AND IN LYMPHANGIOGENESIS

Sebastian Kempf, Zumer Naeem, Yanis Afir, Jiong Hu, Timo Frömel, **Ingrid Fleming**
Institute for Vascular Signalling, Department for Molecular Medicine, Goethe University Frankfurt, Frankfurt am Main, Germany

In the retina a diol derived from the w-3 polyunsaturated fatty acid (PUFA) docosahexanoic acid, is required for normal vascular development but in high concentrations (in retinas from diabetic mice) was able to induce vascular destabilization and pericyte loss to result in breakdown for the blood retinal barrier. We determined whether a similar effect could be detected in hearts from diabetic Ins2Akita mice. Diabetes was associated with the development of heart failure that was accompanied by a change in the cardiac PUFA profile and a decrease in pericyte alignment with small arteries and capillaries. sEH was mainly expressed in cardiomyocytes and its specific deletion for these cells prevented the pericyte loss and migration. In the heart 6 PUFAs dominated and 12,13-DiHOME had particularly marked effects on pericyte migration and attenuating responses to PDGF. Studies addressing the impact of sEH deletion and overexpression of cardiomyocyte metabolism are ongoing.
We previously reported that the deletion of Cyp2c44 had a marked impact on lymphangiogenesis in a mouse model of breast cancer and studied the phenomenon in more detail. In a spheroid-based assay using embryonic stem cells (mESC) and tumour cells, we observed differential effects of PUFA epoxide/diol on angiogenesis and lymphangiogenesis. Indeed, while w-6 epoxides promoted angiogenesis, w-6 diols promoted lymphangiogenesis, the opposite was the case for the w-6 PUFAs. In the mouse ear the lack of Cyp2c44-/- attenuated lymphatic vessel maturation and severely affected lymphatic valve formation, resulting in micro-leaks. The deletion of the sEH (to increase epoxide and decrease respective diol formation) resulted in a less dense lymphatic network with fewer branching points. In the lacteals of the GI tract sEH was also detected in developing lacteals and its deletion resulted in stunted lacteal growth.
One key observation was that while increased expression of the sEH resulted in pericyte loss in the retina as well as in the heart, lack of the sEH increased smooth muscle cell coverage of lymphatics. These observations clearly demonstrate the importance of CYP and sEH activity for vascular development and stability in different vascular beds.
Support acknowledgement: Deutsche Forschungsgemeinschaft SFB1531, SFB103

15-PGDH AS A BETTER THERAPEUTIC TARGET THAN ASPIRIN IN DECREASING RISK OF INTRACRANIAL ANEURYSM RUPTURE IN MEN AND WOMEN EQUALLY

David Hasan, MD. MSc
Department of Neurosurgery, Duke University

Previously we showed that aspirin (ASA) decreased the risk of rupture of intracranial aneurysm (IA) significantly more in human males than in females. These results were confirmed in experiments using mouse IA model and high dose of ASA (25 mg/kg/d). As of today, no studies assessed the effect of ASA at a low dose (5 mg/kg/d, comparable to 325 mg/d for human) and tested whether such sex differential response still exists. IAs were created in mice by inducing hypertension using Angiotensin II (Ang II) infusion (1000 ng/kg/min) and injection of elastase (35 mUnits) into basal cisterns. ASA was intraperitoneally injected daily 48 hours after aneurysm induction until 21 days. When grouping males and females together, ASA significantly decreased the incidence of aneurysm formation (60% vs 87%, p=0.0164) and rupture (43% vs 68%, p=0.0480) compared to placebo. When analyzing by gender, the incidence of aneurysm formation was significantly lower in males versus females (40% vs 80%, p=0.0302) but not the risk of aneurysm rupture, which was lower in males (33% vs 53%, p=0.2311). qPCR analysis of mouse arteries at baseline showed significantly higher 15-Pgdh mRNA in male intracranial arteries compared to females (1.69±0.12 vs 1.10±0.14, p=0.027). Administering ASA upregulated 15-Pgdh expression in both sexes but maintained the sex difference (2.37±0.30 vs 1.25±0.12, p=0.012). Our result show that low dose of ASA decreases the risk of aneurysm formation in addition to rupture. This effect is significantly stronger in males than females possibly due to higher level of 15-PGDH in male intracranial arteries.
The research is supported by R01NS107293 NIH/ NINDS

IDENTIFICATION AND CHARACTERIZATION OF NOVEL F2-ISOPROSTANE METABOLITES

Ginger L. Milne[1], Marina S. Nogueira[2], Stephanie C. Sanchez[1], Benlian Gao[1], Warda Amin[1], Sarah Thomas[1], Gong Yang[2], Camille Oger[3], Jean-Marie Galano[3], and Thierry Durand[3]
[1]Department of Medicine, Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232-6602 USA; [2]Department of Medicine, Division of Epidemiology, Vanderbilt University Medical Center, Nashville, TN 37232-6602 USA; 3Institute des Biomolécules Max Mousseron (IBMM) – UMR 5247, Université de Montpellier, Montpellier, France

F2-isoprostanes (F2-IsoPs) are formed from the free radical oxidation of arachidonic acid and are widely used as biomarkers of endogenous oxidative damage. Sixty-four F2-IsoP isomers are generated during lipid peroxidation; however, most studies have focused only on one isomer, 15-F2t-IsoP (commonly referred to as 8-iso-PGF2a). Immunoassay and mass spectrometric (MS) techniques have been developed to quantify 15-F2t-IsoP in biological fluids. Even though this molecule has a short half-life of only a few minutes, most studies quantify 15-F2t-IsoP directly rather than its' urinary metabolite (15-F2t-IsoP-M). An increasing body of evidence demonstrates the efficacy of 15-F2t-IsoP-M quantification. For example, 15-F2t-IsoP-M but not 15-F2t-IsoP was positively associate with age and postmenopausal status in a large population study of middle-aged and older women. Urinary 15-F2t-IsoP-M also show associations with plasma antioxidant levels, carbohydrate intake, glycemic index, and environmental exposures while urinary 15-F2t-IsoP do not. The utility of 15-F2t-IsoP measurement is further limited by the fact that this molecule can be also generated enzymatically via the cyclooxygenases. On the other hand, 5-series F2-IsoPs are only generated via nonenzymatic mechanisms. And, it has been demonstrated that these molecules are present in greater abundance in plasma, tissue, and urine than 15-series F2-IsoP. Yet, 5-series F2-IsoP metabolism remains unexplored. We have undertaken experiments to identify and characterize metabolites of 5-epi-5-F2t-IsoP and 5-epi-5-F2c-IsoP, two abundantly produced 5-series F2-IsoPs, in human liver microsomes. Using targeted and untargeted metabolomic approaches, glucuronide and sulfate metabolites of F2-IsoPs have been identified by MS. Metabolism of these molecules by specific enzymes has also been tested. Collectively, these findings indicate that F2-IsoP metabolism is more complex than previously appreciated. The presence of these metabolites in human urine is under investigation. We anticipate that the completion

of this work will redefine F2-IsoP metabolism and outline a strategy to comprehensively evaluate endogenous formation of these important molecules.

E2F7 DRIVES AUTOTAXIN/ENPP2 TRANSCRIPTION VIA CHROMOSOME LOOPING: REPRESSION BY P53 IN MURINE BUT NOT HUMAN CARCINOMAS

Gabor Tigyi[1,2], Kuan-Hung Lin[1,3], Sue Chin Lee[1], Mélanie A. Dacheux[1], Derek D. Norman[1], Andrea Balogh[1,2], Hsinyu Lee[3]
[1]Department of Physiology, College of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee, 38163, USA; [2]Institue of Translational Medicine, Semmelweis University, Budapest, H-1094, Hungary; [3]Department of Life Sciences, College of Life Science, National Taiwan University, Taipei, 10617 Taiwan

Dedicated to the Memory of Kenneth Honn, PhD.
Dysregulation of the autotaxin (ATX, Enpp2)-lysophosphatidic acid (LPA) signaling in cancerous cells contributes to tumorigenesis and therapy resistance. We previously found that ATX activity was elevated in p53-KO mice compared to wild type (WT) mice. Here, we report that ATX expression was upregulated in mouse embryonic fibroblasts from p53-KO and p53R172H mutant mice. ATX promoter analysis combined with yeast one-hybrid testing revealed that WT p53 directly inhibits ATX expression via E2F7. Knockdown of E2F7 reduced ATX expression and chromosome immunoprecipitation showed that E2F7 promotes Enpp2 transcription through cooperative binding to two E2F7 sites (promoter region -1393 bp and second intron 996 bp). Using chromosome conformation capture, we found that chromosome looping brings together the two E2F7 binding sites. We discovered a p53 binding site in the first intron of murine Enpp2, but not in human ENPP2. Binding of p53 disrupted the E2F7-mediated chromosomal looping and repressed Enpp2 transcription in murine cells. In contrast, we found no disruption of E2F7-mediated ENPP2 transcription via direct p53 binding in human carcinoma cells. In summary, E2F7 is a common transcription factor that upregulates ATX in human and mouse cells but is subject to steric interference by direct intronic p53 binding only in mice.

APPLIED RESOLUTION PHARMACOLOGY IN PERIODONTAL DISEASE TREATMENT

Thomas E. Van Dyke
Forsyth Institute and Harvard University Faculty of Medicine

Periodontitis, the most common inflammatory disease of man, is an infectious/inflammatory disease associated with dysbiosis of the commensal oral microbiome. The mildest, reversible form of the disease, gingivitis, is ubiquitous affecting >90% of people, but periodontitis, with irreversible tissue destruction, affects more than 47% of the US population. Importantly, recent studies demonstrate a relationship between oral infectious/inflammatory conditions, in particular periodontal disease, and risk for systemic diseases, including cardiovascular disease and Type 2 diabetes. Endogenous lipid mediators of resolution, including analogues under development for human use, have provided new approaches for the management of periodontitis. Preclinical studies have demonstrated that specialized proresolving lipid mediators (SPMs) are potent when topically applied in the prevention and treatment of periodontitis demonstrating significant regeneration of soft tissues and bone lost to disease. A Phase 1 clinical trial in humans reveals these compounds are safe and early efficacy data in humans reveals that a specific lipoxin analog reduces gingivitis and established periodontitis. These first in human studies demonstrate safety and potential efficacy of a pro-resolving mimetic for the treatment of periodontal diseases in man. To understand the relationship of SPMs to regeneration, studies of periodontal ligament stem cells have revealed modulation of stem cell biology by SPMs. The inflammatory mechanistic link between systemic conditions, including cardiovascular disease, is revealed to be modifiable with SPMs. Support acknowledgement: Supported in part by USPHS Grant DE025020 from the National Institute of Dental and Craniofacial Research.

PGI_2 REGULATION OF ALLERGEN-INDUCED ILC2 MEMORY RESPONSE

Weisong Zhou, Jian Zhang, Shinji Toki, Allison E. Norlander, Stokes Peebles
Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

Group 2 innate lymphoid cells (ILC2) are innate leukocytes that are activated in an antigen-independent fashion by release of IL-33, TSLP, and IL-25 as a result of challenge with protease containing antigens such as the fungus Alternaria or papain. We have published that that PGI2 inhibits lung ILC2 activation and type 2 cytokine (IL-5 and IL-13) production in vitro and in vivo, and that this is specific to signaling through the PGI2 receptor IP. However, the impact of PGI2 signaling on ILC2 memory responses remains unknown.
In this study, IL-13 reporter (tdtomato) wild type (WT) mice and IL-13tdtomato IP KO mice were intranasally challenged in a first phase with either Alternaria extract or PBS for 4 consecutive days. Thirty days later, in a second phase, the mice were intranasally challenged for 3 consecutive days with papain or PBS.
We found that IP KO mice had increased IL-13 responses following Alternaria challenge compared to WT mice when measured 1 day after the last of 4 consecutive challenges, and that the IL-13 responses were non-detectable in PBS challenged WT and IP KO mice. These results confirmed our previously published results. When the mice were harvested 30 days after the end of the Alternaria challenge, the IL-13 responses were undetectable in both WT and IP KO mice, reflecting a return to pre-challenge baseline. When mice were challenged with papain in the second phase, only IP KO mice that had previously been challenged with Alternaria extract had detectable IL-13 responses, whereas WT mice that had been previously challenged with Alternaria extract did not. WT and IP KO mice that had been challenged with PBS in the first phase and challenged with papain in the second phase also had undetectable IL-13 responses. These results suggest that PGI2 signaling inhibits the ILC2 memory response to different protease containing antigens. The heightened ILC2 memory responses in IP KO mice were associated with increased eosinophilia in the lung compared to WT mice.
These results indicate that PGI2 receptor signaling has an inhibitory function in the generation of ILC2 innate immune memory.
Support acknowledgement: This study was supported by National Institute of Health grants R56 AI076411, R01 AI 070672, R01 AI 059108, GM 015431 and Veteran Affairs grant 1I01BX000624.

Volume 249 September 2023 ISSN 0163-7258

17th International Conference on Bioactive Lipids in Cancer, Inflammation, and Related Diseases

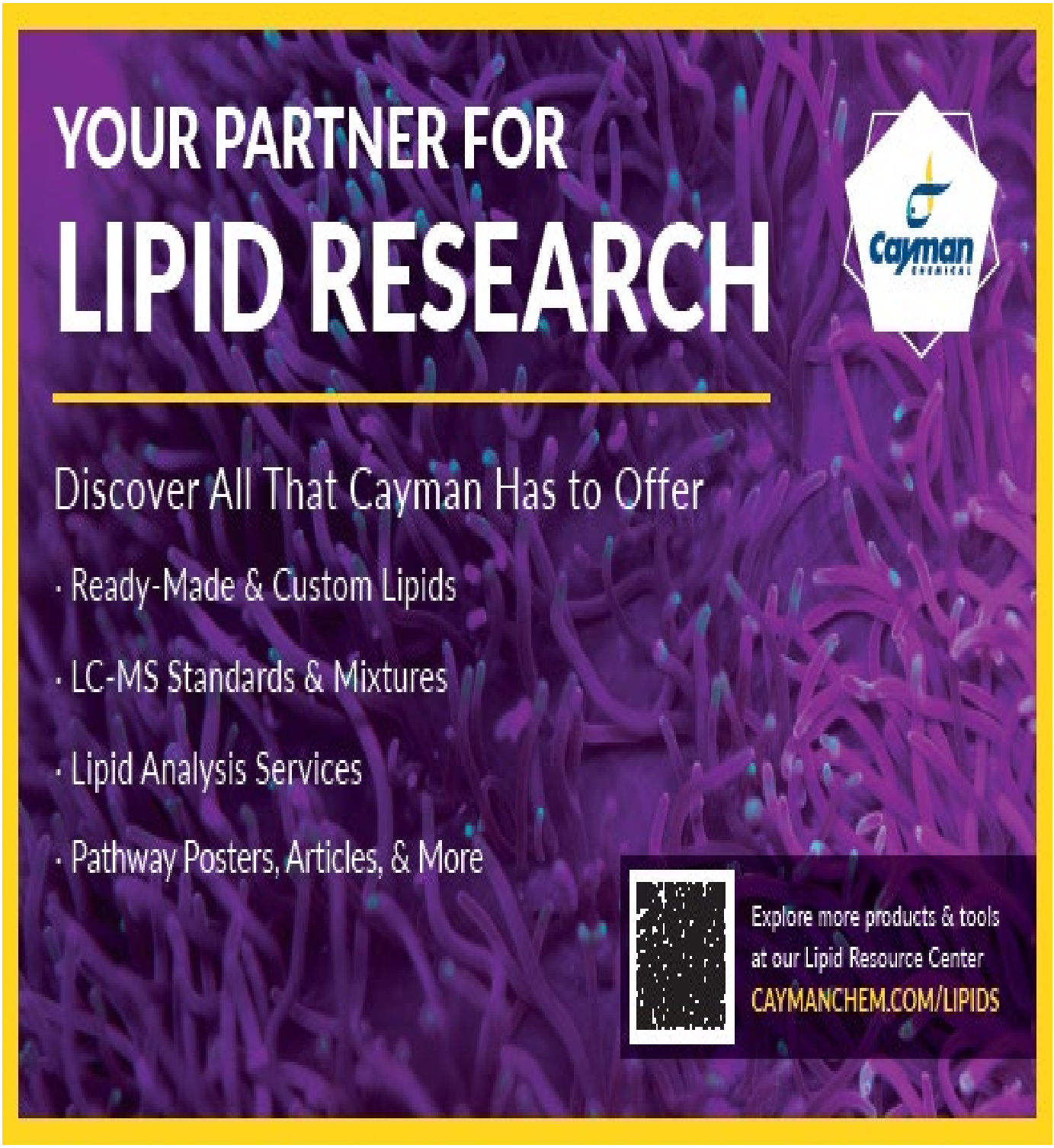
Pharmacology & Therapeutics
An International Review Journal
Executive Editor-in-Chief: S. J. Enna
Executive Editors: M.J. Curtis, J.P. Hardwick, P. Holzer, B.A. Teicher, L. Wecker

The VSI: Bioactive Lipids articles are now online at:
https://www.sciencedirect.com/journal/pharmacology-and-therapeutics/special-issue/10B5BF66865

Edited by: Dr. Darryl Zeldin
Dr. Kenneth Honn
Ms. Caryn Volpe

Sponsors

We are grateful for your support of the 19th International Winter Eicosanoid Conference!



NEW YORK MEDICAL COLLEGE
A MEMBER OF TOURO UNIVERSITY

DEPARTMENT OF PHARMACOLOGY

****

NIH National Institute of Environmental Health Sciences
Your Environment. Your Health

****