September 21, 2018

Lexington Convention Center

21st Cardiovascular Research Day 2018

University of Kentucky

Abstract Book

HealthCare® GILL HEART & VASCULAR INSTITUTE

Saha Cardiovascular Research Center

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SCHEDULE FOR THE DAY

Friday, September 21
University of Kentucky Cardiovascular Research Day
Lexington Convention Center | Bluegrass Ballrooms

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Guest Check–In Begins | Bluegrass Prefunction

10:00 am
Scientific Session I | Bluegrass Ballroom I
Moderators: Julie Pendergast, Ph.D. and Mary Sheppard, M.D.

10:00 am
Welcoming Comments
Alan Daugherty, Ph.D., D.Sc.
Director, Saha Cardiovascular Research Center

10:05 am
Research Blitz
Aida Javidan | University of Kentucky
Jeff Chalfant | University of Kentucky
Eva Gatineau | University of Kentucky
Bradley Wright | University of Kentucky
Hannah Russell | University of Cincinnati
David Henson | University of Kentucky
Chia-Hua Wu | University of Kentucky
Hisashi Sawada | University of Kentucky
Brooke Ahern | University of Kentucky
Jeff Chen | University of Kentucky
Ya Wang | University of Kentucky
Shayan Mohammadmoradi | University of Kentucky

10:15 am
Trainee Presentations

Hsuan Peng | Graduate Student | Abdel-Latif Lab | Saha Cardiovascular Research Center
Polymer Enhance Mesenchymal Stem Cell Retention in the Heart after Transplantation: Potential Therapeutic Applications

Dylan Colli | Undergraduate | Kekenes-Huskey Lab | Department of Chemistry
A Matched-filter Based Algorithm for Subcellular Classification of T-system in Cardiac Tissues

10:45 am
Faculty Presenter
Scott Gordon, Ph.D.
Assistant Professor
Saha Cardiovascular Research Center and Physiology
Lipoproteins in Cardiovascular and Metabolic Diseases

11:05 am
Faculty Presenter
Ming Gong, Ph.D.
Professor, Physiology
Circadian Rhythms of Blood Pressure and Vascular Function in Diabetes

11:25 am
Featured Speaker
Dianna Milewicz, M.D., Ph.D.
President George H.W. Bush Chair of Cardiovascular Medicine
Vice Chair, Department of Internal Medicine
Director, Division of Medical Genetics
Director, Medical Scientist Training Program (MSTP)
Director, John Ritter Research Program
University of Texas Health Science Center at Houston
Genes Predisposing to Thoracic Aortic Aneurysms and Dissections Implicate Mechanotransduction as a Primary Driver of the Disease

12:00 pm
Lunch | Scientific Session II | Bluegrass Prefunction

12:30 pm
Featured Speaker
Mary McDermott, M.D.
Jeremiah Stamler Professor of Medicine
Northwestern University Feinberg School of Medicine
Peripheral Artery Disease and Functional Impairment: From Basic Science to Clinical Trials

1:00 pm
Break

1:15 pm
Poster Session | Bluegrass Ballroom II
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Odd Numbered Posters
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Even Numbered Posters

3:15 pm
Scientific Session III | Bluegrass Ballroom I
Moderators: Donna Arnett, Ph.D. and Nancy Webb, Ph.D.

3:15 pm
Trainee Presentations
Yasir Alsiraj, Ph.D. | Fellow | Cassis Lab | Department of Pharmacology and Nutritional Sciences
Inhibition of Neprilysin Attenuates AngII-induced Abdominal Aortic Aneurysms (AAAs) in Hypercholesterolemic Male Mice

Oluwabukola Omotola | Graduate Student | Pendergast Lab | Department of Biology
Estrogen regulates daily metabolic rhythms in female mice

3:45 pm
Distinguished Alumni Presentation
Gregory Graf, Ph.D.
Professor
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Cholesterol Metabolism in the Metabolic Syndrome

4:10 pm
Featured Speaker
Jerome Rotter, M.D.
Distinguished Professor of Pediatrics, Medicine, and Human Genetics
Director, Division of Genomic Outcomes, Translational Genomics and Population Sciences
University of California, Los Angeles
Clinical Implications of the Genomic Relationship between Lipids and Heart Disease

4:45 pm
Networking Reception | Bluegrass Prefunction

6:00 pm
Dinner and Awards Ceremony | Bluegrass Prefunction

FEATURED SPEAKER

Mary McDermott, M.D.

Jeremiah Stamler Professor
Northwestern University Feinberg School of Medicine
Senior Editor, JAMA

Mary M. McDermott M.D. is the Jeremiah Stamler Professor of Medicine and Professor of Preventive Medicine at Northwestern University Feinberg School of Medicine. Dr. McDermott is a general internist and a clinician investigator whose research focuses on lower extremity peripheral artery disease (PAD). Dr. McDermott's research, funded by the National Heart Lung and Blood Institute (NHLBI), has objectively defined the magnitude and significance of functional impairment, functional decline, and mobility loss in people with PAD including among PAD patients without exertional leg pain. She was the first investigator to demonstrate that home-based exercise programs improve walking performance in people with PAD and have a durable effect on walking performance. More recently, Dr. McDermott's NHLBI-funded research program consists of randomized clinical trials designed to identify medical therapies and novel exercise interventions to improve lower extremity functional performance and prevent functional decline in people with PAD. Dr. McDermott is an elected member of the American Society of Clinical Investigation and the American Association of Physicians. She is an American Heart Association Distinguished Scientist. She is a Senior Editor for JAMA.

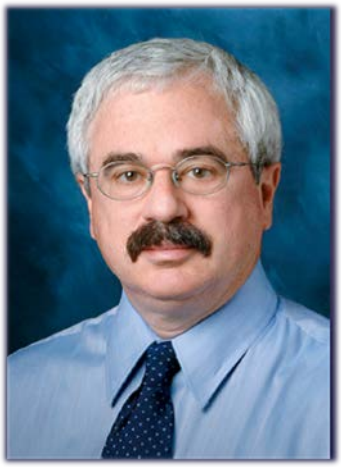
FEATURED SPEAKER

Dianne Milewicz, M.D., Ph.D.

President George H.W. Bush Chair of Cardiovascular Medicine
Vice Chair, Department of Internal Medicine
Director, Division of Medical Genetics
Director, Medical Scientist Training Program (MSTP)
Director, John Ritter Research Program
University of Texas Health Science Center at Houston

Dianna M. Milewicz, M.D., Ph.D. is the President George H.W. Bush Chair of Cardiovascular Medicine, Director of the Division of Medical Genetics and Vice-Chair of the Department of Internal Medicine at McGovern Medical School at the University of Texas Health Science Center at Houston (UTHealth). She completed her postgraduate training in internal medicine, specialized further in cardiology and medical genetics, and forged a career in translational studies focused on genetic predisposition to vascular diseases. She has been inducted into the American Society of Clinical Investigation and the Association of American Physicians. She has received numerous honors and awards for her research, including the Antoine Marfan Award, the Doris Duke Distinguished Clinical Scientist Award, the Belgium Princess Lilian Award, and the University of Texas Presidential Scholars Award for Excellence in Research. She has sought to rapidly and efficiently translate her research findings into improved clinical care and her patient advocacy is evident by her appointment as Chair of several Boards, including that of the Marfan Foundation, John Ritter Foundation, Genetic Aortic Disease Association of Canada, Thoracic Aortic Disease Coalition and Texas American Heart Association. Dr. Milewicz is also committed to mentoring the next generation of physician scientists. She has been the Director of the Medical Scientist Training Program offered jointly by the McGovern Medical School at UTHealth and UT MD Anderson Cancer Center for over 15 years and has mentored numerous junior faculty initiating their research careers.

FEATURED SPEAKER



Jerome Rotter, M.D.

Professor
Department of Pediatrics, Medicine and Human Genetics
Director, Division of Genomic Outcomes, Translational Genomics and Population Science
University of California, Los Angeles

Jerome I. Rotter, M.D., FACP, FACMG is Director of Research and Co-Director of the Medical Genetics Institute, Director of the Division of Medical Genetics (Department of Medicine) and Director of the Common Diseases Genetics Program at Cedars-Sinai. He holds the Cedars-Sinai Board of Governors Chair in Medical Genetics. Dr. Rotter is also Principal Investigator of the National Heart Lung and Blood Institute's (NHLBI) MESA (Multi-Ethnic Study of Atherosclerosis) Family Study and Associate Director of National Institutes of Health (NIH) projects and center grants on inflammatory bowel diseases, coronary artery diseases and treatment of lipid disorders. Dr. Rotter received his bachelor's and medical degrees from University of California, Los Angeles (UCLA). He completed his internship in medicine at Harbor-UCLA Medical Center, followed by a residency in internal medicine at Wadsworth VA Hospital in Los Angeles and a fellowship in medical genetics at Harbor-UCLA Medical Center.

2018 DISTINGUISHED ALUMNI SPEAKER

Gregory Graf, Ph.D.

Professor
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky

Dr. Gregory Graf holds a BS in Animal Sciences from Texas A&M University and a Ph.D. in Physiology from the College of Medicine at the University of Kentucky. Following postdoctoral training in Molecular Genetics in the laboratory of Dr. Helen Hobbs at the UT Southwestern Medical Center in Dallas, he returned to the University of Kentucky and joined the faculty in the Division of Pharmacology and Experimental Therapeutics in the Department of Pharmaceutical Sciences. Dr. Graf previously served as Assistant Dean for Translational Research for the College of Pharmacy and the UK Center for Clinical and Translational Science.

The goal of Dr. Graf's research program is to identify novel proteins and pathways that directly influence risk factors for metabolic diseases that include dyslipidemia, hyperglycemia, insulin resistance, and inflammation. Cholesterol has long been known to increase risk for cardiovascular disease. It is now clear that disturbances in cholesterol metabolism also contribute to obesity-related phenotypes such as insulin resistance, inflammation, and nonalcoholic fatty liver disease. Our present focus is to understand the mechanism by which disruptions in sterol homeostasis influence these risk factors and to determine if accelerating cholesterol elimination will improve obesity-related metabolic dysfunction.

FACULTY PRESENTATIONS

Ming Gong, M.D., Ph.D.

Professor
Department of Physiology
University of Kentucky

Ming C. Gong is a Professor of Physiology and a member of the Saha Cardiovascular Research Center at the University of Kentucky College Of Medicine. Dr. Gong received her Ph.D. training at the Peking Union Medical College and the University of Virginia. Research in Dr. Gong's laboratory focuses on the circadian rhythm dysregulation of vascular function and blood pressure in obesity/diabetes and the molecular mechanisms of aortic aneurysm. Her major research contributions include the elucidation of the important roles of clock genes in vascular contractile function, blood pressure circadian rhythm and aortic aneurysm. Dr. Gong serves as a reviewer for NIH and AHA grant proposals and many scientific journals.

Scott Gordon, Ph.D.

Assistant Professor
Saha Cardiovascular Research Center
Department of Physiology
University of Kentucky

Scott Gordon received his B.S. from the State University of New York College at Brockport and his Ph.D. from the University of Cincinnati College of Medicine's Pathobiology and Molecular Medicine Program. Dr. Gordon is a newly appointed Assistant Professor in the Saha Cardiovascular Research Center and the Department of Physiology at the University of Kentucky. The focus of his laboratory is lipoprotein physiology in cardiovascular and metabolic disease. Specific areas of interest are mechanisms related to intestinal lipid absorption and vascular inflammation in atherosclerosis. Dr. Gordon recently transitioned to UK from a postdoctoral fellowship at the NIH where he studied the composition and function of lipoproteins and received the prestigious Lenfant Biomedical Fellowship and the NIH Fellows Award for Research Excellence. Dr. Gordon is also the recipient of an NHLBI Career Transition Award.

Saha Awardees

The Saha Cardiovascular Research Center is pleased to announce the recipients of the 2018 Saha Awards for Cardiovascular Research and Education. The Saha Awards are given to encourage and support staff and students with an interest in and dedication to cardiovascular medicine. Each award includes an unrestricted $1000 prize and a certificate.

The 2018 Recipients are:

Medical Student Award: **Rahul Annabathula**

University of Kentucky Nursing Student Award: **Jessica Harman**

Eastern Kentucky Nursing Student Award: **Nikita Tamara Watts**

EVENT SUPPORTERS

Gill Foundation of Texas

●

The Saha Fund for Cardiovascular Research and Education

●

The Estate and Family of Mrs. Hager Koostra

●

Mr. and Mrs. Bob Allen

2018 POSTER JUDGES

Douglas Andres
Donna Arnett
Alan Daugherty
Frederick de Beer
Maria de Beer
Brian Delisle
Joyce Evans
John Fowlkes
Ming Gong
Scott Gordon
Gregory Graf
Jiao Guo
Zhenheng Guo
Peter Kekenes-Huskey
Sangderk Lee
Zhenyu Li
Robert Lodder
Analia Loria
Hong Lu
Mary McDermott
Dianne Milewicz
Fredrick Onono
Phillip Owens
Abhijit Patwardhan
Julie Pendergast
Charlotte Peterson
Debra Rateri
Jerome Rotter
Jonathan Satin
Travis Sexton
Ying Shen
Mary Sheppard
Preetha Shridas
Susan Smyth
Venkateswaran Subramanian
Lisa Tannock
Ryan Temel
Sean Thatcher
Michael Tranter
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Nancy Webb
Jeremy Wood
Frederique Yiannikouris
Changcheng Zhou

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2018 ABSTRACTS

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J. Matthew Thomas, MS [1] • Julie S. Pendergast, PhD [2] • W. Scott Black, MD [3] • Philip A. Kern, MD [4] • Jody L. Clasey, PhD [1]
Kinesiology and Health Promotion University of Kentucky [1] • Biology University of Kentucky [2] • Clinical Sciences University of Kentucky [3] • Medicine University of Kentucky [4]

Fat mass, and not heart rate recovery is associated with cardiorespiratory fitness in young, sedentary adults

Graduate Student

Peak oxygen uptake resulting from maximal graded exercise testing is considered a measure of cardiorespiratory fitness. Post-exercise heart rate recovery (HRRec) measures have been used as a clinical indicator of health and mortality in older adults. However, the relationship between HRRec and cardiorespiratory fitness in young, sedentary adults has not been fully elucidated.
PURPOSE: To examine the association between peak oxygen uptake (VO_2; $ml \cdot kg^{-1} \cdot min^{-1}$) and HRRec responses following a progressive maximal graded exercise test (MaxGXT; treadmill); and body composition measures in young, sedentary adults. **METHODS:** We examined peak VO_2 and absolute ($beats \cdot min^{-1}$) and relative (%) HRRec measures at 1, 3, and 5 mins post MaxGXT in 41 young (mean ± SD, age = 26.7 ± 6.5) adults (27 females). All subjects were sedentary (< 2hrs weekly structured exercise), non-smokers, free of known cardiovascular disease risk and medications. Body composition measures including fat mass (kg), fat-free mass (kg), mineral-free lean mass (kg), and percentage body fat (%) were determined by total body DXA scans. Pearson's correlation analysis was used to determine if significant ($p < 0.05$) correlations were observed between peak VO_2, absolute HRRec and relative HRRec, and body composition measures. **RESULTS:** No significant correlations were observed between peak VO_2 (36.0 ± 8.7) and absolute HRRec at 1 min (29.7 ± 8.0; r = 0.22), 3 mins (62.6 ± 11.3; r = -0.01) or 5 mins (71.2 ± 12.0; r = 0.03). Similarly, there were no significant correlations between peak VO2 and relative HRRec at 1 min (15.6 ± 4.3; r = 0.11), 3 min (32.8 ± 6.0; r = -0.14) or 5 min (37.2 ± 6.1; r = -0.11). Peak VO_2 was significantly correlated with percentage body fat (34.0 ± 8.7; r = -0.77; p<.001) and fat mass (26.0 ± 11.2; r = -0.59; p<.001), but not significantly correlated with fat free mass (48.5 ± 12.3; r = 0.22) or mineral free lean mass (45.9 ± 11.8; r = 0.22). **CONCLUSION:** Although heart rate recovery measures have been used as a clinical indicator of health and mortality in older adults, it may not be a valid measure of cardiorespiratory fitness in sedentary, young adults. Furthermore, our data suggests that while peak VO_2 may be influenced by absolute and relative adiposity, it is not associated with measures of the fat free body.

Supported by the University of Kentucky Pediatric Exercise Physiology Laboratory Endowment, the University of Kentucky, and the NIH National Center for Advancing Translational Sciences, TL1TR001997, UL1TR000445, 1U54RR032646-01 and UL1TR001998.

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Hisashi Sawada, MD, PhD [1] • Debra Rateri, MS [1] • Bradley Wright, [1] • Jessica Moorleghen [1] • Deborah Howatt, [1] • Mark Majesky, PhD [2] • Alan Daugherty, PhD, DSc [1]
Saha Cardiovascular Research Center University of Kentucky [1] • Center for Developmental Biology and Regenerative Medicine University of Washington [2]

Smooth Muscle Origin-specific Effects of LRP1 Deletion on Angiotensin II-induced Ascending Aortic Aneurysm

Fellow

Objective: Low-density lipoprotein receptor-related protein 1 (LRP1) plays a critical role in maintaining aortic wall integrity. LRP1 deletion in smooth muscle cells (SMCs) augments angiotensin II (AngII)-induced ascending aortic aneurysms. SMCs in the ascending aorta originate from both the second heart field (SHF) and cardiac neural crest (CNC). The purpose of this study was to determine whether LRP1 depletion in these two SMC origins has differential effects on AngII-induced ascending aortic aneurysm.

Methods and Results: Mef2c-Cre was used to delete LRP1 in SMCs of SHF origin; while Wnt-1-Cre was utilized to delete LRP1 in SMCs of CNC origin in mice. Saline or AngII (1,000 ng/kg/min) was infused for 28 days into 12 - 14 week-old male mice with LRP1 depletion in either SHF or CNC origin as well as their wild type littermates. No mice died in saline-infused groups of either genotype. In the AngII-infused mouse group, LRP1 depletion in SMCs of SHF origin led to 38% death due to ascending aortic rupture, compared to a 4% rupture rate in wild type littermates ($p = 0.002$). In the survivors of AngII-infused group, LRP1 deletion in SMCs of SHF origin resulted in larger ascending aortic diameter compared to wild type littermates (1.6 ± 0.1 vs 2.0 ± 0.1 mm, $p < 0.05$), as measured by ultrasonography. In contrast to the increases of aortic rupture and luminal dilation in mice with LRP1 depletion in SMCs of SHF origin, LRP1 deletion in SMCs of CNC origin did not affect AngII-induced aortic rupture rate or luminal dilation, compared to wild type littermates. To explore potential signaling mechanisms on how LRP1 depletion in SMCs of SHF origin augments AngII-induced ascending aortic aneurysm, mice were infused with either saline or AngII for 24 hours, and ascending aortic tissues were harvested for Western blot analyses. Aortic LRP1 protein abundance was decreased in mice with LRP1 depletion in SMCs of SHF origin regardless of infusion. Although SMAD2 and ERK signaling contribute to aortic wall integrity, this short interval of AngII infusion did not change these activities in the aorta of SHF-SMC specific LRP1 deleted mice.

Conclusion: LRP1 expression in SHF, but not in CNC, -derived SMCs exerts a critical role in the augmentation of AngII-induced ascending aortic aneurysm.

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Ailing Ji, PhD [1] • Xuebing Wang [1] • Victoria Noffsinger [1] • Maria de Beer, PhD [2] • Frederick de Beer, MD [3] • Nancy Webb, PhD [4]
Saha Cardiovascular Research Center University of Kentucky [1] • Physiology University of Kentucky [2] • Internal Medicine University of Kentucky [3] • Pharmacology and Nutritional Sciences University of Kentucky [4]

Hepatic Lipidation of SAA is Dependent on ABCA1 but not SR-BI

Staff

Objectives: Serum amyloid A (SAA) is an inflammatory mediator whose concentration in plasma is increased in individuals with acute or chronic inflammation. Circulating SAA is produced and secreted largely by the liver and is present in plasma mainly associated with HDL. While accumulating evidence suggests that lipid-free and HDL-associated SAA have different activities, the pathways by which SAA acquires lipid and is incorporated into HDL are poorly understood. Plasma SAA is cleared more rapidly than the other major HDL apolipoproteins, but pathways involved in its plasma clearance have not been defined. In this study we examined how SAA is lipidated and how such lipidation relates to the formation of nascent HDL particles. We also examined the role of hepatocytes in SAA clearance.

Approach and results: To investigate lipidation of endogenously expressed SAA, primary hepatocytes were prepared from mice 6 hours after i.p. injection of 1 ug/g lipopolysaccharide or 24 hours after i.v. injecting $1x10^{11}$ particles of an adenoviral vector expressing SAA. To study exogenous SAA lipidation, primary hepatocytes from SAA-deficient mice were incubated with 5 – 10 ug/ml purified mouse SAA. Culture media were collected after 0, 4, 8, 18 or 30 hours, separated by non-denaturing gradient gel electrophoresis, and analyzed by Western blotting to determine the lipidation status of SAA and apoA-I, the major apolipoprotein on HDL. Based on the migration of lipidated species, both endogenously expressed and exogenously added SAA were lipidated to form similar nascent particles that were distinct from apoA-I-containing particles. Moreover, the lipidation of apoA-I was not altered when SAA was over-expressed. Like apoA-I, formation of SAA-containing particles was dependent on ABCA1, but not SR-BI. In 37° C cell-association assays, both lipid-free and HDL-associated SAA bound primary hepatocytes to a greater extent compared to HDL-associated apoA-I or apoA-II, suggesting that SAA is selectively taken up by primary hepatocytes. Cell mediated protein degradation assays demonstrated significantly more SAA degradation by hepatocytes compared to apoA-I, indicating that the liver is a critical organ not only responsible for SAA biosynthesis but also for SAA clearance. Unlike SAA lipidation, hepatocyte uptake and degradation of SAA was not dependent on ABCA1.

Conclusions: SAA is lipidated in an ABCA1-dependent manner to form nascent particles that are distinct from apoA-I-containing particles, indicating that SAA is not incorporated into HDL during HDL biogenesis. SAA is selectively removed from HDL and degraded after binding to hepatocytes. These findings provide new insights into SAA metabolism and function.

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Jeff Chen [1] • Deborah Howatt [1] • Jessica Moorleghen [1] • Alan Daugherty, PhD, DSc [1]
Saha Cardiovascular Research Center University of Kentucky [1]

Gonadectomy Does Not Prevent Progressive Aortic Dilation in a Marfan Syndrome Mouse Model

Graduate Student

Objective:
Marfan Syndrome is a genetic disorder cause by mutations in fibrillin-1, a gene that encodes a component of the extracellular matrix. This syndrome results in dilation of the proximal thoracic aorta. Using two mouse models of Marfan syndrome with reductions in fibrillin-1 abundance, we demonstrated previously that aortic dilation is greater in male than in female mice in both models. In this study, we investigate the effect of sex hormone removal on progression of aortic dilation in fibrilin-1 hypomorphic mice which develop severe and progressive ascending aortic dilation.

Method and Results:
Ascending aortic diameters were measured using a standardized 2D trans-thoracic ultrasonography protocol. Differences in aortic diameters between male and female FBN1mgR/mgR mice were detected as early as 6 weeks of age (1.79 ± 0.11 vs 1.40 ± 0.12 mm; $p = 0.025$) while there was no significant diameter differences between sexes of wild type littermates (1.11 ± 0.12 vs 1.10 ± 0.12 mm; $p = 0.94$). Ascending aortas were cryosectioned at the point of greatest dilation. Elastin fragmentation, as visualized by elastin autofluorescence, demonstrated that male FBN1mgR/mgR mice exhibited increased elastin fragmentation (2.8 vs 1.9 breaks/100uM $p = 0.03$). Successful orchiectomy and ovariectomy and performed on 8 week old mice reduced testosterone concentrations below limits of detection in male mice and resulted in uterine atrophy of female mice, respectively. Aortic diameters measured 4 weeks after surgery demonstrated no difference in aortic diameters between gonadectomized and sham FBN1mgR/mgR mice of either sex.

Conclusions:
We demonstrated that FBN1mgR/mgR male mice exhibited increased aortic dilation and elastin fragmentation, compared to their females. After gonadectomy, aortic diameters were unaffected by 4 weeks of removal of sex hormones. Future experiments will determine the effects gonadectomy earlier in life, and more prolonged observation intervals.

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Bailey Stone[1] • Adrien Mann[1] • Sierra Paxton[2] • Ryan E. Temel[2] • A. Phillip Owens III[1]
[1]Heart, Lung, and Vascular Institute; Division of Cardiovascular Health & Disease Department of Internal Medicine University of Cincinnati • [2]Saha Cardiovascular Research Center and Department of Physiology University of Kentucky

MicroRNA-33a/b Inhibition Attenuates Microvessicle and Monocyte Tissue Factor Activity in the Plasma of Atherosclerotic Non-human Primates

Undergraduate

Objective: Hypercholesterolemia is associated with increased cardiovascular morbidity and mortality via progression of atherosclerotic disease. Dyslipidemia is also associated with a prothrombotic state due to increases in the procoagulant protein tissue factor (TF), which is highly expressed in atherosclerotic plaques. We recently demonstrated that acute hypercholesterolemia can induce the activation of coagulation with time-dependent increases in monocyte TF activity and microvesicle (MV) TF activity in the circulation of both mice and monkeys. Our objective for the current study was to determine the effect of chronic dyslipidemia and subsequent correction of hypercholesterolemia with either standard chow or antagonism of the lipid metabolism regulating microRNA-33 (miR-33) on the activation of coagulation in non-human primates (NHPs).

Methods and Results: Thirty six male cynomolgus monkeys (Macaca Fascicularis) were fed a fat and cholesterol enriched diet (37%, 0.3 mg/kcal cholesterol) for a period of 20 months, at which time a subset was euthanized and atherosclerotic development characterized (n = 12). The remaining monkeys were switched to a standard 'chow' diet and treated for a further 6 months with either saline (n = 12) or the miR-33a/b antagonist RG428651 (anti-miR-33a/b; n = 12). Blood was collected and citrated plasma processed at day 0 (baseline), 20 months (peak experimental), and 26 months (intervention). Total plasma cholesterol was significantly elevated with 20 months of cholesterol enriched diet (491% increase compared to baseline; $P < 0.001$). MV TF (baseline: 0.75 ± 0.2 pg/mL; 20 months: 1.62 ± 0.3 pg/mL; $P < 0.001$) and monocyte TF activity (baseline: 26.6 ± 3.8 pg TF/mg protein; 20 months: 79.8 ± 5.8 pg TF/mg protein; $P < 0.001$) were significantly augmented at 20 months compared to baseline. After 6 months of intervention, vehicle saline and anti-miR-33a/b attenuated plasma cholesterol by 84% and 79% respectively and approached baseline cholesterol measurements ($P < 0.001$). Interestingly, while the hypocholesterolemic conditions of vehicle significantly reduced MV TF (vehicle: 1.08 ± 0.2 pg/mL) and monocyte TF activity (17.6 ± 4.1 pg TF/mg protein), anti-miR-33a/b treatment significantly decreased MV TF (anti-miR: 0.24 ± 0.1 pg/mL) and monocyte TF activity (anti-miR: 10.4 ± 4.4 pg TF/mg protein) past the original baseline.

Conclusions: Our results demonstrate that chronic hypercholesterolemia augments plasma MV TF and monocyte TF activity in NHPs. The prothrombotic state induced by hypercholesterolemia was attenuated by 'chow feeding' with additional significant benefit derived from the miR-33a/b antagonist RG428651. Future studies will examine whether the benefit of anti-miR-33a/b is derived from decreased oxidized LDL activation of TLR4 or HDL-C, both of which can affect TF expression.

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Yu Zhong, PhD [1] • Xufang Mu [2] • Ming Gong, MD, PhD [1] • Zhenheng Guo, PhD [2]
Department of Physiology University of Kentucky [1] • Pharmacology and Nutritional Sciences University of Kentucky [2]

Endothelial Mineralocorticoid Receptor Mediates Aldosterone plus Salt-Induced Abdominal Aortic Aneurysm

Staff

Objective—We recently reported that administration of mice with aldosterone (Aldo) or deoxycorticosterone acetate (DOCA) plus salt induces AAA via mineralocorticoid receptor (MR). The current study defines the specific roles of endothelial MR in Aldo-salt induced AAA.

Approach and Results—A tamoxifen inducible endothelial cell (EC)-specific MR knockout mouse model (iECMRKO) knockout mouse model were developed. The iECMRKO mice were protected from Aldo-salt-induced AAA. Mechanistically, EC-specific MR deletion suppressed aortic elastin degradation, matrix metalloproteinase-2 (MMP-2) and MMP-9 upregulation, macrophage and neutrophil infiltration. Surprisingly, flow cytometry analysis showed that neutrophils, but not macrophages were decreased in iECMRKO mice in the aorta 1 week after Aldo-salt administration. Treatment of C57BL/6 mice with an anti-PMN antibody selectively suppressed Aldo-salt-induced circulating neutrophils, and protected mice from Aldo-salt-induced AAA. In cell cultures, Aldo-induced endothelial adhesion molecules (E-selectin, P-selectin, and ICAM-1, but not VCAM-1) mRNA expressions were abolished in MR-deficient ECs. Importantly, Aldo-salt-induced ICAM-1 but not VCAM-1 protein upregulation was abolished in aortas from iECMRKO mice.

Conclusions—Endothelial MR plays an important role in Aldo-salt-induced AAA. Moreover, ICAM-1, but not VCAM-1, and neutrophils, but not macrophages, mediate the early processes of Aldo-salt-induced and endothelial MR-mediated AAA development.

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Jacqueline Leachman [1] • Carolina Dalmasso , PhD [2] • Celia Ritter [3] • Xiu Xu [2] • Jason Backus [4] • Lisa Cassis, PhD [2] • Analia Loria, PhD [2]
Pharmacology and Nutritional Sciences University of Kentucky [1] • Pharmacology and Nutritional Sciences University of Kentucky [2] • Biology University of Kentucky [3] • University of Kentucky [4]

Male Mice Exposed to Postnatal Neglect Show Increased Adipose Tissue-Derived Aldosterone

Graduate Student

We have previously shown that mice subjected to maternal separation and early weaning (MSEW), a model of early life stress, display exacerbated obesity-induced hypertension, heart rate and sympathetic tone when fed a high fat diet (HF), most likely via the overactivation of the renin-angiotensin system (RAS).

In this study, we investigated the influence of MSEW on downstream RAS components, such as aldosterone. MSEW was performed by separating the pups from their mother for 4 to 8 hours during postnatal days (PD) 2 to 16 and weaned 4 days early. Control mice remained undisturbed and were weaned at PD 21. Eight-week-old mice were fed on a low fat diet (LF) or HF (10 or 60 % fat Kcal) for 12 weeks. Each litter was represented by one male randomly assigned to each diet. In the last week of experiment, transcutaneous glomerular filtration rate (GFR) and metabolic function were measured in all mice. Male MSEW and control mice fed a HF displayed similar metabolic parameters, including water intake (3.4±0.3 vs. 3.4±0.2 ml/day), diuresis (1.1±0.1 vs. 1.0±0.2 ml/day), proteinuria (3.5±0.7 vs. 2.9±0.5 mg/day) and GFR (0.76±0.02 vs. 0.79±0.03 ml/min/100g BW). However, MSEW increased kaliuresis (0.20±0.04 vs. 0.11±0.01 mmol/day, $p<0.05$) and urinary aldosterone (25±4 vs. 11±1 ng/g crea, $p<0.05$); however, plasma aldosterone was similar between groups (257±3 vs. 233±3 pg/ml), the correlation between plasma and urinary levels were significant only in MSEW mice ($R^2=0.75$, $p<0.05$). Furthermore, only MSEW mice showed a positive correlation between increased adiposity (36±1 vs. 24±4, %) and urinary aldosterone (slopes= 2.5±0.9 vs. -0.1± 0.1, $p<0.05$). Taken together, these data suggest that increased AngII may stimulate aldosterone synthesis in adipose tissue. Thus, peripheral RAS activation most likely contributes to the development of neurogenic hypertension in obese male mice.

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"Mixing Cup" Windows App for Temporal Interpolation of Cardiac Cines

Leon Lin[1] • Michael Winkler, MD[2] • Mohammed Issa, MD[2]
Stanford University[1] • University of Kentucky[2]

Student

The matrix of imaging data captured during cardiac imaging exams (ultrasound, SPECT, PET, CT, and MRI) typically has a temporal axis which can be converted into cines. Firstly, motion-based interpolation can heighten frame rate and smooth transitions to improve the quality and realism of cines, especially cardiac cines which capture the rapid motion of the heart. Secondly, different scanner and post processing software vendors' products output different frame rates, output formats, and storage matrices which can be onerous to store or share due to file size constraints. We designed "Mixing Cup" to be an easily and freely shareable, simple to learn Windows applet to apply interpolation, file conversion, and video compression to medical cines.

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Jennifer Wayland [1] • Laura Peterson [2] • Brianna Levitan [2] • Jon Satin, PhD [2] • John McCarthy, PhD [2]
Biology University of Kentucky [1] • Physiology University of Kentucky [2]

Cardiac Phenotypes in Muscle-Specific Ribosomal Protein RPL3L Knockout

Undergraduate

It has recently been discovered that changes in the composition of proteins associated with the ribosome can influence the mRNAs translated by the ribosome. In other words, as described by Shi *et al,* certain mRNAs can be preferentially translated by specialized ribosomes (1). Skeletal and cardiac muscle-specific ribosomal protein RPL3-like substitutes for its ubiquitously expressed paralog RPL3 on ribosomes. In skeletal muscle, previous research has found that RPL3L is not highly expressed until postnatal development is nearly complete, while its paralog RPL3 is expressed at steadily decreasing levels as development progresses. This led to the current study investigating whether or not RPL3L is required for maintenance of cardiac and skeletal muscle.

In order to determine the function of RPL3L in striated muscle, we generated an RPL3L knockout mouse using the FAST gene modulation system described by Tanaka *et al* (2). Wild type and knockout mice aged seven months underwent ECG telemetry for three weeks. Two weeks into the observation period, they were injected with isoproterenol in order to investigate the potential effect of RPL3L knockout on β-adrenergic signaling in the heart. Echocardiograms were performed on another cohort of animals of the same age to determine if the structure of the heart was altered in the RPL3L knockout mouse. One month later, the animals were sacrificed and single cardiomyocytes were isolated and analyzed with ImageJ to determine cell size.

We found that during hours of rest and inactivity, there were no significant differences in several ECG measurements of electrical activity between the RPL3L knockout and wild type animals. However, when challenged with the β-agonist isoproterenol, the knockouts temporarily achieved a significantly higher heart rate ($p<0.05$), indicating a higher sensitivity to β-adrenergic stimulation. A trend was found for larger cardiomyocyte area in the knockout, suggesting a hypertrophic response on the cellular level. Interestingly, despite the increased individual cardiomyocyte area, the left ventricular wall was significantly thinner in the knockouts as measured by echocardiogram. This collection of results implies that knockout of RPL3L leads to various phenotypic changes at the cellular level in the heart, though the mechanism which causes these changes is not yet clear.

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Stuart Pearce [1] • Qingzhong Xiao, MD, PhD [1]
William Harvey Research Institute -Clinical Pharmacology University of London [1]

Role of Neutrophil Elastase in Abdominal Aortic Aneurysms and Thoracic Aortic Dissection

Graduate Student

Abdominal Aortic Aneurysms (AAA) affect 4-5% of men over 65, and Thoracic Aortic Dissection (TAD) is a life-threatening aortic pathology where 75% of patients die within 2-weeks post-onset. Relatively little is known about the underlying mechanisms, which warrants further investigation. Neutrophil Elastase (NE) is an enzyme with roles in priming of the immune system, clearance of large pathogens and remodelling of extra-cellular-matrix proteins, all influential in AAA and TAD. Our recent study suggests a causal role for NE in hyperlipidemia-induced atherosclerosis. However, little is known regarding implications of NE in AAA and TAD. This Study aims to investigate the role of NE within both pathologies.

Gene-expression of NE and AAA-associated markers, MMP-2 and MMP-9, were significantly up-regulated by CaCl2-and AngII-treatment in the cultured vascular smooth muscle cells, endothelial cells and macrophages. In both AngII- and CaCl2-induced AAA mouse models, reduction of aortic expansion in NE-knockout mice was observed, compared with wild-type littermates. TAD experiments reaffirmed the functional importance of NE, with death abolished in NE-knockout mice. Histological analysis is ongoing in order to determine the phenotypical changes produced by loss of the NE gene within these models. Preliminary translational work is also underway, with an Audit of Aneurysm patients' blood profiles. Additionally, peripheral blood and aortic tissues were harvested from surgical repair patients with AAA for NE expression analysis. Initial results show an alteration in proportions of White Blood cell populations, namely macrophages and lymphocytes within expansive aneurysms.

This study suggests NE could be a regulator of aortic expansion and dissection, and therefore a potential target for AAA and TAD treatment. Further work is still needed to elucidate the causal mechanism by which NE takes its effect on AAA and TAD.

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Victoria Noffsinger [1] • Maria de Beer, PhD [2] • Andrea Trumbauer [3] • Preetha Shridas, PhD [4] • Madison Rich [1] • Patricia Wilson, MS [1] • Ailing Ji, PhD [1] • Xuebing Wang [5] • Lisa Tannock, MD [6] • Nancy Webb, PhD [7] • Frederick de Beer, MD [6]
Saha Cardiovascular Research Center University of Kentucky [1] • Physiology University of Kentucky [2] • CVRC University of Oklahoma [3] • Internal Medicine University of Kentucky [4] • Internal Medicine University of Kentucky [6] • Pharmacology and Nutritional Sciences University of Kentucky [7]

Characterization of Transgenic Mice with Inducible, Tissue-specific Expression of Serum Amyloid A

Staff

Objectives: Acute phase serum amyloid A (SAA) comprises a family of secreted proteins that are thought to play an important role in innate immunity. During an acute inflammatory response, SAA is highly induced in the liver and is present in plasma mainly associated with HDL. While accumulating evidence suggests that lipid-free and HDL-associated SAA have distinct activities, the factors that regulate the equilibrium between the two forms of SAA are unknown. We recently reported that SAA3 is the major isoform induced in adipose tissue of mice injected with lipopolysaccharide (LPS).[1]However, plasma levels of SAA3 were ~5-fold lower compared to SAA1.1/2.1 in LPS-injected mice, suggesting that the tissue source of SAA may influence the extent to which SAA reaches the circulation associated with HDL. In this study we developed transgenic mice with doxycycline-inducible, tissue-specific SAA expression to investigate the *in vivo* fate of SAA derived from liver versus adipose tissue.

Methods and Results: Mice harboring a doxycycline-inducible mouse SAA1.1 transgene were generously provided by Dr. JP Simons (Kings College, London), in which SAA1.1 expression is regulated by the reverse tetracycline-dependent transcriptional activator (rtTA).[2] The SAA1.1 transgenic mice were crossed with mice that express rtTA under the control of the AT-specific adiponectin promoter [3] to produce SAA1.1-TG^{fat} mice, or with mice that express rtTA driven by the liver-enriched activator protein promoter, PLAP [4], to produce SAA1.1-TG^{liver} mice. The transgenic strains were crossed with mice deficient in all 3 mouse acute phase SAA isoforms[5] to produce mice with SAA1.1 expression only in adipocytes and hepatocytes, respectively. SAA induction in the transgenic mice was highly dependent on doxycycline dose and was reversible, returning to baseline values within 48h after removing doxycycline. RT-PCR and Western blotting confirmed tissue-specific SAA1.1 expression in SAA1.1-TG^{fat} and SAA1.1-TG^{liver} mice. Interestingly, despite achieving similar tissue mRNA and protein expression with 1 mg/ml doxycycline, plasma levels of SAA were >40-fold higher in SAA1.1-TG^{liver} compared to SAA1.1-TG^{fat}mice. Virtually all of plasma SAA detected in both SAA1.1-TG^{liver} and SAA1.1-TG^{fat} mice was found associated with HDL.

Conclusions: We have developed novel mouse strains with inducible, tissue-specific SAA expression. These mice will be used to investigate the metabolic fate and *in vivo* activity of SAA derived from liver and adipose tissue.

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Ya Wang, PhD [1] • Congqing Wu, PhD [2] • Hong Lu, MD, PhD [2] • Alan Daugherty, PhD, DSc [2]
Cardiovascular Key Laboratory of Zhejiang Province Zhejiang University [1] • Saha Cardiovascular Research Center University of Kentucky [2]

Conversion of Angiotensin II to Angiotensin A Diminishes its Effects on Abdominal Aortic Aneurysms

Graduate Student

Angiotensin (Ang) A is formed by the decarboxylation of the N terminal residue of AngII. This study determined whether this one amino acid change impacted effects of AngII on abdominal aortic aneurysm (AAA) formation in mice. Computational analyses implicated that AngA had comparable binding affinity to both AngII type 1 and 2 receptors as AngII. To compare effects of these two octapeptides in vivo, male low-density lipoprotein receptor (*Ldlr)* or apolipoprotein E (*Apoe*) deficient mice were infused with either AngII or AngA (1 μg/kg/min) for 4 weeks. While AngII infusion induced AAA consistently in both mouse strains, the equivalent infusion rate of AngA did not lead to AAA formation. We also determined whether co-infusion of AngA would influence AngII-induced aortic aneurysm formation in male *Apoe*$^{-/-}$ mice. Co-infusion of the same infusion rate of AngII and AngA did not change AngII-induced AAA formation. Since it was reported that a 10-fold higher concentration of AngA elicited comparable vasoconstrictive responses as AngII, we compared a 10-fold higher rate (10 μg/kg/min) of AngA infusion into male *Apoe*$^{-/-}$ mice with AngII (1 μg/kg/min). This rate of AngA led to abdominal aortic dilation in 3 of 10 mice, but no aortic rupture, whereas the 10-fold lower rate of AngII infusion led to abdominal aortic dilation or rupture in 8 of 10 mice. In conclusion, AngA, despite only being one amino acid different from AngII, has diminished effects on aortic aneurysmal formation, implicating that the first amino acid of AngII has important pathophysiological functions.

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Lei Cai, PhD [1] • Peter Hecker [2] • Tong Li, MD, PhD [1] • Joseph Layne, PhD [3] • Zhen Wang, PhD [1] • Sierra Schlicht[1] • Courtney Burkett [1] • Tara Keenan [1] • Nishita Meshram, MS [2] • Morgan Kelley [4] • Ching-Yi Hu, MS [2] • Shahrzad Hashemi, MS [2] • Imroz Apon[4] • Waleed Aljaaidi [4]• Irina Shalaurova, [5] • Margery Connelly, PhD [5] • Ryan Temel, PhD [1]

CVRC and Department of Physiology University of Kentucky [1] • Department of Pharmacology and Nutritional Sciences University of Kentucky [2] • Novartis, Boston, MA [3] • University of Kentucky [4] • Research and Development, Labcorp (Liposcience), Raleigh, NC [5]

Impact of MicroRNA-33a/b Antagonism on Atherosclerosis Regression and Stabilization in a Nonhuman Primate Model

Staff

The two members of the microRNA-33 (miR-33) family miR-33a and miR-33b coordinately regulate lipid metabolism with their host genes sterol regulatory element binding transcription factor 2 (SREBF2) and SREBF1. Antagonism of miR-33 in mice reduced atherosclerosis by stimulating reverse cholesterol transport and dampening plaque inflammation. However, these studies were of limited translational value since mice express only miR-33a. We and others published that miR-33 antagonism in nonhuman primates (NHPs), which like humans express both miR-33a and miR-33b, resulted in a favorable plasma lipid profile including increased HDL cholesterol (HDLC). Based upon these results, we hypothesized that antagonizing miR-33a/b would regress or stabilize atherosclerotic lesions in NHPs. Male cynomolgus monkeys were fed an atherogenic diet and after 20 months a subset (progression; n=12) was euthanized to characterize atherosclerosis development. The remaining animals were switched to a standard NHP "chow" diet and treated for 6 months with saline (vehicle; n=12) or the miR-33a/b antagonist RG428651 (anti-miR-33; n=12). Due to chow being a hypocholesterolemic diet, both the vehicle and anti-miR-33 groups displayed rapid, sustained, and similar decreases in cholesterol and particle concentrations of VLDL and LDL. Treatment with anti-miR-33a/b significantly increased HDLC and large HDL particle (HDLP) concentrations and decreased medium HDLP concentration. The HDL changes were associated with a significant elevation in the hepatic protein level of ABCA1, an essential factor in nascent HDL production and a miR-33 target. Progression, vehicle, and anti-miR-33 groups showed no difference in right coronary artery (RCA) lesion area, lesion/wall ratio, and percentage of lesion occupied by α-actin+ smooth muscle cells. CD68+ macrophages/foam cells in the RCA lesions were abundant in the progression group but were almost absent in the vehicle and anti-miR-33 groups. Cholesteryl ester, a marker of foam cell content, was also reduced in the thoracic aorta to a level in the two regression groups that was similar to that observed in healthy NHPs. In conclusion, our results indicate that in spite of seemingly beneficial changes in circulating HDL levels, miR-33a/b antagonism for 6 months did not stimulate plaque regression or stabilization. This work was supported by NIH grants P20 GM103527 and R01 HL111932.

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Maria Kraemer, PhD [1] • Courtney Hammill, MS [1] • Baoxiang Yan [1] • Fredrick Onono, PhD [1] • Susan Smyth, MD, PhD [2] • Andrew Morris, PhD [1]
Saha Cardiovascular Research Center University of Kentucky [1] • Division of Cardiovascular Medicine University of Kentucky [2]

Regulation and Function of Lipoprotein Associated Bioactive Lysophospholipid Mediators of Atherosclerosis

Fellow

Lysophosphatidic acids (LPAs) are a family of bioactive radyl hydrocarbon substituted derivatives of glycerol 3 phosphate. LPAs are ligands for at least 6 G-protein coupled receptors. In the cardiovascular system LPA promotes pro inflammatory signaling pathways in multiple blood and vascular cell types. LPA and genes involved in LPA metabolism and signaling have been linked to atherosclerosis in preclinical models and are associated with heritable cardiovascular disease risk in humans. LPA accumulates in atheromas and can be visualized by mass spectrometry imaging in the lipid rich core suggesting the hypothesis that LPA associates with atherogenic low density lipoproteins. However, although LPA is present in isolated LDL, nothing is known about the source, regulation and biological activity of LDL-associated LPA. We have used mouse models and mass spectrometry methods to examine effects of diet and genetically induced hyperlipidemia on circulating levels and distribution of LPA in plasma. While more than 90% of plasma LPA was associated with serum albumin in the blood of wild type mice fed either normal chow or a western diet we observed a 3-4 fold increase in LPA pools associated with low density (LDL and VLDL) pools in hyperlipidemic mouse models (LDLr-/-, ApoE-/-, and PCSK9 treated C57Bl6) fed a western diet. Mass spectrometry analysis revealed diet dependent differences in the molecular species composition of these LDL and albumin associated LPA pools. These increases in LDL associated LPA were not observed in mice with genetic deficiency of autotaxin which is a secreted lysophospholipase D that is well established to be responsible for generation of plasma LPA in mice and humans. LPA and autotaxin's substrate lysophospholipids are abundant in isolated human LDL. However LDL associated LPA levels were not increased after incubation with recombinant autotaxin under conditions where the enzyme was highly active against exogenous substrates. Taken together, these data support the hypothesis that LDL associated LPA could contribute to cardiovascular disease processes including atherosclerosis. While autotaxin is necessary for generation of LDL associated LPA in vivo this may not involve direct hydrolysis of LDL associated substrates.

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Michihiro Okuyama, MD, PhD [1] • Weihua Jiang [2] • Lihua Yang [2] • Aida Javidan [2] • Venkateswaran Subramanian, PhD [2]
Saha Cardiovascular Research Center University of Kentucky [1] • Saha Cardiovascular Research Center University of Kentucky [2]

Inhibition of Hippo-YAP Signaling Does Not Affect Angiotensin II-induced Abdominal Aortic Aneurysms in Male LDL Receptor Deficient Mice

Fellow

Objective: Hippo-YAP signaling pathway is well known to regulate cell survival, proliferation and apoptosis. Mammalian STE20-like protein kinase 1 (MST1), a core component of the Hippo pathway, promotes cell apoptosis and inhibits cell proliferation. Recent studies showed MST1 inhibition, or activation of its downstream YAP signaling improves cardiac regeneration. However, the functional role of Hippo-YAP in the development of aortic aneurysms is still unknown. The purpose of this study is to examine the effect of Hippo-YAP signaling inhibition by XMU-MP-1 (inhibitor of MST1 and 2) on AngII-induced abdominal aortic aneurysm (AAA) formation in mice.

Methods and Results: Male LDL receptor -/- mice (weeks old; n=6-7 per group) were fed a fat-enriched diet (21% wt/wt milk fat; 0.15% wt/wt cholesterol) for 5 weeks. The MST 1 and 2 inhibitor, XMU-MP-1 (3 mg/kg/day) or vehicle was administered daily by gavage for 5 weeks. After 1 week of high fat feeding and MST inhibitor dosing, the mice were infused subcutaneously with AngII (1,000 ng/kg/min) by osmotic minipumps for 4 weeks. AngII increased systolic blood pressure similarly in both groups (pre-infusion- Vehicle: 144 ± 4 versus XMU: 145 ± 5 mmHg; post-infusion- Vehicle: 183 ± 5 versus XMU: 185 ± 13 mmHg; P = not significant). AngII infusion led to increased aortic luminal dilation in both groups as measured in-vivo by ultra-sonography (pre-infusion- Vehicle: 0.92 ± 0.04 versus XMU: 0.93 ± 0.07 mm; post-infusion- Vehicle: 1.66 ± 0.03 versus XMU: 2.19 ± 0.22 mm; P = not significant). Ex-vivo measurement showed equivalent abdominal aortic expansion (Vehicle: 1.45 ± 0.19 versus XMU: 2.23 ± 0.44 mm; P = not significant) in both groups after AngII infusion for 4 weeks.

Conclusion: These findings suggest that Hippo-YAP signaling inhibition by XMU-MP-1 does not affect the development of AngII-induced AAAs.

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Carolina Dalmasso, PhD [1] • Jacqueline Leachman [1] • Lisa Cassis, PhD [1] • Analia Loria, PhD [1]
Pharmacology and Nutritional Sciences University of Kentucky [1]

Female Mice Exposed to Postnatal Neglect Display Angiotensin II-dependent Obesity-induced Hypertension

Fellow

We have previously demonstrated that female mice subjected to maternal separation with early weaning (MSEW), a model of early life stress, show exacerbated diet-induced obesity and hypertension. The goal of this study was to test whether female MSEW mice display angiotensin II (AngII)-dependent hypertension. MSEW was achieved by repeated, daily separations from the dam and weaning at postnatal day 17; normally reared controls (C) were weaned at postnatal day 21. C and MSEW female weanlings were placed on low fat (LF) of high fat (HF) diet (10% and 60% fat kcal, respectively) and implanted with radiotelemetry after 18 weeks. MSEW did not change 24-hr mean arterial pressure (MAP) in female LF-fed mice, however it increased MAP in HF-fed mice ($p<0.05$). Plasma renin concentration was reduced in MSEW fed HF diet when compared to control HF-fed females. Angiotensin converting enzyme 1 (ACE1) mRNA expression and AngII peptide levels were significantly increased in perigonadal adipose tissue from HF-fed MSEW mice ($p<0.05$); however, no differences between groups were observed in liver and kidney. Chronic enalapril treatment (2.5 mg/kg/day, drinking water, 7 days) reduced MAP in both C and MSEW HF-fed groups ($p<0.05$) and abolished the differences due to MSEW. The acute administration of AngII (1, 10 and 50 ug/kg, s.c.) to awake mice showed that MSEW significantly increased AngII-induced elevations in MAP, either in untreated or enalapril-treated conditions. Altogether, obesity increases BP in female MSEW via elevated circulating AngII and enhanced AngII sensitivity. Moreover, adipose tissue-derived AngII production most likely contributes to exacerbate obesity-induced hypertension in female MSEW mice.

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Shayan Mohammadmoradi, MS [1] • Hong Lu, MD, PhD [1] • Alan Daugherty, PhD, DSc [1]
Saha Cardiovascular Research Center University of Kentucky [1]

High Mobility Group Box 1 Enhances Human Aortic Adventitial Fibroblast Migration

Graduate Student

Background and Objective: Migration of adventitial fibroblasts may play a critical role in vascular remodeling, a feature of aortic aneurysmal formation and development. High-mobility group box 1 (HMGB1) is a nuclear protein that acts as a damage-associated molecular pattern and has been reported to contribute to vascular remodeling. The purpose of this study was to determine whether HMGB1 contributed to migration of aortic adventitial fibroblasts.

Methods and Results: Human aortic adventitial fibroblasts were grown to 80–90% confluence and made quiescent by serum starvation for 24 hours. Subsequently, these cells were cultured in fresh medium without stimulation or were incubated with 1, 10 and 100 ng/mL of recombinant HMGB1 for 24 hours. A wound healing assay was used to investigate cellular migration and HMGB1 induced cellular migration. Transwell permeable inserts with an 8.0 μm PET membrane were used to further assess cell migration. Human aortic adventitial fibroblasts in 100 μl serum-free medium were placed above the inserts and 500 μl of HAoAF conditioned medium from cells incubated with HMGB1 (100 ng/mL) for 24 hours was placed below. After 24 hours of incubating at 37 °C, the number of cells migrated to the bottom wells were assessed using EVOS FL Cell Imaging System. There was an approximate 100% increase in the number of cells that migrated toward the conditioned medium compared to control media.

Conclusion:HMGB1 increases human aortic adventitial fibroblast migration. We will determine by which mechanism that HMGB1 contributes to migration of adventitial fibroblasts and whether this process contributes to aortic aneurysms.

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David Henson[1] • Ayman Samman Tahhan, MD [2] • Arshed Ali Quyyumi, MD [2] • Vincent Venditto, PhD [1]
Pharmaceutical Sciences University of Kentucky [1] • Cardiology Emory University [2]

Graduate Student

Apolipoprotein A-I (ApoA-I) is a target of IgG autoantibody induction in patients, but the role of these antibodies has not been fully elucidated. Previous research has characterized anti-ApoA-I IgG antibodies targeting delipidated ApoA-I as a biomarker of cardiovascular progression, but only a moderate association was observed. We hypothesize that free anti-ApoA-I IgG is a single component of the anti-ApoA-I response and characterization of anti-ApoA-I antibody profiles will be more predictive of adverse cardiovascular disease outcomes. Given the relative concentrations of ApoA-I and anti-ApoA-I antibodies in circulation, we developed an ELISA assay to quantify ApoA-I bound to IgG as a soluble immune complex (IC) in sera samples. This ELISA assay was used to screen plasma from 359 patients with coronary artery disease (CAD) and 103 blood donor samples. Data from this assay showed blood donor patients have significantly higher levels of ApoA-I/IgG ICs as compared to patients with CAD ($p < 0.0001$). Analysis of outcomes in patients with CAD shows that patients in the lowest tertile for ApoA-I/IgG IC values have an increased risk for death and myocardial infarction as compared to patients in the highest tertile with an hazard ratio of 1.89 (95% CI: 1.02 - 3.52; $p = 0.04$) after adjustment for 6 common cardiovascular risk factors. Pearson correlation analysis between ApoA-I/IgG ICs in the 359 patients with CAD found no relationship between ApoA-I/IgG ICs and 26 common clinical measures. In addition the relationship between ApoA-I/IgG ICs and both total ApoA-I concentration and total IgG concentration were evaluated in the 103 blood donor patients. No significant relationship between ApoA-I/IgG ICs and total ApoA-I concentration (113 ± 44 mg/dL, $r^2 = 0.0075$) was observed. A weak correlation was observed between ApoA-I/IgG IC level and total IgG concentration (12 ± 4 mg/mL $r^2 = 0.089$). Initial characterization of ApoA-I/IgG IC from patients indicates that ApoA-I/IgG ICs are enriched in the anti-inflammatory IgG4 antibody as compared the subclass ratio in total serum (36% and 7%, $p = 0.0381$). The enrichment in the anti-inflammatory IgG4 provides a potential cause of the protective effect of ApoA-I/IgG ICs in patients. The identification and further characterization of ApoA-I/IgG ICs has the potential to guide clinical diagnosis and intervention strategies in patients with atherosclerotic cardiovascular disease.

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Shiksha Joshi[1] • Sandipan Shringi[2] • John Suffredini, MD [2] • Maya Guglin, MD, PhD [1] • Navin Rajagopalan, MD [1]
Cardiology, Internal Medicine University of Kentucky [1] • Internal Medicine University of Kentucky [2]

Long term IV Inotropes in Advanced Heart Failure are Associated with Decrease in Hospitalized Days and Improved Functional Class

Staff

Background
The role of IV inotropes in the treatment of hospitalized acutely decompensated heart failure patients with end organ hypo-perfusion is well established. However, continuous IV inotropes in ambulatory patients remains controversial. Some studies suggest that long term IV inotrope infusions did not improve functional class or mortality in advanced heart failure patients. Overall, the current data on long term IV inotropes in advanced heart failure is limited and the topic needs further study with larger groups of patients.

Methods
This was a retrospective study which included patients diagnosed with low output advanced heart failure and treated with long term IV inotrope therapy at the University of Kentucky. Consecutive patients on home IV milrinone, who remained on it for at least 6 months, were included. We collected demographic, hemodynamic, biochemical, medical therapy, imaging, device interrogation and hospitalization data of patients with advanced HF from up to 6 months before starting IV inotrope and compared it to up to 6 months after initiating therapy.

Results
A total of 70 patients on continuous IV milrinone were included. Mean age was 57.5 +/- 12.5 years, 55 (78.5%) were male. 35 (50%) had ischemic and 35 (50%) had non-ischemic cardiomyopathy. Mean number of hospitalized days six months prior to initiating inotrope therapy was 10.18 days compared to4.97 days six months after the therapy (p=0.002). Mean Cardiac index by Fick, six month before and after was 1.80 L/min/m2 and 2.23 L/min/m2 respectively (p<0.001). Mean cardiac output by Fick, before and after was 3.72 L/min and 4.57 L/min respectively (p<0.001). Mean wedge pressure, six months before and after was 22.39 mmHg and 17.65 mmHg respectively (P=0.007). Mean pulmonary artery pressure, six month before and after was 35.78 mmHg and 29.78 mmHg respectively (P=0.002). Mean NYHA Class, six month before and after was 3.32 and 2.67 respectively (P<0.001).

Conclusion
Based on statistical analysis of the data, we conclude that the long-term IV use of milrinone is associated with improvement in hemodynamics and functional class of the patients along with a statistically significant decrease in the number of hospitalized days.

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Bradley Wright [1] • Hisashi Sawada, MD, PhD [1] • Jessica Moorleghen [1] • Deborah Howatt [1] • Debra Rateri [1] • Mark Majesky, PhD [2] • Alan Daugherty, PhD, DSc [1]
Saha Cardiovascular Research Center University of Kentucky [1] • Department of Pediatrics University of Washington [2]

Differential Effects of LRP1 in AngII-induced Ascending Aortic Pathologies Between Male and Female Mice

Undergraduate

Objective: Low-density lipoprotein receptor-related protein 1 (LRP1), a transmembrane protein, is important in maintaining elastin fiber integrity of the aortic wall. Smooth muscle cells (SMCs) of the ascending aorta are composed of the inner medial layers from the cardiac neural crest and the outer layers from the second heart field (SHF). LRP1 depletion in SMCs of male mice augments angiotensin II (AngII)-induced ascending aortic dilation and rupture attributed specifically to SMCs of SHF origin. The purpose of this study was to determine whether depletion of LRP1 in SHF-derived SMCs (SHF-SMC LRP1) has differential effects on AngII-induced aortic dilation, rupture, and elastin fragmentation between male and female mice.

Methods and Results: Female LRP1 floxed mice were bred to male LRP1 floxed mice with Mef2c-Cre transgene to generate SHF-SMC LRP1 +/+ and -/- mice. Male and female mice at 12-14 weeks of age were infused with either saline or AngII (1,000 ng/kg/min) for 28 days (n = 12-31). Ascending aortic diameter (AoD) was measured by ultrasound, rupture was determined by necropsy, and elastin fragmentation was assessed by Movat's staining. In male mice of both genotypes, AoD and elastin breaks were increased by AngII infusion, and AoD was positively correlated with elastin fragmentation ($r^2 = 0.48$, $p < 0.001$). SHF-SMC LRP1 deletion augmented aortic dilation, rupture rate, and elastin fragmentation in AngII-infused male mice. In females, AngII infusion increased AoD in SHF-SMC LRP1 -/- mice, but not in their wild type controls. AngII-induced elastin fragmentation did not differ between SHF-SMC LRP1 +/+ and -/- female mice, and no correlation between AoD and elastin fragmentation was detected ($r^2 = 0.01$, $p = 0.88$). Despite the extent of AngII-induced elastin fragmentation that was equivalent to that in males, aortic rupture is lower in female than in male LRP1 deleted mice (9 vs 38%, $p = 0.02$).

Conclusion: In male mice, AngII infusion promotes dilation and rupture of ascending aorta that is augmented in SHF-SMC LRP1-/- mice and positively associated with elastin fragmentation. However, AngII only promotes ascending aortic dilation in female mice with SHF-SMC LRP1 deletion, despite elastin fragmentation being equivalent between SHF-SMC LRP1 +/+ and -/- mice.

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Manpreet Sira [1] • Shiksha Joshi [1] • Maya Guglin, MD, PhD [2]
University of Kentucky [1] • Internal Medicine - Cardiology University of Kentucky [2]

Outcomes of In-Hospital Cardiac Arrests: Where Can We Do Better?

Graduate Student

Background: Survival in cardiac arrest is poor. In-hospital cardiac arrest has a greater potential for survival than out-of hospital cardiac arrest, because it is usually witnessed, and professional help is readily available. We reviewed in-hospital cardiac arrests occurring during one calendar year in order to identify the areas of improvement.

Methods: We analyzed 598 hand-written code sheets from January 2017 to December 2017, and excluded respiratory (intubation only) codes, codes in patients with Do not resuscitate status, pediatric codes, and out-of-hospital arrests. We then explored survival to ROSC versus survival to discharge based on the diagnosis, location of the code within the hospital, initial rhythm, workdays/hours vs weekends/off hours. Chi square test was used for comparison.

Results: Of the 598 codes, 236 codes were excluded due to being a respiratory code, a pediatric code, an out-of-hospital arrest, patient being DNR, or the family stopping the code mid-way. The final sample consisted of 369 codes in 287 individual patients with 53 patients having multiple codes during a single admission. There were 178 males (61.8%) and 110 females, and the mean age was 60.0±14.1. Mean duration of the code was 29.9±17.8 minutes. 96.5% of cardiac arrests were witnessed.273 out of 369 codes resulted in survival post-code (74%), however, only 71 out of 288 patients survived to discharge (24%). Of these 288 patients, 8 patients received VA ECMO during their stay of which 3 survived. Survival to ROSC versus survival to discharge are presented in a table.

Conclusions: Over 50% of IHCAs occur in patients with chronic conditions with poor prognosis, with 10-12% survival to hospital discharge. Significant proportion of IHCA occur secondary to medical manipulations/equipment failure.

There is no difference in survival to either ROSC or discharge in males versus females, weekdays/regular hours vs weekend/off hours, patients with or without end stage renal disease. Women have a trend towards better survival to discharge (P=0.06).

Patients who experience cardiac arrest on telemetry have higher chances to survival than patients in the ICU or off telemetry

Initial shockable rhythm improves survival to ROSC but not to hospital discharge.

In cardiac arrests due to acute reversible conditions, only 41.4% survive to discharge. This group should be the focus of effort to improve outcomes. Different forms of mechanical circulatory support should be considered.

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Peter Hecker [1] • Lei Cai, PhD [2] • Donna Wilcock, PhD [3] • Elizabeth Head, PhD [4] • Peter Nelson, MD, PhD [5] • Ryan Temel, PhD [6]
Pharmacology and Nutritional Sciences University of Kentucky [1] • Saha Cardiovascular Research Center University of Kentucky [2] • Physiology and Sanders-Brown Center on Aging University of Kentucky [3] • Sanders-Brown Center on Aging and Department of Pharmacology and Nutritional Sciences University of Kentucky [4] • Pathology, Division of Neuropathology and Sanders-Brown Center on Aging University of Kentucky [5] • Physiology and The Department of Pharmacology and Nutritional Sciences University of Kentucky [6]

Intracranial Atherosclerosis and Vascular Hallmarks of Cognitive Impairment and Dementia in Nonhuman Primates fed an Atherogenic Diet

Graduate Student

Vascular cognitive impairment and dementia (VCID) is the second most common cause of dementia trailing Alzheimer's disease. Poor cardiovascular health facilitates poor brain health and improvements in vascular outcomes may potentially delay or prevent onset of VCID. Notably among the many vascular hallmarks of VCID, intracranial atherosclerosis (ICAS) is a public health concern for both its role in stroke and subsequent cognitive dysfunction. Reducing low-density lipoprotein (LDL) concentration with statins is a primary therapeutic approach to stabilize atherosclerotic vascular disease (AVD), but statins only reduces ischemic stroke risk by ~20% and do not appear to reduce VCID. This suggests that treatment of hypercholesterolemia alone is not an optimal approach for reducing VCID and additional therapies are likely needed to promote the regression and/or stabilization of ICAS. To date, there is a paucity of animal models that develop ICAS making it difficult to test novel therapies to reduce ICAS burden and downstream hallmarks of VCID. During a study to determine the impact of microRNA-33 (miR-33) antagonism on cardiovascular AVD, we fortuitously discovered that our nonhuman primate (NHP) model had ICAS and other neurovascular hallmarks of VCID. Indeed, evaluation of intracranial arteries revealed that after 20 months on an atherogenic diet over 50% of NHPs (n=63) developed ≥1 atherosclerotic lesions within the circle of Willis (COW), the main arterial network that supplies blood to the brain. Atherosclerotic lesions from sections of COW were characterized by necrotic cores surrounded by macrophages, smooth muscle cell migration into the intima, and a fibrous cap that was rich in collagen. Furthermore, preliminary data collected on a subset of animals (n=5) presented, in addition to ICAS, evidence of neurovascular hallmarks of VCID such as: gross ischemic lesions, gross infarcts, brain arteriolosclerosis (B-ASC), microinfarcts and microhemorrhages. Moreover as a surrogate for direct measures of reactive gliosis, the density of positive immunohistochemistry (IHC) staining for IBA1+ microglia and GFAP+ astrocytes was assessed on brain sections from 2 animals: one animal with ICAS and a control animal with no AVD. This assessment suggested an increased neuroinflammatory response in the animal with ICAS when compared to the control as evidence by increased density of IHC+ reactive glial cells and changes in glial morphology often associated with a neuroinflammatory state. We are currently analyzing a complete set of intracranial arteries and brains from our NHPs in hopes that our model will become useful in determining causes and therapies for ICAS and VCID.

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Chia-Hua Wu [1] • Deborah Howatt [1] • Jessica Moorleghen [1] • Craig Vander Kooi, PhD [2] • Alan Daugherty, PhD, DSc [1] • Hong Lu, MD, PhD [1]
Saha Cardiovascular Research Center University of Kentucky [1] • Department of Molecular and Cellular Biochemistry University of Kentucky [2]

Conserved Sequence in the Loop Region of AGT Affects Plasma AGT Concentrations but Has no Effects on Ang II-mediated Functions

Graduate Student

Background and Objective: Angiotensinogen (AGT) is the unique substrate that is the source of all angiotensin peptides. The loop region of AGT protein formed by residues 291-301 is highly conserved and contains strictly conserved solvent accessible hydrophobic residues W292 and V299 along with strictly conserved S298. In this study, we determined whether depletion of these conserved sequence in mouse AGT affects AngII-mediated functions.

Methods and Results: Surface plasmon resonance analysis showed that mutation of W292 in AGT loop region decreased its binding affinity to megalin, a protein that is abundant in renal proximal tubules. We then determined whether mutation of W292 affects AngII-mediated functions in mice. Hepatocyte-specific AGT deficient (hepAGT-/-) mice having low plasma AGT were injected with adeno-associated viral vectors (AAV) encoding the mutated AGT (W292A). Repopulation of this mutated AGT led to increases of plasma AGT concentrations, blood pressure and atherosclerosis, which were comparable to hepAGT+/+ mice. We then determined whether depletion of the entire conserved sequence (291-301) would affect AngII-mediated functions. Administration of AAV encoding AGT with substitution of the loop region with GA linker only moderately increased plasma AGT concentrations, although mRNA abundance of AGT in liver was comparable with its abundance in hepAGT +/+ mice. Despite the much lower concentrations of plasma AGT, depletion of the conserved sequence in the loop region significantly increased blood pressure and atherosclerosis to values that were equivalent as in hepAGT+/+ mice.

Conclusion: The highly conserved sequence in the loop region of AGT may affect AGT protein metabolism, but does not affect AngII-mediated functions.

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Rebika Khanal [1] • Lance Johnson, PhD [1] • Jason Brandon, PhD [1] • Brandon Farmer, MD, PhD [2]
Physiology University of Kentucky [1] • College of Medicine University of Kentucky [2]

APOE and Fatty Acid Metabolism: E4 Increases Astrocyte Lipid Droplet Formation

Undergraduate

Cardiovascular disease (CVD) is the leading cause of death amongst both men and women in the United States. Apolipoprotein E (APOE) is present in both the periphery and the brain, and is associated with circulating lipoproteins, especially very low density lipoprotein and high density lipoproteins. ApoE is well known for its connection to both Alzheimer's disease (AD) as well as cardiovascular diseases (CVD). In humans, there are three common isoforms of apoE: E2, E3, and E4. The E4 isoform, compared to E2 and E3, is associated with an increased risk for both the development of AD as well as CVD. It has become clear that AD is influenced by both metabolic and vascular factors – both of which precede and may contribute to dementia. Interestingly, E4 is associated with deficiencies in both areas; there is both decreased cerebral glucose metabolism and lower cerebral blood flow in E4 individuals. Specifically, In the periphery, it is known that the lipid metabolism is altered by the E4 isoform of APOE, however the precise mechanism of how it is altered is unknown, and very little is know about E4's effects on brain fatty acid metabolism. Therefore, we used mouse models that express human apoE, to explore the mechanism of lipid uptake and storage in E4 mice and E4-expressing astrocytes compared to E3 and E2. Our data show that E4-expressing astrocytes form significantly more lipid droplets than E3-expressing astrocytes when treated with sub-millimolar concentrations of oleic acid. However, when treated with palmitic acid *in vivo*, there was no significant difference in the uptake of fatty acid amongst various tissue types in E2, E3, E4, and Knockout (KO) mice. Palmitic acid was administered *in vivo* and allowed to perfuse into the heart, liver, kidney, skeletal muscle as well as subcutaneous fat, visceral fat, and brown adipose tissue. These findings are an important step toward elucidating the precise mechanism(s) of APOE's effects on fatty acid metabolism in the brain and the periphery in order to better understand the role of this important genetic risk factor in lipid metabolism, vascular disease and dementia.

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Samuel Slone [1] • Sarah Anthony [2] • Lisa Green [3] • Michelle Nieman [4] • John Lorenz, PhD [4] • Michael Tranter, PhD [2]
Physiology and Systems Biology University of Cincinnati [1] • Internal Medicine University of Cincinnati [2] • Pharmacology and Systems Physiology University of Cincinnati [3] • Physiology University of Cincinnati [4]

Inhibition of The RNA Binding Protein Hur Reduces Cardiac Cell Death Following Ischemia/Reperfusion Injury

Graduate Student

Despite medical advances, cardiac ischemia/reperfusion (I/R) injury (myocardial infarction) remains a leading cause of morbidity and a huge economic burden in the United States. Total damage to the heart following I/R injury is directly linked to the percentage of individual cardiac myocytes that undergo apoptotic cell death. In addition to cell death that occurs during the initial infarct, it is widely accepted that reperfusion, the return of oxygenated blood following ischemic injury, introduces reactive oxygen species (ROS) that further activate downstream pro-apoptotic pathways. We have previously shown the RNA binding protein HuR to be activated downstream of p38 MAPK in cardiac myocytes following pressure overload-induced hypertrophy; since p38 MAPK is known to be downstream of ROS, we postulated that HuR is a potential mediator of apoptotic gene expression during cardiac reperfusion injury.

To address the functional role of HuR in I/R, we utilized an inducible cardiomyocyte-specific HuR deletion mouse (iCM-HuR$^{-/-}$) and subjected these mice to 30 minutes of LAD (left anterior descending artery) ligation followed by 24 hours of reperfusion. Analysis of infarct size showed that iCM-HuR$^{-/-}$ mice had a significantly smaller infarct compared to control mice (41% infarct/risk in iCM-HuR$^{-/-}$ vs. 51% infarct/risk in control, N=3, $P<0.05$). This result was recapitulated using simulated I/R (simI/R) *in vitro*on neonatal rat ventricular myocytes (NRVMs) and H9C2 cells (myoblast derived rat left ventricular cardiomyocytes) by subjecting them to 6 hrs of simulated ischemia (glucose deprivation and hypoxia at ≤1% O_2) followed by 2 or 24 hrs of re-oxygenation (and reintroduction of glucose/serum). Similar to our *in vivo* results, siRNA-mediated knockdown or pharmacological inhibition of HuR significantly reduced simI/R-induced cell death and caspase-3 activity.

In conclusion, our results suggest that ablation of HuR results in a significant reduction in infarct size following I/R injury through a reduction in caspase 3-dependent apoptotic cell death. This implies a promising role for pharmacological inhibition of HuR as a therapeutic target for cardiomyocyte protection during ischemia/reperfusion injury.

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Aubrey Melton [1] • Renee Donahue, MS [2] • Ahmed Abdel-Latif, MD, PhD [3]
Biology Berea College [1] • Saha Cardiovascular Research Center University of Kentucky [2] • Gill Heart Institute University of Kentucky [3]

Altered Immune Cell Profile Contributes to Increase Risk of Heart Disease in Aging Mice

Undergraduate

Cardiovascular disease accounts for one of every four deaths in the United States, making it the leading cause of death of men and women. Many factors contribute to cardiovascular disease risk including high blood pressure, high cholesterol, smoking and aging. As you age, your risk of heart disease doubles due to factors such as heart enlargement, wall thickening and valve stiffening. It is well known that after cardiac tissue damage, macrophages play an important role in the balance of pro- and anti-inflammatory processes which have a direct effect on angiogenesis, vascularization, scar spread, and functional outcome. We hypothesize that as we age, the cardiac macrophage immune cell profile may be altered, which can play an important role in developing increased risk for heart disease. Therefore, the goal of the present study was to characterize the basal cardiac immune cell profile of 23-24 month aged mice and compare it to that of 6-8 week young mouse hearts. We used immunohistochemical methods to examine the level of endothelial formation (Isolectin B4), the macrophage profile (Iba-1 and CD206), and scar size and fibrosis (Masson's Trichrome and Picrosirius Red). Analyses show a significant increase in blood vessel formation and anti-inflammatory macrophage numbers and a visual decrease in fibrosis in young mice compared to the aged mice. This may contribute to the increased risk of heart disease seen as we age. Quantification of fibrosis and pro-inflammatory macrophages is ongoing.

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Mohammad Javad Mollakazemi, MS [1] • Dibyajyoti Biswal, MS [1] • Joyce Evans, MS [1] • Abhijit Patwardhan, PhD [1]
Department of Biomedical Engineering University of Kentucky [1]

Cardiac-synchronized EEG: Effects of Cognition and Tempo of Music

Graduate Student

Introduction: It is known that listening to songs can entrain cardiac rhythms suggesting that neural changes in response to listening to songs, in turn, affect cardiac rhythms.

Methods: Eigen decomposition of cardiac-synchronized EEGs was used to investigate effects of phase, tempo and cognition of auditory stimuli. For evaluating effects of tempo, slow and fast tempo songs were used, and for cognition, each subjects' favorite song was used. To further investigate the role of cognition, phase of local spectra of subject's favorite song and fast tempo song was scrambled while preserving magnitude spectra. ECG and six EEGs were recorded as subjects listened to songs. The R waves of ECG were localized, and the EEGs in 300 ms segments ending at each R wave were extracted. To see difference between EEGs synchronized to different portion of cardiac cycle, 300 ms segments starting at each R wave were also extracted. Eigen decomposition of covariance of data matrix of EEG segments was performed. The same process was repeated for post R peak EEG segments. The number of eigenvalues needed to reconstruct 80% of variance of data, based on spectral decomposition, was computed for all trials by obtaining cumulative sum of eigenvalues of covariance matrix (Qsum).

Results and Discussion: All eigenvalues of covariance matrix of post R peak EEG segments in all songs were larger than pre R peak ones. This difference was more significant and consistent in temporal EEGs. The second eigenvalue of P3 was affected significantly by all songs and this change was bigger in pre R peak EEG (23.21% average) than post R peak (16.33% average). For both pre and post R peak EEGs, within the ten largest eigenvalues, among all songs the fast local phase randomized (LPR) caused the largest increase and the slow song caused the largest decrease. In pre R peak data, T3 had the lowest average change rate in ten largest eigenvalues while had the highest average change rate in post R peak data than other five EEGs. These results suggest that EEGs recorded at T3 are more sensitive to phase of cardiac cycle. The Qsum for post R peak data was higher than pre R peak for all EEGs in all songs, indicative of increased complexity of EEGs in post-R-peak than pre-R-peak EEGs. T4 had the largest Qsum in favorite song and the lowest Qsum in slow song. T4 is close to audio cortex and is on the right hemisphere that it is hypothesized that effect of music is larger. In favorite LPR song, Qsum decreased significantly in T4, which increased in fast LPR song. This opposite direction of change when listening to randomized versions of the songs possibly was a result of cognition because subjects, after listening to the favorite-LPR, realized that it is their favorite song but this was not the case for fast-LPR song. The larger Qsum for favorite song indicates an increase in dimension of EEGs from T4 suggesting that more complex cerebral activity may exist when subjects listen to songs that they like.

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Yasir Alsiraj, PhD [1] • Sean Thatcher, PhD [1] • Mark Ensor, PhD [1] • Lisa Cassis, PhD [1]
Pharmacology and Nutritional Sciences University of Kentucky [1]

Inhibition of Neprilysin Attenuates AngII-Induced Abdominal Aortic Aneurysms (AAAs) in Hypercholesterolemic Male Mice

Fellow

Objective: Abdominal aortic aneurysm (AAA) is a symptomatic deadly vascular disease of elderly men. Plasma levels of brain natriuretic peptide (BNP), which is degraded by the metalloendpeptidase, neprilysin, have been suggested as biomarkers of incident AAA. However, it is unclear if neprilysin plays a role in AAA development. Entresto®, a neprilysin inhibitor in combination with an angiotensin receptor blocker, has demonstrated efficacy in human heart failure and is under investigation for treatment of other cardiovascular diseases. In this study, we examined the effect of the neprilysin inhibitor, sacubitril, on AngII-induced AAAs in male *LDLr*$^{-/-}$ mice.

Methods and Results: Male (8-12 weeks of age) *LDLr*$^{-/-}$ mice were fed a Western diet (Teklad TD88137) for the duration of the study. Vehicle or sacubitril (S, 1, 6 or 20 mg/kg/day) were administered by osmotic minipump for one week, and then minipumps containing vehicle or S (at respective doses) in combination with AngII (1,000 ng/kg/min) were implanted for 28 day delivery. Body weights were similar in all groups. Sacubitril decreased systolic blood pressure (measured by tail cuff during week 3 of AngII infusions) in a dose-dependent manner, with maximal effects S6 mg/kg/day (vehicle, 150 ± 5; S1 mg/kg/day, 142 ± 7; S6 mg/kg/day, 118 ±5; S20 mg/kg/day, 122 ± 5 mmHg). Sacubitril dose-dependently reduced suprarenal aortic lumen diameters (day 28: vehicle, 1.8 ± 0.02; S1, 1.9 ± 0.2; S6, 1.6 ± 0.2; S20, 1.2 ± 0.1 mm; $P<0.05$) and maximal AAA diameters at study endpoint (vehicle, 2.3 ± 0.2; S20, 1.0 ± 0.1 mm; $P<0.05$). AAA incidence (89% in vehicle-infused mice) was significantly reduced by S20 mg/kg/day (20%). Similarly, sacubitril reduced atherosclerosis in a dose-dependent manner (vehicle, 8 ± 1.3; S1, 9.2 ±1.1; S6, 5.6 ±1.4; S20, 2.7 ± 0.4 % lesion surface area; $P<0.05$). Interestingly, AngII-induced reductions in plasma renin concentrations were reversed by sacubitril (vehicle, 3.5 ±0.1; S20, 22.3 ± 3.2 ng/ml; $P<0.05$).

Conclusions: These results demonstrate that inhibition of neprilysin protects against AngII-induced atherosclerosis and AAAs in male *LDLr*$^{-/-}$ mice. Future studies will determine mechanism of action in neprilysin inhibition, and whether combination with an angiotensin receptor blocker is an effective therapeutic in the prevention and treatment of atherosclerosis and AAA progression.

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Ahmed Al-Darraji, PharmD [1] • Himi Tripathi, PhD [2] • David Henson [3] • Renee Donahue, MS [2] • Lakshman Chelvarajan, PhD [2] • Erhe Guo, PhD [4] • John Gensel, PhD [5] • Vincent Venditto, PhD [3] • David Feola, PharmD [3]
Pharmacology and Nutritional Sciences University of Kentucky [1] • SAHA Cardiovascular Research Center University of Kentucky [2] • College of Pharmacy University of Kentucky [3] • Department of Physiology University of Kentucky [5]

Graduate Student

Liposomal Azithromycin as Immunotherapy in Ischemic Heart Disease

Introduction: Macrophages are the primary immune cells organizing the early inflammatory stage and the later repairing events as well. The two activation states of macrophages (inflammatory vs. reparatory) with distinct functions made macrophages an attractive therapeutic target to early resolving inflammation through dominating the reparatory phenotype. We recently demonstrated that azithromycin (AZM), an antibiotic with immunomodulatory properties, improves cardiac remodeling and recovery post-MI through sifting macrophages to the reparatory state. However, the findings were in a clinically irrelevant model of AZM therapy (pre-MI treatment and relatively high dose). It is known in MI settings that liposomes are a potent drug delivery system to enhance the efficacy and reduce the off-target effects, caused by specific accumulation in the damaged heart. Therefore, we hypothesized that the liposomal AZM (Lazm) treatment, started after MI, triggers inflammation resolution and improves cardiac recovery as a result of intensified accumulation of AZM in the injured myocardium. **Methods and results**: Mice were treated with Lazm (40 or 10 mg/kg/day) or vehicle starting after MI for 7 days. It was noticed that the inflammatory macrophages ($CD45^+/Ly6G^-/F4\text{-}80^+/CD11c^+/CD86^+$) were significantly decreased with a concomitant elevation in reparatory macrophages ($CD45^+/Ly6G^-/F4\text{-}80^+/CD11c^+/CD206^+$) in the heart of Lazm treatment groups, leading to a significant decrease in inflammatory/reparatory macrophage ratio. Furthermore, the pro-inflammatory neutrophils (N1) ($Ly6G^+/CD11b^+/CD206^+$) were remarkably declined in the same group. The pro-inflammatory monocytes ($Ly6C^{hi}$) were also decreased, while the anti-inflammatory monocytes ($Ly6C^{lo}$) were increased in the heart of Lazm mice. Alongside with changes in immune cells, gene analysis data show that the mRNA levels of inflammatory genes (IL-1β, TNF-α, MCP-1, IL-6, and iNOS) were significantly downregulated, and the anti-inflammatory genes (IL-10, TGF-β, YM-1, Fizz1, and arginase-1) were upregulated in the heart and the blood with Lazm treatment. Finally, it was observed also that the apoptosis in the peri-infarct zone of Lazm treated mice is also significantly decreased. **Conclusion:** Lazm is a potent treatment in resolving the post-MI over exaggerated inflammatory response and early starting the reparatory phase, leading to more protection against acute ischemic injury. This is the first study demonstrates the immune modulation properties of liposomal AZM which have not been examined before and have potential wide therapeutic applications beyond the cardiovascular field, and also provide novel and clinically relevant cardioprotective therapeutics for post-AMI injury. Long-term follow-up studies are warranted to examine the therapeutic applications associated with enhanced immunomodulatory potential of Lazm.

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Kelly Jones [1] • Eva Gatineau, PhD [1] • Ming Gong, PhD [2] • Frederique Yiannikouris, PhD [1]
Pharmacology and Nutritional Sciences University of Kentucky [1] • Physiology University of Kentucky [2]

Adipose PRR deficiency Increases Blood Pressure via an AngII-dependent Mechanism in Female Mice and Through the Autonomic Nervous System in Male Mice

Graduate Student

Obesity and lipodystrophy models, two opposite ends of adipose tissues dysfunction, can lead to hypertension and constitute relevant tools to decipher mechanisms involved in blood pressure control. Since sex related difference exists in blood pressure control, we aimed to determine whether the mechanism leading to hypertension in adipocyte prorenin receptor (PRR) KO mice was similar in male and female mice.

Adipocyte PRR KO and control littermate (CTL) male (n=8 to 9 mice/group) and females mice (n=6 to 7 mice/group) were fed a high fat diet. The systolic blood pressure (SBP) was evaluated by radiotelemetry. Adipose-PRR KO significantly increased SBP in both male and female mice (Day SBP, CTL male=122.2±1.9 mmHg, KO male=128.7±2.4 mmHg, P=0.046; CTL female=120.4±0.9 mmHg, KO female=125.5±1.9 mmHg, P=0.03). Acute losartan injection, an AT_1R inhibitor, blunted elevated SBP in both CTL female and male mice. Interestingly, losartan decreased SBP to a greater extent in adipose PRR KO mice compared with control mice but only in female mice (Delta SPB, CTL male=-7.9±1.5 mmHg; KO male=-6.4±3.1 mmHg, P=0.68; CTL female=-5.3±1.9 mmHg; KO female=-14.2±0.9 mmHg, P=0.015). To assess the contribution of the autonomic nervous system, male and female mice were injected with propranolol or atropine. The tachychardic response to atropine was significantly greater in adipose-PRR KO male mice compared with control male mice (Delta Heart Rate, CTL male=+114.2±12.1 bpm; KO male=+156.0±11.1 bpm, P=0.023) but did not change in adipose-PRR KO female mice compared with control female mice (Delta Heart Rate, CTL female=+84.8±10.1 bpm; KO female=+89.8±21.3 bpm, P=0.85).

In conclusion, our data strongly suggest that the elevation of SBP in adipose PRR KO mice is mediated by an AngII-dependent mechanism in female mice and by the activation of the autonomic nervous system in male mice. These results support the importance of sex-specific approach for the development of personalized drugs in hypertension treatment.

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Ebubechi Adindu [1] • Baoxiang Yan, MS [2] • Sony Soman, PhD [2] • Courtney Hammill, MS [2] • Andrew Morris, PhD [2] • Fredrick Onono, PhD [2]
University of Kentucky [1] • Internal Medicine University of Kentucky [2]

Beneficial Effects of Phosphatidylcholine Depletion is Independent of Autotaxin Inhibition in Hyperlipidemic Mouse Models of Breast Cancer.

Undergraduate

Autotaxin (ATX) is a secreted lysophospholipase D that converts lysophosphatidylcholine (LPC) to the bioactive lysophosphatidic acid (LPA). LPA is a pleiotropic growth-factor lysophospholipid that promotes cancer cell survival, growth, migration, invasion, metastasis, and resistance to chemotherapy and radiotherapy. Inhibition of LPA signaling using ATX inhibitors decreases initial tumor growth and subsequent lung metastatic nodules in the highly aggressive and metastatic 4T1 breast cancer mouse model. We tested the efficacy of ATX inhibition in the hyperlipidemic estrogen receptor (ER)-positive E0771 breast cancer mouse model using a novel small-molecule ATX inhibitor, PAT-505 [3-((6-chloro-2-cyclopropyl-1-(1-ethyl-1H-pyrazol-4-yl)-7-fluoro-1H-indol-3-yl) thio)-2-fluorobenzoic acid sodium salt]. PAT-505 is a potent, selective, noncompetitive inhibitor that robustly reduces liver fibrosis in a Stelic Mouse Animal Model of nonalcoholic steatohepatitis (NASH) fed choline-deficient, high-fat diet. Six-week old female C57Bl6 low density lipoprotein receptor knockout mice (LDLr-/-) were fed a high fat diet (60% kcal fat) for 16 weeks. Subsequently, E0771 syngeneic mammary cells were injected into the inguinal mammary fat pads of mice fed continuously on high fat diets and treated with either PAT-505 or vehicle control. Primary tumor growth was monitored using orthogonal caliper measurements and tumor volumes estimated from width2 × length/2. The number of metastatic nodules in the lungs were counted after staining with India ink. Contrary to our expectation, treatment with PAT-505 resulted in no significant effect on primary tumor growth and an increased number of metastatic nodules compared with vehicle-treated mice. ATX produces majority of circulating LPA from LPC which is derived from the highly abundant phosphatidylcholine (PC). We measured the concentration of PC in various components of mouse diet fat sources and determined that casein is the main source of PC when present in rodent diets. Using tandem mass spectrometry we established that using "vitamin-free" test casein or replacing casein with amino acids in rodent diets significantly decreases the relative abundance of PC in these diets. In high fat diet mouse models of breast cancer we discovered that feeding mice with diets in which casein is replaced with amino acids decreases primary tumor growth. However, treatment with PAT-505 reverses the gains of casein replacement and instead leads to enhanced tumor growth and metastasis. These studies suggest that dietary casein promotes primary tumor growth in hyperlipidemic E0771 models of cancer and that targeting ATX inhibition is not efficacious in these models. Our results show that under certain hyperlipidemic conditions consumption of dietary casein can promote primary tumor growth. We also provide evidence emphasizing the need for further investigation in ascertaining the impact and utility of ATX inhibitors in breast cancer treatment.

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P. Deng[1] • M.C. Petriello[1] • J. B. Hoffman[1] • A. J. Morris[1] • B. Hennig[1]
Superfund Research Center University of Kentucky[1]

Effects of PCB126 and dietary inulin on circulating lipids and metabolites in mice

Fellow

Polychlorinated biphenyls (PCBs) are persistent environmental pollutants that can promote inflammation and atherosclerosis in preclinical models. The prebiotic inulin has antinflammatory effects and may lower circulating lipids which is a risk factor for cardiovascular disease. We used a mouse model of experimental atherosclerosis to examine effects of the most potent dioxin-like pollutant PCB 126 and dietary inulin on circulating lipids and metabolites. Low density lipoprotein receptor knockout (LDLr-/-) mice were fed with control western diet or inulin supplemented diet with or without oral gavage of PCB 126. Plasma samples were collected 2-days after PCB 126 exposure. Non-targeted metabolomic analysis using liquid chromatography-high resolution mass spectrometry identified 1871 metabolites. In control diet groups, a set of 5 metabolites were significantly decreased in PCB 126 exposed mice, which were further identified as bile acids. Interestingly, the effect of PCB126 exposure on plasma bile acids was not observed in inulin-fed mice. We also identified unique effects of PCB126 exposure and dietary inulin on the plasma lipidome. PCB 126 elicited a two-fold increase in Cer(d42:2), which was decreased by dietary inulin along with two other ceramides, Cer(d42:1) and Cer(d40:1). These results suggest short-term PCB 126 exposure lead to systemic alterations in bile acid and sphingolipid metabolism, which could be attenuated by dietary inulin intake. Further studies of these effects might identify mechanisms underlying the proinflammatory cardiovascular disease promoting actions of PCBs and the possible protective effects of dietary inulin.

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Harry Chanzu, PhD [1] • Subershan Wigna-Kumar, [2] • Smita Joshi, PhD [1] • Sidney W. Whiteheart, PhD [1]
Molecular and Cellular Biochemistry University of Kentucky [1] • Paul Laurence Dunbar High School, Lexington KY [2]

Role of the Proteoglycan, Serglycin, in Platelets α-Granule Biogenesis

Fellow

Occlusive cardiovascular diseases (e.g., strokes and myocardial infarctions, MI), a major cause of death and disabilities, are influenced by platelet secretion. Upon injury to a blood vessel, platelets activate and secrete cargo stored in membranous compartments, called granules. While secretion is needed for normal clotting, excessive release leads to occlusive thrombosis. Serglycin is an intra-granular, chondroitin sulfate proteoglycan produced by hematopoietic cells, including platelets. We hypothesize that its negative charges are crucial for the storage of platelet granule proteins. Previous studies (Woulfe, DS et *al*, 2008) showed that Serglycin knockout (KO) mice exhibit a bleeding phenotype, defective aggregation, and α-granule packaging defects. We have reexamined these animals and shown that Serglycin KO platelets have α- granule secretion defects. Consistent with previous data, there is a defect in platelet factor 4 (PF4) levels but not in the α-granule membrane protein, P-Selectin. Antibody array analysis has defined other defects in α-granule cargo packaging. Levels and release of serotonin from dense granules and β-hexosaminidase from lysosomes were unaffected suggesting that Serglycin is only important for α-granules. Ultrastructural studies, by electron microscopy, show random and disperse α-granule cargo distribution in Serglycin KO platelets, while the wild-type platelets have a fibrous granular content. In sum, these studies are illuminating the physiological function of Serglycin in platelets and defining its role in granule cargo packaging and release.

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Devi Thiagarajan, [1] • Weihua Jiang, [1] • Venkateswaran Subramanian, PhD [3]
Saha Cardiovascular Research Center University of Kentucky [1] • Department of Physiology, Saha Cardiovascular Research Center University of Kentucky [3]

Calpain-2 knockdown prevents AngII-induced cytoskeletal protein degradation and protects ECM integrity in primary aortic smooth muscle cells.

Staff

Background and Objective: Integrity of extracellular matrix (ECM) remains compromised in both abdominal aortic aneurysm (AAA) patients as well as in animal models. Defective ECM could be due to the loss of cytoskeletal proteins that bridges the contractile filaments with ECM, through proteases. Earlier, using a pharmacological inhibitor and genetic deficient mice, we identified that calpain-2 (a class of calcium-activated, intracellular cysteine proteases) plays a critical role in AngII-induced AAA formation in mice. Also, we demonstrated that calpain-2 is involved in the fragmentation of cytoskeletal proteins, Filamin A and Talin. Here, we explore the impact of calpain-2 mediated-cytoskeletal protein fragmentation in compromising the ECM integrity, *in vitro*.

Methods and Results: Primary human and mouse aortic smooth muscle cells (SMCs) were subjected to siRNA mediated silencing. Markers for cytoskeletal proteins (Filamin A and Talin1) and ECM proteins (Elastin and collagen) were studied using western blot, ELISA and immunofluorescence (IF). To examine the role of cytoskeletal structural protein in ECM protein stability, we did siRNA mediated silencing of Filamin A and Talin1 and observed reduced level of ECM proteins- Elastin and collagen. In addition, silencing of calpain-2 prevented AngII-induced fragmentation of Filamin A and Talin1 along with preserved ECM proteins compared to control cells.

Conclusion: These results indicate that calpain-2 as a potential protease involved in cytoskeletal protein degradation, thereby affecting the ECM integrity in AAAs.

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Seonwook Kim [1] • Lihua Yang [1] • Ryan Temel, PhD [2] • Richard Lee [3] • Mark Graham [3] • Sangderk Lee, PhD [2]
Saha Cardiovascular Research Center University of Kentucky [1] • Saha Cardiovascular Research Center, Department of Pharmacology and Nutritional Sciences University of Kentucky [2] • Cardiovascular Antisense Drug Discovery Group, Ionis Pharmaceuticals [3]

HB-EGF is a Novel Stimulator for the Hepatic Lipid Production

Staff

Study goal: Unbalanced hepatic lipid production is a risk factor for the development of non-alcoholic fatty liver disease (NAFLD) or hypertriglyceridemia. Previous reports showed that the circulatory heparin-binding EGF-like growth factor (HB-EGF), which is upregulated by obesity-associated oxidative stress, was correlated with circulatory lipid level and risk of coronary artery disease. The HB-EGF ASO administration induced a striking downregulation of circulatory TG and cholesterol levels in a mouse model of hyperlipidemia. Concordantly there was sufficient protection against atherosclerosis and NASH phenotypes in the model. In this study, we tested the hypothesis that the HB-EGF is a novel stimulator of lipid synthesis in the liver.

Experiment and Result: In human-derived liver cell line HepG2, the recombinant HB-EGF stimulated an increase of the lipid synthetic gene expression regulated by SREBP1 signaling and upregulation of the apoB expression in the cells. In C57BL/6 mice, the administration of recombinant HB-EGF via tail-vein injection increased the production of VLDL-TG secretion from the liver. In contrast, the administration of the HB-EGF antisense oligonucleotide (ASO) for six weeks or bolus injection of an EGFR blocker BIBX1382 induced significant suppression of hepatic VLDL production. The HB-EGF ASO administration downregulated lipid synthetic gene expressions in a mouse model.

Conclusion: The study results indicate that the HB-EGF is an autonomous stimulator for the hepatic VLDL secretion via SREBP1 in the liver tissue. Further study on the regulatory mechanism for the SREBP1 by the HB-EGF signaling and its pathological roles in the development of NAFLD or hypertriglyceridemia will be necessary.

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Congqing Wu, PhD [1] • Yinan Wei, PhD [2] • Zhenyu Li, MD, PhD [1]
Saha Cardiovascular Research Center University of Kentucky [1] • Department of Chemistry University of Kentucky [2]

Inflammasome Activation Triggers Blood Coagulation through Pyroptosis

Fellow

Disseminated intravascular coagulation (DIC) is a fatal complication of systemic bacterial infection. Blood coagulation induced by bacterial infection is often attributed to host inflammatory response to bacterial virulence factors, yet mechanisms for initiation of the coagulation cascade have not been fully elucidated. Here we identify inflammasome activation as a trigger for coagulation induced by Gram-negative bacterial products. Specifically, canonical inflammasome activation elicited by the conserved type III secretion system (T3SS) rod protein EprJ from E. coli induces systemic coagulation through caspase-1 activation, whereas noncanonical inflammasome activation by lipopolysaccharide (LPS), a major outer membrane component of Gram-negative bacteria, produces similar effects via caspase-11. Cleavage of gasdermin D (Gsdmd) by caspase-1 or caspase-11 drives macrophage pyroptosis, leading to the release of tissue factor (TF), an essential initiator of coagulation cascades. Deficiency of Gsdmd or inhibition of TF abolishes inflammasome-mediated coagulation. Our data reveal a novel mechanism of DIC in sepsis and demonstrate inflammasome connecting inflammation with thrombosis.

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Preetha Shridas, PhD [1] • Maria C de Beer, PhD [2] • Nancy R Webb, PhD [3]
Internal Medicine University of Kentucky [1] • Physiology University of Kentucky [2] • Pharmacology and Nutritional Sciences University of Kentucky [3]

High-Density Lipoprotein Inhibits Serum Amyloid A–Mediated Reactive Oxygen Species Generation and NLRP3 Inflammasome Activation

Objectives: Interleukin-1beta (IL-1β) has been implicated in inflammatory diseases, including atherosclerosis and abdominal aortic aneurysm (AAA). Production of bioactive IL-1β is controlled by the inflammasome, a multi-protein complex that regulates caspase-1 activity. Serum Amyloid A (SAA) is an acute-phase protein whose levels in circulation is elevated in individuals with chronic inflammation. We previously reported that deficiency of SAA protects mice from angiotensin II (AngII)-induced AAA. Here we report that reduced AngII-induced AAA in SAA-deficient mice is accompanied by significant reductions in plasma IL-1β, indicating that SAA is required for inflammasome activation in AngII-infused mice. The objective of this study is to investigate mechanisms involved in SAA-mediated inflammasome activation.

Methods/Results: SAA dose-dependently induced both caspase-1 activation and IL-1β secretion in J774 macrophage-like cells incubated with 0-25 mg/ml purified mouse SAA. The ability of SAA to induce IL-1β secretion was significantly reduced in bone marrow-derived macrophages deficient in NLRP3. A caspase-1inhibitor, Z-YVAD-FMK, significantly suppressed IL-1β secretion induced by SAA, whereas the P2X7-receptor antagonist, AA38079, had no effect. Inhibition of reactive oxygen species (ROS), cathepsin-B activation, and cellular potassium efflux by N-acetyl-L-cysteine, CA-074, and glyburide, respectively, blocked NLRP3 inflammasome activation by SAA. Pre-incubating SAA with HDL prior to cell treatments completely abrogated SAA-mediated inflammasome activation. In contrast, HDL did not alter inflammasome activation triggered by ATP.

Conclusions: SAA-mediated NLRP3 inflammasome activation in macrophages is dependent on ROS generation, release of cathepsin-B, and potassium efflux, and is independent of the P2X7 receptor. Moreover, our data identify a novel mechanism by which HDL may exert cardioprotective effects.

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Oluwabukola Omotola, [1] • Sandra Legan, PhD [2] • Julie Pendergast, PhD [1]
Biology University of Kentucky [1] • Physiology University of Kentucky [2]

Estrogen Regulates Daily Metabolic Rhythms in Female Mice

Graduate Student

Introduction: After menopause, women are susceptible to obesity comorbidities such as metabolic syndrome and cardiovascular disease, suggesting that estrogen regulates these processes. Shift work, which disrupts circadian rhythms, also increases the risk of obesity and cardiovascular disease in women.

Objective: In this study, we sought to examine the role of estrogen in regulating daily rhythms associated with the development of obesity. High-fat diet feeding in male mice, which causes diet-induced obesity, disrupts eating rhythms and the molecular timekeeping rhythm in the liver. Since previous studies demonstrated that female mice are resistant to diet-induced obesity, we aimed to determine whether daily metabolic rhythms were also protected from the effects of high-fat feeding in females.
Methods: Female C57BL/6J PERIOD2::LUCIFERASE mice (6 weeks old) were ovariectomized and implanted subcutaneously with either a physiological dose of 17β-estradiol (E2) or vehicle (sesame oil) in silastic tubing. At 7 weeks old, females were single-housed in cages in light-tight boxes in 12L:12D and fed low-fat diet (10% kcal fat) for 1 week and then high-fat diet (45% Kcal fat) for 1 week. Eating behavior and locomotor activity rhythms were continuously measured with infrared video cameras and sensors, repsectively. The timing, or phases, of molecular timekeeping rhythms were measured in explanted central and peripheral tissues at the end of the experiment.
Results and Discussion: Consistent with previous studies, we found that ovariectomized females became obese, while estrogen replacement in ovariectomized mice inhibited high-fat diet-induced weight gain and adiposity. Ovariectomy also reduced the amplitudes of the eating behavior rhythms in female mice, such that they resembled those of male mice consuming high-fat diet. In contrast to ovariectomized females and males, whose daily rhythms of eating behavior were abrogated during high-fat feeding, estrogen-treated ovariectomized females had high-amplitude eating behavior rhythms on high-fat diet. Estrogen protects the daily locomotor activity rhythm during high-fat feeding in female mice while ovariectomized females in contrast had low activity even on low-fat diet. In addition, the phase of the liver molecular circadian rhythm was advanced (by 3.5h) in oil-treated ovariectomized mice, but not in estrogen-treated ovariectomized mice. Molecular rhythms in explanted suprachiasmatic nuclei, pituitary, lung, kidney, aorta, spleen, and white adipose tissue did not differ between oil- and estrogen-treated ovariectomized mice.
Conclusion: Together our data show that estrogen protects the integrity of daily metabolic rhythms in female mice during high-fat feeding.

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Mohamed El-Helw, MD [1] • Lakshman Chelvarajan, PhD [2] • Greg Milburn [3] • Autumn L. Conge [3] • Kenneth Campbell, PhD [3] • Mariusz Z. Ratajczak, MD, PhD [4] • Ahmed Abdel-Latif, MD, PhD [5]
Gill Heart Institute University of Kentucky [1] • Saha Cardiovascular Research Center University of Kentucky [2] • Department of Physiology University of Kentucky [3]• Stem Cell Institute at James Graham Brown Cancer Center University of Louisville [4] • Gill Heart Institute and Division of Cardiovascular Medicine University of Kentucky [5]

Fellow

Identification of Very Small Embryonic Like Stem Cells (VSELS) in Human Heart Tissue Among Young and Old Individuals

Abstract:

Very small embryonic-like stem cells (VSELs) are promising cellular substrate for regenerative therapies such as heart disease. However, their presence in cardiac tissue has not been described before. The purpose of our study was to determine whether human heart tissue contains VSELs and whether their prevalence changes with age.

Methods:

We examined the frequency of human VSELs, defined as CD133+/SSEA4+/CD45- in the epicardium and endocardium of human heart tissue obtained from the Gill Heart and Vascular Institute-Cardiovascular Biorepository. Our sample included 15 subjects (age ranging from 9 to 76 years). Subjects were categorized into young (less than 39 years), middle-age (40-59 years), and old (≥ 60 years). VSELs were quantified as number of cells per high power field (HPF) in at least 20 consecutive fields for both epicardium and endocardium sections in each subject

Results:

Our careful histological evaluation identified small number of VSELs in cardiac tissue in all subjects examined. VSELs were more prevalent in epicardial compared to endocardial tissue. Interestingly, and in agreement with prior reports regarding VSELs in other tissues, the prevalence of VSELs was dramatically reduced with age reaching a nadir in subjects greater than 40 years (5.9 ± 0.4 cells/HPF at age of 9 years down to 0.2 ± 0.07 cells/HPF at the age of 76 years).

Conclusions:

This is the first report documenting the existence of human cardiac VSELs. Our data suggest significant reduction of cardiac VSELs with age suggesting potential depletion of their pool in adults and older individuals. Future studies examining the significance and regenerative potential of cardiac VSELs are underway.

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Michael Petriello, PhD [1] • Richard Charnigo, PhD [2] • Marian Pavuk, PhD [3] • Linda Birnbaum, PhD [4] • Bernhard Hennig, PhD [5] • Andrew Morris, PhD [1]
Cardiovascular Medicine University of Kentucky [1] • Biostatistics University of Kentucky [2] • CDC Agency for Toxic Substances and Disease Registry [3] • NCI at NIEHS [4] • Animal and Food Sciences University of Kentucky [5]

Serum Levels of Dioxin-Like Pollutants Are Positively Associated with the Cardiometabolic Disease Risk Biomarker TMAO in Leaner Individuals

Staff

Cardiovascular disorders are largely caused by genetic and environmental factors. Well-studied lifestyle dependent determinants of increased CVD risk include smoking, physical inactivity, and poor nutrition, but emerging data now implicate exposures to environmental pollutants as an important contributor to inter-individual variability in CVD risk. It is also critical to identify biomarkers of toxicant exposure and cardiometabolic disease. Emerging biomarkers such as trimethylamine-N-oxide (TMAO) have been associated with increased risk of coronary artery disease and diabetes. Recently, we published that in preclinical models, exposure to dioxin-like PCBs can increase circulating levels of TMAO. In our preclinical studies, dioxin-like PCBs strongly increase the enzyme responsible for TMAO production, FMO3, resulting in amplified increases in TMAO. We have now begun to investigate if these associations between pollutant exposure and TMAO are evident in the highly exposed Anniston, Alabama population. We have used mass spectrometry to quantitate TMAO in plasma of the Anniston, Al cohort, and determined that higher body burden of pollutants is significantly associated with increased circulating TMAO. These associations are evident only in women and diminish as BMI increases. To study mechanisms we have characterized a mouse model that develops atherosclerosis but not adipose tissue expansion. We examined the effects of PCB 126 on markers of systemic inflammation and atherosclerotic lesion size. Exposed mice exhibited significantly increased plasma cytokine levels and accelerated atherosclerotic lesion formation. More works need to be completed to determine the role of TMAO and FMO3 in these processes (Supported in part by NIEHS/NIH grants P30ES026529 and P42ES007380).

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Eva Gatineau, PhD [1] • Ryan Temel, PhD [2] • Gregory Graf, PhD [3] • Frederique Yiannikouris, PhD [2]
Pharmacology and Nutritional Sciences University of Kentucky [1] • Pharmacology and Nutritional Sciences University of Kentucky [2] • Pharmaceutical Sciences University of Kentucky [3]

Elevated Soluble Form of Prorenin Receptor Contributes to Lipid Synthesis in Liver and Adipose Tissue of Mice

Fellow

Non-alcoholic fatty liver disease is strongly associated with obesity and metabolic syndrome. Our laboratory previously demonstrated that adipose prorenin receptor (PRR) KO mice have no adipose tissue and elevated hepatic triglycerides and cholesterol contents. The deletion of PRR in adipose induced a counter regulatory increase of hepatic PRR mRNA abundance and increased plasma soluble PRR (sPRR).

To determine whether hepatic PRR regulated endogenous hepatic lipid synthesis, PRR floxed male mice fed a standard diet were injected with saline or a single dose of an adeno-associated virus thyroxine-binding globulin-Cre (CTL, N=6; KO, N=5). The deletion of PRR in liver led to a significant decrease of hepatic TG levels (CTL, 615 ± 196 mg/g prot; KO, 114 ± 18 mg/mg prot, $P<0.05$) and PPARgamma gene expression in liver. However, plasma total cholesterol levels were significantly higher in KO mice compared with CTL mice (CTL, 143 ± 10 mg/dL; KO, 383 ± 14g mg/dL, $P<0.05$). Hepatic total cholesterol levels increased in KO mice compared with CTL mice (CTL, 40 ± 3 mg/mg prot; KO, 102 ± 6 mg/mg prot, $P<0.05$). This elevation was associated with an increase in SREBP-2 and HMGCoA-reductase gene expression. Similar to adipose PRR KO, liver PRR KO induced a counter regulatory increase of plasma sPRR levels (CTL, 5942 ± 199 pg/ml; KO, 8903 ± 559 pg/ml, $P<0.05$) and elevated total adipose sPRR levels (CTL, 36.2 ± 8.7 pg/ml; KO, 92.7 ± 22.3 pg/ml, $P<0.05$).

To determine whether circulating sPRR regulated endogenous hepatic and adipose lipid synthesis, C57BL/6J mice (n=7 to 8 mice/group) were infused with saline or sPRR. In addition, PRR was silenced in pre-adipocytes or in HepG2 cells and the cells were treated with sPRR.

Results showed that silencing of PRR in HepG2 cells did not change SREBP-2 gene expression but sPRR treatment significantly up-regulated SREPB2 mRNA expression in control HepG2 cells (Veh, 1.00 ± 0.04; sPRR, 1.36 ± 0.08, $P<0.05$). Similarly, sPRR infusion increased SREPB2 mRNA expression in the liver of mice (Veh, 1.01 ± 0.07; sPRR, 1.23 ± 0.07, $P<0.05$). The silencing of PRR in primary adipocyte decreased PPAR-gamma gene expression. The treatment with sPRR increased PPARgamma gene expression in control cells but does not rescue PPARgamma gene expression in KO cells.

Together, our results demonstrated that the lack of PRR in the liver induced a counter regulatory increase of plasma sPRR originate from the adipose tissue. sPRR could participate to cholesterol synthesis via the stimulation of SREBP-2 pathway independently of the presence of the full length PRR whereas the stimulation of PPARgamma pathway via sPRR is PRR-dependent. Futures studies will investigate the mechanism by which PRR and sPRR regulate SREBP-2 and PPARgamma.

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Jeffrey Chalfant, MS [1] • Deborah Howatt [2] • Julie Pendergast, PhD [1]
Biology University of Kentucky [1] • Saha Cardiovascular Research Center University of Kentucky [2]

Constant light exposure increases atherosclerosis in ApolipoproteinE-deficient mice

Graduate Student

Circadian rhythms are 24-hour oscillations of almost every biological process in the body. The circadian system coordinates these rhythms of physiology and behavior with environmental cycles. Disruption of circadian rhythms increases the risk for cardiovascular disease (CVD). However, the mechanisms linking circadian disruption and CVD are largely unknown. In this study, we investigated the effects of constant light exposure, which chronically disrupts circadian rhythms, on atherosclerosis. We studied C57BL/6J *ApolipoproteinE*-deficient *(ApoE*$^{-/-}$) mice because they spontaneously develop atherosclerotic lesions. At 7 weeks old, male *ApoE*$^{-/-}$ mice were singly housed in light-tight boxes in 12L:12D and fed low-fat diet. Locomotor activity and eating behavior rhythms were continuously monitored using passive infrared sensors and infrared video cameras, respectively. At 8 weeks old, mice were either kept in control 12L:12D or housed in constant light for 12 weeks. At 20 weeks old, atherosclerotic lesion area and serum lipids were measured. *ApoE*$^{-/-}$ mice housed in constant light had locomotor activity and eating behavior rhythms that were arrhythmic or the rhythms were severely disrupted. Chronic exposure to constant light also increased atherosclerosis in male *ApoE*$^{-/-}$ mice compared to those in 12L:12D. Total serum cholesterol did not differ between mice in 12L:12D and constant light. However, the cholesterol lipoprotein profile analyzed by FPLC showed that VLDL/LDL was increased in male *ApoE*$^{-/-}$ mice in constant light compared to 12L:12D. Together, these data demonstrate that chronic circadian disruption with constant light exposure increases atherosclerosis and disrupts the distribution of lipoproteins in male *ApoE*$^{-/-}$ mice.

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Megan Jay [1] • Shannon Jones [1] • Hannah Russell [1] • Adrien Mann [1] • Nathan Robbins [1] • Deborah Howatt [2] • Alan Daugherty, PhD, DSc [2] • A. Phillip Owens III, PhD [1]
Internal Medicine University of Cincinnati [1] • Saha Cardiovascular Research Center University of Kentucky [2]

Thrombin Activation of Platelet Protease-activated Receptor 4 Augments Atherosclerosis

Graduate Student

Objective: Platelet activation has been shown to play a critical role in both formation and propagation of atherosclerosis. Protease-activated receptors (PARs) 1 and 4 mediate signaling by the coagulation protease thrombin. While PAR1 and 4 mediate thrombin activation on human platelets, mouse platelets contain PAR4 with PAR3 acting as a cofactor. Recent studies have demonstrated direct thrombin inhibitors (DTIs) reduced atherosclerosis in hypercholesterolemic mice. In this study, we examined the effect of PAR4 deficiency on the development of atherosclerosis.

Methods and Results: Male low-density lipoprotein receptor deficient (Ldlr-/-) mice (8-12 weeks old) that were on a Par4+/+ or Par4-/- background (n = 5 – 20 each group) were fed a fat and cholesterol-enriched diet for 12 weeks to induce hypercholesterolemia and chronic atherosclerosis. PAR4 deficiency attenuated aortic sinus (65% decrease; P = 0.001) and aortic arch (71% decrease; P = 0.001) atherosclerosis with no effects on total plasma cholesterol concentrations or lipoprotein distributions. Macrophage (CD68) and platelet (platelet factor 4) accumulation was also attenuated with PAR4 deficiency (P < 0.05). Bone marrow transplantations were utilized to examine non-marrow or marrow-derived effects (n = 15 for each of 4 chimeric groups). The reductions in atherosclerosis, macrophage accumulation, and platelet infiltration were attributable to hematopoietic-derived PAR4 (P < 0.05). To determine if PAR4 mediated all effects of thrombin signaling, Ldlr-/-/Par4+/+ or Ldlr-/-/Par4-/- mice were fed a fat and cholesterol-enriched diet, supplemented with placebo or the DTI dabigatran etexilate (30 g/kg diet) for 12 weeks. Dabigatran etexilate administration reduced atherosclerosis in PAR4+/+ mice (P < 0.05) but not in PAR4-/- mice (P = 0.75).

Conclusion: We demonstrated whole body and hematopoietic PAR4 deficiency resulted in significantly attenuated aortic sinus and root atherosclerosis, as well as decreased macrophage and platelet accumulation. Thrombin inhibition did not alter atherosclerosis in PAR4 deficient mice. Together, these results suggest that thrombin amplification of atherosclerosis in mice is likely due to activation of PAR4 on hematopoietic cells.

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Kelsey Conrad, MS [1] • Shannon Jones [1] • Robert Helsley, PhD [2] • Rebecca Schugar, PhD [2] • Zeneng Wang, PhD [2] • Stanley Hazen, MD, PhD [2] • J. Mark Brown, PhD [2] • A. Phillip Owens III, PhD [1]

Department of Internal Medicine University of Cincinnati [1] • Department of Cellular and Molecular Medicine Cleveland Clinic [2]

Gut Microbiota and Circulating Trimethylamine N-oxide (TMAO) Are Associated with Aortic Aneurysm Formation

Graduate Student

Background: The gut microbiota is a metabolically active endocrine organ critical to the maintenance of cardiovascular health. Dietary sources of choline are metabolized by microbial enzymes to form trimethylamine (TMA) and liver-specific flavin-containing monooxygenase 3 (FMO3) converts TMA to the pro-inflammatory molecule trimethylamine N-oxide (TMAO). Clinical trials have correlated high levels of circulating TMAO to an increased risk of cardiometabolic diseases. However, this meta-organismal pathway has not been evaluated in the context of abdominal aortic aneurysm (AAA). The objective of this study was to investigate plasma TMAO levels in AAA patients and determine the effects of a high choline diet with and without antibiotics on the development of AAA in a mouse model.
Methods: Plasma samples from humans with fast growing (n=85) or slow growing AAAs (n=84), and normal (non-aneurysmal) aortas (n=115) were analyzed for plasma TMAO via liquid chromatography tandem mass spectrometry. *C57BL/6J* male (n=20) and female (n=20) mice were fed either a defined chow diet (n=10 each sex) or a choline-rich diet (1.2%; n=10 each sex) for 5 weeks. After 1 week of diet, basal abdominal ultrasounds were performed and angiotensin II (AngII; 1,000ng/kg/min) was infused for 28 days via osmotic minipumps. Termination ultrasounds were performed on day 27 and mice were sacrificed on day 28. Aortas were evaluated for aneurysm incidence and plasma was analyzed for the metabolites TMA, TMAO, and choline. To investigate the role of the gut microbiota in the initiation of AAA, vehicle water (n=20) or water containing broad-spectrum antibiotics (n=20) were provided to male *Ldlr*$^{-/-}$ mice (6 weeks). Following 1 week of water treatment, mice were fed either a defined chow diet (n=10 each water treatment) or a choline-rich diet (n=10 each water treatment). After one week of feeding, AngII was infused for 4 weeks. Mice were then sacrificed on day 28.
Results: Circulating plasma TMAO was significantly elevated in a step-wise fashion with the rate of aneurysm growth versus non-aneurysmal control patients (fast growing>slow growing>normal patients; $P<0.001$). Administration of a choline-rich diet augmented AAA incidence ($P<0.02$) and aortic diameter ($P<0.001$), but not ascending aortic area in both male and female *C57BL/6J* mice versus placebo-fed mice. Plasma levels of TMA, TMAO, and choline were significantly elevated in choline-fed mice versus normal chow ($P<0.05$). Finally, administration of broad-spectrum antibiotics to eliminate the gut microbiota significantly decreased aortic diameter ($P<0.005$), AAA incidence ($P<0.05$), and ascending aortic area ($P<0.01$) in choline-fed Ldlr$^{-/-}$ mice versus control.

Conclusions: Our results indicate increases in circulating TMAO augments the growth status of aneurysms in human patients as well as the incidence of AAA in C57BL/6J mice and the incidence of aortic arch aneurysm and AAA in *Ldlr*$^{-/-}$ mice in a gut microbiota-dependent manner.

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Dibyajyoti Biswal, [1] • Abhijit Patwardhan, PhD [1]
Biomedical Engineering University of Kentucky [1]

Development of Visual Feedback Tool for Control of Breathing to Investigate Role of Respiration in Autonomic Responses during Listening to Music

Graduate Student

To further investigate the role of respiration in autonomic responses during listening to music, control of respiratory pattern would be useful by eliminating the confound of changes in the pattern. Towards this goal, we implemented a visual feedback based approach using LabVIEW® for ease of voluntary control of breathing. The developed tool was tested using three cases, quiet breathing (eupnea, trial1), simulated hypo and hyperpnea (trial2) and during listening to song (trial3). Quality of control of respiration during the cases was assessed by normalized root mean square error (NRMSE®) and correlation coefficient (R) between 'target' and 'control' signals in both time and frequency domains (auto-spectra). During experiment the test subject sat in a comfortable chair and was asked to minimize movement. An Inductotrace® was used to obtain abdominal and chest respiratory signals that were digitized at a rate of 1000 samples/second simultaneously using a commercial system (Windaq) and the developed program in LabVIEW. Short segments of respiration (2 minutes each for eupnea and simulated hypo and hyperpnea) and approximately 4 minutes during listening to song were recorded to test for ease of use and to assess the quality of control that could be achieved. During first part of these trials, the subject breathed normally or simulated hypo and hyperpnea or listened to a song (using circumaural headphones) while keeping eyes closed. The sum of abdominal and chest signals was recorded and used as the 'target' signal for next part of that trial. During second part 'target' signal was displayed from right to left as a solid line while the breathing (sum of the abdominal and chest signals) was also displayed scrolling from left to right. The subject was instructed to breathe such that the two signals overlaid each other. To estimate the quality of control, the data recorded by the commercial system were used. During analysis, the target and the control signals were aligned using cross-correlation. From the aligned signals R and NRMSE were calculated. From the aligned target and control, auto-spectra were calculated using Welch's method with 100 second window length, Hanning window and 50% overlap. R values for trial1, trial2 and trial3 were 88.23%, 93.96% and 92.51% respectively. NRMSE in time domain for these were 58.85, 27.27 and 63.16 units. Similarly, R value for the trials in frequency domain were 99.90%, 94.36% and 99.71% respectively. NRMSE in frequency domain were 0.25, 0.39 and 0.75 respectively. The subject reported relative ease in following the target pattern except mentioned that on occasion the end expiratory volume seemed to be off. Further refinement in baseline correction is likely to alleviate this concern. High R values and low NRMSE in both domains during all trials shows that the subject was able to follow the pattern closely using the developed tool.

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Aida Javidan, MS [1] • Weihua Jiang, MS [1] • Lihua Yang, MS [1] • Venkateswaran Subramanian, PhD [2]
Saha Cardiovascular Research Center University of Kentucky [1] • Physiology University of Kentucky [2]

Enhanced Autophagy by Celastrol Supplementation Profoundly Increases AngII-induced Abdominal Aortic Aneurysm Formation in Male and Female Mice.

Graduate Student

Background and Objective: Abdominal Aortic Aneurysms (AAAs) are permanent dilations of the abdominal aorta with greater than 80% mortality after rupture. Currently, there are no proven non-surgical therapeutics to blunt AAA progression and rupture. In addition, the prevalence of AAAs is 4–5 times greater in males than females. Recent findings demonstrated that autophagy–related genes are markedly upregulated in human AAAs. Autophagy refers to the cellular self-eating degradation process, which regulates cellular homeostasis by recycling damaged organelles and long-lived proteins. However, its functional role in AAA development is still unclear. The purpose of this study is to test the effect of Celastrol supplementation as an autophagy enhancer on AngII-induced AAAs in mice.

Methods and Results: Male and female LDL receptor -/- mice (8-10 weeks old; n= 10 per group) were fed a fat-enriched diet (21% wt/wt milk fat; 0.15% wt/wt cholesterol) supplemented with or without Celastrol (10mg/kg/day) for 5 weeks. After 1 week of high fat diet feeding mice were infused with AngII (male - 500 or female - 1000 ng/kg/min) for 28 days by osmotic minipumps. Western blot analyses showed a significant increase in autophagy protein (LC3-II) with Celastrol supplementation. Celastrol supplementation significantly reduced body weight and had no effect on AngII-induced systolic blood pressure in both male and female mice. Celastrol supplementation profoundly increased AngII-induced aortic luminal dilation in both male (Control = 1.25 ± 0.05 versus Celastrol = 1.55 ± 0.09 mm, $P<0.05$) and female (Control = 1.05 ± 0.04 versus Celastrol = 1.64 ± 0.13 mm, $P<0.05$). Ex vivo measurement of maximal diameter of abdominal aortas revealed that Celastrol supplementation significantly increased AAA formation in both male (Control = 1.01 ± 0.09 versus Celastrol = 1.62 ± 0.14 mm, $P<0.05$) and female (Control = 0.90 ± 0.03 versus Celastrol = 1.74 ± 0.25 mm, $P<0.05$) mice. Celastrol supplementation also significantly increased aortic arch area (Control = 18.15 ± 1.12 mm^2 versus Celastrol = 24.90 ± 0.91 mm^2, $P<0.05$) in male mice.

Conclusion: These findings suggest that enhanced autophagy by Celastrol supplementation profoundly increases AAA in both male and female mice.

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Michael Franklin, MS [1] • Deborah A. Howatt [1] • Jessica J. Moorleghen [1] • Hong Lu, MD, PhD [1] • Alan Daugherty, PhD, DSc [1]
Saha Cardiovascular Research Center University of Kentucky [1]

Pharmacological Inhibition of Lysyl Oxidase Induces Aortic Dissection in Juvenile but not Mature Mice

Staff

Background and Objective: Aortic dissection is a devastating cardiovascular disease associated with high mortality. The pathological mechanisms of this disease have not been defined. Lysyl oxidase (LOX) is the enzyme that initiates the irreversible covalent cross-linking of collagen and elastin in vascular tissues. In this study, we compared effects of inhibiting LOX on aortic dissection between juvenile and mature mice for both males and females.

Methods and Results: In an initial study, 4 week old male and female C57BL/6 mice were administered β-aminopropionitrile monofumarate (BAPN; ~1g/kg/d in drinking water), a LOX inhibitor, or control (drinking water alone) for 4 weeks. In mice administered BAPN, 4 male and 1 female mice died of aortic rupture. The intimal areas of ascending aortas and whole arches in male mice administered BAPN were significantly larger than controls, but there was no significant difference between measurements of female mice administered BAPN compared to controls. To examine if LOX inhibition induces aortic pathologies associated with aortic dissection in mature mice, 25 week old male and female C57BL/6 mice were administered either drinking water alone or BAPN (~1 g/kg/d in drinking water). No mice died of aortic rupture in these mature mice. We also found no significant differences in intimal area measurements between BAPN and control groups.

Conclusion: These results demonstrate the advancement of BAPN induced aortic pathologies associated with aortic dissection in juvenile, but not mature mice. It will need to be confirmed whether LOX inhibition-induced aortic dissection has sexual dimorphism.

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Hannah Russell [1] • Keith Saum, PhD [1] • Alexandra C Sundermann [2] • Shannon M Jones [1] • Anders Wanhainen, PhD [3] • Todd L Edwards, PhD [4] • Lori A Holle [5] • Alisa S Wolberg, PhD [5] • A. Phillip Owens, III, PhD [1]
Department of Internal Medicine University of Cincinnati [1] • Vanderbilt University [2] • Uppsala University [3] • Vanderbilt University [4] • University of North Carolina at Chapel Hill [5]

Fibrinogen Depletion Attenuates Angiotensin II-induced Abdominal Aortic Aneurysm

Graduate Student

Background: Fibrinogen and fibrin provide physical and biochemical support to a developing clot and is defined as one of the most crucial independent risk factors for cardiovascular diseases (CVDs). In addition to clot formation, fibrinogen promotes wound healing and powerful inflammatory and immune responses by engagement of leukocytes. Increased circulating fibrinogen and fibrin degradation products are correlated with increased diameter and progression of abdominal aortic aneurysm (AAA). However, a causal link between fibrinogen and AAA has not yet been established. The objective of this study was to determine the role of fibrinogen depletion in a mouse model of AAA.
Methods and results: To determine whether aneurysm resulted in a procoagulant environment, we examined plasma levels of thrombin generation by calibrated automated thrombography (CAT), thrombin anti-thrombin (TAT), and fibrinogen in control and AAA plasma from mice and humans. Patients and mice with AAA had significant elevations in thrombin generation, TAT, and fibrinogen versus saline controls (mice) and control patients (human). To determine the effect of fibrinogen, in vivo, low density lipoprotein receptor deficient (*Ldlr*$^{-/-}$) mice were injected with scrambled anti-sense oligonucleotide (ASO) or β-fibrinogen ASO (30 mg/kg) 3 weeks prior to experimentation and throughout the study. Fibrinogen ASO treatment achieved > 90% depletion of fibrinogen. After 3 weeks, mice were fed a fat and cholesterol enriched diet (42% milk fat; 0.2% cholesterol) 1 week prior to and throughout infusion with angiotensin II (AngII; 1,000 ng/kg/day) for 28 days. Fibrinogen ASO attenuated abdominal diameter (33% decrease; $P = 0.001$), and inflammatory cytokines (>75% decreased IL-1 and IL-6; $P = 0.001$) versus scrambled ASO control. Further, fibrinogen depletion significantly attenuated aneurysm incidence and rupture-induced death ($P < 0.05$). Fibrinogen is known to promote inflammatory response by activating and recruiting leukocytes. To investigate the contribution of this effect in abdominal aortic aneurysm, Fib$\gamma^{390\text{-}396A}$ mice carrying a mutant form of fibrinogen incapable of binding leukocyte αMβ2-integrin were fed a fat- and cholesterol-enriched diet (42% milk fat; 0.2% cholesterol) 1 week prior to and throughout infusion with angiotensin II (AngII; 1,000 ng/kg/day) for 28 days. Mutant mice showed attenuated aortic size and aneurysm incidence ($P = 0.036$) compared to controls.
Conclusions: Our results demonstrate that AAAs augment procoagulant markers in both humans and mice. Importantly, fibrinogen depletion attenuates AAA incidence, diameter, rupture-induced death, and inflammation, and impairing the inflammatory capabilities of fibrinogen reduces aortic size and aneurysm incidence. Therefore, reduction of plasma fibrinogen may be a novel treatment strategy in patients with AAA.

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Brooke Ahern [1] • Bryana Levitan [2] • Mihir Shah [1] • Andrea Sebastian [1] • Douglas Andres, PhD [3] • Jonathan Satin, PhD [1]
Physiology University of Kentucky [1] • Physiology, Gill Heart and Vascular Institute University of Kentucky [2] • Molecular and Cellular Biochemistry University of Kentucky [3]

Cardiac Specific Rad Ablation Enhances Cardiac Function

Graduate Student

Objective: The aim of this study is to test the hypothesis that cardiac specific Rad ablation (cRadKO) provides positive inotropic support, and thus provide protection against systolic heart failure. The underlying mechanism includes chronic PKA-like modulation of L-type calcium channel current ($I_{Ca,L}$). Given that chronic elevated $Ca^{2+}$is associated with pathological remodeling it is also essential to evaluate myocardial structure and function with chronic Rad-deletion.

Methods: We engineereda cardiac-restricted inducible Rad knockout mouse (cRadKO). We examined L-type calcium current ($I_{Ca,L}$) using the whole cell configuration of the patch clamp technique. We assessed global and local cellular calcium handling using Fura-2 AM and Fluo-4 AM, respectively. Cardiac structure and function was assessed *in vivo*with a longitudinal echocardiography study. Thoracic aortic constriction (TAC) to simulate pressure overload was performed with induction of knock out occurring before and after surgery. I_{CaL}, cellular calcium handling, and cardiac structure and function were then analyzed.

Results: cRadKO displayed an increase in$I_{Ca,L}$compared to wild-type (WT) (maximal conductance: cRadKO = 254 ± 19 pS/pF, n=15; WT= 144 ± 12 pS/pF, n=18; p<0.0001). $I_{Ca,L}$activated at more negative voltages (activation midpoint: cRadKO = -18.3 ± 1.0 mV, n=15; WT=-8.1 ± 1.9 mV, n=18; $p<10^{-4}$). $I_{Ca,L}$kinetics are accelerated in cRadKO concordant with the upstroke velocity of Ca^{2+}(transient velocity 48.7 ± 2.4 AU/s, n=49; WT=38.1 ± 3.5 AU/s, n=37; p=.014). Sarcoplasmic reticulum Ca^{2+}load was not different between WT and cRadKO. *In vivo*, cRadKO increased ejection fraction 7d after injection of tamoxifen (cRadKO = 76 ±2%, n = 16; WT = 59 ±4%, n=7; p=.001). Improved function was stable for 15 months (cRadKO = 72 ±3%, n=14; WT = 59 ±3%, n=6; p<.01). TAC caused reduced EF and chamber dilatation in WT; cRadKO induced either 2wk prior to TAC or at the time of TAC surgery maintained significantly elevated ejection fraction without a change in heart dimensions.

Conclusion: Rad ablation provides safe, stable positive inotropic support through a L-type calcium channel-specific mechanism mimicking PKA-modulation of $I_{Ca,L}$. This increased trigger Ca^{2+}drove increased cellular calcium dynamics culminating in improved systolic function. This enhancement provides protection from cardiac hypertrophy and rescue from cardiac pressure overload.

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Dylan Colli, [1] • Ryan Blood, [1] • Aparna Sankarankutty, [2] • Frank Sachse, PhD [2] • Michael Frisk, PhD [3] • William Louch, PhD [3] • Peter Kekenes-Huskey, PhD [1]
Chemistry University of Kentucky [1] • Bioengineering Nora Eccles Harrison Cardiovascular Research and Training Institute; University of Utah [2] • Medicine University of Oslo [3]

A Matched-filter Based Algorithm for Subcellular Classification of T-system in Cardiac Tissues

Undergraduate

In ventricular cardiomyocytes of various mammals, transverse tubules (TTs) are found in a dense, regular pattern that aligns with sarcomeres and Z-lines. In many cardiac disease etiologies, the system of TTs (T-system) is perturbed, which is believed to promote spatially heterogeneous, dyssynchronous Ca2+ release and inefficient contraction. Confocal microscopy has become the de facto standard for characterization of T-system. Several algorithms for detecting and classifying these subcellular structures have emerged. In general, current algorithms were commonly built for the analyses of isolated cells and do not detect subcellular heterogeneity of the T-system. Here we present a matched-filter based algorithm to characterize T-system at the subcellular level in isolated cardiomyocytes and millimeter-scale myocardial sections. The algorithm utilizes “filters” representative of intact TTs, longitudinal tubules, and T-system absence. Application of the algorithm to cardiomyocytes isolated from sham, myocardial infarction (MI), and thoracic aortic banding (TAB) rat models confirmed and quantified locally-heterogeneous TT structure and structural remodeling. We find significant ($p<0.05$, Welch’s t-test) increases in longitudinal tubule density within isolated myocytes intermediate (10±2%, all data reported as mean ± SD, n=3) and proximal to infarct (12±3%, n=3) vs. sham (4±2%, n=5). The algorithm also detected decreases in tubule striations within 5° of myocyte minor axis for isolated TAB (36±9%, n=3) and MI cardiomyocytes located intermediate (37±4%, n=3) and proximal (34±4%, n=3) to infarct vs. sham myocytes (57±12%, n=5). Application of algorithm bootstrapping to rabbit MI tissue revealed distal sections comprised 26.8±1.9% TTs while proximal sections comprised 14.5±1.0% TTs, a 45.9% decrease. The matched filter approach introduced here therefore provides a robust and scalable computational technique for characterizing T-system morphology from isolated cells through millimeter scale cardiac tissue preparations.

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M Abdul Mottaleb, PhD [2] • Michael Petriello, PhD [1] • Maria Kraemer, PhD [2] • Gia Mudd-Martin, PhD [3] • Debra Moser, PhD [3] • Andrew Morris, PhD [1]
Division of Cardiovascular Medicine and the Lexington VAMC University of Kentucky [1] • Division of Cardiovascular Medicine and the Lexington VAMC, College of Medicine University of kentucky [2] • College of Nursing University of Kentucky [3]

Exposure to Polyfluoroalkyl Substances is Positively Associated with Serum Cholesterol in a Population Undergoing a Heart Healthy Intervention

Faculty

Exposure to certain per- and polyfluoroalkyl substances (PFAS) have been shown to positively associate with total and/or low density lipoprotein-associated (LDL) cholesterol in cross-sectional epidemiological studies. Examining how this association is modulated in individuals undergoing lipid lowering therapies may provide additional evidence for a role of PFAS as a risk factor for cardiovascular disease.

We examined the association between 6 PFAS and circulating total and LDL cholesterol levels in individuals undergoing a heart healthy intervention study.

We developed high throughput plate-based extraction and LC-MS analysis methodologies to quantitate PFAS in 350 KY individuals that underwent a holistic heart health intervention study. Serum and demographic information was collected at recruitment and post study commencement. Bivariate statistics and logistic regression modelling were used to examine associations of circulating PFAS and cholesterol before and after intervention, and if effectiveness of treatment modulated the observed associations.

Overall, total cholesterol and LDL cholesterol levels significantly decreased following the intervention. In parallel, PFOS, PFOA, PFHxS, and PFHpA, significantly decreased post intervention. Interestingly, PFOS as well as the combined sum of 6 compounds (Total PFAS), significantly positively associated with total cholesterol only in post-intervention samples (Pearson correlation coefficient 0.132; p=0.0241, 0.115; p=0.0497 respectfully). After adjustment for multiple covariates including gender, BMI, smoking, race, education level, and age; Total $PFAS_{post}$ was still significantly positively associated with total cholesterol in post intervention samples. We also determined that individuals that responded to the treatment more favorably, were those that exhibited a significant positive association between Total PFAS and total cholesterol.

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Travis Sexton, PhD [1] • Tracy Macaulay, PharmD [2] • Leigh Ann Callahan, MD [3] • Richard Charnigo, PhD [4] • Susan Smyth, MD, PhD [2]
Saha Cardiovascular Research Center University of Kentucky [1] • Gill Heart and Vascular Institute University of Kentucky [2] • Pulmonary Critical Care and Sleep Medicine University of Kentucky [3] • Statistics University of Kentucky [4]

Ticagrelor Reduces Thromboinflammatory Markers in Patients with Pneumonia

Staff

Despite advances in supportive treatment and antibiotic therapy for pneumonia, significant improvement in the early and late mortality have yet to be realized and increased rates of adverse cardiovascular events remain an issue. Recently, a role for platelets in inflammatory and immune responses has been identified in addition to their established contribution to hemostasis and thrombosis. Data generated from preclinical animal models and retrospective analysis of clinical interventions suggests that anti-platelet therapy may improve outcomes in patients hospitalized with pneumonia. The XANTHIPPE study was undertaken to establish the effect of ticagrelor on markers of inflammation and thrombosis in patients with pneumonia and to explore its safety in this patient population.

Patients (n = 60) admitted to our institution for pneumonia were randomized within 1 day of hospitalization (or diagnosis) to receive placebo or ticagrelor (180 mg loading dose followed by 90 mg twice daily) for up to 7 days. The primary endpoint was change in platelet-leukocyte aggregates between baseline and 24 hours. Secondary endpoints included change in platelet function, biomarkers of inflammation and thrombosis, lung function, and adverse events during and following their hospitalization.

Among subjects with pneumonia not taking a P2Y12 antagonist at baseline, ticagrelor lowered the percent of leukocytes with attached platelets 11.75% at 24 hours compared to a 10.90% increase in placebo control patients (P = 0.0244). Furthermore, ticagrelor lowered plasma IL-6 levels 83% at 24 hours (P = 0.0226 versus placebo control group). Ticagrelor had a transient effect on markers for NETosis while placebo had no significant effect. Pneumonia patients receiving ticagrelor required less supplemental oxygen and lung function tests also numerically improved, although statistical significance was not achieved.

Our findings indicate a mechanistic link between platelets, leukocytes, and lung injury in settings of pneumonia and sepsis and suggest possible therapeutic approaches to reduce complications of pneumonia by targeting P2Y12 signaling.

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Siying Xu[1], Rupinder Kaur[1], Lisa Bennett[1], Sarah Edwards[1], and Gregory Graf, PhD[1]
Pharmaceutical Sciences University of Kentucky[1]

WIF-B9 Cells: a Novel Cell Culture Model to Investigate Regulation of the Hepatic ABCG5 ABCG8 Sterol Transporter

Undergraduate

Background: ABCG5 and ABCG8 form a heterodimer (G5G8) that is responsible for 70-90% of biliary cholesterol secretion. G5G8 is thought to function at the apical (canalicular) surface of hepatocytes and "pump" cholesterol in bile through the hydrolysis of ATP. WIF-B9 cells are a rat hepatoma cell line, express endogenous G5G8, and polarize in cell culture to from apical, canalicular-like domains. In vivo studies suggest that fibroblast growth factor 15 (FGF15), and its human ortholog FGF19, promote biliary cholesterol secretion by redistributing G5G8 to the canalicular surface in liver.

Hypothesis: WIF-B9 cells are a useful in vitro model to study the regulation of G5G8 trafficking in response to FGF15/19.

Methods: WIF-B9 cells were cultured to polarity (10-14 days) on glass coverslips and imaged by differential interference contrast (DIC) and immunofluorescence microscopy to observe the subcellular localization of endogenous G5G8. In addition, G5G8 responsiveness to LXR agonist, FGF15/19 on G5G8 distribution and mRNA and protein expression were analyzed.

Results: Endogenous G5G8 was detected at the mRNA and protein level and increased in response to LXR agonist treatment. Endogenous G8 was primarily detected in sub-apical regions in polarized WIF-B9 cells. FGF15/19 did not alter G8 distribution with acute treatment at concentrations near the physiological range.

Conclusions: WIF-B9 cells express endogenous G5G8 and respond, at the transcriptional level, to known regulators of G5G8 expression in vivo. However, the distribution of the transport complex was not altered by FGF15/19. WIF-B9 cells are useful in vitro model for studies of G5G8 regulation but may not reproduce all features of complex trafficking.

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Himi Tripathi, PhD [1] • Ahmed Al-Darraji, PharmD [1] • Erhe Gao, PhD [2] • Lakshman Chelvarajan, PhD [1] • Bryana Levitan [1] • Andrew J Morris, PhD [3] • Susan S Smyth, MD, PhD [4] • Ahmed Abdel-Latif, MD, PhD [4]
Gill Heart Institute and Division of Cardiovascular Medicine University of Kentucky [1] • The Center for Translational Medicine Temple University School of Medicine, Philadelphia, PA [2] • Gill Heart Institute and Division of Cardiovascular Medicine, The Lexington VA Medical Center, Lexington, KY University of Kentucky [3] • Gill Heart Institute and Division of Cardiovascular Medicine, The Lexington VA Medical Center, Lexington, KY University of Kentucky [4]

Role of Lipid Phosphate Phosphatase-3 in Cardiac Inflammation

Staff

Introduction: The increased inflammatory response following myocardial infarction (MI) can have harmful effects on cardiac function. This pathological inflammation aggravates tissue damage and is correlated with the development of heart failure. Lysophosphatidic acid (LPA), produced by autotaxin (ATX), regulates monocytosis and promotes inflammation. Signaling of lipid substrate including LPA is dephosphorylated and terminated by Lipid phosphate phosphatase 3 (LPP3). The role of ATX/LPA signaling nexus in cardiac inflammation is poorly understood.

Hypothesis: We investigated the possible role of LPP3 in cardiac and systemic inflammation and resulting adverse cardiac remodeling post-MI.

Methods: We utilized a mouse model with inducible deletion of LPP3 and littermate controls. $LPP3^{fl/fl}$ and $LPP3^{-/-}$ mice (6-8 weeks of age) underwent MI or sham surgery. Inflammatory cell content was assessed using flow cytometry. Cardiac function and scar size were assessed by echocardiography and Mason Trichrome staining, respectively.

Results: MI was associated with increased number of cardiac neutrophils ($CD45^+/CD115^-/Ly\text{-}6G^+/C^+$), inflammatory monocytes ($CD45^+/CD115^+/CD11b^+$) and increased number of cardiac and spleen macrophages during peak post-MI inflammation, as assessed by flow cytometry. This increase in inflammatory cells and inflammation may be the consequence of the significant increase in bone marrow and spleen progenitor cell count and proliferation. Moreover, $LPP3^{-/-}$ mice cardiac functional recovery was also reduced and increased infarct size.

Conclusion: LPP3 plays an important role in modulating inflammation and targeting ATX/LPA signaling can represent a novel therapeutic target for coronary heart diseases.

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Shannon Jones [1] • Hadi Khalil, PhD [2] • Zhenling Liu [1] • Yanli Zhao, PhD [1] • Jeffery Molkentin, PhD [3] • Onur Kanisicak, PhD [1]
Pathology University of Cincinnati [1] • Pediatrics Cincinnati Children's Hospital Medical Center [2] • Pediatrics Cincinnati Children's Hospital Medical Center, Howard Hughes Medical Institute [3]

Cardiac Fibroblasts Play a Critical Role during both Fibrosis and Reverse Remodeling

Graduate Student

Cardiac fibrosis is a grim consequence of almost all myocardial injuries. In myocardial infarction (MI), what starts as a protective scarring process to prevent ventricular wall rupture becomes a pathological remodeling of the tissue with the accumulation of excess extracellular matrix (ECM) proteins. Eventually, this adaptation impedes the mechanical and electrical properties of the myocardium resulting in heart failure. Previously, cardiac fibrosis was assumed irreversible; however clinical approaches using ACE inhibitors, β-blockers, and ventricular assist devices have all shown signs of "reverse" remodeling resulting in improved cardiac function. Recently, cardiac fibroblasts (CFs), and especially their activated mature form, myofibroblasts (MFs), have emerged as potential therapeutic targets in preventing both acute and chronic cardiac fibrotic disease states. However, the molecular and cellular mechanisms defining the role of MFs in longstanding fibrosis as well as reverse remodeling and resolution of cardiac fibrosis are still unknown. Up until very recently, the main limitation has been the inability to specifically study and manipulate the activities of CFs or MFs in vivo given a lack of cell type-specific genetic tools. To address this issue we recently generated a novel mouse model that permits lineage tracing of all MFs in the heart after injury or stress stimulation, which we used to address the fate of MFs after injury resolution. MFs were lineage traced with a tamoxifen inducible periostin allele knockin of the MerCreMer cDNA (PostnMCM), with a Rosa26-eGFP dependent reporter. PostnMCM x R26-eGFP mice were transiently injured with the combined infusion of angiotensin II and phenylephrine (Ang/PE) infusion for 2 weeks, during which time tamoxifen was also given to trace all newly formed MFs. Mice were then allowed to "rest" for 2 weeks or longer with no Ang/PE as the fibrotic response regressed, and the fate of the $eGFP^+$ cells was assessed. The data show that immediately after 2 weeks of Ang/PE infusion nearly all the eGFP+ periostin lineage-traced myofibroblasts were αSMA positive and have an activated myofibroblast gene expression profile. However, when the fibrotic response regressed weeks later, a number of periostin-lineage traced eGFP+ cells were still present in the heart and these cells showed a phenotypic and molecular reversion back to CFs with a loss of myofibroblast marker genes. These results suggest that CFs are very unique cell types that can differentiate to MFs then back again to resident CFs.

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Murong Ma, MS [1] • Chia-Hua Wu, PhD [2] • Ya Wang, PhD [3] • Congqing Wu, PhD [2] • Deborah A Howatt [2] • Jessica J Moorleghen [2] • Craig Vander Kooi, PhD [4] • Alan Daugherty, PhD, DSc [2] • Hong Lu, MD, PhD [2]
Pharmacology and Nutritional Sciences University of Kentucky [1] • Saha Cardiovascular Research Center University of Kentucky [2] • The Cardiovascular Key Lab of Zhejiang Province[3] • Department of Molecular and Cellular Biochemistry University of Kentucky [4]

Sequences Proximate to the Renin Cleavage Site in Angiotensinogen Do Not Affect Angiotensin II-mediated Functions

Graduate Student

Objective: Angiotensinogen (AGT) cleavage by renin is the rate limiting step to produce angiotensin (Ang)II, a critical contributor to hypertension and atherosclerosis. Human AGT can not be cleaved by mouse renin as demonstrated by a transgenic mouse model expressing human AGT. Amino acids at positions 11 and 12, adjacent to the renin cleavage site in AGT, have been proposed to regulate renin cleavage between human and mouse. This study determined whether these two residues affect renin cleavage and the consequent AngII-mediated functions.

Methods and Results: Hepatocyte-specific AGT deficient (hepAGT-/-) mice have low plasma AGT but high renin concentrations. This mouse model injected with adeno-associated viral vectors (AAV) encoding AGT was used to repopulate AGT. We first determined whether repopulation of human AGT using AAV recapitulate phenotypes of human AGT transgenic mice. HepAGT-/- mice were injected with AAV having a null insert or encoding human AGT, while wild type littermates (hepAGT+/+) were injected with the null AAV. Administration of AAV encoding human AGT led to high plasma human AGT concentrations without affecting plasma mouse renin concentrations, and had no effect on blood pressure and atherosclerosis. In a subsequent study, AAV encoding mutated mouse AGT with Leu11Val and Tyr12Ile that were same as the two residues in human were injected into hepAGT-/- mice. Repopulation of the mutated mouse AGT resulted in increased plasma mouse AGT concentrations and reduced renin concentrations that were comparable to their concentrations in hepAGT+/+ mice injected with the null AAV. Consequently, AAV-driven expression of mutated mouse AGT increased blood pressure and atherosclerosis in hepAGT-/- mice that were comparable to their magnitudes in hepAGT+/+ mice injected with the null AAV.

Conclusion: The two amino acids proximate to renin cleavage site do not affect AngII-mediated effects.

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McKenzie Crist [1] • Sarah Anthony [1] • Adrienne Guarnieri [1] • Lindsey Lanzillotta [1] • Lisa Green [1] • Phil Owens, PhD [1] • Michael Tranter, PhD [1]
Internal Medicine University of Cincinnati [1]

Human Antigen R regulates brown adipose function via UCP1 expression

Graduate Student

Obesity, a heterogeneous metabolic disease characterized by an excessive accumulation of body fat, has proven to be a rising economic burden in the United States and can lead to heart disease, cancer, arthritis, and other comorbidities. Obesity is primarily an imbalance in energy expenditure, with more energy being taken in than is burned. While white fat primarily stores energy, brown fat (BAT) expends energy through thermogenesis. In thermogenesis, the electrochemical gradient in oxidative phosphorylation is dissipated, primarily by the uncoupling protein UCP1, allowing energy to be released as heat. The RNA-binding protein Human antigen R (HuR) has been shown to regulate many pathways involved in cellular metabolism, inflammation, and adipocyte differentiation. In this study, we examined the role of HuR as a mediator of UCP1.

We utilized adipocyte-specific HuR deletion (adipo-$HuR^{-/-}$) mice, generated by crossing HuR fl/fl mice with adipo-cre mice. When subjected to cold stress (4C), these mice were observed to be less cold tolerant compared to their control littermates. Additionally, adipo-$HuR^{-/-}$ mice had lower UCP1 expression in their BAT than controls. Next, because UCP1 has been shown to be induced by beta-3 (B3) adrenergic receptor activation, we treated wild type mice with the B3 agonist CL316,243, with and without pharmacological HuR inhibition. Mice treated with both B3 agonist and HuR inhibitor had blunted UCP1 expression compared to those treated with just B3 agonist, indicating that HuR is necessary for B3-induced upregulation of UCP1.

In conclusion, our results indicate that HuR regulates brown adipose function via B3 induced expression of UCP1.

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George Chalhoub[1] • Travis Sexton, PhD [1] • Susan Smyth, MD, PhD [2]
Saha Cardiovascular Research Center University of Kentucky [1] • Gill Heart and Vascular Institute University of Kentucky [2]

Thrombinflammatory and Endovascular Integrity Biomarkers in the Setting of Sepsis

Graduate Student

Background

Sepsis is an exaggerated response to an infection that results in systemic microvascular leakage and multiple-organ failure. Sepsis accounts for 10% of in-hospital mortality rates in the US. However, current therapies do not properly tackle all aspects of this immune dysfunction; rather, they focus on aggressively treating the underlying infection or the resulting symptoms. Growing evidence indicates that platelets are key effectors in many inflammatory diseases, including sepsis. Thrombocytopenia - low platelet counts - is a common complication of sepsis and a biomarker for disease severity. The primary objective of this pilot study is to determine if platelet count and platelet function correlate with vascular integrity, changes in inflammation, sepsis sources, and patient outcomes.

Methods

All hospitalized adult patients meeting the definition of severe sepsis using the Sepsis Related Organ Failure Assessment (SOFA) were eligible for enrollment in the registry / biobank. Blood samples at baseline and discharge were collected on enrolled patients as well as daily clinical information. Platelet activity was tested using Adenosine Diphosphate (ADP) and Thrombin Receptor Activating Peptide (TRAP) induced light transmission aggregation. Plasma was stored for subsequent biomarker analysis. Biomarkers to be analyzed include: IL-6, IL-1 beta, IL-10, MIP1-alpha, MIP1-beta, sCD40L, TNF-alpha, and endovascular integrity markers Angiopoietin 1 and 2. Follow up information was collected via electronic medical record or phone call.

Results

A total of 86 patients have been recruited to the sepsis biobank. A preliminary biomarker analysis was performed and the results will be presented.

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Hsuan Peng [1] • Anuhya Gottipati, PhD [2] • Lakshman Chelvarajan, PhD [1] • Calvin Cahal, [1] • Eman Elsawalhy [1] • Bryana Levitan [1] • Ahmed Abdel-Latif, MD, PhD [1] • Bradley Berron, PhD [2]
Gill Heart Institute University of Kentucky [1] • Department of Chemical and Materials Engineering University of Kentucky [2]

Polymer Enhance Mesenchymal Stem Cell Retention in the Heart after Transplantation: Potential Therapeutic Applications

Graduate Student

Introduction: Mesenchymal stem cell (MSC) transplantation yields cardiac protective and functional preservation post-myocardial infarction (MI) in human and animal studies. However, MSC mediated therapeutic benefits in cardiac repair are strongly correlated with their engraftment efficiency. To enhance MSC based cellular therapy, we developed a 100nm biodegradable gelatin polymer (gelMA) cell surface coating to improve MSC retention without compromising survival and metabolic activity.

Methods: GFP+ bone marrow derived MSCs (6-8 weeks in age) were enriched *in vitro* to a homogeneous population (CD90+, Sca-1+, CD44+. CD45-) at passage 5. Prior to transplantation, MSCs were coated with gelMA using the atom transfer radical polymerization technique, and directly injected in peri-infarct border immediately after MI (induced by left anterior descending artery ligation) in wildtype C57BL/6J mice.

Results: Flow cytometry and immunohistochemistry confirmed a significant increase in cardiac MSC retention 7 days post-MI. We observed significant reduction in cardiac neutrophils ($CD115^-$/Ly-$6G^+$/C^+), inflammatory monocytes ($CD115^+$/$CD11b^+$) and macrophage (F4/80+/CD11b+) in the early phase post-MI. Echocardiographic studies conducted 30 days after MI showed significant increase in left ventricular ejection fraction, significant reduction in left ventricular end-systolic and end-diastolic diameters in mice treated with coated MSCs compared to those treated with vehicle (PBS) or uncoated MSCs.

Conclusion: Our data provide first evidence for the successful use of a biodegradable/biocompatible cell coating to enhance the retention and anti-inflammatory effects of transplanted MSCs. Our strategy enhanced the therapeutic benefits of MSCs and could provide the basis for more successful regenerative therapies.

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Kaitlyn Samuels[1] • Isabel Derera[1] • Bret Smith, PhD[1] • Brian Delisle, PhD[1]
Physiology, University of Kentucky[1]

Investigating Cardiac Gene Expression Changes from Acquired Epilepsy as a Possible Mechanism for SUDEP

Undergraduate

Sudden Unexpected Death in Epilepsy (SUDEP) is defined as the sudden, unexplainable death of an individual with a chronic seizure disorder, and accounts for approximately 17% of epilepsy-related deaths. Peri-ictal autonomic irregularities leading to cardiorespiratory arrest have been shown to be the immediate cause of SUDEP, however the underlying mechanisms of this process are largely unknown. Autonomic abnormalities, including tachycardia or bradycardia, cardiac arrhythmias, blood oxygen desaturation, and apnea occur over time in individuals with epilepsy, implicating seizure-associated central autonomic reactive neuroplasticity and/or cardiac remodeling as potential drivers for increased SUDEP risk. Thus, a deadly form of cardiovascular disease develops as a result of recurrent seizures in epilepsy. We hypothesize that the development of spontaneous seizures coincides with altered gene expression in cardiomyocytes. Hearts from pilocarpine-treated mice, a model of acquired temporal lobe epilepsy (TLE) via the pilocarpine-induced status epilepticus, were collected after a prolonged period of spontaneous seizure expression. These hearts were utilized for RNA-seq experiments to determine the cell-type specific gene expression changes that occur in concert with eliptogenesis.

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Xufang Mu[1] • Shu Liu[2] • Zhuoran Wang[2] • and Zhenheng Guo, PhD[1]
Department of Pharmacology and Nutritional Sciences University of Kentucky[1] •
Department of Physiology University of Kentucky[2]

Androgen Mediates the High Susceptibility of Male Mice to Aldosterone and High Salt-induced Abdominal Aortic Aneurysm

Graduate Student

Abdominal aortic aneurysm (AAA) has a high mortality rate when ruptured, and male sex is a nonmodifiable risk factor for AAA in human. Our lab recently reported a novel mouse AAA model, in which aldosterone infusion in the presence of high salt intake induces an age-dependent AAA. However, it is unknown whether this AAA model shows a sex difference similar to human. In this study, we investigated whether the male mice are more susceptible to aldosterone plus high salt-induced AAA. When the results showed yes, we further investigated whether androgen mediates the increased susceptibility in male mice.

To determine whether there is sex difference in aldosterone plus high salt-induced AAA, 10-month-old male and female wild-type C57BL/6 mice were infused with aldosterone and salt for 4 weeks to induce AAA. We found that female mice were effectively protected from the AAA induction: none of the nine female mice developed AAA. In contrast, 7 of the 10 male mice developed AAA independent of blood pressure level. The AAA in the male mice was associated with breakages of aortic elastin. Interestingly, the female mouse heart and kidney were also protected from aldosterone and high salt-induced fibrosis. To further investigate if the male sex organ plays a role in the formation and progression of AAA, we investigated whether orchiectomy protects male mice from aldosterone and high salt-induced AAA. Ten-month-old male mice received sham (n=4) or orchiectomy (n=15) surgery and then infused with aldosterone in the presence high salt intake. Results showed that orchiectomy powerfully protected the male mice from AAA: only one of the 15 orchiectomized mice developed AAA. In contrast, 3 of the 4 sham-operated mice developed AAA. We then tested whether loss of the male sex hormone testosterone mediates the protective effect of orchiectomy. The following four groups of 10-month-old mice were administered with aldosterone and high salt: 1) normal male mice, 2) orchiectomized male mice, 3) orchiectomized male mice plus DHT pellet; and 4) male mice administered with androgen receptor degradation enhancer ASC-J9. The results demonstrated that DHT restored the high AAA incidence in the orchiectomized male mice. The ASC-J9 mimicked the orchiectomy significantly decreased the incidence of AAA. Taken together, our results demonstrated that male mice are much more susceptible to aldosterone and high salt-induced AAA. Androgen and its receptor mediate the high susceptibility in male mice. These results suggest the aldosterone plus high salt-induced AAA is a useful mouse model for the study of mechanisms underlying the sex difference in AAA, and implicate androgen and its receptor as a potential therapeutic target in AAA treatment.