

Ohio Valley Society of Toxicology Annual Meeting Program



October 18th, 2019

Procter & Gamble
Mason Business Center (MBC)
8700 Mason Montgomery Road
Mason, OH 45040



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MEETING AGENDA

8:00 – 8:45 am	Registration and Continental Breakfast and Poster Set-up P&G MBC Atrium
9:00 - 9:15 am	Welcoming Remarks Dr. Matt Doyle, Global Head of Product Safety, Environmental Science & Sustainability, Procter & Gamble
9:20 - 9:40 am	Overview of Safety at P&G Dr. George Daston, Distinguished Research Fellow, Procter & Gamble, and Vice-President SOT
9:45 - 9:55 am	Meeting Logistics Lisa Navarro, Ph.D., Director of Regulatory, Flavor Producers, and OVSOT President, 2019-2020
10:00 - 11:15 am	Doctoral Student Platform Presentations and Judging
11:15 - 12:15 pm	Poster viewing and judging (P&G MBC Atrium)
12:15 - 1:00 pm	Lunch (P&G MBC Atrium)
1:00 - 2:00 pm	Tox on the Clock
2:00 - 2:45 pm	Post-Doctoral Platform Presentations and Judging
2:45 - 3:00 pm	Cookie Break (P&G MBC Atrium)
3:00 - 4:00 pm	Keynote: Dr. Karen VanderMolen and Dr. Vincent Sica, P&G Trace Analytical Capability Core - <i>The Importance of Analytical Partnerships in Evaluating Botanical Safety.</i>
4:00 - 4:45 pm	Presentation of Awards and Closing Remarks



PARKING INFORMATION

OVSOT 2019 Annual Meeting logistics:

Address: Procter and Gamble, Mason Business Center 8700 Mason
Montgomery Road Mason, OH 45040

Date: Friday, October 18th

***If using Google Maps, address will bring you directly to the **RED circle** on the map to a circular drive that rounds a flagpole and has the main entrance to the Mason Business Center. **Please park in Visitor lot 10 and enter the building at the RED circle.** You will enter the P&G lobby. Look for an OVSOT sign-in table to register and obtain your Visitor security badge for the day.





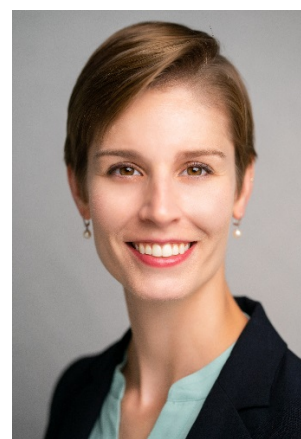
Keynote Speakers

The Importance of Analytical Partnerships in Evaluating Botanical Safety

As complex mixtures, botanicals present unique challenges when assessing safe use, particularly when traditional toxicological studies are not available in the literature. Obtaining data for developmental and reproductive toxicity can be particularly difficult. In these instances, partnering with analytical chemists is a valuable avenue to avoid time- and cost-intensive animal studies. Using a multi-detector approach, analytical chemistry can provide an accurate, constituent-based profile of the botanical ingredient, including both qualitative and quantitative information on all constituents above a pre-defined threshold of toxicological concern. These data help drive an *in silico* safety assessment to identify suspected toxicants and assess the potential for deleterious effects using structure activity relationships. Additionally, plausible mechanisms can then be tested using *in vitro* models (e.g., receptor binding assays). Using this combination of literature data, *in silico*, and *in vitro* tools can provide assurance of safety while reducing the use of animals.

Karen M. VanderMolen, PhD

Dr. VanderMolen is an analytical chemist turned toxicologist who specializes in the safety assessment of complex mixtures, with an emphasis on natural products. She joined The Procter & Gamble Company as a product safety toxicologist after completing her PhD in Medicinal Biochemistry at the University of North Carolina at Greensboro. Both her academic and industry experience include the evaluation of fungal and botanical dietary supplements, and more recently the incorporation of natural products into assembled consumer goods such as diapers, toilet tissue, and feminine hygiene products. Her research focuses on leveraging alternative toxicological tools, such as *in silico* and *in vivo* approaches, to evaluate complex materials for which traditional toxicology data rarely exist in the literature.



Vincent P. Sica, PhD

Dr. Sica is an analytical chemist that specializes in the qualitative and quantitative analyses of small molecules. Both his academic and industrial experiences include the evaluation of fungal and botanical dietary supplements using mass spectrometry. At P&G, he works closely with the toxicologists in safety to provide a comprehensive constituent characterization of botanicals used in consumer goods, such as dietary supplements and beauty care products. His research supports the use of alternative toxicological tools, such as *in silico* and *in vivo* approaches, to evaluate complex materials for which traditional toxicology data rarely exist in the literature.



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Title

Phase-specific Influences of Electronic Cigarette Flavorings and Acrolein on Platelet Aggregation

Authors

Andre Richardson^{1,2}, Tatiana Krivokhizhina, Ph.D.², Stanley D'Souza, Ph.D.³, Sanjay Srivastava, Ph.D.^{2,4}, Daniel J. Conklin, Ph.D.^{1,2,4}

Affiliation and Address

Dept. Pharmacology & Toxicology¹, Diabetes and Obesity Center², Dept. Physiology³, Div. Environmental Medicine⁴

Abstract

The platelet aggregometry assay is the gold standard platelet function assay that measures the ability of different stimuli to induce platelet-platelet aggregation *in vitro*. There is evidence showing that tobacco- and electronic cigarette (e-cig)-derived constituents such as acrolein and e-cig flavorings exacerbate platelet activation -- a prothrombotic condition. Because little is known about how tobacco constituents affect the phases of platelet aggregation, we investigated direct effects of acrolein and e-cig flavorings on the phases of adenosine diphosphate (ADP)-induced platelet aggregation. By knowing what phase is more affected, we will more fully understand the mechanism of these pro-thrombotic agents. Human platelet rich plasma (PRP) was obtained via centrifugation of whole blood from healthy volunteers for use in a platelet aggregometry assay. A ChronoLog aggregometer measured aggregation photometrically post-ADP (2.5 or 10 μ M) induction over 5 min. Prior to ADP, PRP was incubated with increasing concentrations of e-cig flavorant compounds (e.g. cinnamaldehyde, and eugenol) or acrolein for five min at 37°C. Platelet poor plasma (PPP) was used as the reference sample. Using a paired Student's *t*-test, the percent responses of phase 1 and phase 2 of flavorings or acrolein were compared with respect to their control. Statistical significance was accepted where $p < 0.05$. At the concentrations tested of cinnamaldehyde, we observed no change in overall aggregation as well as the phase 1 and phase 2 responses compared with ADP-only control. Eugenol displayed an antiplatelet response of ADP-induced platelet aggregation. Furthermore, acrolein has no influence on ADP-induced aggregation. However, at 10 and 30 μ M of acrolein, we observed varying biphasic responses. Under the conditions tested, though we did not find any consistent changes in the biphasic activity of acrolein or the flavorants compared with control, it is important to understand how the constituents of tobacco smoke and e-cig aerosols may impair platelet function. This method of analyzing platelet function will help evolve the field of platelet aggregometry testing, to provide better details of biphasic activity in laboratory testing of platelet function, and to understand prothrombotic actions associated with smoking.



Title

The Role of Dietary Zinc in Cadmium-Enhanced Non-Alcoholic Fatty Liver Disease

Authors

Jamie L. Young¹, Bin Zhou², Li Zhou², Wenqian Zhou², Hongbo Men², Lu Cai^{1,2}

Affiliation and Address

¹Department of Pharmacology and Toxicology, ²Pediatric Research Institute, Department of Pediatrics, University of Louisville School of Medicine, Louisville, KY 40202 USA

Abstract

Environmental exposure to cadmium (Cd) is implicated in the development of several metabolic syndrome (MetS)-related diseases including non-alcoholic fatty liver disease (NAFLD), however, the ability of early-life, in utero Cd exposure to cause obesity-induced MetS in adulthood is poorly understood. Therefore, we developed an in vivo two-hit model to study the effect of whole life, low dose Cd exposure and high fat diet (HFD) on MetS with an emphasis on NAFLD. Additionally, we investigated the impact of dietary zinc supplementation and deficiency on disease outcome as both obesity and Cd disrupt zinc homeostasis and zinc deficiency is common in both obese and NAFLD patients. Adult male and female C57BL/6J mice fed normal diets (ND) were exposed to 0, 0.5 or 5 ppm Cd-containing drinking water for 1 week before breeding. Pregnant dams and offspring (F1) were continuously exposed to the same Cd concentration. At weaning, offspring were fed ND or HFD. Male mice were further grouped into diets containing 10, 30, or 90 mg zinc/4057 kcal, representing deficient, normal and supplemented zinc diet, respectively. Body weights and water consumption were recorded weekly. Prior to sacrifice changes in body fat composition were assessed using DEXA scan technology. Insulin resistance was characterized via intraperitoneal glucose tolerance tests, and serum insulin, triglycerides (TG) and cholesterol (TC) levels. Animals were sacrificed 10 or 24 weeks after weaning. Blood samples were collected and liver tissue was snap-frozen, fixed for histopathological analyses or used for metals analysis. Liver to body weight ratios, plasma aminotransferase, and hepatic TG and TC were determined. Thus far we show body weight was impacted by diet, Cd exposure and altered dietary zinc. HFD altered body composition in males and females whereas Cd exposure altered body composition in males only. Glucose handling was altered in HFD fed males and to a lesser degree in females whereas Cd nor altered dietary zinc impacted glucose handling. HFD caused liver injury in males and Cd exposure exacerbated this injury. Overall, results from this study will provide insight into the mechanisms by which whole life, low dose Cd exposure enhances HFD-induced MetS and discern a potential therapeutic role for zinc in MetS.



Title

Influence of Surface Modification of Carbon Nanotube-Coated Scaffolds on Keratinocyte Cell Growth

Authors

Soham D Parikh¹, Courtney E.W. Sulentic² & Sharmila M

Affiliation and Address

Center for Nanoscale Multifunctional Materials, Department of Mechanical & Materials Engineering, Wright State University, Dayton OH, United States¹; Department of Pharmacology & Toxicology, Wright State University, Dayton, OH, United States²

Abstract

Traumatic injuries involving skin affect approximately 5 million people every year and cause financial burden of \$20 billion in the USA alone. Current therapeutics for wound healing often do not effectively close the wound or require extensive post-operative care. Different materials with carbon nanotubes (CNTs) have been utilized for effective adhesion, growth, and differentiation of bone, muscle, and cardiac cells; however, this has not been extensively studied in keratinocytes. The proposed project aims to determine whether the use of CNTs attached to carbon scaffolds are capable of sustaining keratinocytes for future development of novel skin grafts and/or bandage development. CNTs can provide excellent mechanical and electrical properties, biocompatibility, nano-roughness and wettability that may help in cell adhesion, growth, and differentiation for cell scaffolding. However, loose CNTs can migrate, causing in-vivo toxicity. To suppress this risk, our team has developed biomimetic scaffolds with multiscale hierarchy where carpet-like CNT arrays are covalently bonded to larger biocompatible substrates. We found that the hydrophobic CNT-coated scaffolds initially reduce cell proliferation. However, altering wettability of the scaffolds using chemical and surface plasma treatments result in normal growth curves of different cell types including keratinocytes, indicating that cell growth can be modified through wettability control. CNT-coated fiber scaffolds can resemble a band-aid and could support cell growth and could maintain normal proliferation while healing progresses. In this study, various cytotoxicity, cell proliferation and viability, and cellular migration assays will be conducted to understand keratinocyte cell growth on these CNT-coated scaffolds. Due to their large surface area, these scaffolds have a great potential to be coated with multiple growth factors and anti-bacterial agents, which would be ideal for wounds requiring extensive skin regeneration. While detailed cell growth and toxicity analyses are underway, preliminary results support future potential of bio-mimetic scaffolds in tissue engineering.



Title

Lung Fibroblasts from Idiopathic Pulmonary Fibrosis Patients Produce an Oxidizing Extracellular Redox Potential

Authors

Yuxuan Zheng, Tom Burke, Jeffrey Ritzenthaler, Igor Zelko, Victor van Berkel, David Nunley, Andrew Halayko, Jesse Roman, Walter Watson

Affiliation and Address

Department of Pharmacology & Toxicology, Department of Medicine, Department of Cardiovascular & Thoracic Surgery, University of Louisville School of Medicine, Louisville, KY

Abstract

Idiopathic pulmonary fibrosis (IPF) is associated with increased deposition of extracellular matrix proteins, and lung fibroblasts are responsible for producing these pro-fibrotic proteins. One of the signals that evokes pro-fibrotic gene expression is a shift in the extracellular cysteine/cystine redox potential ($E_h(\text{Cys}/\text{CySS})$) toward more positive (more oxidizing) potentials. Recently, we found that mouse lung fibroblasts can regulate their own extracellular $E_h(\text{Cys}/\text{CySS})$ and that changes in the activity of the CySS transporter Slc7a11 can change the value at which $E_h(\text{Cys}/\text{CySS})$ is maintained. Our hypothesis is that human lung fibroblasts from patients with IPF express low levels of SLC7A11 and produce an oxidizing extracellular $E_h(\text{Cys}/\text{CySS})$. IPF fibroblasts were isolated from explants of 6 IPF patients receiving lung transplant. Non-IPF fibroblasts were isolated from relatively normal lung tissues adjacent to cancerous tissues of 6 lung cancer patients. SLC7A11 mRNA levels were measured by qPCR. Concentrations of Cys, CySS, glutathione (GSH), glutathione disulfide (GSSG) and mixed disulfide (CySSG) were measured by HPLC. Nernst equation was used to determine the redox potentials. The data showed that extracellular $E_h(\text{Cys}/\text{CySS})$ was more oxidized for lung fibroblasts from IPF patients than those from non-IPF donors. Oxidation of $E_h(\text{Cys}/\text{CySS})$ in IPF fibroblasts was not due to decreased expression of SLC7A11. However, higher SLC7A11 expression was correlated with more reduced extracellular $E_h(\text{Cys}/\text{CySS})$ and more reduced intracellular $E_h(\text{GSH}/\text{GSSG})$ in non-IPF fibroblasts, but not in IPF fibroblasts. Therefore, SLC7A11-dependent regulation of extracellular and intracellular redox environments is lost in lung fibroblasts from IPF patients, suggesting that SLC7A11-independent mechanisms are responsible for aberrant redox regulation in this important effector cell of pulmonary fibrosis.



Title

Δ Np63 α suppresses cell invasion by modulating Rac1 activity

Authors

Amjad A. Aljagthmi, Natasha T. Hill, Mariana Cooke, Marcelo G. Kazanietz, Martín C. Abba, Weiwen Long, Madhavi P. Kadakia.

Affiliation and Address

Wright State University, Biochemistry and Molecular Biology department.
3640 Colonel Glenn Hwy. Dayton, OH 45435 USA

Abstract

Δ Np63 α , a member of the p53 family of transcription factors, is overexpressed in a number of cancers and plays a role in proliferation, differentiation, migration and invasion. Δ Np63 α has been shown to regulate several microRNAs that are involved in development and cancer. We identified miRNA miR-320a as a positively regulated target of Δ Np63 α . Previous studies have shown that miR-320a is downregulated in colorectal cancer and targets the small GTPase Rac1, leading to a reduction in non-canonical WNT signaling and epithelial mesenchymal transition (EMT), thereby inhibiting tumor metastasis and invasion. We showed that miR-320a is a direct target of Δ Np63 α . Knockdown of Δ Np63 α in HaCaT and A431 cells downregulates miR-320a levels and leads to a corresponding elevation in PKC γ transcript and protein levels. Rac1 phosphorylation at Ser71 was increased in the absence of Δ Np63 α , whereas overexpression of Δ Np63 α reversed S71 phosphorylation of Rac1. Moreover, increased PKC γ levels, Rac1 phosphorylation and cell invasion observed upon knockdown of Δ Np63 α was reversed by either overexpressing miR-320a mimic or Rac1 silencing. Finally, silencing PKC γ or treatment with the PKC inhibitor Gö6976 reversed increased Rac1 phosphorylation and cell invasion observed upon silencing Δ Np63 α . Taken together, our data suggest that Δ Np63 α positively regulates miR-320a, thereby inhibiting PKC γ expression, Rac1 phosphorylation, and cancer invasion.



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Title

Silver Nanoparticle-Induced Differential Biodistribution and Inflammatory Responses in Healthy and Obese Models

Authors

Lisa Kobos¹, Jonathan Shannahan¹, Saeed Alqatani^{1,2}, Li Xia¹, Vincent Coltellino¹, Riley Kishman¹

Affiliation and Address

¹ School of Health Sciences, Purdue University

² National Center for Pharmaceuticals, King Abdulaziz City for Science and Technology

Abstract

Silver nanoparticles (AgNPs) are commonly used as an antibacterial agent in many applications, including in medical devices and implants. Despite this, most of the research regarding AgNP toxicity has been performed in healthy models, and the toxicity of AgNPs in diseased conditions is poorly characterized. Our current evaluation utilized both healthy and obese models to determine differences in AgNP toxicity between subpopulations. We hypothesized that AgNPs would have differential biodistribution and that exposure would induce greater inflammation in obese mice due to their underlying disease state. Healthy and obese C57BL6 mice were injected via the tail vein with 2mg/kg AgNPs. After 24 h, the organs were collected and assessed for Ag content and expression of inflammatory genes including IL-6, TNF- α , TGF- β , IL-1 β , and others. AgNPs were found to induce differential total and relative inflammatory responses in healthy and obese mice. While AgNP exposure consistently caused an increase in the expression of inflammatory genes within the spleen of healthy mice, obese mice frequently saw a decrease in expression. Both healthy and obese mice had an approximately equal inflammatory response in the liver. AgNPs were found to localize primarily to the liver and the spleen, though silver was present in all organs that were tested, including the brain. Biodistribution differences were observed between models. More silver was found in the liver, kidney, heart, and serum of the obese mice than the healthy mice. For the intestines and lungs, more silver was detected in the healthy than the obese. The aorta, spleen, and brain contained the same amount of silver between groups. These findings indicate that AgNPs behave differently in healthy and obese physiological environments, and that prevalent underlying diseases may impact NP biodistribution and toxicity.



Title

Developing Analytical-Based Methodologies to Study the Absorption, Distribution, Metabolism, and Excretion (ADME) of Silver Nanoparticles in Red Blood Cells

Authors

Praveen K. Alla^{1,2}, Kelsey L. Hood¹, Arathi S.L.Paluri¹, John D. Ryan, Peter K. Lauf², Norma C. Adragna², Ioana E. Pavel¹

Affiliation and Address

¹Wright State University, Department of Chemistry

²Wright State University, Department of Pharmacology and Toxicology

Abstract

Multiple national agencies such as the National Nanotechnology Initiative and the National Institute for Occupational Safety and Health outlined the need to improve the risk assessment and control associated with heavy use of nanomaterial such as silver nanoparticles. This work addresses this knowledge gap by developing analytical-based methodologies for the identification of key pharmacokinetic parameters: the absorption (A), distribution (D), metabolism (M), and elimination (E) of AgNPs from human red blood cells (RBCs). To achieve this, citrate-capped AgNPs of 5- 10 nm in diameter were incubated with RBCs in 5% glucose solution. (A) Graphite furnace atomic absorption spectroscopy (GFAAS) measurements revealed that $48 \pm 5\%$ of the administered AgNPs were accumulated in cells. (D) CytoViva hyperspectral imaging of the RBCs showed that $\sim 70\%$ of the up taken AgNPs were adhered to the cell membrane components. In addition, Raman Hyperspectral imaging revealed molecular interactions between AgNPs and hemoglobin (diminished oxyhemoglobin Raman peaks), and thereby, suggested the formation of deoxyhemoglobin. (M) AAS measurements on cell extracts indicated that AgNPs inhibit the Rb^+ influx and reduce the K^+ ion concentration in RBCs. (E) GFAAS methodologies are currently explored for examining the elimination rate laws of AgNPs. Overall, the results of this study suggest AgNPs may remain concentrated in the circulatory system in spite of their negative charge and biocompatible capping agent.



Title

Particulate Hexavalent Chromium Induces Cytotoxicity and Genotoxicity in Female and Male Fin Whale Primary Fibroblasts

Authors

Idoia Meaza^{1,2}, Rachel M. Speer^{1,2}, Jennifer H. Toyoda^{1,2}, John Pierce Wise^{1,2}

Affiliation and Address

The Wise Laboratory of Environmental and Genetic Toxicology¹ University of Louisville,
School of Medicine, Pharmacology and Toxicology²

Abstract

Hexavalent chromium is a global health concern, a known human carcinogen as well as reproductive and developmental toxicant. Although it is clear that DNA damage, manifested as strand breaks and chromosomal changes plays a central role, the mechanism underlying its carcinogenicity is uncertain. Chromium is a ubiquitous marine pollutant and high chromium levels have been reported in North Atlantic whales including fin whales. Whale species represent a good model for toxicological studies because despite the fact that marine mammals accumulate high amounts of pollutants due to their long lifespan and deep diving behavior, they are known to be less susceptible to develop cancer than humans. Studying the toxicity of chromium in whale cells might be key to acquire a better understanding of the underlying toxicity of chromium. Therefore, we hypothesized that acute (24 h) and prolonged (120 h) exposures of particulate Cr(VI) to fin whale male and female cells induces both cytotoxic and genotoxic effects. To investigate this, cytotoxicity was measured by the clonogenic assay also known as colony forming assay, which measures the ability of cells to proliferate and form colonies after the treatment. Clastogenicity was measured using the chromosome aberration assay. Intracellular chromium levels were also determined with Graphite Furnace Atomic Absorption Spectroscopy. As hypothesized, particulate Cr(VI) induced a cytotoxic and genotoxic response in a concentration dependent manner in cells from both sexes. Both acute and prolonged exposure induced similar amounts of cytotoxicity, but prolonged exposure induced less genotoxicity than acute exposure. Intracellular chromium was lower after prolonged exposure than acute exposure. Overall, compared to previous data in human cells, both fin whale sexes showed a resistance to Cr(VI), as prolonged particulate Cr(VI) exposures in human cells showed an increased the amount of toxicity. This outcome suggests marine mammals may have developed cellular mechanisms to counterbalance chromium induced toxicity. In the future, assessing cellular mechanisms activated after the Cr(VI) exposure, such as, Cr transport, DNA repair and cell death pathways, could provide insights into why fin whale cells are resistant to genotoxic agents.



Title

Chromate-Induced Loss of E2F1 Inhibits RAD51 Response in Homologous Recombination Repair

Authors

^{1,2}Rachel M. Speer, ³Xixi Zhou, ³Ke Jian Liu, ¹Cynthia L. Browning, ⁴Kazuya Kondo, ^{1,2}John Pierce Wise, Sr.

Affiliation and Address

¹Wise Laboratory of Environmental and Genetic Toxicology; ²University of Louisville, Louisville, KY; ³University of New Mexico, Albuquerque, NM; ⁴University of Tokushima, Japan.

Abstract

Lung cancer is the leading cause of cancer death, however the mechanisms of how lung carcinogens cause cancer are poorly understood. Metals including hexavalent chromium [Cr(VI)] are known to induce chromosome instability, an early event in lung cancer. Failure of homologous recombination repair is a key mechanism of structural chromosome instability. We have shown particulate Cr(VI) causes DNA double strand breaks and prolonged exposure impairs the effector step of homologous recombination targeting the RAD51 protein. It is currently unknown how Cr(VI) impairs RAD51 function. This study considers the effects of particulate Cr(VI) on transcription of RAD51 specifically focusing on its primary transcription factor, E2F1. The objective of this study was to show prolonged exposure to particulate Cr(VI) inhibits RAD51 expression by affecting E2F1-mediated transcription of RAD51. We found exposure to particulate Cr(VI) reduced RAD51 and E2F1 mRNA and whole cell and nuclear protein levels. Next we showed E2F1 knockdown inhibited the RAD51 response to particulate Cr(VI) by reducing RAD51 expression and nuclear foci formation. Finally, *in vitro* Cr-binding showed Cr(III), not Cr(VI) can bind to acetylated lysine as a potential physico-chemical mechanism of Cr-inhibited function. This work was supported by the National Institute of Environmental Health Sciences [ES016893 to J.P.W], the Jewish Heritage Foundation for Excellence [JPW], and the NIEHS T32 Training Grant [5T32ES011564-12 to JPW and RMS].



Title

Prolonged Particulate Hexavalent Chromium Exposure Disrupts Centrosome Regulation Proteins and Causes Centrosome Amplification

Authors

Jennifer H. Toyoda¹, Julieta Martino¹, Amie Holmes¹, Kazuya Kondo², and John Pierce Wise, Sr.¹

Affiliation and Address

¹Wise Laboratory of Environmental and Genetic Toxicology, University of Louisville

²Department of Oncological Medical Services, The University of Tokushima, Japan

Abstract

Hexavalent chromium [Cr(VI)] is a well-known lung carcinogen with environmental and occupational exposure risks. A key mechanism for Cr(VI) carcinogenesis is the induction of chromosome instability (CIN), including changes in chromosome number which can arise due to centrosome amplification. Our study shows supernumerary centrosomes in human lung tumors from chromate workers and in human lung cells after 120h Cr(VI) exposure. Our hypothesis is Cr(VI) disrupts proteins that regulate centrosome duplication, leading to centriole disengagement and centrosome amplification. During interphase in normal cells, centriole engagement blocks premature centrosome duplication. Our findings show increasing concentrations and prolonged exposure to Cr(VI) increased centriole disengagement in human lung interphase cells. We also measured decreased protein levels of the separase inhibitors, securin and cyclin B1, which are key to regulating the centrosome cycle. Together, these data demonstrate prolonged Cr(VI) exposure induces centriole disengagement, centrosome amplification, and causes decreased levels of proteins that inhibit centriole disengagement. Ongoing studies using securin and separase siRNA knockdown are elucidating the role of these proteins in the Cr(VI) carcinogenic mechanism. This work was supported by NIEHS grant R01ES016893 (J.P.W.) and T32-ES011564 (J.H.T.).



Title

Differential Effects of the AhR on Immunoglobulin Expression in a human B-cell line

Authors

Mili Bhakta, Clayton Alex-Buckner, Kaulini Burra, Courtney Sulentic

Affiliation and Address

Department of Pharmacology and Toxicology Wright State University

Abstract

Inhibition of antibody expression and secretion by the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is well established in animal models. This inhibition is mediated by the aryl hydrocarbon receptor (AhR). However, the effect of TCDD on human B cells is less clear. Therefore, the objective of this study was to evaluate the effects of TCDD on Ig expression, via ELISA analysis and real-time PCR, in a human B-cell line (CL-01). CL-01 cells were generated from a Burkitt lymphoma patient. These cells can be stimulated by CD40L and IL-4 to secrete Ig and undergo class switch recombination. TCDD significantly inhibited IgG secretion but had no effect on IgM secretion. TCDD also altered Ig heavy chain (IGH) transcription, producing a significant decrease in γ 1-4 transcripts and an increase in α 1 and α 2 transcripts. Co-treatment with an AhR antagonist not only reversed the effects of TCDD but also enhanced stimulation-induced γ 1-4 expression and IgG secretion, perhaps due to the presence of endogenous AhR ligands in the media. These results support a role of the AhR in regulating human IGH expression.



Title

Effects of intranasal carnosine on alpha-synuclein-positive neurons in the Thy1-aSyn mouse model of Parkinson's disease

Authors

Josephine Brown¹; Mei-Ling Bermúdez; Lauren Baker^{1,2}; Kim Seroogy³; and Mary Beth Genter¹

Affiliation and Address

¹Department of Environmental Health; ²Neuroscience Undergraduate Program; ³Department of Neurology; University of Cincinnati, Cincinnati, OH, United States

Abstract

Parkinson's disease (PD) is a common and debilitating neurodegenerative disorder. Early symptoms of PD include motor dysfunction and decreased olfaction. Toxic aggregation of α -synuclein (aSyn) is a hallmark of PD neuropathology and includes aSyn accumulation in the olfactory bulb (OB) and substantia nigra pars compacta (SNpc), which correlates with olfactory and motor impairments, respectively, in PD patients. Thy1-aSyn transgenic mice exhibit these and other features of PD, making these mutant mice a suitable model for studying drug intervention in PD. Carnosine is an endogenous dipeptide and was found to produce beneficial effects in PD patients. We have previously shown that treatment with 2.0 mg/d intranasal (IN) carnosine for 2 months improved motor behavior in Thy1-aSyn mice. Here, we tested the following hypotheses:

(1) Thy1-aSyn mice will have increased aSyn(+) neurons in the brain and (2) IN carnosine will decrease the number of aSyn(+) somata in the mouse brain. Two-month-old wild-type and Thy1-aSyn mice received IN vehicle or 2.0 mg/d carnosine treatment for 2 months. Brains were collected, sectioned, and immunostained for aSyn; and labeled cells were counted using design-based stereology in the SNpc and OB. In the SNpc, Thy1-aSyn mice exhibited more aSyn(+) perikarya compared to wild-type controls ($p < 0.001$). Carnosine-treated Thy1-aSyn mice demonstrated fewer aSyn(+) somata compared to vehicle-treated animals ($p = 0.003$). Moreover, the number of aSyn(+) somata in carnosine-treated Thy1-aSyn mice were reduced to vehicle-treated wild-type levels in the SNpc ($p = 0.28$). Counts of the olfactory bulb glomerular layer suggest Thy1-aSyn mice have fewer aSyn(+) cells ($p < 0.001$), with no treatment effect. These results suggest that intranasal administration of carnosine decreases somatic aSyn in the SNpc, which may underlie the improved motor function found previously in Thy1-aSyn model of Parkinson's Disease.



Title

Effects of Angiotensin II Type 1 A Receptor (AT1aR) on renal and urinary biomarkers of acute kidney injury in Two-kidney One Clip model of Renovascular Hypertension.

Authors

Anhar Hosawi, Sanjeev Dhakal, Laale Alawi, Harshal Sawant, Unmesha Thanekar, Nadja Grobe, Khalid M. Elased

Affiliation and Address

Boonshoft School of Medicine, Wright State University, Dayton, OH

Abstract

Renin angiotensin system (RAS) plays a key role in the pathogenesis and progression of hypertension and renal injury. Angiotensin-II (Ang-II) type 1 receptor (AT1R) mediates the action of Ang-II to increase blood pressure, fluid retention and aldosterone secretion. Ang-II is involved in cell injury, vascular remodeling and inflammation. These effects could be counteracted through its catabolism into vasodilator peptide, Ang (1-7) by angiotensin converting enzyme 2 (ACE2) and/or neprilysin (NEP). Our previous studies demonstrated urinary shedding of renal ACE2 and NEP in the *db/db* diabetic mice which could act as biomarker for diabetic kidney disease (DKD). The aim of the study is to test the hypothesis that there is increased urinary ACE2 and NEP shedding in the two-kidney one clip (2K1C) model of renovascular hypertension. Further, we investigated the effects of deleting AT1aR on these RAS components and on kidney injury molecule-1 (KIM-1). Renovascular hypertension was induced in AT1aR knock out (AT1aKO) and WT mice by 2K1C surgery. Blood pressure (BP) measurements by radio-telemetry revealed significant increase in 2K1C mice ($p < 0.001$ vs. sham). Deleting AT1aR significantly decreased BP in 2K1C mice compared to 2K1C WT mice ($p < 0.001$). Renal expression of ACE2 and NEP were significantly decreased in the clipped kidney of WT and AT1aKO group ($p < 0.05$). In contrast to diabetes, there was no detectable urinary ACE2 and NEP expression and activity in 2K1C mice. However, urinary KIM-1 expression was increased in 2K1C mice. Although deleting AT1aR attenuated albuminuria and hypertension, it did not alter urinary KIM-1 expression. These results suggest that the decreased renal NEP and ACE2 in the clipped kidney of 2K1C model may worsen kidney injury via impaired Ang (1-7) formation. In conclusion, urinary KIM-1 could serve as an early indicator of non-diabetic acute kidney injury. Urinary ACE2 and NEP could be used as biomarkers of DKD.



Title

Associations between Mood and Manganese Exposure in Welders

Authors

Mahsa Servati, Khunsha Ahmed, Humberto Monsivais, Ulrike Dydak

Affiliation and Address

School of Health Sciences, Purdue University, 550 Stadium Mall Drive, West Lafayette, IN 47907

Abstract

Introduction

Chronic manganese (Mn) exposure can lead to cognitive, psychiatric and motor deficits, with mood disorders being reported as one of the earliest symptoms in humans exposed to Mn. The purpose of this study was to assess whether mood changes in welders exposed to Mn were associated with Mn exposure or neuroimaging markers, such as elevated brain Mn levels and thalamic γ -aminobutyric acid (GABA) levels, assessed by magnetic resonance imaging (MRI) and spectroscopy (MRS).

Methods

Forty-four male welders were recruited from a local manufacturer. Each subject underwent an MRI/MRS exam to assess brain Mn deposition, as measured by the R1 relaxation rate, and thalamic GABA levels. The Brief Symptom Inventory (BSI) was administered to assess nine domains of mood, as well as a global severity index. Individual exposure to Mn was assessed using personal air sampling and a work history questionnaire. Spearman Rank Tests were performed to test for associations between raw mood scores and R1 values, thalamic GABA levels and Mn exposure in the welders. Furthermore, the standardized t-scores for the mood values were compared against normative standards.

Results

Comparing the BSI t-score of the Global Severity Index (GSI), a summary score indicating the subject's distress level, against the male adult non-patient norm disclosed that 49% of the welders were classified as "clinical cases". The subdomains of somatization (SOM), obsessive-compulsive (OC) behavior and interpersonal sensitivity (IS) were significantly associated with elevated past-three months Mn exposure (SOM, IS: $\rho > 0.3$, $p < 0.05$) and/or toenail Mn levels (SOM, OC: $\rho \geq 0.3$, $p < 0.05$). Thalamic GABA levels were significantly associated with depression scores ($\rho = -0.44$, $p = 0.02$), and Mn levels of the Caudate Nucleus were found to be inversely associated with 5 of the 9 mood domains, as well as the overall GSI (all $\rho < -0.3$, $p < 0.05$).

Conclusion

In agreement with previous literature we report increased changes in mood and associations with increased Mn exposure in a typical occupational welding setting in the US with Mn exposure levels around 0.1 mg/m³. The association of depression scores with thalamic GABA is in line with reports of thalamic lesions being implicated with depression. However, the findings of an inverse relation to brain Mn levels will need to be investigated further. Factors, such as alcohol consumption and shift work will need to be considered as confounders.



Title

A Pilot Project to Apply a Collaborative Framework to Brownfields Redevelopment in Northern Kentucky

Authors

Yvonne Sene¹, Emmanuella Asiedu¹, Andrew Maier², Mark Maddaloni², Jacqueline Patterson³, Christine Perdan Curran¹

Affiliation and Address

1. Northern Kentucky University Department of Biological Sciences, Highland Heights KY
2. Cardno ChemRisk, Cincinnati OH
3. University of Cincinnati College of Medicine

Abstract

A brownfield is a site formerly used for industrial or commercial purposes where future use could be affected by contamination. The goal of this pilot project is to apply and further develop a collaborative framework to enhance community engagement in chemical risk assessment and decision-making for contaminated site redevelopment. The project began with an intensive search of the scientific literature to identify best practices in community-engaged research and risk communication. We also reviewed publicly available documents on over 300 potential redevelopment sites in Northern Kentucky. Following consultation with environmental experts and government agencies, we narrowed the list of sites to 10 for in-depth consideration. The site furthest along with redevelopment plans was the Marianne Theater, a historic building in Bellevue, KY. We are now working with city leaders and residents to develop messaging regarding the building and its value to the community. We will rely on information gained about three other historic theater projects from other area of Kentucky as well as data gathered on successful and unsuccessful brownfield redevelopment projects. The ultimate goal is to identify critical success factors and obstacles that hinder effective redevelopment. A key lesson learned in the early stage of the project was the importance to communicate more than environmental information to gain wider acceptance of a project within a community and stronger financial support to complete the remediation and renovations.



Title

Expression of copper transport protein-2 (CTR2) in the blood-CSF barrier: Effect of lead exposure in vitro

Authors

Luke (Luqing) Liu, David Du, and Wei Zheng*

Affiliation and Address

School of Health Sciences, Purdue University, West Lafayette, IN 47907

Abstract

Copper (Cu) is an essential metal for living organisms; either deficiency or overload of Cu can cause cellular dysfunctions. Thus, the Cu homeostasis is tightly regulated by multiple copper transport proteins. Copper transport protein-2 (CTR2) has recently been suggested to regulate the function of copper transport protein 1 (CTR1) so to maintain the stable intracellular Cu level. However, the existence of CTR2 in the blood-cerebrospinal fluid (CSF) barrier (BCB) in brain choroid plexus, which regulates material movements between the blood and CSF, was virtually unknown. This study was designed to explore the existence of CTR2 in the BCB; furthermore, the effect of in vitro exposure to lead (Pb), a toxic metal abundantly accumulated in the choroid plexus, on the expression of CTR2 was investigated. Immortalized choroidal epithelial Z310 cells and freshly isolated mouse plexus tissues were used to identify the expression of CTR2. Both qPCR and confocal immunofluorescent data demonstrated the presence of CTR2 in Z310 cells as well as in plexus tissues; in Z310 cells, CTR2 appeared to be localized in cytoplasm and stored in vesicles, while in plexus tissues, CTR2 signals were primarily present in the cytosol between nuclei and brush boarder facing the CSF. When Z310 cells were incubated in a Pb-containing medium at 2, 5, and 10 μM for 24 hrs, the levels of CTR2 expression, as quantified by immunofluorescence, were reduced by 35.50%, 37.14%, and 52.10%, respectively, as compared to controls ($p < 0.05$, $n = 4$). The confocal data also revealed that incubation of plexus tissues in a Pb-containing artificial-CSF significantly reduced the fluorescent signals associated with CTR2. Moreover, CTR2 signals in Pb-treated plexus tissues appeared to be concentrated in areas beneath the epithelial microvilli, suggesting an intracellular trafficking of CTR2 towards the CSF-facing epithelial surface. These observations provide the first-hand evidence that CTR2 exists in the blood-CSF barrier, and Pb exposure not only reduces the expression of CTR2, but also causes it to translocate toward the epithelial microvilli. Mechanistic studies to investigate the function of CTR2, in coordination with CTR1, in regulating Cu transport by the BCB, and the consequence of Pb exposure are currently in progress. (Supported in part by NIH/NIEHS R01 ES027078)



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Title

Activation of Th1/Th17 Pathways are Associated with Steroid Resistance in Mouse Models of Allergic Airway Inflammation

Authors

Brandon W. Lewis¹, Devine Jackson¹, Manel Guessas¹, Sonia Guessas¹, Josh Walum¹, YS Prakash^{3,4}, and Rodney D. Britt Jr^{1,2}.

Affiliation and Address

¹The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH.² Department of Pediatrics, Ohio State University, Columbus, OH. ³Department of Physiology and Biomedical Engineering, ⁴Department of Anesthesiology and Perioperative Medicine, Mayo Clinic, Rochester, MN

Abstract

Introduction: Pediatric asthma is a chronic pulmonary inflammatory disease that is characterized by airway hyperresponsiveness (AHR), restricted airflow, and increased immune cell infiltration. The adaptive immune response to environmental insults, such as allergens, is thought to heavily influence asthma phenotype and severity. Previous studies have shown increases in Th1 and Th17 effector T cells in patients with steroid resistant severe asthma, suggesting that Th1 and Th17 inflammation promotes steroid resistance. In the present study, we tested the hypothesis that induction of Th1/Th17 inflammation, via the bacterial second messenger c-di-GMP, will reduce corticosteroid sensitivity in newborn mice chronically challenged with allergens.

Methods and Results: Newborn mice were intranasally sensitized and challenged with saline, mixed allergen (MA; 10 µg *Aspergillus*, *Alternaria*, ovalbumin, and house dust mite), or c-di-GMP+MA for 3X/week for 7 weeks. During the last two weeks, mice were IP injected with vehicle, 1 mg/kg Fluticasone propionate (FP), and 3 mg/kg FP. The following day, lungs were harvested, and T cell populations were assessed by flow cytometry. Lung function and cytokine measurements were performed. Mice exposed to MA and c-di-GMP+MA exhibited substantially increased AHR. In contrast to MA challenge, the effects of c-di-GMP+MA on AHR were not altered with 1 or 3 mg/kg FP. Additionally, c-di-GMP+MA mice exhibited robust Th1 and Th17 responses as indicated by increased IFN γ and IL-17A levels and IFN γ - and IL-17A-producing CD4⁺ T cells. While AHR remains increased, treatment with 3 mg/kg FP suppressed Th1 but not Th17 CD4⁺ T cells. In regard to signaling pathways, STAT1 phosphorylation was significantly increased in c-di-GMP+MA, while treatment with 1 mg/kg FP and 3 mg/kg FP reduced STAT1 phosphorylation.

Conclusions: Taken together, these data show that c-di-GMP+MA induces a Th1/Th17-skewed immune environment that may facilitate steroid resistance. Steroids had differential effects in c-di-GMP+MA mice as evidenced by dampening of Th1 cells while AHR persists in the presence of steroids. Further understanding the role of Th1/Th17 inflammation in allergic airway inflammation will enhance understanding of steroid resistance in severe asthma.



Title

An Endocrinized Fibroblast Growth Factor 1 Variant Reverses Nonalcoholic Fatty Liver Disease via Activating AMPK in Type 2 Diabetes

Authors

Qian Lin¹, Zhifeng Huang³, GenXiang Cai², Paul N. Epstein, Kupper A. Wintergerst, Daniel J. Conklin, Lu Cai¹, Yu Li^{*2}, Yi Tan^{*1}

Affiliation and Address

¹Department of Pediatrics, University of Louisville, Louisville, KY, USA; ²University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China.

Abstract

Nonalcoholic fatty liver disease (NAFLD) is common in type 2 diabetes (T2D) patients. Our recently engineered FGF1 partial agonist (FGF1^{ΔHBS}) exhibits reduced proliferative potential, while preserving the full metabolic activity of native FGF1. This study showed that administration of FGF1^{ΔHBS} to 2-month-old db/db mice for 2 months lowered blood glucose, improved insulin sensitivity, lowered liver weight, lipid deposition, and inflammation and improved liver function. Simultaneously, FGF1^{ΔHBS} prevented diabetes-induced oxidative stress, promoted nuclear translocation of antioxidant transcription factor Nrf2 and elevated downstream antioxidant genes. In addition, FGF1^{ΔHBS} inhibited activity and/or expression of hepatic lipogenic genes SREBP-1, FAS and SCD-1. Furthermore, FGF1^{ΔHBS} restored hepatic fatty acid oxidation signaling including elevated CPT-1α, PPARα and PGC-1α, increased phosphorylation of AMPK along with AMPK substrates ACC1 and SREBP1. This demonstrates association of FGF1^{ΔHBS} modified lipid metabolism with activation of AMPK signaling. Mechanistically, hepatic cells treated with palmitate mimicked T2D phenotype of hepatic oxidative damage and lipid disorder, all of which could be reversed by supplementing cells with FGF1^{ΔHBS}. Nrf2 knockdown by SiRNA abolished anti-oxidative actions of FGF1^{ΔHBS} but did not affect FGF1^{ΔHBS} actions on lipid metabolic disorder. Whereas, AMPK inhibition, using Compound C or SiRNA knockdown, abolished both the ability of FGF1^{ΔHBS} to prevent lipid metabolic disorder and produce antioxidative protection. Remarkably, 3 months administration of FGF1^{ΔHBS} to 9-month-old db/db mice completely reversed late NAFLD and activated Nrf2 and AMPK pathways. Most importantly, liver-specific AMPK knockout almost completely abrogated the preventive effects of FGF1^{ΔHBS} on NAFLD induced by HFHS diet. Our findings indicate that in addition to glucose-lowering and insulin-sensitizing effects, FGF1^{ΔHBS} can reverse NAFLD in T2D by activating AMPK, which prevents hepatic lipotoxicity by inhibiting lipogenesis and activating lipid oxidation.



Title

Alternative Splicing as a Key Mechanism in Arsenic-Induced Squamous Cell Carcinoma: Evidence from RNA-Seq and Proteomic Analysis

Authors

Ana P. F. Cardoso¹, Mayukh Banerjee¹, Daniel W. Wilkey¹, Laila Al-Eryani^{1,2}, Mohammed A. A. Sayed¹, Juw W. Park¹, Michael Merchant¹ and J. Christopher States¹

Affiliation and Address

¹ University of Louisville, Louisville, KY; and ² National Cancer Institute, Bethesda, MD.

Abstract

Background: Chronic exposure to arsenic, a group I carcinogen, leads to multi-organ cancers, skin being the primary target, although, the molecular etiology remains elusive. Arsenic exposure has been reported to induce differential mRNA splicing. Abrogation of normal alternative splicing patterns have been linked to several cancers, as the expression of many cancer-related genes are regulated by alternative splicing. We hypothesized that differential mRNA splicing in protein coding genes could be a driving force in an established cell line model of arsenic-induced skin carcinogenesis. Methods: HaCaT cells exposed to 0 (As⁻) or 100 (As⁺) nM NaAsO₂ were maintained for 7 weeks in independent quadruplicates. RNA and proteins were isolated from each sample. RNA-seq was performed followed by analysis of the data by rMATS algorithm to identify significant differential splicing events between the As⁻ and As⁺ samples. In addition, we performed ESI tandem mass spectrometric analysis to quantify differential proteome expression in whole cell lysates of the same samples. Results: 1,149 skipped exon events were significantly different between As⁻ and As⁺, of which 61.5% resulted in protein coding changes. From proteomic analysis, 227 differentially expressed proteins were found. Gene ontology (GO) analysis of the alternatively spliced genes found enrichment in several splicing related pathways (83 unique genes). In accordance, GO analysis of the proteomic profile also showed splicing as a major enriched category (37 unique proteins). Comparison of over-represented pathways from alternative splicing and proteomic data revealed 3 common molecules: Splicing Factor SWAP (*SFSWAP*), DEAD-Box Helicase 42 (*DDX42*) and Serine/Arginine Repetitive Matrix 2 (*SRRM2*). These genes are related to mRNA splicing and are associated with cancer. Conclusion: Presence of overlapping unique genes/proteins in the two datasets that are involved in the same splicing regulation pathways suggests that global modulation of mRNA splicing by arsenic exposure plays a role in arsenic-induced carcinogenesis. Funding: NIH grants R01ES027778, R21ES023627, P20GM103436, R15GM126446, P20GM113226.



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Title

KRAS Pathway based Targeted Therapy Responses in Pancreatic Cancer

Authors

Abdullah Althiaban, Anita Thyagarajan, Ravi P. Sahu

Affiliation and Address

Wright State University Boonshoft School of Medicine, Dayton, OH 47907

Abstract

Pancreatic cancer is among the leading causes of cancer-related deaths. There have been advancements in the treatment approaches for pancreatic ductal adenocarcinoma (PDAC), yet the response rates in PDAC patients were relatively low and/or short-lived. Therefore, there is an urgent need to introduce novel approaches to improve the efficacy of current therapies. As mutations in the KRAS oncogene have been found to be the most prevalent, yet difficult to target, strategies to target the downstream signaling cascades (Ras-Raf-MEK-ERK) have been explored for PDAC. Notably, therapies targeting MEK and ERK kinases have been associated with promising outcomes. Here, we discuss the recent updates with particular focus on MEK and ERK-based approaches for PDAC.



Title

INSULIN-like growth factor-1(IGF-1) impacts p53 function in UVB-irradiated human keratinocytes and skin epidermis

Authors

Abdulrahman Alkawar¹ and Michael G. Kemp¹

Affiliation and Address

Dept. of Pharmacology and Toxicology, Wright State University Boonshoft School of Medicine

Abstract

Skin cancer is the most prevalent human malignancy and is primarily caused by ultraviolet (UV) wavelengths of sunlight. However, the fact that most skin cancers occur in people over the age of 60 indicates that advantaged age is a second skin cancer risk factor. Why geriatric skin is prone to developing skin cancers is not clear, but several studies have shown that dermal fibroblasts in geriatric skin express lower levels of the hormone insulin-like growth factor-1 (IGF-1) than young adult skin and that deficient IGF-1 signaling negatively impacts how epidermal keratinocytes respond to UVB radiation. A major regulator of the cellular response to UVB-induced DNA damage response is the tumor suppressor protein p53, and a previous study indicated that p53 is not properly activated in UVB-irradiated human keratinocytes deficient in IGF-1 signaling. Using cultured human keratinocytes in vitro, we show here that several downstream transcriptional targets of p53, including the cell cycle-dependent kinase inhibitor p21, the translesion synthesis polymerase pol eta, and the DNA repair factors XPC and DDB, are not properly induced following UVB exposure in cells deprived of IGF-1. Using discarded human skin from routine panniculectomies, we show that the topical application of an IGF-1 receptor antagonist similarly abrogates the ability of UVB exposure to properly induce p21 protein. In a skin sample from a geriatric individual, we further demonstrate that the injection of recombinant IGF-1 into the skin partially restores the UVB-dependent induction of pol eta at the mRNA level. Because these various p53 target gene products are involved in delaying entry into S phase, accurately replicating UVB photoproducts, and removing UVB photoproducts from genomic DNA, our findings indicate that the inability of IGF-1-deficient geriatric skin properly activate p53-response genes may predispose geriatric skin to mutagenesis and carcinogenesis.



Title

Alternative Non-invasive Insulin Delivery for Management of Diabetes

Authors

Abeer Najjar, Khalid M. Elased

Affiliation and Address

Department of Pharmacology & Toxicology, Wright State University, Boonshoft School of Medicine, 3640 Colonel Glenn Hwy. Dayton, OH, 45435

Abstract

The prevalence of diabetes is increasing worldwide. Many of type 2 diabetic patients and all type 1 diabetic patients require insulin for the management of hyperglycemia. Insulin syringe and pumps were modernized for minimal invasive subcutaneous insulin delivery. However, patients are hesitant or defer to embrace multiple insulin injections, due to concern of needle phobia, lipodystrophy, and hypoglycemia. Therefore, minimally invasive alternatives delivery for insulin is desirable. Administration of insulin to the lungs was the first reported novel approach to deliver insulin. In 2006 Exubera®, was the first inhaled insulin approved by the FDA. However, in 2007 it was withdrawn due to poor sale and failure to gain acceptance from patient and physicians. The innovation for inhaled insulin continued, in 2014 Afrezza was approved by the FDA. Afrezza indicated as combination therapy for T1DM and T2DM together with long-acting insulin. Afrezza is a powder ultra-rapid acting insulin with fast absorbance and elimination rate that leads to reduce the incidence of hypoglycemia. This may improve patient satisfaction and acceptability of this therapy. However, there is a black box warning of acute bronchospasm risks. Afrezza is contraindicated in chronic pulmonary disease situation such as asthma or COPD. The aim of the study is to give an updated overview of the effectiveness and safety of inhaled insulin profile based on key and recent clinical trials and find if there is any evidence of pulmonary function safety issues that would be a concern for the use of Afrezza. Additionally, the study reviews some of the different insulin technology that is under investigation. This includes oral, buccal, peritoneal and transdermal delivery of insulin. In conclusion, alternative routes of administration of insulin could improve the patient's quality of life and compliance.



Title

Phase-specific Influences of Electronic Cigarette Flavorings and Acrolein on Platelet Aggregation

Authors

Andre Richardson^{1,2}, Tatiana Krivokhizhina, Ph.D.², Stanley D'Souza, Ph.D.³, Sanjay Srivastava, Ph.D.^{2,4}, Daniel J. Conklin, Ph.D.^{1,2,4}

Affiliation and Address

Dept. Pharmacology & Toxicology¹, Diabetes and Obesity Center², Dept. Physiology³, Div. Environmental Medicine⁴

Abstract

The platelet aggregometry assay is the gold standard platelet function assay that measures the ability of different stimuli to induce platelet-platelet aggregation *in vitro*. There is evidence showing that tobacco- and electronic cigarette (e-cig)-derived constituents such as acrolein and e-cig flavorings exacerbate platelet activation -- a prothrombotic condition. Because little is known about how tobacco constituents affect the phases of platelet aggregation, we investigated direct effects of acrolein and e-cig flavorings on the phases of adenosine diphosphate (ADP)-induced platelet aggregation. By knowing what phase is more affected, we will more fully understand the mechanism of these pro-thrombotic agents. Human platelet rich plasma (PRP) was obtained via centrifugation of whole blood from healthy volunteers for use in a platelet aggregometry assay. A ChronoLog aggregometer measured aggregation photometrically post-ADP (2.5 or 10 μ M) induction over 5 min. Prior to ADP, PRP was incubated with increasing concentrations of e-cig flavorant compounds (e.g. cinnamaldehyde, and eugenol) or acrolein for five min at 37°C. Platelet poor plasma (PPP) was used as the reference sample. Using a paired Student's *t*-test, the percent responses of phase 1 and phase 2 of flavorings or acrolein were compared with respect to their control. Statistical significance was accepted where $p < 0.05$. At the concentrations tested of cinnamaldehyde, we observed no change in overall aggregation as well as the phase 1 and phase 2 responses compared with ADP-only control. Eugenol displayed an antiplatelet response of ADP-induced platelet aggregation. Furthermore, acrolein has no influence on ADP-induced aggregation. However, at 10 and 30 μ M of acrolein, we observed varying biphasic responses. Under the conditions tested, though we did not find any consistent changes in the biphasic activity of acrolein or the flavorants compared with control, it is important to understand how the constituents of tobacco smoke and e-cig aerosols may impair platelet function. This method of analyzing platelet function will help evolve the field of platelet aggregometry testing, to provide better details of biphasic activity in laboratory testing of platelet function, and to understand prothrombotic actions associated with smoking.



Title

TIP60 upregulates Δ Np63 α to promote cellular proliferation

Authors

Andrew J. Stacy, Jin Zhang, Akshay Hira, Michael P. Craig, and Madhavi Kadakia

Affiliation and Address

Dept. of Biochemistry and Molecular Biology, Wright State University.

Abstract

An estimated 5.4 million cases of non-melanoma skin cancer (NMSC) are reported in the U.S. at an associated cost of \$4.8 billion. Δ Np63 α , a proto-oncogene in the p53 family of transcription factors, is overexpressed in squamous cell carcinoma (SCC) and associated with poor prognosis and survival. Δ Np63 α elicits its tumorigenic effects, in part, by promoting cellular proliferation and cell survival. Despite its importance to SCC, the upstream regulation of Δ Np63 α is poorly understood. In this study, we identify TIP60 as a novel upstream regulator of Δ Np63 α . Using a combination of overexpression, silencing, and stable expression approaches in multiple cell lines, we showed that TIP60 upregulates Δ Np63 α expression. Utilizing a pharmacological inhibitor and cycloheximide treatment, we showed that TIP60 catalytic activity is required for stabilization of Δ Np63 α protein levels. We further showed that TIP60 inhibits Δ Np63 α ubiquitination and proteasomal degradation by immunoprecipitation of ubiquitinated Δ Np63 α with and without TIP60 overexpression. Stabilization of the Δ Np63 α protein was further associated with TIP60-mediated acetylation. Finally, we demonstrated that TIP60-mediated regulation of Δ Np63 α increases cellular proliferation by promoting G2/M progression by performing MTS assays and flow cytometry. Taken together, our findings provide evidence that TIP60 may contribute to SCC progression by increasing Δ Np63 α protein levels thereby promoting cellular proliferation.



Title

Overexpression of hsa-miR-186 induces anchorage-independent growth and chromosomal alterations in arsenic-exposed human keratinocytes: A preliminary study

Authors

Angeliki Lykoudi, Ana P. F. Cardoso, Jiguo Wu*, Sandra S. Wise and J. Christopher States

Affiliation and Address

Dept. Pharmacology and Toxicology, University of Louisville, Louisville, KY, USA,
*Guangdong Provincial Key Laboratory of Tropical Medicine, Southern Medical University, Guangzhou

Abstract

Background: Chronic arsenic (As) exposure through drinking water is a global health issue, affecting >200 million people. As is a group I human carcinogen and a clastogen causing chromosomal instability (CIN). Skin is the primary target organ for As toxicity. miRNA dysregulation and CIN are suggested mechanisms of As carcinogenesis. hsa-miR-186 is overexpressed in As-induced squamous cell carcinoma relative to premalignant hyperkeratosis. Predicted targets of miR-186 are cell cycle proteins, thus overexpression of miR-186 likely leads to CIN and aneuploidy, which are features of cancer. We showed that overexpressing miR-186 in HaCaT cells increased numerical and structural chromosomal abnormalities. **Hypothesis:** miR-186 overexpression drives malignant transformation of HaCaT cells by induction of CIN and potentially accelerates As-induced transformation. **Methods:** Stable clones of HaCaT transfected with miR-186 expression vector or empty vector were maintained under puromycin selection. Selected clones exposed to 0 or 100 nM As, were cultured for 10 weeks. Anchorage independent growth was tested in soft agar colony formation assay. Giemsa banding was used to produce karyotypes and cytogenetic analysis for CIN was performed. **Results:** HaCaT overexpressing miR-186 and exposed to As exhibited signs of anchorage independent growth since single cells formed clusters of cells in agar while HaCaT transfected with vector control did not. As-exposed HaCaT that overexpress miR-186 also contained additional chromosomal material in one of the four copies of chromosomes 1 and 11; increases in double minute chromosomes; two copies of the abnormal isochromosome 9q and one extra chromosome 18. HaCaT authenticity was validated by the presence of reported marker chromosomes and STR mapping. **Discussion:** Consistent chromosomal abnormalities and increased growth ability in agar were observed in As exposed miR-186 overexpressing keratinocytes, suggesting contribution of CIN to malignant transformation. Only one clone was tested per group and these karyotypic changes might be that clone's feature and not an effect of As toxicity. Thus, future research using additional clones is warranted.



Title

Study Colloidal Silver Nanoparticles: A more effective bactericidal agent than chlorine against waterborne Gram-negative bacteria

Authors

Ashley Wilcox, Marjorie Markopoulos, John Ryan, Alex Gilman, Ioana Pavel

Affiliation and Address

Wright State University, 3640 Colonel Glenn Hwy, Dayton, OH 45435

Abstract

The main aim of this study was to determine the antimicrobial and bactericidal activity of unfunctionalized, silver nanoparticles (eAgNP) of ~10 nm in diameter against well-established water indicator organisms: *Escherichia coli*, *Klebsiella variicola*, and *Pseudomonas aeruginosa*. This was achieved by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of eAgNP, which were synthesized electrochemically, size-selected, concentrated, and purified by tangential flow filtration. The MIC values for *E. coli*, *K. variicola*, and *P. aeruginosa* were 0.75-4.02, 1.09-4.08, and 1.55-5.39 mg/L, respectively. The MBC values for the same bacteria were 1.51-3.96, 1.55-5.39, and 2.18-4.99 mg/L, respectively. When tested against chlorine, the MIC values increased 1000-, 2000-, and 3000-fold, respectively, for mentioned bacteria. The MBC values increased 1000-, 1500-, and 1500-fold, respectively. ICP-OES was then used to track potassium leakage in order to determine cellular membrane damage. CytoViva Hyperspectral Imaging was then performed to confirm the proposed mechanistic aspects associated with the attachments of the negatively charged eAg to the cellular membrane and their subsequent structural disruption.



Title

Creatine Protects Fibroblasts From Stress Induced Senescence.

Authors

Avinash S Mahajan¹, Michael G Kemp¹, Christine M Rapp¹, Jeffrey B Travers^{1,2}

Affiliation and Address

1. Dept. of Pharmacology and Toxicology, Wright State University.
2. Dayton Veterans Affairs Medical Center, Dayton, OH

Abstract

Dermal fibroblasts provide structural support to the skin by producing collagen beneath the epidermis. Fibroblasts also produce insulin like growth factor-1 (IGF-1), which binds to IGF-1 receptors (IGF-1Rs) on keratinocytes to activate signaling pathways that regulate cell proliferation and cellular responses to genotoxic stressors like ultraviolet B radiation found in sunlight. Our group has determined that the lack of IGF-1 expression due to fibroblast senescence in the dermis of geriatric individuals is correlated with an increased incidence of skin cancer in geriatric patients. The present studies were designed to test the hypothesis that pro-energetics like creatine can protect fibroblasts against senescence. To that end, we used an experimental model of senescence in which primary human fibroblasts are treated with hydrogen peroxide in vitro, with senescence measured by staining for beta-galactosidase activity (+beta-gal). Our studies indicate that pretreatment with creatine protects human fibroblasts from H₂O₂-induced senescence. These studies suggest a potential strategy for protecting fibroblasts in geriatric skin from undergoing stress-induced senescence, which may maintain IGF-1 levels and limit mutagenesis and carcinogenesis in epidermal keratinocytes.



Title

E-Cigarette vaping liquids and a flavoring chemical perturb bone, cartilage and vascular development in zebrafish embryos

Authors

Beas Bhattacharya¹, Vedang Narain¹, Maria Bondesson¹

Affiliation and Address

Dept. of Intelligent Systems Engineering, Indiana University, Bloomington

Abstract

Electronic cigarettes are an increasingly popular smoking device, especially among adolescents and youth. E-cigarettes are perceived to be a safer alternative to tobacco cigarettes leading to that pregnant mothers may replace tobacco smoking with e-cigarette use. However, whether these products have any side effects on the developing embryo has not been thoroughly evaluated. We tested cinnamon flavored e-liquids (with 12 mg/ml nicotine and without nicotine) and the flavoring chemical cinnamaldehyde on zebrafish embryonic development. We studied possible effects on hatching success, bone, cartilage and blood vessel development in 3-4 days old transgenic zebrafish larvae. Our study showed that the e-cigarette liquids significantly affected craniofacial bone and cartilage development. Exposure also resulted in structural malformations in intersegmental blood vessels. Hatching frequency was significantly delayed by vaping liquid exposure. Presence of nicotine in the e-liquid only slightly augmented the perturbations identified. Exposure to the favoring chemical cinnamaldehyde induced similar malformations as the vaping liquids, but at ten times lower concentration. Exposure to the humectant propylene glycol, present in e-liquids, did not cause any visible effect on hatching or bone development in zebrafish larvae at the concentrations tested. In conclusion, we show that exposure to cinnamon flavored e-cigarettes or the flavoring chemical cinnamaldehyde during embryonic development cause significant structural malformations in bone, cartilage and vasculature.



Title

Assessing the role of chronic arsenic in disrupting the EGFR signaling axis

Authors

Christine Kim, Brian P. Ceresa, Ph.D.

Affiliation and Address

Department of Pharmacology and Toxicology, University of Louisville

Abstract

Lung cancer is the leading cancer in the U.S., and Kentucky leads the nation with the highest lung cancer death rates. Although smoking is associated with lung cancer development, 20% of patients who die from lung cancer in the U.S., have never smoked, suggesting there are other risk factors. One of these risk factors is arsenic exposure. Fifteen percent of the U.S. population drinks domestic well water, and in many areas, this unregulated well water contains levels of arsenic that exceeds the Environmental Protection Agencies (EPA) recommended levels. Though there is a strong association between arsenic exposure and lung cancer development, the clear mechanism is unknown. Further, previous studies have shown acute arsenic exposure increases expression of the epidermal growth factor receptor (EGFR), a cell surface receptor tyrosine kinase that is associated with many different types of cancer, including lung cancer. We hypothesize that chronic arsenic exposure disrupts the EGFR endocytic trafficking, leading to increased receptor expression. This project examines the impact of chronic "environmentally relevant" levels of arsenic on the EGFR expression, distribution and trafficking. A non- malignant human bronchial epithelial cell line, Beas-2B cells were exposed to 100 nM sodium arsenite for 24 weeks. This concentration was chosen, as it is the average blood arsenic level in individuals who are exposed to high levels of arsenic. The chronic arsenic exposure increased EGFR protein expression levels and its activity, increased transcription and protein levels of TGF α , and altered the distribution of the EGFR. TGF α , unlike the other EGFR ligands, diverts the stimulated receptor from lysosomal degradation. Consistent with an increased protein level of TGF α , we observed increased cell surface level of EGFR in response to chronic arsenic exposure.



Title

Corticosteroids Reduce Effects of IL-17A on Inflammation and Proliferation in Human Pediatric Airway Smooth Muscle Cells

Authors

Devine Jackson¹, Brandon W. Lewis¹, Elise Coneglio¹, Manel Guessas¹, and Rodney D. Britt Jr^{1,2}.

Affiliation and Address

¹The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH. ² Department of Pediatrics, The Ohio State University, Columbus, OH.

Abstract

Introduction: Asthma is characterized by chronic inflammation, airway hyperresponsiveness (AHR), remodeling, restricted airflow, and acute exacerbations. Airway smooth muscle (ASM) structure and function plays a critical role in airway constriction and inflammation. IL-17A has been linked to driving AHR and steroid resistance as evidenced by previous studies showing increased IL-17A and IFN γ levels in sputum and bronchial biopsies of patients with severe asthma. However, little is known about the role of IL-17A, and its interaction with IFN γ , in severe pediatric asthma. In the present study, we assessed the effects of IL-17A on production of chemokines and proliferation in human pediatric ASM.

Methods and Results: Human pediatric ASM was isolated from airways of donors or patients undergoing tracheal reconstruction surgery (0-21 years old; NCH IRB approved). Cultured ASM were treated with vehicle (0.01% DMSO), 10 nM fluticasone propionate (FP), 10 ng/mL IL-17A, and/or 25 ng/mL IFN γ for 24 h. Cells were harvested to measure mRNA levels of pro-inflammatory genes (CXCL1, CXCL2, CXCL3, and CXCL10). Media was collected to measure CXCL10 levels by ELISA. CXCL1, 2, and 3 mRNA levels were increased by IL-17A, while CXCL10 was increased by only IFN γ . Treatment of FP inhibited IL-17A-induced CXCL1, 2, and 3 mRNA levels but IFN γ -induced CXCL10 mRNA expression remains increased. Similarly, IFN γ -induced secretion of CXCL10 was reduced by FP, but this anti-inflammatory effect was absent in cells treated with IL-17A/IFN γ . Additionally, IL-17A increased pediatric ASM proliferation, however these effects were inhibited by treatment with FP.

Conclusions: Our data suggest that effects of IL-17A on pediatric ASM chemokine production and proliferation are sensitive to corticosteroids. However, interactions between IL-17A and IFN γ may contribute to increased steroid resistance in pediatric ASM. While the effects of IL-17A on pediatric ASM may be corticosteroid-sensitive, interactions with additional pathways may promote ASM inflammation in severe asthma.



Title

Sulforaphane Improves Right Ventricular Function and Reduces Pulmonary Vascular Remodeling in Pulmonary Arterial Hypertension

Authors

Yin Kang^{1,2}, Guangyan Zhang^{1,2}, Sheng Wang³, Jiapeng Huang², Jun Cai¹, Lu Cai¹, Bradley B. Keller²

Affiliation and Address

¹Pediatric Research Institute, ² Cardiovascular Innovation Institute, University of Louisville,

³Department of Anesthesiology, Guangdong Cardiovascular Institute, Guangzhou, China

Abstract

Right ventricular (RV) dysfunction is the main determinant of mortality in patients with pulmonary arterial hypertension (PAH) and there are no therapies designed to prevent RV injury in PAH. Inflammation is known to play an important role in the pathogenesis and progression of PAH and in diabetes-induced cardiac injury. Sulforaphane (SFN), a potent Nrf2 activator, has shown significant anti-inflammatory effects and cardiac protection in preclinical diabetic models. Therefore, we investigated the preventive effects of SFN in a murine model of acquired PAH induced by the combination of SU5416 (vascular endothelial growth factor receptor inhibitor), and 10% hypoxia (SuHx) for 4 weeks in male mice. Mice were randomized to be treated with the anti-inflammatory nutraceutical agent SFN at a daily dose of 0.5 mg/kg, 5 days per week for 4 weeks, or to vehicle control. Transthoracic echocardiography was performed sequentially during PAH progression to characterize chamber specific altered RV and left ventricular (LV) function along with relevant measures of histology, protein, and gene expression. SuHx induced progressive RV, but not LV, diastolic and systolic dysfunction and RV remodeling, fibrosis, and inflammation. SFN prevented SuHx-induced RV dysfunction and remodeling, reduced RV inflammation and fibrosis, upregulated Nrf2 expression and its downstream gene NQO1 and reduced the inflammatory mediator NLRP3. SFN also reduced SuHx induced pulmonary vascular remodeling, inflammation and fibrosis. Thus, SuHx-induced RV and pulmonary dysfunction, inflammation, and fibrosis can be prevented or attenuated by SFN creating the opportunity for further studies to investigate SFN and the role of Nrf2 and NLRP3 pathways in preclinical and clinical studies.



Title

Why Do Whales Have Lower Cancer Rates: Whale Cells Avoid Particulate Chromate Induced Homologous Recombination Repair Inhibition

Authors

Haiyan Lu, Cynthia L. Browning, John Pierce Wise, Sr.

Affiliation and Address

¹Pediatric Research Institute, ² Cardiovascular Innovation Institute, University of Louisville,

³Department of Anesthesiology, Guangdong Cardiovascular Institute, Guangzhou, China

Abstract

Whales are long lived species living in a complex environment that puts them at risk for long-term exposure and accumulation of environmental contaminants. However, they have low cancer rates, which may be due to better DNA protective mechanisms. How whales evade carcinogenesis is unknown. Particulate hexavalent chromium (Cr(VI)) is a human lung carcinogen. Cr(VI) induces DNA double strand breaks in human cells. Homologous recombination (HR) repair protects against these breaks by repairing them and preventing them from causing genomic instability and carcinogenesis. Prolonged particulate Cr(VI) induces DNA double strand breaks and suppresses HR repair in human lung cells. Whales are exposed to high Cr levels. This study focuses on the effect of particulate Cr(VI) exposure in whale lung cells to determine if they exhibit protective mechanisms against Cr(VI)-induced chromosome instability. Our study show Cr(VI) induced a concentration-dependent increase in DNA double strand breaks after both acute (24 h) and prolonged (120 h) exposure in whale lung cells, but did not inhibit HR repair in whale lung cells. Acute (24 h) Cr(VI) exposure caused G2/M arrest in human lung cells, but G1 arrest in whale lung cells. Prolonged (120 h) Cr(VI) exposure cause G2/M arrest in both human lung cells and whale lung cells. Future investigation of the differences in how human and whale cells respond to chemical carcinogens may provide valuable insight into mechanisms of preventing chemical carcinogenesis. The work was supported by the National Institute of Environmental Health Sciences [ES016893 to J.P.W].



Title

A Pilot Study of Proteomic Profiles in Patients who Develop Ventilator Acquired Pneumonia

Authors

Hima P. Yenuga¹, Ravi P. Sahu¹, William C. Grunwald, Jr.¹, Thomas A. Sensing², Claire E. Hardman³, Jennifer M. Lynde², John S. Bruun², Rachel L. Armstrong², Angela S. Shoen³, and John K. Bini², David R. Cool¹

Affiliation and Address

¹Department of Pharmacology & Toxicology, Wright State University Boonshoft School of Medicine; ²Department of Surgery, Wright State University Boonshoft School of Medicine; ³Boonshoft School of Medicine, Wright State Physicians

Abstract

Ventilator associated pneumonia (VAP) is the most common nosocomial infection that leads to an increase in mortality rates of ICU patients. The patients in this study were in the ICU and required ≥ 48 hours of mechanical ventilation and had a Clinical Pulmonary Infection Score ≥ 6 . In this study we recruited 16 subjects who were on ventilator for more than 48 hrs, obtained serum and sputum from the patients, frozen at -80°C and stored until use. Of those, 6 developed pneumonia (VAP) and the rest were considered as Non-VAP. Our first goal was to analyze cytokine levels, Platelet Activating factor acetylhydrolase (PAFAH), and Pentraxin 3, in the sputum as well as serum samples from these patients. Platelet Activating factor acetylhydrolase (PAFAh) is an enzyme that inactivates platelet activating factor, which can cause some cytokines to be released. Pentraxin 3 has been described as an early marker of lung injury. Using the Bio-Rad BioPlex 200 system, we analyzed 27 cytokines in sputum and serum. Of these, we found that 6 were significantly increased in the sputum of VAP patients, while only 1 was significantly increased in the serum from VAP patients. The PAFAh assay revealed no significant difference between VAP and non-VAP patient sputum or serum, though there was a trend for lower PAFAh in VAP patients compared to non-VAP. Analysis for Pentraxin 3 indicated that it was present in both sputum and serum. However, there was no significant difference between VAP and non-VAP patients. Analysis of the sputum samples by mass spectrometry revealed no obvious profile differences between VAP and non-VAP. However, the presence of a polymeric compound was observed, mostly in VAP patients, though its reason for its presence was not determined. Sequencing of fragmented peptides from the sputum also revealed that one of the peptides was from pneumonia bacteria. In conclusion, the results strongly suggest that a larger study is needed, in addition to a broad analysis of cytokines, other enzymes and proteins need to be explored as potential markers for early diagnosis and potential new treatments of ventilator acquired pneumonia.



Title

Protective Effect of LGG and Its Supernatant against Myocardial Dysfunction in Obese Mice Exposed to Intermittent Hypoxia is Associated with the Activation of Nrf2 Pathway

Authors

Hui Xu, Jiqun Wang, Jun Cai, Wenke Feng, Yonggang Wang, Quan Liu, Lu Cai

Affiliation and Address

Pediatric Research Institute, Department of Pediatrics, University of Louisville, Louisville, KY 40202
USA

Abstract

Prolonged intermittent hypoxia (IH) has been shown to impair myocardial function (mainly via oxidative stress and inflammation) and modify gut microbiota in mice. Gut microbiota plays an important role in health and disease, including obesity and cardiovascular diseases (CVD). Probiotics refer to live microorganisms that confer health benefits on the host after administration in adequate amounts. Research on novel probiotics related therapies has evoked much attention. In our previous study, both *Lactobacillus rhamnosus* GG (LGG) and LGG cell-free supernatant (LGGs) were found to protect against alcohol-induced liver injury and steatosis; however, the effects of LGG and LGGs on cardiac tissues of obese mice exposed to IH have not been determined. Here we exposed high-fat high-fructose diet (HFHFD)-induced obese mice to IH, to establish a model of obesity with obstructive sleep apnea (OSA). Mice were divided into four groups: (1) HFHFD for 15 weeks; (2) HFHFD for 15 weeks with IH in the last 12 weeks (HFHFD/IH); (3) and (4) HFHFD/IH plus oral administration of either LGG (10^9 CFU bacteria/day) or LGGs (dose equivalent to 10^9 CFU bacteria/day) over the 15 weeks, respectively. Compared to HFHFD mice, HFHFD/IH-mice showed heart dysfunction with significant cardiac remodeling and inflammation; all pathological and functional alterations were prevented by treatment with both LGG and LGGs (no significant difference between LGG and LGGs in this respect). The cardioprotective effect of LGG and LGGs against IH/HFHFD was associated with up-regulation of nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated antioxidant pathways. Our findings suggest a cardioprotective effect of LGG and LGGs in obese mice with OSA.



Title

Arsenic as a developmental inhibitor in alternative model organisms

Authors

JA Coral^{1,4}; JM Tennessen^{2,4}; M Bondesson^{1,4}; JR Shaw^{3,4}

Affiliation and Address

1: Department of Intelligent Systems Engineering; 2: College of Arts & Sciences
3: School of Public & Environmental Affairs; 4: Indiana University, Bloomington, IN

Abstract

Embryonic and early life-stage development is critical to the prolonged fitness of an organism. Chemical exposure during key developmental time points can modify crucial molecular pathways, via changes in gene regulation, resulting in an array of downstream effects. These effects can manifest in a multitude of fashions, scaling from biochemical to morphometric. In an effort to understand not only the broad array of metabolic outcomes of chemical exposure during development, but also how these chemical pathways evolved across taxa, we have developed the PhyloTox program. This methodology utilizes alternative model organisms (*C. elegans*, *D. pulex*, *D. melanogaster*, *D. rerio*, and *X. tropicalis*) and human cells to build a phylogenetic network of molecular pathways during embryonic/larval/early stage development. Understanding both the pathways and the evolution of the pathways can offer insight into not only important biomolecular events that can drive downstream effects, but also the extent of conservation of critical chemical response pathways across the evolutionary hierarchy. To begin, we have exposed a number of alternative model organisms (*D. pulex*, *D. melanogaster*, *D. rerio*) to trivalent arsenic (As(III)). As(III) is known to cause toxicity in a variety of organ systems and impact numerous molecular pathways. The variety of toxic outcomes demonstrates a multiple-pathway attack on a physiological system. The complexity of arsenite toxicity is further demonstrated by hormetic response at numerous receptors, including the glucocorticoid and estrogen receptors. Herein we demonstrate arsenic as a developmental inhibitor. Correlating with increasing concentrations, *D. rerio* demonstrated decreased growth and hatch rates, as well as a bimodal toxicity curve. *D. melanogaster* exhibited decreased larval growth rates manifesting in increased time to pupation, and decreased eclosion success percentages. *D. pulex* acute toxicity curves have also been generated. Taken together, these results present evidence of arsenic as a developmental pathway disruptor. We are currently following these organism level experiments with metabolomics and transcriptomic analysis at a sublethal level to further understand the biomolecular implications of arsenic exposure during early stage development.



Title

An Environmental Toxicology Assessment of Heavy Metal Accumulation in American Alligators in Florida

Authors

John P. Wise, Jr.¹, Haiyan Lu¹, Idoia Meaza¹, Sandra S. Wise¹, Tayler Croom-Perez¹, Rachel Speer¹, Jennifer Toyoda¹, Abdulmehdi Ali², Lu Cai¹, Ke Jian Liu², James T.F. Wise³, Jamie L. Young¹, and John Pierce Wise, Sr.¹

Affiliation and Address

¹School of Medicine, University of Louisville; ²Health Sciences Center, University of New Mexico;

³College of Pharmacy, University of Arizona

Abstract

Heavy metal pollution in surface waters and sediments in Florida been reported at elevated levels since the 1980s, but little is known about the potential impacts of these metals on wildlife or the environment. Previous reports indicate elevated levels of Al, Ga, La, Mn, Fe and Zn in water samples and elevated levels of Cr, Cu, Mn and Zn in sediment samples collected from three different sites in Florida. American alligators (*Alligator mississippiensis*) are the apex predators for this ecosystem and may serve as a useful indicator species for ecosystem health. Here, we report levels of heavy metals and quantified accumulation of DNA damage and chromosome instability in blood and scute samples collected from wild, free-ranging alligators. We compared data from all three sites, including Lake Woodruff which is considered a “pristine” environment. Our data show approximately 3-fold higher amounts of DNA strand breaks (measured by the comet assay) present in alligator blood cells from a coastal site than Lake Woodruff alligators. These alligators also exhibited moderate to high levels of aneuploidy in blood cells. In contrast, these alligators were similar or lower for most metal levels, except for gold in alligator blood and aluminum in alligator scute. Future studies will assess genotoxic biomarkers present in alligator scute and use alligator skin cells in culture to evaluate heavy metal-induced cytotoxicity and genotoxicity.



Title

Effect of Tobacco Product-Derived Saturated and Unsaturated Aldehydes on Circulating Angiogenic Cells

Authors

Jordan Finch, Andre Richardson, Pawel Lorkiewicz, Zhengzhi Xie, Daniel J. Conklin

Affiliation and Address

Univ. of Louisville; 580 S. Preston St. Louisville, KY 40202

Abstract

Tobacco smoking is the most significant modifiable risk factor in the development of cardiovascular disease (CVD). One mechanism by which mainstream cigarette smoke (MCS) contributes to CVD is the development of endothelium dysfunction. Endothelium function is in part maintained by circulating angiogenic cells (CACs). Both saturated (formaldehyde, FR; acetaldehyde, AC) and unsaturated (crotonaldehyde, CR) aldehydes are abundant toxins in MCS and/or electronic cigarette (e-cig) aerosol. However, tobacco products generate varying levels of specific aldehydes, and the effects of these aldehydes on endothelium repair is unknown. Thus, we performed a series of exposure studies to determine the endothelium-related effects of MCS, e-cig aerosol, and constituent aldehydes. Urinary aldehyde metabolites were measured post-exposure (6h) to determine whether levels of exposure to FR (5 ppm), AC (5 ppm), or CR (1 ppm) were comparable to levels generated in MCS (3R4F) or e-cig aerosol (blu⁺). Flow cytometry was used to measure the levels of stem cells, specifically Sca-1⁺ and Flk-1⁺/Sca-1⁺ cells, in blood of mice exposed (4d or 2wk) to air control, aldehyde, or tobacco product. Sca-1⁺ cells were decreased after 4 days of exposure to MCS, but CACs were unchanged. Interestingly, 4-day e-cig aerosol exposure had no effect on levels of either cell type. However, 4-day exposure to FR increased CACs, yet two weeks of exposure decreased both cell types. AC exposure caused changes in Sca-1⁺ cells only after a 2-week exposure. Similarly, exposure to CR showed an increase only in Sca-1⁺ cells. Taken together, these data indicate that MCS likely contributes to the development of CVD by altering overall levels of stem cells (Sca-1⁺). FR and AC both showed contrasting changes in CACs after 4-day and 2-week exposures; although these saturated aldehydes are abundant in both MCS and e-cig aerosol, neither constituent recapitulated the effects seen in the complete mixtures, indicating that the complex composition of these aerosols may counteract effects of individual constituents. Nonetheless, the data from these exposure studies suggest that constituent aldehydes affect different targets that likely contribute to overall MCS- and e-cig-induced cardiovascular toxicity. Future studies will endeavor to define the mechanisms by which aldehydes as shared constituents of tobacco-based products induce cardiovascular toxicity.



Title

Effect of UV-B radiation on dermal skin cells and their derived exosomes in diabetic neuropathy

Authors

Kartheek Pothana, Jinju Wang, Yanfang Chen

Affiliation and Address

Department of Pharmacology and Toxicology, Boonshoft School of Medicine, Wright State University, Dayton, OH 45435

Abstract

Among many complications of diabetes mellitus, diabetic neuropathy (DN) is more common and problematic situation in modern world and it is the primary cause for diabetic foot ulcer formation leading to leg amputation in severe conditions. Long-term exposure of high glucose to neurons causes starvation (by endoneurial micro-angiopathy) and apoptosis, leading to degeneration of nerve fibers resulting in DN. Peripheral nerves consists of Schwann cells (SCs) which are the regeneration units containing paracrine neurotrophic substances (nerve growth factor and brain derived neurotrophic factor) with which they repair neurons.

Ultraviolet-B (UV-B) is radiation emitted by sun and ranges from 280-315nm in the spectrum and can penetrate till the epidermal layer of skin i.e., keratinocytes. It was well known that many components like cytokines, hormones, etc. helps or participate in cell communication but recently it was found that multi-vesicular particles (MVPs) like exosomes (Exs) also participate in cell to cell communication. Exs are the bilipid layered and encloses components of cells like DNA, RNA, miRNA, lncRNA, circRNA, proteins, amino-acids and is the smallest of MVPs ranging from 30-150nm. The function of Exs varies according to the condition of its parent cell. Our group had proved that Exs from progenitor cells provide beneficial effect in different injury conditions. As the UV-B induce stress and stimulate to produce and release Exs from keratinocytes, which communicate SCs, present in the dermal layer of skin. If UV-B can modulate the release and composition of Exs from keratinocytes under diabetic condition then they could provide protective effect on SCs, by which SCs can help in the repair of neurons and reduce diabetic neuropathy by an unknown mechanism. To analyze this, aims were designed, 1. To determine whether UV-B modulates the release and expression levels of miR-132, miR-221, miR-222, miR-182, H13 lncRNA-miR130b (which promote proliferation of SC) in EXs from Keratinocytes under diabetic condition. 2. To evaluate if the EXs from Keratinocytes under diabetic condition & UVB, show protective effect on Schwann cells in diabetic neuropathy. We expect that the expression levels of miRNAs, lncRNAs from keratinocytes under diabetic and UV-B condition in Exs provide beneficial effect in SCs treating DN.



Title

Vascular Toxicity of Mainstream Cigarette Smoke in Mice: Role of Crotonaldehyde and TRPA1

Authors

Lexiao Jin, M.D.^{1,2}. Daniel J. Conklin, Ph.D.^{1,2}.

Affiliation and Address

Department of Pharmacology and Toxicology; 2. American Heart Association – Tobacco Regulation and Addiction Center

Abstract

Introduction Tobacco smoking is the greatest preventable cause of cardiovascular diseases (CVD), such as stroke and atherosclerosis. MCS (mainstream cigarette smoke) is a significant source of 2 unsaturated aldehydes -- acrolein and crotonaldehyde (CR).

Aldehydes are estimated to account for >90% of the non-cancer risk of smoking. **Objective** The purpose of this study was to better understand the vascular toxicity of MCS by testing the contribution of CR in mice in vivo, and the mechanistic role of TRPA1 (transient receptor potential ankyrin-1) in vascular effects.

Method and Results Mice (male, C57BL/6 or TRPA1-null) were exposed to HEPA-filtered air (control), MCS (3R4F; 12 cigarettes, 6h/d) or to CR (Kin-Tek; 1ppm, 6h/d) for 4 days or 12 weeks (5d/week). Following exposures and euthanization, thoracic aorta was removed and vascular reactivity was measured ex vivo, and group data were compared using Student's t-test with significance set at $P < 0.05$. In the acute MCS study, aorta function was altered by MCS with differences in either sensitivity (EC_{50}) or efficacy of contractions by phenylephrine (PE) and relaxations of acetylcholine (ACh) and sodium nitroprusside (SNP) -- effects not observed after 12-weeks of MCS exposure indicating acclimation. In contrast, either 4-day or 12-weeks of CR exposure, led to similar and sustained changes in both vascular EC_{50} and efficacy (PE decreased; ACh and SNP increased) compared with air group. As TRPA1 is known to contribute to neurogenic inflammation of MCS and CR, 4-day exposures of TRPA1-null mice to MCS or CR were performed. After 4-day MCS exposure, ACh-induced % relaxation (but not EC_{50}) was significantly decreased in TRPA1-null mice compared with air group. However, after 4-days of CR exposure in TRPA1-null mice, no changes were observed in aorta reactivity. To test for direct effects of CR in blood vessels, precontracted superior mesenteric artery (SMA) were relaxed by CR. The CR-induced relaxation was impaired in the presence of TRPA1 antagonist or in SMA of TRPA1-null mice. **Conclusions** The vasculature is targeted by MCS and CR via complex mechanisms. Augmented smooth muscle relaxation appeared as an early sign of vascular toxicity, and perhaps, represents compensatory changes that may lead to vascular disease. Notably, TRPA1 receptor plays a complex role in the acute vascular effects. Future studies are required to understand the aldehyde contribution to CVD risk of tobacco smoking.



Title

Metabolic Syndrome Enhances Nanoparticle-Induced mTOR Signaling via Alterations in the Biocorona

Authors

Li Xia, Sherleen Adamson, Saeed Alqahtani, Lisa Kobos, Jonathan Shannahan*

Affiliation and Address

School of Health Sciences, Purdue University, West Lafayette IN 47907, United States

Abstract

Nanoparticles (NPs) interact with biomolecules forming biocoronas (BC) in biological environments. The BC is known to impact biological responses affecting NP biomedical applications. Metabolic syndrome (MetS) is an increasingly prevalent condition within our society and results in the formation of unique BCs. In this study, we examined differential cell signaling that may occur in MetS by using the BC. Rodent cell lines (rat endothelial cells and mouse macrophages) and human relevant Fe₃O₄ NPs without or with BCs were utilized. NPs were incubated in water, 10% normal serum, or 10% MetS serum to form BCs. Endothelial cells were exposed to NPs for 3, 12, or 24 h and differential cell signaling was discovered using a RNA-Seq approach. Functional enrichment of the differentially expressed genes identified time-dependent alterations in the activation of a number of pathways including DNA damage repair, oxidative stress, inflammation, and mTOR signaling. Specific examination of mTOR signaling demonstrated BC-dependent alterations within the pathway consisting of enhanced phosphorylation of AKT as well as total and phosphorylated mTOR. Additionally, markers of apoptosis (cleavage of caspase-3 and cytochrome c levels) were elevated only in cells exposed to NPs with a MetS BC. mTOR signaling induces apoptosis via inhibition of autophagy. Macrophages were exposed to NPs without or with BCs and decreased gene expression of LC3, a marker of autophagy, was observed for only NPs with a MetS BC. Treatment with rapamycin, an inhibitor of mTOR, inhibited alterations in LC3 expression. To understand up-stream regulation of mTOR, we utilized pharmacological inhibitors to reduce NP interactions with cell surface scavenger receptors. CD36 was determined to be the primary facilitator of NP internalization and is increasingly expressed on cells due to MetS. Treatment of cells with a CD36-specific ligand was determined to enhance mTOR gene expression to a similar degree as exposure to NPs with a MetS BC. Lastly, a number of processes down-stream of mTOR (insulin sensitivity and lipogenesis) were determined to be specifically modified due to exposure to NPs with a MetS BC. Together these findings demonstrate that the formation of a BC within MetS may result in enhanced mTOR signaling due to increased scavenger receptor interactions leading to exacerbated responses and progression of MetS associated diseases.



Title

TCDD Exposure During Embryonic and Neonatal Development and Implications in Cardiac Disease

Authors

Matthew de Gannes, Sheryl Koch, Jack Rubinstein, Jacek Biesiada, Mario Medvedovic, Liang Niu, Chia-I Ko, Alvaro Puga

Affiliation and Address

University of Cincinnati, 160 Panzeca Way, Cincinnati OH

Abstract

Congenital heart disease (CHD) is the leading birth defect worldwide, yet the precise etiological factors underlying CHD remain largely unknown. Epidemiological studies estimate that <15% of cases of CHD can be traced to known Mendelian inheritance, suggesting that environmental agents operative during heart formation may be crucial contributors to the disease. Exposure to tetrachlorodibenzo-*p*-dioxin (TCDD) in the young has been associated with disease conditions in adult fish, birds and mammals, including higher incidence of congenital heart abnormalities. TCDD is a potent ligand for the Ah receptor (AHR) – a sensor of environmental signals as well as morphogenic and detoxification pathways. Our lab has previously shown that Ah receptor ablation or activation by TCDD similarly disrupt molecular networks involved in heart formation and function. DNA methylation dynamics have previously been shown to be important in orchestrating cardiac development and disease. Here, we hypothesize that TCDD exposure during cardiac development alters DNA methylation and gene expression in genes essential for cardiac function. A stable cell line differentiated from mouse embryonic stem (ES) cells that expresses the selection markers puromycin resistance and eGFP under the control of the cardiomyocyte marker *Nkx2-5* was treated with either 5nM TCDD or DMSO (control) after 0, 24, 48, and 72 hours of culture. Differentiated cells treated with TCDD after 24, 72, and 96 hours were harvested for RNA-seq and methyl-seq analyses where they were compared with untreated ES cells and control differentiated cells after 24 hours of culture. Here, we reveal the effects of TCDD on the expression of cardiac genes, DNA methylation patterns, and relevant pathways affected. Continued work in this area will further elucidate the complex epigenetic patterns and mechanisms surrounding the development of congenital heart disease.



Title

Arsenite can Displace Ainc from the Zinc Finger Domains of Alternative Splicing Regulator ZRANB2

Authors

Mayukh Banerjee¹, Ana P.F. Cardoso¹, Angeliki Lykoudi¹, Daniel W. Wilkey², Walter H. Watson², Nichola C. Garbett², Michael M. Merchant², Jianmin Pan³, Shesh N. Rai³, J. Christopher States¹

Affiliation and Address

Departments of ¹Pharmacology and Toxicology, ^{2,3}Bioinformatics and Biostatistics, University of Louisville, Louisville, KY, USA

Abstract

Background: Exposure to arsenic, a class I carcinogen, affects 200 million people globally. Skin is the major target organ but molecular etiology remains unclear. Using an established HaCaT cell line model of arsenic-induced squamous cell carcinoma, we demonstrated occurrence of global dysregulation of both miRNA expression and alternative splicing during carcinogenesis. Hsa-miR-186, an intronic miRNA encoded within the ZRANB2 gene, was highly induced in squamous cell carcinoma tissue samples compared to premalignant hyperkeratosis. ZRANB2, an alternative splicing regulator has two C4 zinc knuckle motifs that are integral to its structure and splicing function. Zinc fingers are known targets for arsenic binding and zinc displacement. Hypothesis: Arsenite (As^{3+}) displaces zinc (Zn^{2+}) from ZRANB2, altering its structure and function, resulting in increased transcription of ZRANB2 as a homeostatic response and a concomitant increase hsa-miR-186 expression.

Methods: ZRANB2 expression in HaCaT cells acutely exposed to As^{3+} (0 or 5 μM ; 0- 72 h) was examined by immunoblotting. $\text{As}^{3+}/\text{Zn}^{2+}$ binding (One-site specific binding non-linear model) and mutual displacement experiments (log(inhibitor) vs. response non-linear model) were performed with synthetic apo-peptides corresponding to each ZRANB2 zinc finger motif. A combination of intrinsic fluorescence, UV spectrophotometry, zinc colorimetric assay and ESI-MS/MS was used to characterize metal binding/mutual displacement. TRA2 β splicing was monitored by RT-qPCR. **Results:** As^{3+} exposure induced ZRANB2 expression between 3-24 h ($p < 0.05$; ANOVA). As^{3+} was found to bind to, as well as displace Zn^{2+} from, each zinc finger motif ($p < 0.05$; ANOVA). Also, Zn^{2+} displaced As^{3+} from As^{3+} -bound zinc finger motif ($p < 0.05$; ANOVA). ZRANB2-directed TRA2 β splicing was impaired ($p < 0.05$; ANOVA). **Conclusions:**

Arsenic exposure displaces Zn^{2+} from ZRANB2 zinc fingers, changing its structure and compromising splicing of its targets. ZRANB2 expression increases in response. The elevated miR-186 expression observed in As^{3+} induced squamous cell carcinoma is likely a consequence of induced ZRANB2 expression in response to chronic As^{3+} induced ZRANB2 disruption.



Title

Photodynamic therapy induces Microvesicle particles production

Authors

Oladayo A. Oyebanji¹, Langni Liu¹, Christine M. Rapp¹, Jeffrey B. Travers^{1,2}

Affiliation and Address

1. Dept. of Pharmacology and Toxicology, Wright State University.

2. Dept. of Dermatology, Wright State University, Dayton OH.

Abstract

Photodynamic therapy (PDT) involves the use of light at an appropriate wavelength acting on a photosensitizing chemical to cause cell death via generation of reactive oxygen species. PDT has been useful in the management of skin conditions (like acne, psoriasis) and cancers like superficial skin, esophageal and non-small cell lung cancers. In addition to these therapeutic effects, previous murine studies from our group have demonstrated that topical PDT induces immunosuppression in vivo. Thus, PDT of skin can generate a systemic effect through unknown mechanisms. Microvesicle particles (MVPs) are small extracellular membrane-enclosed particles believed to mediate cell-to-cell communication via the transport of bioactive signaling substances. These present studies tested if PDT can generate MVP release. Our studies used in vitro (HaCaT; keratinocyte cell line), ex vivo (human skin explants) and in vivo (mice) models. PDT increased MVP release in both keratinocytes in vitro as well as treatment of human explants ex vivo. Murine studies also revealed a significant increase in skin MVPs following PDT treatment. These studies reveal a consistent production of MVPs following PDT and thus, provide an insight into a possible novel mechanism whereby PDT exerts its effects; via generation of MVPs.



Title

Developing the zebrafish posterior lateral line as a predictive toxicity model

Authors

Rachna Sachanandani^{1,5}, Savini Thrikawala¹, Cliff Stephan², Nghi Nguyen², Mary Sobieski², Richard S Judson³, Divya Chilukuri⁴, Sara Flores⁴, Jan-Åke Gustafsson¹, Maria Bondesson^{1,5}

Affiliation and Address

¹Department of Biology and Biochemistry, Center for Nuclear Receptors and Cell Signaling, University of Houston, Houston, TX. ²Center for Translational Cancer Research, Texas A&M Health Science Center, Institute of Biosciences and Technology, Houston, Texas. ³National Center for Computational Toxicology, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, North Carolina. ⁴College of Natural Sciences and Mathematics, University of Houston, Houston, TX. ⁵Department of Intelligent Systems Engineering, Indiana University, Bloomington, IN

Abstract

The vertebrate zebrafish (*Danio rerio*) embryo is an emerging model for screening teratogenic compounds. We aim to understand the developmental toxicity effects of environmental pollutants by using zebrafish embryos to identify compounds that negatively affect neuromasts of the posterior lateral line (PLL), a system of tactile sense cells in fish. The zebrafish neuromasts are mechanoreceptors that respond to the flow of water, and are structurally and functionally similar to human inner ear hair cells. Posterior neuromasts are positioned along the zebrafish body through migration of the neuromast primordium formed near the otic vesicle. Transgenic zebrafish embryos expressing fluorescence in neuromasts (GW57A) were used in a primary high throughput screen (HTS) of 294 unique compounds from Environmental Protection Agency's (EPA's) ToxCast phase I chemical inventory, primarily consisting of pesticides and antimicrobials. The HTS screen and follow-up confirmatory tests confirmed 22 of the 294 compounds as PLL development disruptors. A univariate analysis was performed on the confirmed hits to identify ToxCast in vitro biochemical and cellular assays that significantly correlated with the identified in vivo PLL disruptors. Thirteen in vitro assays were identified, of which several assays for serotonin signaling were retained. We were able to confirm the involvement of the serotonin pathway in PLL development by dosing zebrafish larvae with known serotonin pathway disruptor compounds. In conclusion, the transgenic zebrafish line expressing fluorescence in neuromasts is a reliable model for HTS screening of large libraries of compounds to identify PLL development disruptors. In addition to that, we identified and confirmed the serotonin pathway as one of the potential pathways through which these compounds disrupt PLL development. This suggests that we can use the zebrafish PLL development disruption to also predict and identify compounds that disrupt the serotonin pathway. This abstract does not necessarily reflect EPA policy.



Title

Exacerbation of Nanoparticle-Induced Pulmonary Inflammation in a Mouse Model of Metabolic Syndrome

Authors

Saeed Alqahtani,¹ Lisa Kobos,¹ Li Xia,¹ Jonathan Shannahan¹

Affiliation and Address

¹ School of Health Sciences, Purdue University, West Lafayette IN

Abstract

Metabolic Syndrome (MetS) is increasingly prevalent within our society and is an established risk factor for the development of many chronic diseases including type 2 diabetes, cardiovascular disease, cancer, and others. Specifically, MetS is a collection of conditions including increased blood pressure, high blood sugar, obesity, and dyslipidemia. Growing evidence suggests that exposures contribute to chronic diseases and that individuals with these chronic diseases respond more robustly to exposures. We hypothesize that MetS-associated dysregulation of lipids contributes to exacerbated inflammatory responses following exposures. Further, we hypothesize that commonly prescribed statins, which modulate lipids, will decrease susceptibility. To address this hypothesis, a silver nanoparticle (AgNP) exposure was utilized to evaluate pulmonary inflammation in a mouse model of diet-induced MetS. Mice were provided with either a normal control diet (10% of calories from fat) or a high-fat western diet (60% of calories from fat) for 20 weeks. A subset of mice while on these diets were treated with atorvastatin for the final 10 weeks to modulate lipids. Mice were exposed to 50 μg of AgNPs via oropharyngeal aspiration, and acute toxicity endpoints were evaluated 24 hours after exposure. Mice on the MetS diet demonstrated increased body weight, and cholesterol levels compared to mice on the normal diet. Statin treatment did not significantly alter diet-induced changes in circulating lipids. Cytometry analysis of bronchoalveolar lavage fluid demonstrated increased inflammatory cell influx in both healthy and MetS mice. Neutrophilic influx was elevated in MetS mice compared to healthy mice following exposure. Additionally, MetS enhanced inflammatory mRNA expression and protein levels of monocyte chemoattractant protein-1, macrophage inflammatory protein-2, and interleukin-6. Stain therapy reduced these markers of inflammation (neutrophilic influx and inflammatory gene expression) in mice suffering from MetS following exposure. A lipidomics approach was utilized to investigate lipid mediators of pulmonary inflammation. This assessment demonstrated reduced levels of lipids involved in inflammatory resolution (EPA, DHA, and their metabolites-resolvins, maresins, and others) in the MetS model compared to healthy following AgNP exposure. Statin treatment was determined to inhibit these MetS-induced lipid alterations following AgNP exposure and lowered responses to those observed within the healthy model. Taken together, our data suggests that MetS exacerbates the acute pulmonary toxicity induced by AgNP exposure via a disruption of lipid mediators of inflammatory resolution leading to enhanced pulmonary inflammation. Based upon this data it is likely that individuals suffering from diseases characterized by lipid dysregulation may be increasingly susceptible to the adverse health effects of exposures.



Title

Analyzing Estrogenicity of Bisphenol Analogues using Transgenic Zebrafish

Authors

Silvia Karim¹, Caroline Pinto², Ruixin Hao², Marina Grimaldi³, Patrick Balaguer³, Maria Bondesson¹.

Affiliation and Address

¹Department of Intelligent Systems Engineering, Indiana University, Bloomington, IN 47408, USA.

²Center for Nuclear Receptors and Cell Signaling, Department of Biology and Biochemistry, University of Houston, Houston, Texas 77204, USA.

³Institut de Recherche en Cancérologie de Montpellier, INSERM U1194, Institut régional du Cancer de Montpellier, Université de Montpellier, 34298 Montpellier Cedex 5, France.

Abstract

Bisphenol A (BPA) has been widely used to make epoxy resin, polycarbonate plastics and other materials over the past decade. It is one of the most common environmental pollutants. Exposure to BPA has possible hazardous effects on the human brain, heart, liver, and blood, partly because of its estrogenic activity. BPA analogues have been synthesized to be considered as replacement molecules for BPA. These analogues need to be thoroughly evaluated for their estrogenic activity. The transgenic *Tg(5×ERE:GFP)* zebrafish is used to study agonistic and antagonistic estrogenic effects of bisphenols *in vivo* (Gorelick and Halpern, *Endocrinology* 2011 152(7)2690-703). To investigate estrogenic activity of BPA analogues, three days old zebrafish embryos were exposed to a concentration range of BPA, BPC-CL, BPAF, BPC and BPE, incubated and imaged at day four by fluorescence microscopy. The fluorescence in images were quantified by ImageJ. The exposures to BPA, BPAF, BPC and BPE efficiently induced GFP expression first in the heart valves and at higher doses in the liver, whereas BPC-CL activated GFP expression in the liver and brain. The GFP expression in fish was compared to estrogen response in reporter cells that express the zebrafish estrogen receptors driving expression of an ERE-luciferase reporter. BPC-CL preferentially activated *esr2a*, whereas BPA, BPAF, BPC and BPE preferentially activated *esr1*. We conclude that transgenic estrogen reporter fish in combination with estrogen reporter cells efficiently can be used to assess estrogenic capacity of environmental pollutants.



Title

National Secular Trends in Ambient Air Volatile Organic Compound Levels and Biomarkers of Exposure in the United States

Authors

Stacey L. Konkle, Kristina M. Zierold, Kira C. Taylor, Daniel W. Riggs, and Aruni Bhatnagar

Affiliation and Address

Christina Lee Brown Envirome Institute, University of Louisville School of Medicine, Louisville, KY, United States

Abstract

BACKGROUND: Exposure to air pollution is a leading cause of global mortality. Volatile organic compounds (VOCs) are constituents of ambient air that could exert adverse health effects.

OBJECTIVE: To examine the relationship between VOC levels in ambient air and individual-level VOC exposures, as assessed by urinary VOC metabolites.

METHODS: Secular trends in 11 ambient air VOCs (2005 to 2013) and individual-level metabolites of 14 VOCs (2005 to 2014) were assessed using National Monitoring Programs (NMP) and National Health and Nutrition Examination Survey (NHANES) data, respectively. To isolate environmental exposure, individuals reporting exposure to tobacco smoke were excluded. Quantile regression models were used to assess secular trends in VOC exposure, and survey-weighted regression models were built to identify factors associated with VOC exposure.

RESULTS: All annual mean ambient air VOC levels decreased from 2005 to 2013 (Range: 12.5%-77.2%). However, 11 of the corresponding VOC metabolites increased during the same time (Range: 0.3%-53.6%). There was a proportional change in patterns of VOC exposure across NHANES waves, with the middle quantiles of exposure showing the most increase. VOC exposures were significantly associated with age, sex, race, education, and physical inactivity, but not with secular VOC trends.

DISCUSSION: In the United States, individual exposure to several VOCs increased between 2005-2014 despite a decline in ambient air VOC levels. This inverse relationship suggests that ambient VOCs are not the primary source of VOC exposure, therefore, decreasing ambient VOCs alone may not be sufficient to protect against the adverse health effects associated with VOC exposure. Our study supports a shift in the relative importance of volatile chemical products on VOC exposures.



Title

Chromate-Induced Changes in the Fibroblast Secretome and its Effects on Epithelial Cells

Authors

Taylor J. Croom-Perez, Idoia Meaza, and John Pierce Wise, Sr.

Affiliation and Address

Wise Laboratory of Environmental and Genetic Toxicology, Department of Pharmacology and Toxicology, School of Medicine, University of Louisville, Louisville, Kentucky

Abstract

Lung cancer continues to be the leading cause of cancer death in the U.S. One of the key strategies to combating it is to better understand its causes. Hexavalent chromium [Cr(VI)] is a human lung carcinogen of major public health concern because exposure to it is common in the workplace and in the general environment. Our studies focus on investigating the mechanisms of Cr(VI)-induced carcinogenesis, which are currently poorly understood. Pathology data from Cr(VI) exposed workers show a key factor of Cr(VI)-induced lung cancer is the accumulation of Cr in the stroma, rather than the epithelia, while the tumors that form are all epithelial in origin. These outcomes support that fibroblast signaling to epithelial cell may be an important mechanism for Cr(VI) carcinogenesis. However, the impact of Cr(VI) on the contents of the fibroblast secretome has not been studied, therefore this study focuses on determining the effects of exposing epithelial cells to the secretome of chromate exposed fibroblast cells. To do this, human lung fibroblasts were exposed to hexavalent chromium for 120 h and then the conditioned media, containing the fibroblast secretome, was cleared and concentrated. Epithelial cells were then treated with the fibroblast concentrated conditioned media for 1 week and for 6 weeks. At each endpoint the epithelial cells were harvested and changes in cell survival, mitotic index, chromosome damage, and anchorage independent growth were determined. Preliminary results suggest that compared to non-chromate exposed fibroblast cells, no significant changes after 1 week of secretome exposure were seen in the epithelial cells. Initial analysis shows effects on cell survival and mitotic index after a 6-week exposure to the secretome of the chromate-induced transformed fibroblast cells, suggesting that fibroblast exposure to chromium induces changes in the contents of the cells' secretome that are passed to epithelial cells and alter growth characteristics. This research provides initial evidence that cell-to-cell interaction is effected by chromium and suggests that this cellular signaling mechanism could be important in Cr(VI) carcinogenesis. Grant support for this study provided by grants from NIEHS R01ES016893 (JPW), KLCRP-C17 (JPW) and NIEHS 2T32ES011564-11A1 (JPW and TCP)



Title

Protective effect and mechanism of metallothionein on myocardial injury induced by high-fat diet and therapeutic dose of arsenic trioxide

Authors

Tingwen Ge^{1,2}, Youxi Yu¹, Qiuju Liu¹, Jiuwei Cui¹, Lu Cai²

Affiliation and Address

¹The First Hospital of Jilin University, Changchun, Jilin 130021, China. ²Pediatric Research Institute, Department of Pediatrics, the University of Louisville, Louisville, KY 40202, USA

Abstract

Objective: Arsenic trioxide is a common clinical anti-tumor drug. However, the cardiotoxicity caused by its application seriously hindered the strategy of treatment. Unfortunately, patients with tumors also commonly suffer from heart damage induced by a high-fat diet or abnormal lipid metabolism, which greatly limits the choice of arsenic trioxide as an anti-tumor treatment. Metallothionein is an effective endogenous antioxidant, which can effectively protect cardiac myocytes from oxidative stress injury. Therefore, the purpose of this study was to prove whether metallothionein can protect cardiac myocytes and inhibit the occurrence of cardiac toxicity under the double attack of high-fat diet and treatment dose of arsenic trioxide.

Methods: MT-TG transgenic and WT mice were exposed to a high-fat diet and a normal diet. Subsequently, arsenic trioxide (5mg/kg/day) was injected for one month.

Results: Metallothionein has a protective effect on myocardial oxidative stress injury, which may be achieved by directly inhibiting oxygen free radicals. In addition, metallothionein protects myocardial cell apoptosis by inhibiting the P53 signaling pathway and inhibiting myocardial cell apoptosis. Finally, metallothionein can inhibit the recruitment of immune cells by inhibiting the activation of the NF- κ B inflammatory signaling pathway and inhibiting its downstream inflammatory cytokines, adhesion molecules and chemokines, thus achieving the protective effect of myocardial inflammatory response and fibrosis.

Conclusion: Metallothionein can protect myocardial cells and inhibit oxidative stress, apoptosis, inflammatory reaction and fibrosis induced by high-fat diet and treatment dose of arsenic trioxide, which provides a theoretical basis for clinical application.



Title

Role Of Macrophage Polarization In Adipose Tissue And Perivascular Adipose Tissue Of Hypertensive Mice

Authors

Venkata sai Usha sri polaki, Jinju Wang, Shuzhen Chen, Ji Bihl

Affiliation and Address

Pharmacology and Toxicology Department, Boonshoft School of Medicine, Wright State University, Dayton, OH 45435

Abstract

Hypertension also known as high blood pressure (HBP), is a long-term medical condition in which the blood pressure in the arteries is persistently elevated. It can lead to severe health complications and increase the risk of heart disease, stroke, and sometimes death.

Perivascular adipose tissue (PVAT) is known as the adipose tissue (AT) surrounding all the blood vessels and plays a critical role in the pathogenesis of the vascular disease. In vascular pathologies, PVAT increases in volume and becomes dysfunctional, with altered cellular composition and molecular characteristics. Macrophages have been found to accumulate in PVAT during hypertension, which might be involved in the inflammation of hypertension.

Inflammation has been shown to play an important role in hypertension; however, the exact mechanisms by which the activated immune cells from which part lead to the development and maintenance of hypertension remain to be elucidated. The objective of this study is to determine the role of the polarization of macrophages in the PVAT of hypertensive mice.

Methods: Renin transgenic (R+) mice (n=6/group) have been used as a hypertensive mice model. The PVAT and AT samples were collected for macrophages isolation. The polarization of macrophages in AT and PVAT was determined by using the flow cytometry. Macrophages were defined as F4/80 positive cells. M1 phenotype was identified as CD86 positive macrophages, while the M2 phenotype was identified as CD206 positive macrophages.

Results: 1) R+ showed high blood pressure when compared to control mice. 2) The numbers of M1 macrophages were increased, while the numbers of M2 macrophages were decreased in both the AT and PVAT of R+ mice. Conclusion: The M1 phenotypes of macrophages in PVAT and AT is increased in hypertensive mice, which might be related to the inflammation of hypertension. Further studies will verify the inflammatory cytokine levels in PVAT and AT in hypertensive mice.



Title

Evaluation of physiologically relevant aryl hydrocarbon receptor ligands on Ig heavy chain expression in a human B-cell line model.

Authors

Valerie Benedict, Courtney Sulentic

Affiliation and Address

Wright State University, Boonshoft School of Medicine, 3640 Colonel Glenn Hwy, Dayton, OH 45435

Abstract

The aryl hydrocarbon receptor (AhR) is a transcription factor that affects immune cell differentiation and function in animal models. AhR may serve as an environmental sensor since it not only binds to toxicants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) but also to endogenous, dietary and bacterial ligands such as 6-formylindolo[3,2-*b*]carbazole (FICZ) and indole. In animal studies, different AhR ligands have produced different and even opposite functional effects on T cell subtypes, which may be due to transient vs. persistent activation of the AhR. The objective of this study was to determine if AhR ligands differentially modulate the expression of Ig heavy chain constant regions (*IGHC*) (i.e. μ , γ , α , ϵ) in a human B-cell line (CL-01). TCDD inhibited IgG secretion and γ 1-4 transcript levels but increased α 1 and α 2 transcript levels. Evaluation of the effect of indole and FICZ on *IGHC* expression patterns is currently underway. These studies will determine the sensitivity of individual *IGHCs* to AhR ligands and whether physiologically relevant AhR ligands induce different *IGHC* profiles.



Title

Multi-label model for toxicity prediction

Authors

XiuHuan Yap, Michael Raymer

Affiliation and Address

Bioinformatics Research Group (BiRG), Wright State University

Abstract

Most computational predictive models are specifically trained for a single toxicity endpoint. Since more than 1300 toxicity assays have been reported in the TOXCAST dashboard, achieving high coverage over this growing number of toxicity endpoints remains challenging. Furthermore, single-endpoint models lack the ability to learn dependencies between endpoints, such as those targeting similar biological pathways, which may be used to boost model performance. In this study, we characterize the performance of 3 multi-label classification (MLC) models, namely Classifier Chains (CC), Label Powersets (LP) and Stacking (SBR), on Tox21 challenge data. These MLC models employ the Problem Transformation approach, which is algorithm-independent and thus generally compatible with existing classifiers. Using Logistic Regression as the base classifier and random label partitioning ($k=3$), CC and LP show statistically significant improvement in model performance using Hamming and subset 0/1 scores ($p<0.05$). On the other hand, SBR show significant improvements in micro-averaged F1 and accuracy scores. Additionally, when using label partitioning learned by Louvain community detection method, CC and SBR models retain their performance improvement over single-endpoint models, suggesting that learning marginal dependence is a key contributor to improving model performance. Taken together, MLC models could potentially boost the performance of current single-endpoint predictive models. Future studies include examining the relationships among endpoints that lead to optimal performance for particular MLC methods, testing these models on datasets with a greater number of toxicity endpoints, and using base classifiers that are well-established in predictive toxicology.



Title

Expression of *Kcc2a-S25*, a new splice variant of the neuronal K^+Cl^- cotransporter-2 in endocrine tissues and testicle germ cells

Authors

Yakshkumar Rathod, Shams Kursan, Eduardo Dias-Junior, Lisa Kelly and Mauricio Di Fulvio.

Affiliation and Address

Department of Pharmacology and Toxicology, Wright State University, Boonshoft School of Medicine, Dayton Ohio 45435.

Abstract

The *Slc12a5* gene encodes the K^+Cl^- cotransporter-2 (*Kcc2*), a membrane protein which maintain the intracellular chloride concentration ($[Cl^-]_i$) below thermodynamic equilibrium in neurons. Therefore, *Kcc2* function determines the inhibitory action of activated γ -aminobutyric acid (GABA) type-A ($GABA_A$) and glycine receptors, two Cl^- channels gated by their respective neurotransmitters. Two main splice variants of *Kcc2* have been functionally identified in rodents and human central neurons: *KCC2a* and *KCC2b*, both constitutively active neuronal Cl^- extruders under most physiological conditions. Recent results from our laboratory demonstrated that *Kcc2* has an extra-neuronal expression pattern and function. Indeed, *Kcc2a*, *Kcc2b* and a new splice variant lacking exon 25 (*Kcc2a-S25*) were found at the transcript level in the mouse, rat and human pancreatic endocrine islet whereas immunoreactive *Kcc2* was detected in all endocrine cells of the mammalian islet where it plays a role in insulin secretion. Notably, *Kcc2a-S25* and immunoreactive *Kcc2* mostly, but not exclusively, localized to intracellular granules of chromaffin cells of the adrenal medulla. Therefore, our aim was to test the hypothesis that *Kcc2a-S25* is expressed in endocrine tissues. To this end, we screened *Kcc2a*, *Kcc2b* and *Kcc2-S25* transcript expression pattern in RNA extracts from mouse hypophysis, thyroid, ovary, testicle, adrenal, prostate and adipose tissues. We used conventional RT-PCR and variant-specific primer sets to demonstrate *Kcc2a* and *Kcc2b* mRNA expression and RT-PCR coupled to *MspI*-restriction digestion to demonstrate expression of *Kcc2a-S25* in mouse tissues. To verify expression of *Kcc2* at the protein level, we performed immunofluorescence microscopy using polyclonal antibodies characterized and validated against knock-out tissues. Our findings extend previous data from our laboratory and demonstrate that *Kcc2a-S25* is the splice variant of *Kcc2* expressed in the tissues tested whereas immunoreactive *Kcc2* is found in all endocrine cells of the islet, cells of the adrenal medulla, ovary and germ cells of the testicle.



Title

Protective effects of sulforaphane on type 2 diabetes-induced cardiomyopathy via AMPK-mediated activation of lipid metabolic pathways and Nrf2 function

Authors

Yike Sun, Shanshan Zhou, Hua Guo, Jian Zhang, Tianjiao Ma, Yang Zheng, Zhiguo Zhang, Lu Cai

Affiliation and Address

Pediatric Research Institute, Department of Pediatrics, University of Louisville, Louisville KY 40202 USA

Abstract

Backgrounds: AMP-activated protein kinase (AMPK), particularly the AMPK α 2 isoform, plays a critical role in maintaining cardiac homeostasis. It was reported that sulforaphane (SFN) prevents type 2 diabetes (T2D)-induced cardiomyopathy accompanied by the activation of AMPK. In this study, AMPK's pivotal role in SFN-mediated prevention against T2D-induced cardiomyopathy was tested using mice with global deletion of the AMPK α 2 gene (AMPK α 2-KO).

Methods and Results: T2D was established by feeding mice high-fat diet (HFD) for three months to induce insulin resistance, followed by an intraperitoneal injection of streptozotocin (STZ) to induce mild hyperglycemia in both AMPK α 2-KO and wild-type mice. Then both T2D and control mice were subsequently treated with or without SFN for 3 months while continuously fed HFD or normal diet. Upon completion of the 3-month treatment, five mice from each group were sacrificed (3-month time-point (3M)). The rest of the mice continued normal diet or HFD until termination of the study at sixth months (6M). Cardiac function was examined with echocardiography before sacrifice at both 3M and 6M. SFN prevented T2D-induced progression of cardiac dysfunction, remodeling (hypertrophy and fibrosis), inflammation, and oxidative damage in wild-type diabetic mice, but not in AMPK α 2-KO mice. Mechanistically, SFN prevents T2D-induced cardiac lipotoxicity with activation of AMPK-mediated lipid metabolic pathways and Nrf2-associated antioxidant action probably via Akt/GSK3 β pathway. However, these improving effects of SFN were abolished in AMPK α 2-KO diabetic mice.

Conclusions: AMPK is indispensable for the prevention of T2D-induced cardiomyopathy, and the activation of Nrf2 function by SFN is AMPK-dependent that may be related to the AMPK/Akt/GSK3 β signaling pathways.



Title

Sulforaphane prevents type 2 diabetes-induced nephropathy via AMPK-mediated activation of lipid metabolic pathways and NRF2 anti-oxidative function

Authors

Zhuo Li, Hua Guo, Tianjiao Ma, Shanshan Zhou, Zhiguo Zhang, Lining Miao, and Lu Cai

Affiliation and Address

Pediatric Research Institute, Department of Pediatrics, University of Louisville,
Louisville, 40202, USA

Abstract

Sulforaphane (SFN) prevents diabetic nephropathy (DN) in type 2 diabetes (T2D) by up-regulating nuclear factor (erythroid-derived 2)-like 2 (NRF2). AMP-activated protein kinase (AMPK) can attenuate the pathogenesis of DN by improving renal lipotoxicity, which is also found associated with the activation of NRF2-mediated antioxidants. Therefore, we investigated whether AMPK α 2, the central subunit of AMPK in energy metabolism, is required for SFN protection against DN in T2D, and whether potential crosstalk occurs between AMPK α 2 and NRF2 in this context. AMPK α 2 knockout (Ampk α 2^{-/-}) mice and wild type (WT) (C57BL/6J) mice were fed a high-fat diet (HFD) to induce insulin resistance, followed by streptozotocin (STZ) injection to induce hyperglycemia (T2D model mice). Both T2D and control mice were treated with SFN or vehicle for three months. At the end of the three-month treatment period, five mice from each group were sacrificed (three-month (3M) time point). The remaining mice were only fed either a HFD or normal diet (ND) for three more months and the study was terminated at six months (six-month (6M) time point). Renal function was assessed by measurement of 24h urine albumin before sacrifice at 3M and 6M. SFN significantly attenuated T2D-induced renal dysfunction and pathology accompanied with biochemical changes including proteinuria, renal hypertrophy, renal oxidative damage, inflammation, and fibrosis in WT mice, but not in Ampk α 2^{-/-} mice. Additionally SFN also prevented T2D-induced renal lipotoxicity via AMPK-mediated activation of lipid metabolic pathways and the NRF2 function in WT diabetic mice. Similarly, these protective effects of SFN were also abolished in Ampk α 2^{-/-} diabetic mice. These results suggest that AMPK α 2 plays a central role in preventing DN. SFN prevents DN by the activation of lipid metabolism and NRF2-associated suppression of oxidative stress, and more important is that both of them are AMPK α 2-dependent.



Title

Feel the burn: Effects of sunscreen nanoparticles on cytokine and chemokine production in human skin

Authors

CBuckner, N Panstingel, C Sulentic

Affiliation and Address

Dept. of Pharmacology and Toxicology, Boonshoft School of Medicine, Wright State University, Dayton, OH, USA

Abstract

The incidence of skin cancer, both non-melanoma and melanoma, have been globally increasing over the past decade. Prevention of these cancers focuses on the reduction of exposure to UVA/UVB spectra light by limiting exposure in combination with sunscreen. To provide better UV protection, metal nanoparticles (Nps) such as TiO₂ and ZnO are often a component of sunscreen. However, studies of the potential toxicological effects of NPs is understudied compared to their broad usage. Although initial studies on sunscreen NPs have shown limited effects, potential immunological effects within the skin have not been adequately studied, especially in the context of sun burns. Here we hypothesize that addition of these nanoparticles to human skin either pre-burned with UV light or added post-burn will elicit a differential cytokine and chemokine profile. As a comparison to the sunscreen NPs we also assayed the immunotoxicity of silver NPs (AgNP), which are used in other dermal applications such as antibacterials. To test this hypothesis, post-surgical human skin explants from three patients were sectioned into 5cm X 5cm pieces. Skin samples were then treated as follows: 1) untreated control, UVB irradiated (1000 J/m²) or burn using a metal block heated to 95C, 2) NPs alone (TiO₂ [0.25mg], ZnO [0.25mg], TiO₂ and ZnO, or Ag [2 ug]), 3) first burned either using UVB or a heated metal block and then treated with NPs, or 4) first treated with NPs then exposed to UVB irradiation followed by a second treatment with NPs. Following treatment, total protein was isolated and with a Bradford assay. Protein samples were then analyzed via a BioRad Bio-Plex Pro Human Cytokine 27-plex in triplicate. Samples were ran twice on the Bio-Plex plate to assay for experimental variation. Protein levels of cytokines and chemokines were normalized to the sample's respective total protein concentration. Due to the size of the data set generated by the multiplex plate, we utilized dimensionality reduction algorithms and multivariate statistics to analyze our data. Applying these analytical techniques allowed us to build a cytokine profile dependent on the NP treatments and burn status. Analysis suggests that metal NPs have a moderate effect on cytokine and chemokine production in a scenario that mimics how sunscreens are normally applied. This study highlights the potential immunological effects of metal NPs within sunscreens under sunburn conditions.



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