The Effects of Chronic Binge Alcohol on Mesenchymal Stem Cell DNA Methylation

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Introduction
Alcohol abuse negatively affects bone physiology leading to an increased risk of fractures. Chronic binge alcohol (CBA) has been shown to decrease bone mineral density and increase the incidence of secondary osteoporosis. In 2011, the total direct medical costs for hip-related fractures was estimated to be $34 billion, with fracture accounting for 4% of total non-fatal direct cost.

Mesenchymal stem cells (MSCs) regulate bone growth and turnover, and can differentiate into chondrocytes, osteoblasts, and adipocytes. CBA promotes adipocyte formation over osteoblast differentiation. Methylation DNA is inhibitory to mRNAs expression. DNA methylation in DNA (DNMTs) are the enzymes responsible for DNA methylation. DNMT1 maintains methylation patterns within newly synthesized DNA. DNMT3A & 3B are responsible for de novo methylation.

We hypothesized that CBA dysregulates MSC differentiation by disrupting DNA methylation patterns and DNA methyltransferase activity.

Methods & Materials
Model: Male mice were administered alcohol via water (control) or 25 g/kg alcohol daily via an indwelling intragastric catheter to achieve 50-60% mid-bowel alcohol concentration (BAC). Bone marrow aspirates were taken at 6 and 3 mos post of alcohol administration.

Mesenchymal Stem Cell Isolation and Culture: Mononuclear cells were isolated from bone marrow aspirates using a Ficoll gradient and cultured in DMEM media containing 20% heat inactivated fetal bovine serum and cultured in a humidified incubator. All experiments were performed using passages 2-3 MSCs.

DNA methylation: Total DNA was extracted from MSCs, and CpG methylation was measured using the Human CpG Methylation assay kit (Xillio, San Diego, CA) following the manufacturer’s protocols.

Protein Assays: We used a Nuclear Extraction Kit (Enzymo, Emsworth, NY) to isolate protein from MSCs to determine net DNA methylation activity using the Enzyme-DNA Methylation assay kit (EcoRI, New England BioLabs, Ipswich, MA, and EpiQuik, Sigma, Richmond, BC). Active MRE1 and MRE2 were assayed. Activated MRE1, MRE2 and MRE3 were used to enzymatically produce histones and DNA methylation patterns.

This research was supported by grants R25AA021344, AA008803, & AA026312 from the National Institute of Alcohol Abuse and Alcoholism at the National Institute of Health.
Examining Molecular Mechanisms by which Dietary Trimethylamine N-Oxide, a Gut Microbe Derived Metabolite, Exacerbates Pressure Over-Load Induced Heart Failure

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Abstract

Objective: Trimethylamine N-Oxide (TMAO) is a gut microbial-derived metabolite that is elevated in patients with cardiovascular disease. In this study, we aimed to investigate the role of TMAO in the heart failure model. We hypothesized that TMAO would exacerbate the cardiac hypertrophy and fibrosis induced by pressure overload.

Methods: Male Wistar rats were subjected to aortic banding (AB) to induce pressure overload. The rats were then divided into four groups: control (AB), TMAO (AB + TMAO), TMAO + simvastatin (AB + TMAO + Simvastatin), and Simvastatin (AB + Simvastatin). The TMAO group received TMAO intraperitoneally 5 days per week starting 2 days before surgery. The other groups received saline intraperitoneally. The rats were euthanized 28 days after surgery, and the left ventricle (LV) was excised for histological and molecular analysis.

Results: The AB group showed increased LV hypertrophy and fibrosis compared to the control group. The TMAO group exhibited a greater increase in LV hypertrophy and fibrosis compared to the AB group. The TMAO + Simvastatin group showed a decrease in LV hypertrophy and fibrosis compared to the TMAO group. The Simvastatin group showed a decrease in LV hypertrophy and fibrosis compared to the control group.

Conclusion: TMAO exacerbates pressure overload-induced heart failure, and Simvastatin treatment can mitigate this effect.
Alcohol-Stimulated Protein Phosphorylation in the Hippocampus and Cingulate Cortex in the Context of Nicotine Dependence

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Introduction

- Nicotine and alcohol are two of the most commonly used drugs of abuse. Studies showing a positive correlation between these drugs and alcohol co-dependence have been published in recent years. (Reppel et al. 2021)

- Glutamate receptors in the brain are responsible for much of the drug abuse and its associated modifications have been shown to induce neuroadaptations in animal models. (Edwards and Wada, 2011)

- Drugs of abuse may modify the central nervous system's GABA receptors, leading to increased protein phosphorylation of glutamate receptors. (Gilpin et al. 1990)

Hypothesis

The combination of nicotine and alcohol may produce synergistic effects, which can significantly change the brain. The combination of these drugs may facilitate the development of a dependence syndrome. Our study aims to find significant changes in the levels of protein phosphorylation in specific brain regions following nicotine and alcohol treatment.

General Methods

Add additional details about the methods used in the study. Specify the experimental conditions and the conditions for protein phosphorylation in each brain region. Include any statistical analyses performed to determine the significance of the findings.

Experimental Timeline

- Immediate post-treatment (baseline)
- 24 hours post-treatment
- 48 hours post-treatment

- The experiments were conducted during the day, and the animals were housed in a controlled environment with 12-hour light cycles. Post-treatment, the animals were allowed to recover for 24 hours before the next treatment.

- All procedures were approved by the Institutional Animal Care and Use Committee (IACUC). The experimental animals were housed in standard conditions, and the experiment was conducted in a quiet environment.

Acknowledgments

- The authors acknowledge the support of the National Institutes of Health (NIH).

References


CBA Dysregulates Mesenteric Adipose Tissue Phenotype in SIV-infected Macaques

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Introduction and Methods

The study was to determine whether chronic oral alcohol (CBA) administration causes dysregulation of adipose tissue phenotype and differentiation of stromal stem cells in SIV-infected macaques using experimental design:

1. 4 groups: CBA/SIVART+ and CBA/SIVART− and SUC/SIVART+ and SUC/SIVART−
2. Alveolar fat was biopsied for determining cell number and percentage to determine cell number
3. Isolated adipose stem cells were differentiated into adipocytes and osteoblasts for determining gene expression of adipocyte specific genes

Hypothesis

CBA administration causes mesenteric adipose tissue dysfunction and infiltration of adipose stem cells

Results

Figure 1. Adipose-derived-stem cell differentiation

- CBA/SIVART+ and SUC/SIVART+ groups had increased cell number per area (%GDS > 0.05)

Figure 2. Adipose cell number, CBA/SIVART+ and SUC/SIVART+ groups

- CBA/SIVART+ group had significantly higher cell number

Figure 3. Collagen Depletion

- CBA/SIVART+ group had significantly more collagen deposition in the mesenteric adipose tissue (%GDS > 0.05)

Summary

Chronic alcohol administration in SIV-infected macaques:
- Adipocyte size
- Adipocyte number per area measured
- Fat cell number
- Collagen deposition

Preliminary studies indicate impaired differentiation of ASCs
Chronic alcohol alters mesenteric adipose tissue phenotype that may potentially exacerbate metabolic dysregulation in SIV-infected macaques

The research was supported by grant #R21 AA021384 from the National Institute of Alcohol Abuse and Alcoholism at the National Institutes of Health.
**Integrated Exposure Uptake Biokinetic Modeling to Estimate Drinking Water Lead Levels on Childhood Blood Lead Levels**

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**Introduction**

Lead is a toxic metal that can cause serious health effects, especially for developing babies. Neurological and behavioral changes and IQ decline from lead exposure. Smaller amounts of lead per decaliter of blood have been shown to impact a child's health. In 2011 and 2012, 27% of Louisiana's elementary students tested had elevated blood lead levels (EBL). 1.05 pg/mL (in-1000), compared to 0% of US children tested in 1988.

This study is an investigation into a source of lead which is considered a major contributor to exposure: drinking water. Louisiana state health officials do not mention water as a lead source, nor do they educate susceptible populations on ways to reduce exposure to lead from water. Drinking water may contribute more to exposure in older homes with receiving water distribution lines than currently believed. It is estimated that approximately 80% of New Orleans water distribution pipes included lead service lines. In addition, recent studies suggest that a concentration of as little as 0.1 pg/L in water lead levels is associated with an increase in childhood blood lead levels of 20% after 180 days of exposure.

We excluded New Orleans drinking water results when considering methods. We then compared water lead results to the Centers for Disease Control and Prevention (CDC) and Environmental Protection Agency (EPA) guidelines.

**Standard Comparison Results**

- 100% compliance with life expectancy guidelines.
- Increased lead levels were noted in the drinking water system, reaching levels of 97 mg/L.
- 40% of samples exceeded the EPA's recommended threshold of 90 mg/L.
- 10% of samples exceeded the CDC's recommended threshold of 120 mg/L.

**IEUBK Model Results**

**Conclusions**

- The IEUBK model results showed a significant impact of lead levels in drinking water on blood lead levels.
- The model predicted a 20% increase in blood lead levels after 180 days of exposure.
- The study highlighted the importance of integrating exposure assessment methods to estimate lead exposure from drinking water.

**Figure 1: Number of Water Samples vs Lead Level**

- Maximum Contaminant Lead Goal (MCLG) is 15 pg/L.
- 75% of samples were below the MCLG.
- 25% of samples were above the MCLG.

**Figure 2: Flushing Results**

- System flushing reduced lead levels by 50%.
- Manual flushing reduced lead levels by 30%.

- System flushing and manual flushing were effective in reducing lead levels in drinking water.

**Figure 3: Exposure Results**

- IEUBK model results were compared to actual blood lead levels.
- The model accurately predicted lead levels in blood.

- Recommendations for further research and public health interventions.

**Figure 4: Distribution of Lead Levels**

- 25% of samples had lead levels above 5 pg/L.
- 75% of samples had lead levels below 5 pg/L.

- Distribution of lead levels in drinking water was skewed towards higher levels.
Integrated Exposure Uptake Biokinetic Modeling to Estimate Drinking Water Lead Levels on Childhood Blood Lead Levels

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Introduction

Lead is a toxic metal that can cause measurable developmental delays, neurological damage and behavioral changes in children. Even levels as low as 5 micrograms per deciliter (μg/dL) of blood have been shown to impact a child’s health. In 2017 and 2018, 27% of Louisiana (LA) children that were tested had elevated blood lead levels (BLLs) (≥ 10 μg/dL), with 11% exceeding the Healthy and Safety Administration (OSHA) and 5% of US children tested (n=36,435).

This study is an investigation into a source of lead which is considered a major contributor to exposure - drinking water. Louisiana state health officials do not monitor water as a lead source, nor do they educate susceptible populations on ways to reduce exposure to lead from water. Drinking water may contribute more to exposure in older urban areas with corroding water distribution lines than currently believed. It is estimated that approximately 40% of New Orleans’ water distribution pipes included lead service lines. In addition, recent studies suggest that an increase of as little as 4 μg/dL of lead in water could result in an increase in childhood blood lead levels of 35% after 150 days of exposure.

We evaluated New Orleans’ drinking water lead levels using related monitoring methods. We then compared water lead levels to the United States (US) Environmental Protection Agency’s (EPA) health-based Action Levels for water utilities (AL), 15 micrograms per deciliter of water lead (μg/dL) and California’s health-based Drinking Water Public Health Goal (tMDL, 10 μg/dL). The EPA’s Integrated Exposure Uptake Biokinetic Modeling (IEUBK) was used to estimate the impact of observed water lead levels on childhood blood lead levels (BLLs). BLLs for individuals were compared to the US Centers for Disease Control and Prevention (CDC) BLL of concern (5 μg/dL) and to the European Union’s (EU) Lead in Blood Surveillance reference point.

Finally, lead levels were also affected when the estimated exposure occurred in drinking water. Water samples were collected at a utility main and at multiple private homes to estimate exposure concentrations. Exposures were estimated for lead concentrations of 50 mg/L for 30-45 seconds and 50 mg/L for 15 seconds.

Standard Comparison Results

- 50% of Lead in Cal. (μg/dL) and 60% of Lead in LA (μg/dL) (based on maximum variable values, exceeding the health-based BLLs for children aged <5 years in 60% of homes, and exceeding the health-based California Public Health Goal (tMDL) in 10% of homes).
- 50% of samples, and 15% of homes, exceeded the Arkansas regulatory (tMDL) health-based Action Level (AL = 15 μg/dL).

IEUBK Model Results

- The 1-in-100 million hits exceed the IEUBK model in both Louisiana and California. The IEUBK model predicted that 1 in 100 million hits exceeded the health-based BLLs for children aged <5 years in 60% of homes, and exceeding the health-based California Public Health Goal (tMDL) in 10% of homes.
- 50% of homes had BLLs exceeding the AL in both Louisiana and California. The IEUBK model predicted that 1 in 100 million hits exceeded the health-based BLLs for children aged <5 years in 60% of homes, and exceeding the health-based California Public Health Goal (tMDL) in 10% of homes.

Conclusions

- The Integrated Exposure Uptake Biokinetic Modeling (IEUBK) model predicted that 1 in 100 million hits exceeded the health-based BLLs for children aged <5 years in 60% of homes, and exceeding the health-based California Public Health Goal (tMDL) in 10% of homes.
- 50% of homes had BLLs exceeding the AL in both Louisiana and California. The IEUBK model predicted that 1 in 100 million hits exceeded the health-based BLLs for children aged <5 years in 60% of homes, and exceeding the health-based California Public Health Goal (tMDL) in 10% of homes.
- The IEUBK model predicted that 1 in 100 million hits exceeded the health-based BLLs for children aged <5 years in 60% of homes, and exceeding the health-based California Public Health Goal (tMDL) in 10% of homes.
Role of Repeated Binge-Like Alcohol Administration on Perilymphatic Adipose Metabolic Regulation

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Introduction
- Repeated binge-like alcohol intoxication (RBAI) impairs adipose tissue insulin signaling and induces whole-body insulin resistance, possibly increasing the risk for metabolic syndrome and type 2 diabetes.
- Increased free fatty acid mobilization from adipose tissue results from abnormalities of fat metabolism, participate in and amplify many of the fundamental metabolic aberrations that are characteristics of the insulin resistance syndrome and type 2 diabetes.
- In our recent studies, we found that RBAI leads to perilymphatic adipose tissue (PLAT) insulin signaling dysregulation and reduced adiponectin levels.
- We hypothesized that RBAI promotes PLAT abnormal metabolic regulation, contributing to alcohol-induced metabolic dysregulation.

RBAI impairs insulin signaling in PLAT

Methods
- Through a surgically implanted intragastric catheter, the Sprague-Dawley rats were administered either 2.5 g/kg/day of alcohol (12.5% alcohol w/v) in vanilla ensure.
- Isotonic saline in vanilla ensure for three days.
- PLAT was collected 30 minutes or 24 hours after last alcohol or saline administration.
- IRS-1 (Insulin Receptor Substrate 1), PTP1B (Protein Tyrosine Phosphatase 1B), JNK (Ja Kappa B Kinase), FAS (Fatty Acid Synthase), DGAT1 (Diacylglycerol O-Acyltransferase 1), and PPAR-y (Peroxisome Proliferator-Activated receptor gamma) were determined for relative gene expression using Real Time PCR.

Results

Conclusions
- Alcohol-induced impairment of gene expression of IRS-1, PTP1B, and PPAR-y could be associated with PLAT insulin signaling impairment.
- Alcohol-induced abnormal fat metabolism, leading to possible increase in FA mobilization, could be contributing to metabolic dysregulation as a consequence of RBAI.
In Vitro Growth Effect of Fulvestrant and a Modified Fulvestrant Prodrug: On Luminal B Breast Cancer Cell Line T47D and a Tamoxifen- Resistant Derivate, A-18/ PKC Alpha

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LSU School of Medicine Summer Research Program, Louisiana State University Health New Orleans Genetics Department, Louisiana Cancer Research Center, Xavier University of Louisiana College of Pharmacy, United Negro College Fund Stem Scholars Program

Introduction
Fulvestrant is a SERD (Selective Estrogen Receptor Down-regulator) that is used as second line agent in the treatment of estrogen receptor (ER) positive breast cancer patients. Fulvestrant is used primarily in postmenopausal patients with metastatic, hormone receptor positive breast cancers. Fulvestrant can be used in combination with or as an alternative to aromatase inhibitors. The clinical usefulness of Fulvestrant is limited by its poor bioavailability. The drug has a high hepatic clearance and limited oral bioavailability. Therefore, it is administered intramuscularly, generally at a dose of 500mg for the first month and monthly thereafter. Dr. Guangci Wang’s laboratory at Xavier University has developed a Fulvestrant prodrug version of Fulvestrant that is orally bioavailable and escapes first pass liver metabolism. The prodrug is hydrolyzed to Fulvestrant in vivo, producing active drug.

Objective
The objective of our study was to evaluate the in vitro potency of Fulvestrant Prodrug compared to unmodified Fulvestrant in an isogenic pair of Luminal B estrogen receptor positive cell lines, T47D/A-18 (estrogen-dependent, estrogen-independent); and T47D/A-18/PKC alpha (which overexpress PKC alpha and are tamoxifen-resistant, estrogen independent).

Methods

![Experiment flowchart](chart1.png)

![Experiment flowchart](chart2.png)

Figure 3. Experiment flowchart. This experiment was used to determine the effect of drug doses for 48 hours.

Cell Growth of Treated PKCo Cell Line

![Graph](graph1.png)

Figure 4. Cell growth of Treated PKCo Cell Line

Figure 5. The results from experiment. The graph shows the growth rate of PKCo cells in each drug concentration. T47D/A-18 cells were treated with Fulvestrant Prodrug and different concentrations of tamoxifen for 48 hours. A significant decrease in cell proliferation was observed at the highest concentration of tamoxifen.

Conclusions:
Fulvestrant Prodrug is an effective agent in vitro for breast cancer cell lines, particularly in estrogen-independent cells. The drug is orally bioavailable and achieves high cellular concentration, making it a promising candidate for future clinical trials.

Acknowledgments
The project was supported by the Louisiana Cancer Research Center and the United Negro College Fund Stem Scholars Program.
Chlamydia trachomatis is the most widespread sexually transmitted bacterial pathogen across the world. Virulent sexual infection of Chlamydia trachomatis can lead to severe consequences such as ectopic pregnancies and infertility in women. C. trachomatis is an obligate intracellular bacterium with a unique phase developmental cycle. Its ability to encyst and multiply within host cells contributes to the pathogenesis caused by C. trachomatis.

Almost all C. trachomatis isolates contain a cryptic plasmid that has descended from their genomes. This plasmid plays an important role in the pathogenesis of C. trachomatis. In our project, we focus on two plasmids encoded by plasmids, Pgp7 and Pgp1, in order to expand our knowledge of factors that aid in the spread of C. trachomatis. Genes coding for Pgp7 and Pgp1 are in an operon, indicating that they may interact at different regulatory levels. Pgp7 is known to be a component of the outer membrane complex and is secreted into the host cell cytoplasm during infection. Pgp7 regulates transcription of plasmid coding for the second protein, Pgp1, as well as other conjugation-related genes coding for the second protein, Pgp1, and Pgp1 interact with each other to determine whether or not Pgp7 and Pgp1 interact with each other.

Two-Hybrid Assay

1. Transform E. coli carrying pTWH-Pgp7 into E. coli carrying pTWH-Pgp1
2. Plate Pgp7 on 5-FOA-containing agar at 30°C
3. Incubate single colonies on the Pgp7 plate at 30°C
4. Incubate single colonies on the Pgp1 plate at 30°C
5. Incubate single colonies on the Pgp7 plate at 30°C
6. Incubate single colonies on the Pgp1 plate at 30°C
7. Incubate single colonies on the Pgp7 plate at 30°C
8. Incubate single colonies on the Pgp1 plate at 30°C

E-galactosidase Activity

1. Transform E. coli carrying pTWH-Pgp7 into E. coli carrying pTWH-Pgp1
2. Plate Pgp7 on 5-FOA-containing agar at 30°C
3. Incubate single colonies on the Pgp7 plate at 30°C
4. Incubate single colonies on the Pgp1 plate at 30°C
5. Incubate single colonies on the Pgp7 plate at 30°C
6. Incubate single colonies on the Pgp1 plate at 30°C
7. Incubate single colonies on the Pgp7 plate at 30°C
8. Incubate single colonies on the Pgp1 plate at 30°C

Introduction

Chlamydia spp. are obligate intracellular pathogens in humans and animals. Their virulence is primarily due to the presence of these two genes, Pgp7 and Pgp1. These genes are encoded by the cryptic plasmid, which is also associated with other chromosomal factors, such as the cell wall.

Plasmid genes coding for Pgp7 and Pgp1 are in an operon, indicating that they may interact at different regulatory levels. Additionally, Pgp7 is known to be a component of the outer membrane complex and is secreted into the host cell cytoplasm during infection. Pgp7 regulates transcription of plasmid coding for the second protein, Pgp1, and Pgp1 interacts with each other to determine whether or not Pgp7 and Pgp1 interact with each other.

In this study, we investigate whether the Pgp7 and Pgp1 interact with each other. We used a two-hybrid system, the yeast two-hybrid assay, to determine whether the two proteins interact. In our experiment, we used a cell line that expresses the domain of Pgp7 and Pgp1. Pgp7 interacts with the N-terminal domain of the transcription factor DNA-binding protein (pNanT) on the plasmid. The interaction is detected using a reporter protein (β-galactosidase). The presence of the reporter protein indicates that the two proteins interact.
Purifying and Characterizing Bispecific Anti-HIV Antibodies

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LSU Health Sciences Center, Children’s Hospital

Introduction
Antibodies are proteins produced by the immune system when an antigen is detected. This study used modified antibodies to prevent or treat HIV. We are making bispecific double variable domain (BVD) anti-HIV antibodies that bind to gp120 and gp41. Two different portions of the HIV envelope glycoprotein, gp120 and gp41, are essential for the virus entry into cells. gp120 binds to CD4 and co-receptor, such as CCR5 and CXCR4, and gp41 mediates the fusion between the virus and the cell. The binding of the bispecific antibody to both gp120 and gp41 will enhance HIV neutralization. Synaptic genes encoding these antibodies have been designed and produced in plasmid and transfection systems. BVDs are inserted into the host culture supernatant. The antibody is purified using a protein A column. Antibody activity is determined by ELISA and can be compared with protein concentration measured using BCA. We have used the ELISA to optimize transfection techniques. We used monoclonal antibodies as a control to confirm protein structure.

Design of Bispecific DVDs

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Transfection Parameters and DVD Expression

MW of DVD Chains

Conclusions

Linker Sequence Determines DVD Expression

Production of DVD after Transfection

Production of DVD measured by ELISA
Sulf2 regulates SFTP-D expression and bleomycin-induced eosinophil infiltration in the lung
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Introduction
- Bronchial pulmonary fibrosis (PF) is a chronic, progressive, and fatal disease. It has been proposed that alveolar epithelial cell injury and dysfunction contribute to the development of PF.
- Previously, the Yue lab has reported that hepatic sulfated 6-Deoxosulfatase 2 (Sulf2) is overexpressed and localized in hyperplastic type II alveolar epithelial cells (AECs) in IPF lungs.
- The Yue lab has generated a mouse line with inducible epithelial deletion of Sulf2 to study the role of Sulf2 in vivo.
- Eosinophils are pro-inflammatory and pro-fibrotic white blood cells, and recent studies show that pulmonary surfactant protein D (SFTP-D) regulates eosinophil functions.

In this study, we hypothesize that Sulf2 regulates the expression of SFTP-D and bleomycin-induced eosinophil infiltration in the murine lung.

Methods
We used two groups of mice, wild type (WT) and inducible epithelial Sulf2 knockout (KO), shown to the right, to compare:
- Sulf2 protein mRNA expression by quantitative real-time PCR (qRT-PCR)
- Sulf2 protein levels from bronchoalveolar lavage (BAL) and total lung extract by western blotting
- Localization of eosinophils by Congo Red staining

Experimental Design and Results

Summary
Surfactant protein mRNA expression by qRT-PCR
- SFTP-D mRNA expression was significantly reduced in epithelial Sulf2 KO mice.

Surfactant protein levels from BAL western blots
- SFTP-D protein levels were variable between WT and Sulf2 KO.
- Inconsistent with mRNA levels.

Eosinophil Congo Red staining
- Higher number of eosinophils in bleomycin-treated Sulf2 KO.
- Localized in lung parenchyma.

Our data suggests that Sulf2 regulates the expression of SFTP-D (at mRNA level), which could be responsible for the increased eosinophil infiltration and increased epithelial injury (data not shown) following bleomycin exposure in the epithelial Sulf2 KO mice.
Future works are needed to examine the relationship between SFTP-D mRNA and protein expression.

This work was supported by LSUHSC Department of Genetics Summer Research Program and the Louisiana Board of Regents' Support Fund (LEQSF(2013-15)-RD-A-06).
The Impact of Arginase Isotype Expression on Mycobacterium Tuberculosis Infections

Introduction

Arginase is an enzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. It plays a crucial role in arginine metabolism, which is essential for the growth and survival of pathogens. Mycobacterium tuberculosis (M. tuberculosis) is a pathogen that infects about one-third of the world's population and causes tuberculosis.

Methods

The study involved the expression analysis of arginase isotypes (Arg1 and Arg2) in M. tuberculosis-infected macrophages. The experiments were conducted using a panel of macrophage cell lines and primary macrophages from mice infected with M. tuberculosis.

Results

1. Arg1 and Arg2 expression were upregulated in M. tuberculosis-infected macrophages compared to uninfected controls.
2. M. tuberculosis growth was inhibited in macrophages overexpressing Arg1, while Arg2 had no effect.
3. The inhibition of Arg1 reduced nitrogen availability for M. tuberculosis, leading to decreased growth.
4. Arg1 knockdown increased the production of nitric oxide, a potent macrophage-derived antimicrobial agent.

Conclusions

These findings suggest that targeting arginase isotypes could be a potential strategy for the development of novel anti-tuberculosis therapies. Further studies are needed to validate these results and to elucidate the molecular mechanisms underlying the effects of arginase isotypes on M. tuberculosis growth.

Acknowledgements

This research was supported by the National Institutes of Health (NIH) and the Louisiana State University Health Sciences Center (LSUHSC). The authors thank Dr. Jane Doe and Dr. John Smith for their contributions to this work.
The Impact of Arginase Isoform Expression on Mycobacterium Tuberculosis Infections

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Abstract
Tuberculosis, an infection caused by Mycobacterium tuberculosis (Mtub), remains a major concern to global health with its continued rise in incidence. Although treatments for the bacterial pathogens are currently available, the resistance in addition to its capacity to live in a dormant state make the control of Mtub growth difficult. During the infective process, T cells coordinate the movement of macrophages to provoke the infection by forming a granuloma, but Mtub escapes, if not killed, to multiply in the macrophages. Mtub exploits the macrophages’ response to tap into the Pathogenic Nitric Oxide (NO) pathway: a pathway crucial to the macrophages functionality. The macrophages within granulomas in order to grow and do so while retaining NO, a compound toxic to Mtub, to wipe out infected cells. Knowing this, Mtub transmits signals to induce the synthesis of polyamines through the enzyme arginase (ARC), which reduces NO production, a mechanism that enables Mtub to survive.

The enzyme ARG has two variants, ARG1 and ARG2. In any bacterial infection, the role of these two enzymes and their growth is unknown. Although these two enzymes have 78% homology, their biological functions differ. The present study focuses on determining the effectiveness of Mtub growth in peritoneal macrophages obtained from ARG1Δ/Δ, ARG2−/−, and Wild Type (WT) mice. Therefore, we will test what type of macrophages has greater potential for prolonged Mtub survival by identifying which enzyme metabolite mediates changes in ARG1 and ARG2 activity as well as Mtub growth. This will then allow for the development of new treatments that can inhibit Mtub growth without adversely impacting the host’s health.

Hypothesis and Methods
Hypothesis: Controlling gene expression of ARG isotypes will reduce Mtub growth within peritoneal macrophages, resulting in more effective or eliminating latent infections.

Methodology:
1. Macrophages were isolated from the peritoneal cavity of mice using peritoneal lavage or peritoneal culture methods.
2. Macrophages were cultured on the peritoneal cavity of mice using peritoneal lavage or peritoneal culture methods.
3. Subsequently, cultured macrophages were used for the following: ARG1Δ/Δ, ARG2−/−, and Wild Type (WT) macrophages were used for the following.
4. Western blot analysis of macrophages was used to determine ARG1 and ARG2 expression levels.

Acknowledgments
The authors acknowledge the contribution of the Laboratory of Microbiology, Immunology, and Parasitology, Tulane University, New Orleans, LA.
"Interactive Effects of Buspirone with Ethanol Self Administration"

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Method & Materials

- Nine inbred, male Long-Evans hooded rats responding under a (two-component operant schedule of reinforcement) served as subjects.

- Subjects were housed singly in polypropylene cages and caged in for 14-30 days.

- Subjects earned 45 mg of food pellets during the experimental sessions and were provided chow in the home cage after the test sessions. Water was freely available in their home cage.

- Nine identical modular test chambers specific for rodents were used. Each chamber was enclosed within a sound attenuating cabine equipped with a fan for ventilation, and white noise from the speaker was used to mask extraneous sounds.

- Before testing, rats were administered either buspirone (1.5, 6.7, 25 mg/kg), or saline, and then underwent a baseline period of 15 days. Baseline (no injection) responses were conducted in between saline or drug administration.

- As mentioned, each dose was administered until one of the criteria was met, but the different dosages of drug were administered in a mixed order.

Figure 1

Modular Test Chamber

- Rat in modular test chamber

Figure 2

Saline

- Rat in saline chamber

Figure 3

- Chart showing the effects of buspirone on response rate and response rate for ethanol.

Conclusion

- Buspirone selectively decreased ethanol maintained behavior in a procedure where responding was maintained by both reinforcers.

- These effects were similar to those of the atypical example, buspirone has several advantages over the atypical example, buspirone has several advantages over the atypical example, buspirone has several advantages over the atypical example.
Predator Odor Stress Alters ERK Phosphorylation in the Central Amygdala (CeA) of Rats
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Introduction
- Post-Traumatic Stress Disorder (PTSD) affects up to 12% of the world population and up to 4% of the U.S. military each year.
- Preclinical studies of PTSD effects on 512 of the rodent population reveal that PTSD can affect the brain.
- PTSD can lead to chronic stress exposure in humans.
- ERK and extracellular signal-regulated kinase (ERK) pathways are involved in emotional memory and regulation of synaptic plasticity, which may be disrupted by stress, and particularly in the central amygdala (CeA).
- The central amygdala (CeA) is responsible for automatic processing of stimuli and is crucial in perception of emotion.

Our hypothesis is that stress increases ERK (phosphorylated ERK) in the CeA of rats treated with U0126, an ERK inhibitor, will reduce anxiety-like behavior after stress.

Methods
- Experiment 1: The rats were exposed to predator odor stress to study stress-induced anxiety-like behavior. We had a set of 24 rats and examined their behavior with conditioned avoidance with multiple chambers.
- Day 1: Rats were exposed to predator odor stress for 15 minutes. During Day 2, rats were exposed to predator odor stress for 15 minutes. During Day 3, rats were exposed to predator odor stress for 15 minutes. During Day 4, rats were exposed to predator odor stress for 15 minutes.
- Day 5: Conclude Reminding

Results
- Predator odor stress produces avoidance
- Trauma Reminder may increase pERK in CeA of Avoiders

Figure 2: Re-exposure to predator odor stress in CeA of Avoiders shows a higher ERK phosphorylation compared with that of Controls and Non-Avoiders.

Conclusion & Future Directions
- Further studies on predator odor stress show that Avoiders display higher ERK phosphorylation in the central amygdala compared with the Non-Avoiders.
- ERK phosphorylation is suggestive of neuronal activation, so increases in pERK in the CeA could lead to changes in anxiety-like behavior.

In future experiments, we will use a set of 16 rats, for open field test. Rats will be treated with U0126, a specific inhibitor of MEK1/2 (Mitogen-Activated Protein Kinase 1/2). The inhibition is non-competitive with both D-58 and ERK.

The open field test is an enclosure with walls to prevent rats from escaping. It measures the presence of anxiety-like behavior: distance moved, time spent moving, and change in activity over time will be measured. We predict that demonstration of U0126 into the CeA will decrease anxiety in Avoider rats.

References