Voelcker Biomedical Research Academy

Class of Summer 2016

Graduating Scholar Abstracts

Protein Effects on the Brain and Liver

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Background: Proteins are one of the sources of the mitochondria which is main focus in Dr. Bai's lab and his partners. The focus of Bi Zhu was the effects of the protein on the liver and analyzing the effects in mouse model. We focused on multiprotein complex protein and study the effects on the liver. In the lab we used gel and Blue Native PAGE to see results threw protein study, e.g: gel electrophoresis.

Methods: Blue Native PAGE was developed for the separation of mitochondrial membrane proteins and complexes in the mass range of 10 kDa to 10 MDa. It is used for the one-step isolation of microgram amounts of membrane protein complexes from biological membranes and total cell and tissue homogenates; for clinical diagnostics of human mitochondrial disorders; to determine native masses and oligomeric states; to determine the stoichiometry of a multiprotein complex by an antibody-based gel-shift method; to identify physiological protein–protein interactions; for 2D crystallization and electron microscopy, in-gel activity assays, native electro blotting and immunodetection; for studies of neurotransmitter assembly, protein import and apoptosis research; to isolate supramolecular physiological protein assemblies, and many more tasks. It is a convenient and inexpensive technique based on a few simple principles.

Results: Since the study is in mid process we are currently studying and analyzing results. We are addressing our hypothesis. The results of the gel and protein analysis used in lab are as such. Blue native PAGE, similar to coimmunoprecipitation, has the advantage that detergent must be added only once (for solubilization), and this detergent generally is not harmful to the proteins, owing to the presence of lipid from the solubilized membranes. Unlike coimmunoprecipitation, BN-PAGE does not require antibodies to isolate the desired protein but seems to be less mild because of the anionic properties of added Coomassie dye.

Conclusion: Our focus is using the protein focus in the brain to prevent and/or slow brain decaying.

Funding: Voelcker Biomedical Research Academy at the University of Texas Health at San Antonio

Developing a Standard for Analytical Ultracentrifugation

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Analytical Ultracentrifugation is a first principle technique that lays the foundation for molecular structure research across all disciplines. Previous work has been done to create a protocol using Bovine Serum Albumin as a reference standard for the AUC. This protein has proven to be ineffective as a standard due to its disposition to aggregation, degradation, and heterogeneity. The goal of this experiment was to develop a standard for AUC investigators of all disciplines to ensure that the results obtained are both reliable and precise. The hypothesis stated that DNA with a known sequence, molecular mass, and shape will provide an ideal standard for the Analytical Ultracentrifuge, and can be purified to a very high degree with gel electrophoresis. DNA fragments of precise length were grown up in a transformed pUC8 plasmid with a pPOL1-208-12 insert. Glycerol stocks of this transformed bacteria were plated, and an isolated colony was placed in a large overnight culture. A DNA extraction protocol was performed through a series of vortexing, centrifugation, and denaturing solutions. The samples were then purified in a vertical agarose gel column and fractions were collected. DNA samples were measured in the AUC at 25k, 35k, and 45k rpm in titanium cells at various ratios. The data was then analyzed in the UltraScan III software.

From these preliminary results, the effectiveness of DNA as a reference standard for the AUC is promising. By testing multiple lengths of DNA with varying molecular mass, the full range of the Analytical Ultracentrifuge's capabilities can be examined.

Mesenchymal Stem Cells Improve Survival in a Mouse Model of Severe Pneumococcal Pneumonia

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Background: Community acquired pneumonia is a leading cause of death in the U.S., resulting in about 1 million hospitalizations and about 50,000 deaths each year. This disease is most commonly caused by the bacterium *Streptococcus pneumoniae*. The objective of this study is to observe the effect of adipose-derived human mesenchymal stem cell (AdH MSC) treatment on host survival, bacterial burden, tissue damage, and cardiac microlesion formation during a pneumococcal infection compared to the placebo. We hypothesize that pneumococcal pneumonia challenged mice treated with mesenchymal stem cells will have increased survival, less bacterial burden, decreased tissue damage, and fewer microlesions formed throughout the infection.

Methods: This experimental study used genetically identical 10-week old C57 mice. Each mouse was intranasally infected with *Streptococcus pneumoniae* at a dose of 10^7 CFU. There were two groups of mice: 1) Intervention: the intervention group was given a dose of AdH MSCs (10^6 cells in 100 ul saline) intratracheally, and 2)Control: the control group was given a placebo (saline). Two sets of studies were performed for this experiment. The first set compared the outcomes of mouse survival and blood bacterial burden between the AdH MSC (n=8) and the control (n=5) groups. The second set of mice was used to compare lung bacterial burden, lung tissue damage, and cardiac microlesion formation (48-hours post-infection) between the AdH MSC (n=6) and the control (n=3) groups.

Results: The survival analysis indicates that the AdH MSC mice showed a trend of higher survival rates (29% survival) than the control group (0% survival) after 5 days (p=0.08). The bacterial burden analysis within the blood after 72 hours illustrated that there was a trend towards greater bacterial burden for the AdH MSC group (Med: 5.3 [IQR: 4.6-6.2]) than the control group (Med: 3.8 [IQR: 3.3-4.2];p=0.09). Between the AdH MSC and the control group there was no difference of lung bacterial burden (Med: 4.6 [IQR: 4.0-5.1] vs. Med: 4.8 [IQR:2.3-6.2];p=0.88), lung pathology score (Med: 2 [IQR: 2-3] vs. Med: 3 [IQR: 1-4];p=0.83), nor microlesion number (Med: 5.5 [IQR: 4-7] vs. Med: 9 [IQR: 1-43];p=0.46).

Conclusion: Overall, the AdH MSC group seemed to have a tendency towards higher survival, lower lung damage, and decreased cardiac microlesion formation. Further studies will focus on increasing n values in each group to better define statistical values. Study outcomes and translation of these findings to other species, including humans, may improve survival due to invasive pneumococcal pneumonia.

Effects of Acute Exercise on GNMT in Healthy Males

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Introduction: Studies by our group and others have demonstrated that exercise reduces high grade prostate cancer (PCa). However, the direct molecular mechanisms by which exercise is chemopreventive are unclear. Previous studies have indicated that levels of sarcosine, an N-methyl derivative of the amino acid glycine, differed significantly between localized and metastatic tumors as well as between cancerous and benign prostate tissue. Knocking down glycine N-methyltransferase (GNMT), the enzyme that's responsible for catalyzing glycine to sarcosine, inhibits PCa cell growth.

Purpose: To conduct a proof of concept study on the effects of a single bout of exercise on GNMT concentrations in healthy males.

Methods: In the study, 10 healthy subjects engaged in a 1 hour, single bout of aerobic exercise on a cycle ergometer at 60% max heart rate. Serum was collected pre-exercise and 120 minutes following the intervention. A commercial ELISA kit will analyze the concentrations of glycine N-methyltransferase (GNMT) in the serum (Sigma-Aldrich, St. Louis, Missouri). A student T-test will be conducted with significance set at $p<0.05$.

Results: Data is pending due to technical issues experienced by the vendor regarding development of the ELISA kit. At this point, five male participants have completed the single bout of exercise. The subjects were between 21-26 years (24.6±2.19 years), with an average 60% max heart rate of 117.24±1.31 BPM. Each had a BMI <24.9 kg/m^2 (23.56±1.28 Kg/m^2), and the body fat percentage averaged 16.04±2.10%. We hypothesize that exercise will decrease GNMT in healthy males.

Conclusion: If the hypothesis for this study is confirmed, a similar exercise intervention can then be applied to PCa patients to determine the effect of exercise on GNMT activity. Further, future studies should include quantification of GNMT as a surrogate for sarcosine measurement as an indicator of tumor aggressiveness. Using this data, our group can then elucidate the three-way relationship between GNMT, tumor progression, and exercise in a clinical model.

Effects of Oxygen Levels on Preterm and Term Umbilical-Cord Stem Cells

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BACKGROUND: Recent studies have shown stem cells as being a promising therapeutic agent in regenerative medicine. The umbilical cord provides an excellent source of stem cells due to the fact that the cells can be easily isolated in a non-invasive manner and it is considered medical waste. Studies have demonstrated that umbilical cord mesenchymal stem cells (UC-MSCs) can improve inflammation, fibrosis, and prevent apoptosis. Despite the ubiquitous use of stem cells, research involving the comparison of preterm and term UC-MSCs are lacking. Therefore, our aim was to observe the effects of varying oxygen levels (1%, 21%, and 90% O2) on stem cells and to compare stem cell properties between preterm and term UC-MSCs.

METHODS: Umbilical-cords were obtained from 5 term and 5 preterm neonates. Cells were then isolated from the umbilical cord tissue and met the minimum criteria for MSCs according to The International Society for Cellular Therapy. The cells were then subjected to varying oxygen levels: hypoxia (1% O2), normoxia (21% O2), and hyperoxia (90% O2). Cell motility, proliferation, senescence, viability, colony forming unit efficiency, and cytokine inflammatory expression were assessed.

RESULTS: Under normoxic conditions (21% O2), there were no differences between the preterm and term UC-MSCs. Under hyperoxic conditions (90% O2), however, all cells had a slower motility and lower viability ($p<0.05$). While under hypoxic conditions (1% O2) the term cells had better proliferation. There was no difference between cellular senescence and cytokine expression. Term cells demonstrated more colony forming efficiency compared to preterm cells.

CONCLUSION: Our research showed that there was not a significant difference between preterm and term umbilical-cord stem cells. This work suggests that autologous/allogeneic transfer of umbilical-cord stem cells between preterm and term is possible.

The Effect of Isothiocyanates and Transforming Growth Factor Beta on Cancer Cells

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Breast cancer is an issue that plagues hundreds of thousands of women every year. In this project, the aim is to determine if an isothiocyanate is effective in reducing one's risk of getting breast cancer. Essentially, isothiocyanates have the ability to halt cell growth and initiate cell death specifically in cancer cells. Isothiocyanates are compounds found in some vegetables, known as cruciferous vegetables. Some examples include broccoli, cabbage, cauliflower, brussel sprouts and turnips. To test this, I have infected MDA MB 231 cancer cells with two different drugs, the first being Benzyl Isothiocyanate(BITC) and Phenyl Isothiocyanate(PITC) as well as transforming growth factor beta(TGF-β), which plays an imperative role in cell proliferation, development, differentiation, and overall functionality. I infected 10,000 cells for every concentration of each drug, and for every combination of a drug and TGF-β, making four different groups. The groups were: cells infected with BITC alone, cells infected with BITC and TGF-β, cells infected with PITC alone and cells infected with PITC and TGF-β. The concentrations for each drug respectively include 0, 1, 5, 10, 20 and 50 micromoles. After a 24 hour period, I observed and measured the amount of DNA present in the cells infected. The amount of DNA present suggests the number of cells present. The greater amount of DNA detected, the greater number of cells present. Based on the trials performed, and the results gathered, all four combinations reduced the number of cells present after the 24 hour time period. In every instance, the PITC and TGF-β duo were not as effective at reducing the number of cells as consistently as the others, however the BITC and TGF-β duo worked very well at keeping the number of cells down consistently. TGF-β helped lower the cell number, the drugs alone were not always very effective. Mid-ranged concentrations of both drugs, paired with TGF-β provide the most successful results at reducing the activation of carcinogens and detoxifying them. Evidently, eating foods containing isothiocyanates, is a small step that can be taken toward avoiding breast cancer.

What influences safe medication practices: Investigating the relationship between parents' intentions, self-efficacy, and knowledge.

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Background. Prescription drug misuse frequently occurs among adolescents and young adults. Parental guidance and monitoring play a large role in mitigating drug misuse and, potentially, access to prescription drugs. Indeed, 42% of adolescents who reported misusing drugs identified the source as their parent's medicine cabinet. For this reason, we assessed parents' knowledge, self-efficacy, and intentions about medication practices in the home. We hypothesized that parents with high self-efficacy and knowledge about medication safety and abuse liability would have greater intentions to practice safe medication storage. **Methods.** We developed an online survey based on Social Cognitive Theory. Participants were recruited through flyers and online posts. Data collected was analyzed using bivariate and multivariate analyses. **Results.** The preliminary sample for the current study was 81.3% female, 37.5% Hispanic, and 71.9% had a college degree. Majority of the parents (84.4%) had 1-2 children living in their home. Bivariate analysis indicated a significant association between intentions to practice safe medication storage and self-efficacy ($r=.49$, $p<0.005$), and a nonsignificant relationship between intentions and knowledge of medication safety ($r=.18$, $p>0.05$). A linear regression analysis determined a significant model ($F(1, 30)=4.81$, $p<0.005$), and R^2 showed that 23.9% of the variance in intention is explained by the variance in self-efficacy. **Conclusion.** Our preliminary results partially support our hypothesis suggesting that when parents are more confident that they may keep their medications safe from their children living at home and endorse greater intentions to practice safe medication storage. These findings contribute to the extant literature on parent medication practices in the home – with the goal of reducing prescription misuse among adolescents and young adults.

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An Intergenerational Study of South Texas Veterans and their Access to Health Care

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Background: Underutilization and lack of access to the Veterans Health Administration (VHA) Health Services is a prevalent problem which affects the health of our veterans. About 7 million veterans did not enroll in the VHA program even though 15.6 million are qualified. [1] Due to the United States' engagement in multiple wars and military global presence throughout the years, veterans from all ages may underutilize and unable to access VA Health Services. This study aims to identify intergenerational differences among old and young veterans in terms of: a) VHA access and utilization, b) difficulties in VHA access, and c) recommendations to improve access. Old veterans were those who engaged in World War II (1941) to the end of the Vietnam War (1975). Young veterans were engaged with the operations including the Persian Gulf War, Operation Enduring Freedom (OEF), Operation Iraqi Freedom (OIF), Operation New Dawn (OND) and other more recent operations.

Methods: This is a comparative descriptive study between old (N=15) and young veterans (N=15). The participants completed the Demographics and Military Service Questionnaire, and a study-created Veterans Health and Healthcare Access and Utilization Questionnaire (VHHAUQ). The VHHAUQ included quantitative and qualitative questions related to VHA access and use, and recommendations. The participants also completed the Short Assessment Health Literacy (SAHL) test (which was available in both English and Spanish). The purpose of the health literacy test was to determine potential confounder that could influence important health divisions. Participants received a $10 Subway gift card. Quantitative data was statistically analyzed for central tendencies and differences with $p < 0.5$. Qualitative data was analyzed for emerging themes.

Results: Most of the participants were male (old = 87.7%; young = 66.7%) and served the Army (old = 60%; young = 60%). Young veterans had better health literacy scores compared to the old veterans. Young veterans were more likely to have some VHA disability (mean for old = 34.7%; mean for young = 63.3%). Veterans shared their difficulties related to processing of VHA disability, accessing of VHA services, and quality of VHA service. Older veterans had more difficulties with procuring VHA disability, while younger veterans had more issues with accessing VHA services. Quality of service was an issue articulated by both old and young veterans. The veterans recommended the following strategies to improve services: veteran support programs (i.e. peer-mentoring), improving access to information, and increasing and improving quality of staff.

Conclusion: There are generational differences between the veteran populations (old vs young) in terms of accessing VHA services. To improve utilization of VHA services, issues identified in this study should be addressed, and veterans' recommendations be heeded to increase patient and community engagement. Further investigation of identified issues in a larger veteran population can inform the development of veteran-friendly interventions.

References:

1. Bernard DM, Selden TM. Access to Health Care among Nonelderly Veterans. Med Care 2016; 54 (3) 243-252.

Trm Bystander Activation

Sieon Kim, Wei Liao, Yong Liu, M.D., Ph.D, Nu Zhang, Ph.D

Memory CD8+ T cells are known for exerting effector functions in response to both antigen-specific and non-specific bystander stimulations. In the absence of cognate antigen and in the presence of non-specific bystander inflammation, memory T cells rapidly produce inflammatory cytokines (e.g. IFN-γ). Amongst the factors that contribute to such Trm bystander activation, IL-18 is unique as it is only required for bystander inflammatory responses without apparent involvement in antigen-elicited CD8+ T cell responses. However, such studies on the bystander response of memory CD8+ T cells is almost exclusively based on the results from circulating cells residing in the secondary lymphoid organs. Trm bystander activation in non-lymphoid tissues is expected to do unnecessary immune damage to vital organs, but whether such response happens in a similar manner remains unclear.

Previous studies in Dr. Zhang's lab have shown that Trm cells downregulate IL-18R during differentiation, and that the latter part of the maturation stages of $CD69^+$ Trm cells ($CD69+IL\text{-}18R^{hi}$ and $CD69+IL\text{-}18R^{lo}$) represent the mature Trm population. To further characterize the downregulation of IL-18R, Trm response to bystander inflammation in both *ex vivo* setting was analyzed. Congenically marked naive P14 T cells transferred into B6 recipients followed by LCMV infection. 18 days post infection, P14 T cells were isolated from the spleen and SI-IEL were stimulated ex vivo with 10nM $GP_{33\text{-}41}$ or 20ng/ml IL-12+IL-18 in the presence of Golgi Stop for 4 hours. IFN-γ production was determined by intracellular FACS analysis. The analysis suggested that Trm cells completely lost the response to bystander inflammation while maintaining the capacity to elicit a robust response to cognate antigen during *ex vivo* stimulation. To further validate this finding *in vivo*, IFN-γ-YFP reporter mice were directly infected with LCMV. 20 days post infection, GREAT mice were re-infected with unrelated pathogen *Listeria monocytogenes* (LM) to induce systemic bystander inflammation. 16 hours later, IFN-γ production from polyclonal LCMV-specific $CD8^+$ memory T cells was determined. In contrast, Trms from both kidney and gut completely lost this capacity. These observations suggest that both mucosal and non-mucosal Trm cells have lost the capacity to respond to bystander infection-induced systemic inflammation after $CD69+IL\text{-}18R^{lo}$ maturation.

It was shown that the Trm cells' capability of responding to bystander inflammation is decreased by the downregulation of the protein receptor IL-18K, which happens during the Trm cell maturation. The lab plans to continue the pursuit for the factors necessary for the downregulation of IL-18K, some of which are found to be TBF-β signaling, TBF-β mediated repression of Tcf-1, and the inflammatory signal type 1 IFN. Such studies on the deregulation of IL-18R will eventually connect to the studies regarding certain autoimmune disorders in human, which are often caused by Trm cells' strong response against bystander inflammation in non-lymphoid organs.

Impact of Human Adipose-Derived Mesenchymal Stem Cell Administration on Mouse Cytokine Expression and Behaviors Common to Multiple Psychiatric Disorders

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Mesenchymal stem cells (MSCs) migrate to sites of injury and secrete anti-inflammatory mediators and/or neurotrophic growth factors. The objective of this project was to assess the therapeutic potential for adipose-derived human MSCs administered either by tail vein injection (TVI) or intercerebroventricular (ICV) injection into mice to correct behaviors paralleling these psychiatric disorders. ICV injections were into the third ventricle. Black and tan brachury tufted (BTBR+T/tf) mice were used, since they are reported to exhibit anxiety, and have deficient social and cognitive behaviors, lack corpus callosum and hippocampal commissures, and show hippocampal aplasia. Their response to MSC injections was compared to C57BL/6J mice. Behavior tests included sociability preference, social dominance, light/dark preference, marble burying and water T maze test for cognitive flexibility. Brains were sectioned to examine gross morphology using thionin stain and for later measures of human nucleus and cell surface receptor protein to track cells. Since social behavior is sensitive to changes in serotonergic neurotransmission, hippocampal serotonin transporter density is now being compared. We found that MSC administration slightly improved BTBR social interaction preference and reduced social dominance, but had no impact on their marble burying. In light/dark preference tests, tail vein injection of MSCs reduced time in the light, but only in BTBR mice. In the water T-maze MSC injection by ICV enhanced BTBR acquisition of the location of a hidden platform, but did not help with their reversal learning. Following the battery of behavior tests, and roughly 6-8 weeks after treatments serum cytokines were measured. Serum levels of MCP1, eotaxin, and interleukins 2,3,5 and 9 were increased in BTBR mice ICV injected with MSCs relative to controls.

Confidence to Reducing Drinking Among Patients With DWI Arrest History: A Contingency Management Program

Lianeth Lozano and Charles W. Mathias, Ph.D.

Background: Driving while intoxicated (DWI) offenses are one of the most frequent offenses in the United States. While drunk driving has typically been approached using criminal justice sanctions, recidivism rates remain high and there remains need for more effective intervention. Recent developments, including the transdermal alcohol monitor, allow for use of contingency management (CM) intervention that has been demonstrated to reduce other substance abuse.

Methods: Participants were adults arrested for DWI in Bexar County, TX. The research trial involved comparison of 2 groups: the CM group received weekly incentive for absence of transdermal alcohol, while the control group were incentivized non-contingently (at random, regardless of transdermal alcohol detection). Participants attended the clinic once per week for eight weeks to complete an interview, questionnaires, and have their SCRAM monitor examined. I specifically looked at one component of each visit, the Brief Situational Confidence Questionnaire (BSCQ), which determines the patient's self-confidence to resist the urge to continue alcohol consumption. The BSCQ asks a series of eight questions and provides a scale to show the amount of self-confidence the patient has, this questionnaire allows for us to track the patient's progress throughout the eight weeks.

Results: While participant groups entered the study similar in their confidence to reduce alcohol use, they differed after experiencing their treatment condition. From pre- to post-intervention, the CM group increased 21.71 points on BSCQ (SD = 17.53), compared to Control group increase of only 7.29 points (SD = 22.01). Statistical comparison of groups was at a non-significant trend (t_{27} +=27, p = .06), which is to be expected given this preliminary state of recruitment (n = 28).

Conclusion: These preliminary analyses suggest that confidence to reduce drinking increases following CM treatment. This approach holds promise as a behavioral intervention that is feasible for implementation within the criminal justice system.

How do demographics, psychosocial factors, and access to care, lead to receiving treatment among adolescents and young adults at risk for a substance use disorder?

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Aim. Substance use among adolescents and young adults remains a public health concern in the United States, with nearly 4 million reporting illicit drug use in 2016. In 2014, only 10% of high school students who met criteria for a substance use disorder (SUD) received treatment. To better understand factors associated with receiving treatment for SUD among 12-25 year olds, we conducted an analysis using data from the National Survey on Drug Use and Health (NSDUH).

Methods. We pooled data from the 2013 and 2014 NSDUH respondents aged 12-25 who identified with a SUD. The outcome was whether respondents received treatment for alcohol/illicit drug use in the past year. Predictive factors included: gender, age, marital status, race, income, school enrollment, major depressive episodes, enrolled in government assistance programs, insurance coverage, drug use, and perceived need for treatment. **Results.** Our sample consisted of 6,975 respondents, with 687 identified as receiving treatment in the past year. Logistic regression determined gender (female aOR=0.68;CI:[0.52, 0.88]), age (18-20 aOR=0.65;CI:[0.45, 0.93]), race (black aOR=0.59;CI:[0.41, 0.84]), and income (20-49k aOR=0.72;CI:[0.53, 0.98]) were all significant predictors in receiving treatment. Respondents enrolled in school were less likely to receive treatment (aOR=0.74;CI[0.55, 0.98]). Respondents receiving government assistance (aOR=2.02;CI[1.55, 2.64]) or perceived need for treatment (aOR=1.95;CI[1.09, 3.51]), were more likely to receive treatment. Abuse/dependence of pain relievers (aOR=2.68;CI[1.95, 3.69]), illicit drugs (aOR=3.29; CI[2.46, 4.4]), or marijuana (aOR=1.36;CI[1.1, 1.7]), were significant predictors in receiving treatment; however, abuse/dependence of alcohol was not a significant predictor.

Conclusion. Among 12-25 year olds, perceived need for treatment, and abuse/dependence of prescription pain relievers and illicit drugs were predictive factors of receiving treatment for a SUD. Demographic variables were consistent with previous literature. Our study supports the need to increase access to care for substance and behavioral health treatment.

Funding: Voelcker Biomedical Research Academy at the University of Texas Health Science Center at San Antonio.

Co-occurrence of Illicit Drug Use among Patients with DWI Arrest History

Madison Metzger and Charles W. Mathias, Ph.D.

Background: This research study, entitled Leveraging Transdermal Alcohol Monitoring to Reduce Drinking among DWI Defendants, enrolls individuals who have been arrested and charged with a DWI, DUI, or Obstruction of Highway offense. To determine the success rate of participants' reduction in drinking as a result of receiving incentives, contingency management procedures—having been effective interventions for other substance abuse—were adapted to suit the study population. My interest in the correlation between the study population and drug use was piqued upon conducting Urinary Analyses that yielded a significant number of positive results.

Methods: In order to determine eligibility, potential participants partook in an alcohol screening during which demographic information and alcohol use characteristics were collected by research staff. Those successfully enrolled in the study were fitted with a Secure Continuous Remote Alcohol Monitor which detects transdermal alcohol concentrations emitted through the skin. This data was uploaded to a web interface during each of 8 weekly visits and processed to determine a payment decision. Abstinence or significant reduction in drinking resulted in a monetary reward for the participant.

Urinary analyses were conducted during visits 0 (on-site screening), 4, and 8 using a multi-drug screen test. Tetrahydrocannabinol (THC), Benzodiazepine (BZO), Cocaine (COC), Opioids (OPI), Amphetamines (AMP), and Methamphetamines (mAMP) were among the drugs tested for. Data on these drugs was collected and recorded but did not effect a participant's payment decision focusing on alcohol use.

Results: Out of a total of 45 participants, 13 tested positive for illicit drug use during visit 0. 10/13 participants tested positive for THC, 1/13 tested positive for BZO, 1/13 tested positive for COC, 1/13 tested positive for OPI, 1/13 tested positive for mAMP, and 2/13 tested positive for AMP. 2/13 participants tested positive for 2 or more drugs. Urinary analyses following visit 0 were not considered in this analysis seeing as participants were in varying stages of treatment.

Conclusion: This analysis shows that nearly a third of study participants are engaging in illicit drug use prior to study entry. This high rate of use has prompted quality improvement efforts considering group stratification for positive urinalyses and consideration of how to apply contingencies for illicit substance use in addition to their alcohol intervention.

Regulation of Surfactant Protein Expression by Dexamethasone and a Novel Glucocorticoid in Premature Baboon Lung Explants

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Premature delivery is a major healthcare issue, affecting more than 13% of all births in the US and causing 85% of all newborn morbidities and mortalities. In all babies, proper lung function is crucial at birth. However, as lungs are one of the last organs to develop, premature babies' lungs are not fully developed, which makes them susceptible to respiratory disorders. Premature lungs lack a sufficient amount of surfactant. Surfactant is vital for lung function, as it helps to ensure that the air spaces are expanded and will not collapse. Surfactant consist of lipoprotein molecules made up of phospholipids, cholesterol, and protein, which can be divided into 4 subtypes: A, B, C, and D. The main purpose of the surfactant proteins is promote alveolar stability by lowering the surface tension at the air-liquid interface in the maturing air spaces. However, each surfactant protein exerts several important functions that contribute to overall lung health. Surfactant protein A and D are hydrophilic and are vital for the innate immune system. Both A and D are protective against pulmonary infectious molecules. Surfactant proteins B and C are hydrophobic and regulate lamellar body formation and overall surfactant protein secretion. Dexamethasone is used as a postnatal treatment for increasing surfactant-protein expression in premature babies. Unfortunately, long-term studies showed that postnatal dexamethasone treatment was associated with delayed neurological development. However, without dexamethasone treatment, there is an increased risk of lung disease; therefore, there is an urgent need to find a therapeutic alternative to dexamethasone. Dexamethasone functions in two different ways; in transactivation, dexamethasone binds to the glucocorticoid receptor to directly stimulate gene expression. In contrast, transrepression involves dexamethasone binding to the glucocorticoid receptor to either induce or repress gene expression.

The objective of the study is to compare the effects of a synthetic glucocorticoid, ZK-57740, specific for transactivation, with those of dexamethasone on surfactant protein expression. Premature baboon lung explants (a small piece of whole lung tissue) at 120 day and 165 day gestational age (full term=184 days) were used for this study. Lung explants were cultured in the presence in either dexamethasone (1nM) or ZK-57740 (1nM), for two different time points, 24 and 120 hours, for each gestational age. The tissue was then frozen in liquid nitrogen. Protein samples were isolated from the frozen tissue and its concentration determined using a Nanodrop. Western blot analysis was used to determine the relative expression of surfactant-associated proteins A, B, C, and D in these samples. Protein band intensity was measured using ImageJ and data was expressed as mean ± SE. Comparisons between two groups were using unpaired t-test, and $P<.05$ was considered statistically significant. Surfactant protein A is encoded by two genes, A1 and A2, in humans and baboons. Surfactant protein A1 and A2 are abundant in 165 day lung explants. No significant change in either A1 or A2 was detected after treatment for 24 hours with dexamethasone or ZK-57740 (A1: control 1.94 ± .42 versus dexamethasone 1.5 ± .45 and control 1.94 ± .42 versus ZK-57740 .75 ± .47, A2: control 1.19 ± .33 versus dexamethasone 1.42 ± .41 and control 1.19 ± .33 versus ZK-57740 1.59 ± .83). Ongoing studies will determine levels of the remaining surfactant proteins.

Role of Lysine Demethylase KDM1A in Hypoxia Mediated Functions of Glioblastoma

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Background: Glioblastomas (GBM) are malignant primary brain tumors that affect the brain or the spine. GBM is a grade four glioma which means that it's more aggressive and harder to treat due to its ability to metastasize in the brain rapidly. Treatment for glioblastoma includes: surgery, radiation therapy, and chemotherapy with adjuvant temozolomide. Despite therapy, median survival is 12 to 15 months. Hypoxia-deficiency of oxygen- and hypoxia inducible factors (HIFs) plays a crucial role in the commencement, progression, therapy resistance, and recurrence of GBM. Recent evidence suggested a role of KDM1A-a protein that removes methyl groups on histones- in GBM progression, and KDM1A is highly expressed in glioma stem cells (GSCs). To confirm this, the Sareddy lab pharmacologically inhibited KDM1A expression using NCL-1 and NCD-38 and found that the proliferation and growth of glioma stem cells were considerably reduced *in vivo* and *in vitro*. However, the role of KDM1A in hypoxia remains unknown. The objective of my research was to study and examine the role of KDM1A in hypoxia mediated functions of GBM and test the therapeutic utility of KDM1A specific inhibitors on GBM cells.
Methods: The cell lines I used were U251-WT and U251 KDM1A KO (knockout) and patient derived primary GBM cells: GBM-101310 and GBM-031417. These cells were kept in normoxia and hypoxia (2% 0_2) for 24 h. Then RNA was isolated and used to conduct real time PCR (RT-PCR) with several HIFs (HIF1$\boldsymbol{\alpha}$, HIF2$\boldsymbol{\alpha}$) target genes to determine whether KDM1A could affect the HIF mediated transcription in hypoxic conditions. I then utilized Luciferase Assay to determine if KDM1A KO will affect the HIF-mediated luciferase activity using HIF-luciferase reporter plasmid. Then, I also tested whether KDM1A inhibitor NCD-38 could affect HIF mediated transcription. The cells were kept in normoxic conditions (21% 0_2) as a control group and in the hypoxic chamber with 2% oxygen to resemble hypoxia. After 24 hours, the plate in hypoxia was retrieved and got imaged along with the plate in normoxia. Next I used co-immunoprecipitation (co-IP) with the cell lines U251 and 031417 to see if KDM1A interacts with the HIF1$\boldsymbol{\alpha}$ protein. Magnetic beads were used to bind to the antibody and pull the antibody with whatever it reacts with against a magnetic holder and HIF1$\boldsymbol{\alpha}$ interaction was analyzed using western blotting.
Results: In RT-PCR, U251 KDM1A KO cells in hypoxia were compared to the control group (U251-WT) in hypoxia. The results showed a significant increase in HIF target genes in hypoxia; however, in KDM1A KO cells, I observed a significant downregulation of all HIF target genes compared to U251 cells. This analysis confirms the hypothesis that knocking off the expression of KDM1A works in stopping the transcription of certain HIF genes. Then, the results from co-IP demonstrated that KDM1A interacts with HIF1$\boldsymbol{\alpha}$ in U251 cells under hypoxia. Furthermore, luciferase reporter assays demonstrated that HIF-luc reporter activity was significantly attenuated in KDM1A-KO cells compared to controls. Finally cell viability assays showed that KDM1A inhibitors NCL-1 and NCD-38 significantly inhibited the proliferation of GBM cells in hypoxic conditions.

Conclusion: Based on the results, my data validates the hypothesis that targeting GBM with KDM1A inhibitors could mitigate the hypoxic functions in GBM leading be a possible therapy for patients with GBM.

Effect of maternal PCPA treatment at mid-gestation on adolescent behaviors relevant to autism

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Serotonin is a neurotransmitter critical for normal neurodevelopment and behavior. The precursor to serotonin, tryptophan, crosses the placenta to feed fetal needs during pregnancy and is understood to be the main source of serotonin synthesized by the placenta at mid gestation. The experimental hypothesis was that with pharmacological attenuation of maternal serotonin synthesis during pregnancy, more tryptophan would become available for the fetus and this would improve offspring social behavior. To inhibit maternal serotonin synthesis at mid-gestation, pregnant mice were treated with saline control vehicle or 150 mg/kg of para-chlorophenylalanine (PCPA) by subcutaneous injection daily for 3 days (gestational day 8 - 11). PCPA curbs conversion of tryptophan into serotonin by inhibiting the enzyme tryptophan hydroxylase. At seven weeks of age, offspring from saline and PCPA treated dams were behavior-tested utilizing three-chamber tests for social interaction and social novelty preference, as well as the marble-burying assay for repetitive behaviors, to measure parallels of the two core clinical features of autism. BTBR mice with inherent sociability impairments were used to determine if their deficits could be corrected, and sociable C57BL/6 mice served as a strain control. To ensure scientific rigor, each treatment was performed on two cohorts of 5 dams per treatment and strain, and the sample size for groups was at least 8 offspring from different dams. There was an interaction between sex and body weight of adolescent offspring, such that males from PCPA dams weighed more than females ($p < 0.05$). In sociability preference tests, female offspring from PCPA-treated dams exhibited greater preference for stranger mice versus novel objects, both with measures of time in chamber and time engaged in social sniffing (Tukey's $p < 0.01$). This effect was also echoed in social sniffing by the adolescent male offspring of C57BL/6 but not BTBR mice. Social novelty preference was greater in the female saline group and in the PCPA- exposed males as seen in time spent sniffing. In females, the PCPA treatment significantly reduced the number of marbles buried, but there was no effect of PCPA treatment on this measure in males. It is possible that while maternal serotonin synthesis was inhibited, the subcutaneous injections of PCPA did not cross the placenta and thus did not impact fetal mice ability to synthesize serotonin, as we did not see major detriments to repetitive or social behavior in the PCPA-treated group but instead minor enhancements that were more pronounced in C57BL/6 mice.

The Effect of Beta-Lactams and Macrolides on Cardiac Fibrosis in Pneumococcal Pneumonia Mouse Model

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Background: Pneumonia is the most common infectious cause of death in the world and the eighth leading cause of death in the United States. Beta-lactams (β-lac) and macrolides are the antibiotics of choice to treat patients with community-acquired pneumonia (CAP) but these antibiotics have different mechanisms of action that affect the progression of the disease. Our aim is to investigate the effects of combination therapy by analyzing 5-day survival, bacteremia, and fibrosis in the lung and heart in a pneumococcal pneumonia mouse model. We hypothesize that combination therapy will be more beneficial than monotherapy.

Methods: Using experimental methodology, 12-week old Balb/c mice were intra-tracheally challenged with *S. pneumoniae*, serotype 4 (10^5 CFU). 30 hours after infection, mice received medication or placebo every 12 hours for 5 days. There were four intervention groups divided into 1) beta-lactam (Ampicillin) at a dose of 80mg/kg and macrolide (Azithromycin) at a dose of 50mg/kg (β-lac/M, n=12), 2) beta-lactam alone (β-lac, n=6), 3) macrolide alone (Mac, n=6), and 4) the control group (placebo). Each treatment was administered intraperitoneally and for combination therapy, the second antibiotic was given one hour after the first. These treatments were given for total of five days to all groups of mice. At 5 days after infection, bacteremia, cardiac and lung fibrosis (measured by percent area of collagen deposition by Trichrome staining) were evaluated. Blood was collected every 24 hours to extrapolate bacteremia.

Results: The 5-day survival was 100% for β-lac compared to42% for β-lac/M and 17% for Mac, and 0% for control ($p=0.002$, for all the groups). The β-lac monotherapy and the combination therapy reduced pneumococcal bacteremia 48 hours post infection compared to the macrolide monotherapy. After 18 hours of treatment bacteremia in the β-lac/M (median [IQR] 2 [2-2] log CFU/ml) and the B-lac group (median [IQR] 2 [2-3.7] log CFU/ml) group had cleared compared to the Mac group (median [IQR] 5.52 [4.8-5.95] log CFU/ml). Mice that received the β-lac alone after 5 days had a significant increase in lung fibrosis over the combination therapy and macrolide alone mice ($p<0.001$ for both groups). Similarly, mice that received the beta lactam alone experienced a significant increase in cardiac fibrosis over the combination therapy and macrolide alone groups ($p<0.001$).

Conclusion: Our results suggest that the combination therapy may lower disease severity and fibrosis in the lungs and heart, but had not effect on survival. Translation of these findings in humans may potentially impact the use of antibiotics in severe pneumococcal pneumonia.

Investigating the Effects of Aberrant Calcium Signaling in the Pathogenesis of Tauopathies

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Background: Alzheimer's disease is the most common form of dementia. It is classified as a tauopathy because of the presence of aberrant tau protein neurofibrillary tangles in affected brains. We utilize a *Drosophila melanogaster* model of tauopathy that transgenically expresses a human tau protein. The tau^{R406W} *Drosophila* model is advantageous as it facilitates rapid investigation due to the short fly lifespan, and demonstrates features of human tauopathy including heterochromatin relaxation, DNA damage, cell cycle activation in post-mitotic neurons, and progressive neurodegeneration. Currently in Alzheimer's research, there is evidence suggesting that aberrant regulation of beta-amyloid causes a significant increase in calcium levels during the progression of the disorder. At this time the relationship between calcium levels and tau has not been characterized. To improve our understanding of the role calcium signaling plays in the pathogenesis of tauopathies, especially what role nuclear calcium may play, we utilize the recombinant calcium sensor, GCAmP3.nls, a genetically-encoded calcium indicator with a nuclear localization signal (NLS) and CaMBP4.nls, a calcium blocker. By comparing nuclear calcium levels in transgenic flies expressing GCAmP3.nls with and without tau^{R406W}, we can improve understanding of the role of aberrant calcium signaling in driving neurotoxicity in tauopathies.

Methods: Sectioned, formalin-fixed, paraffin samples of six 10-day old GCamP3.nls tau^{R406W} transgenic flies and six 10-day old GCamP3.nls transgenic flies were stained, using 300 µl of r anti GFP 1^{o} antibody and Alexa488 2^{o} in a 1:200 ratio to target nuclear calcium. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was used to measure neuronal death by detecting DNA fragmentation in six 10-day old tau^{R406W} transgenic flies expressing CaMBP4.nls and six 10-day old transgenic flies expressing CaMBP4.nls.

Results: We find that immunofluorescent staining shows tau transgenic *Drosophila* have markedly less nuclear calcium in neurons of the mushroom body, a region of the fly brain that controls memory, than age-matched controls. Preliminary data demonstrates that genetic reduction of nuclear calcium causes significantly more neuronal death. TUNEL staining shows that 10-day old CaMBP4.nls transgenic flies have 22 ± 2 TUNEL positive nuclei per brain while the 10-day old CaMBP4.nls tau^{R406W} transgenic flies have a significantly higher 40 ± 5 TUNEL positive nuclei per brain ($p=0.04$). The data indicate that depletion of nuclear calcium in tau transgenic *Drosophila* is causal for neuronal death.

Conclusion: These results demonstrate that decreases in calcium signaling in tau transgenic *Drosophila* drives neurotoxicity, providing preliminary evidence that such decreases are characteristic of tauopathies. Additionally, the worsening neurotoxicity associated with the reduction of nuclear calcium levels in tau flies supports a beneficial role for nuclear calcium in protecting the brain against the effects of pathological tau. This data taken together suggests a casual role for the aberrant nuclear calcium signaling in tau transgenic *Drosophila* and could indicate a novel drug target for the treatment of tauopathies.

Family History and the Correlations Between Stress and Resilience In At-Risk Adolescents

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Purpose: Previous research indicates that stressors in adolescent's lives significantly increase their susceptibility to psychiatric disorders. But some may have certain levels of resilience that help to buffer such adverse effects. Resilience is defined as having a seemingly good outcome despite experiencing a high-risk event that can foster psychopathology. While everyone may have some resilience, the levels may vary and can be affected by family history. A family history of substance use disorder, for example, can adversely affect individuals by influencing resilience, perhaps making them more prone to psychiatric disorders and stressors in their lives. This study examines the relationship between resilience, family history, and psychiatric symptoms, specifically whether higher resilience is correlated with lower levels of stress and psychiatric symptoms and the effect of family history on this relationship.

Methods: The specific cohort examined from this study either had (FH+; $n = 218$) or did not have (FH-; $n = 60$) a family history of substance use disorder. Resilience and stress symptoms were measured using multiple self-reports and scaled ratings. The CYRM self report helped diagnose levels of resilience in the participant pool. Stress was measured in three ways: the total number of stressful life events, the cumulative number of objective ratings for these events (weighted), and the cumulative number of subjective ratings for these events. Participants gave the subjective ratings when the stressful life event data was collected; a four-person panel determined the objective ratings. A hierarchical regression and multiple correlation tests were used to examine the two populations' levels of resilience and effect on different stressors in their lives.

Results: FH+ participants were found to have significantly lower levels of resilience to stressors in their lives (M= 106.11, SD= 19.73) than FH- participants (M= 116.71, SD= 16.08, $p < .05$). Levene's Test for Equality of Variances showed that equal variances could be assumed across both groups, $t(276) = 3.85$, $p = .054$. A t-test used to compare the three stress variables (subjective rating, objective rating, and total count) between the two populations revealed that the objective rating (M= -33.26, SD= 9.87) and cumulative event count (M= -11.86, SD= 3.17) were significant, but the subjective rating was not (M= -109.02, SD= 95.33).

Conclusion: The purpose of this study was to compare stress and levels of resilience between the two populations of having a family history of substance use disorder or not. It was revealed through the CYRM scores that those with a family history have significantly lower levels of resilience, meaning they are more susceptible to change from any stressors in their lives. A t-test also showed that those with a family history have significantly higher objective ratings on the stressors in their lives and have a significantly larger cumulative amount of stressful events. This helps show that those with a family history of substance use disorder are more susceptible to psychiatric disorders and stress in their lives. This research has better allowed us to understand how stress affects at-risk adolescents psychologically and will, hopefully, foster better care and prevention for all adolescents.

A Novel Combination Therapy to Treat Triple Negative Breast Cancer

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Background: Triple negative breast cancer (TNBC) is a subtype of breast cancer that is aggressive and has a poorer prognosis within the first five years after diagnosis when compared to other subtypes of breast cancer. Specifically, TNBC lacks estrogen, progesterone, and HER2 receptors meaning the cancer does not respond to hormonal therapy or therapies that target HER2 receptors. This shortage of effective therapies has contributed to a disproportionate share of breast cancer mortality and new therapies are urgently needed. Epigenetic modification of gene expression plays an important role in cancer progression. Histone deacetylases (HDAC) are chromatin modifiers that promote epigenetic changes leading to alteration in gene expression. Earlier studies showed HDAC inhibitors can induce apoptosis of TNBC cells. However, recent clinical trials and mechanistic studies discovered that feedback activation of Leukemia Inhibitory Factor Receptor (LIFR) signaling in TNBC limits the response of HDAC inhibition. It was reported that HDAC inhibition upregulates LIFR expression, and activates JAK1-STAT3 signaling, limiting the HDAC therapy response. Recently, a first in class inhibitor of LIFR, EC359, was developed in our lab.
Objective and Hypothesis: The objective of this study is to test whether the addition of EC359 increases the therapeutic utility of HDAC inhibitor (SAHA) treatment. I hypothesized that combining EC359 with SAHA treatment will enhance HDAC therapy by blocking the activation of the LIFR pathway.

Methods: Drugs tested included EC359 (LIFR inhibitor, 0 to 100 nM), SAHA (HDAC inhibitor 0-10 μM), and combination of both at various concentrations. Throughout the experiments, multiple TNBC cell lines were used including MDA-MB-231, MDA-MB-468, BT549, SUM159, HCC1907, and HCC1806. I tested the efficacy of the new combination therapy using *in vitro* assays including MTT, colony formation, matrigel invasion, cell titer glo, and apoptosis assays. I also conducted mechanistic studies using Western blots, reporter gene assays, and FACS analysis (to examine cell cycle progression). I then performed TNBC xenograft *in vitro* explant assays in order to determine the efficacy of combination therapy on the proliferation of TNBC tumors.
Results: The combination of EC359 and SAHA synergistically reduced the growth of TNBC cells compared to either drug alone. Biochemical studies have confirmed combination therapy significantly reduced STAT3 signaling in reporter gene assays and western analyses. The results supported my hypothesis and combination therapy significantly enhanced apoptosis compared to monotherapy. Further, combination therapy substantially reduced invasion of TNBC cells. Additionally, through the use of human TNBC xenograft tumor tissue explant cultures, I demonstrated that combination therapy has the potential to substantially reduce the proliferation of TNBC tumors.
Conclusions: My results showed that EC359 enhances the therapeutic utility of SAHA by blocking the activation of the LIFR pathway and represents a novel targeted combination therapy for treating TNBC tumors.