2021 ASPET/Charles Ross

Pharmacology Summer Undergraduate Fellows Research Symposium

University of Michigan Medical School
Department of Pharmacology

Thursday August 5th and Friday August 6th 2021
10:00 AM to 12:00 Noon

2901 THSL (Taubman Health Sciences Library)

MEDICAL SCHOOL
PHARMACOLOGY
UNIVERSITY OF MICHIGAN

ASPET
Transforming Discoveries into Therapies

Program:

Thursday August 5th, 2021. 10:00 AM. Room 2901 THSL
Moderators: Drs. Adriana Yamaguchi and Kobina Essandoh

	Presenter	Institution	Rising class	Program	Host Laboratory
1	Harith Palmer	University of Michigan	Junior	ASPET	Dr. Kevin Jones
2	Tuan Kiet Trinh	Kent State University	Junior	ASPET	Dr. Haoming Zhang
3	Rija Awan	University of Michigan	Junior	ASPET	Dr. Mark Cohen
4	Mia Smith	University of Southern California	Sophomore	FCVC	Dr David Jones
5	Israa Zaher	University of Michigan	Senior	FCVC	Dr David Jones

A box Lunch for presenting students and their mentors will be provided after the session in 1301 MSRB III

Friday August 6th, 2021. 10:00 AM. Room 2901 THSL
Moderators: Drs. David Jones and Paula Goforth

	Presenter	Institution	Rising class	Program	Host Laboratory
6	Javier Santiago Perez	University of Puerto Rico Rio Piedras	Senior	Charles Ross	Dr. Colin Greidener
7	Jonida Trako	University of Michigan	Senior	ASPET	Dr. Alan Smrcka
8	Jalaysia Weems	University of Maryland Baltimore County	Senior	Charles Ross	Dr. Emily Jutkiewicz
9	Kyle Browder	Arizona State University	Junior	ASPET	Dr. Mark Cohen
10	Sarina Garcia	University of Texas San Antonio	Junior	Charles Ross	Dr. Mike Holinstat

A box lunch for attendees will be provided after the session in 1301 MSRB III at the conclusion of the symposium

DETERMINING THE ROLE OF DOPAMINERGIC NEURONS IN A ZEBRAFISH MODEL OF PSYCHOSIS BY CHRONIC KETAMINE EXPOSURE

Harith Palmer[1], Nichelle N. Jackson[2], and Kevin S. Jones[2]
[1]Department of Chemical Engineering, University of Michigan and
[2]Department of Pharmacology, University of Michigan Medical School

Ketamine is a commonly used anesthetic that blocks the ion channel of N-methyl-d-aspartate receptors (NMDARs). Therapeutic interest of NMDAR blockers has grown considerably because it can rapidly alleviate the symptoms of depression. However, therapeutic use of ketamine is risky because it can also cause serious psychotomimetic side effects and repeated exposure can be habit forming. Despite growing acceptance of ketamine, there are many unanswered questions about its use as a drug therapy. Our question of focus is, what are the consequences of repeated ketamine exposure to a developing nervous system.

To address this question, we use zebrafish (*Danio rerio*), an animal model in drug abuse research whose behavioral response to ketamine and other NMDAR antagonists is highly homologous to mammals. Here, we examined how chronic ketamine exposure, compared to vehicle treated animals, will influence neuronal activity. Zebrafish express NMDARs as early as 24 hours post fertilization. Here we used a neuronal activity marker, phosphorylated Extracellular Receptor Kinase (pERK) to study how chronic ketamine exposure influences activity in dopaminergic neurons. We used immunohistochemistry (IHC) and multiphoton and light-sheet microscopy to identify tyrosine hydroxylase (TH), an enzyme involved in catecholamine production, specifically dopamine, and pERK staining patterns. Using the TH stain for dopaminergic neurons as a mask, we were able to quantify the average intensity of pERK in fish chronically exposed to ketamine and compare those to average intensity values in vehicle fish.

RECAPITULATING DRUG METABOLISM BY MULTI-UNIT MAMMALIAN CYTOCHROME P450 COMPLEXES IN AMPHIPOL A_{8-35} NANOPARTICLES

Tuan Kiet Trinh[1], Zhiyuan Bo[2], Cian Johnson[3], Haoming Zhang[2]

[1]Department of Chemistry and Biochemistry, Kent State University,
[2]Department of Pharmacology, University of Michigan Medical School, and
[3]Department of Pharmaceutical Sciences, University of Michigan

Cytochromes P450 (CYPs) constitute a superfamily of hemoproteins catalyzing the monooxygenation of inert C-H bonds that are involved in the biosynthesis of steroid hormones and metabolism of therapeutic agents. The underlying basis for such a unique reaction are the interactions of membrane-bound mammalian CYPs with its redox partner comprising NADPH-cytochrome P450 reductase (POR) and cytochrome (Cyt *b5*) where POR and/ or Cyt *b5* transfer essential electrons to CYPs for catalysis. However, the deficiency in the structural information of these complexes, especially in the setting of endoplasmic reticulum (ER) has hampered our understanding of the interaction mechanism(s). To better understand the CYP:POR complexes, we reconstituted multi-unit CYP2B4:POR complexes in amphipol A_{8-35} nanoparticles and investigated the structure in addition to function of these complexes. To mimic the complexes in the ER membranes where excessive CYPs are present, we were able to reconstitute the CYP2B4:POR complexes with varying molar ratios of CYP2B4 to POR. We found that the CYP2B4:POR complexes exhibited higher coupling with increased CYP ratios. At a 3:1 ratio, the complex exhibited a coupling of 53%, compared with 42% for the control complex at 1:1 ratio. These results demonstrated that presence of excess CYP enzymes in the complex enhance the efficiency of drug metabolism, which likely mitigates production of harmful reactive oxygen species. Additionally, we verified the architecture of the complex at 3:1 by negative stain EM and mapped the interactions for protein-protein interactions by chemical crosslinking-liquid chromatography mass spectrometry. As a part of the study, we also prepared a tertiary complex of Cyt *b5*:POR:CYP2B4 in amphipol A_{8-35}. It was found that the tertiary complex exhibited lower catalytic activities, indicative of an inhibitory role of cyt *b5*. These results suggest that reconstitution of the CYP complexes in amphipols leads to homogenous preparations with defined stoichiometry and may play a vital role to elucidate the structure of CYP complex responsible for metabolism of a large number of therapeutic agents.

C-TERMINAL HSP90 INHIBITORS, KU757 AND KU758, ARE EFFECTIVE IN TARGETING AND PREVENTING MIGRATION OF BREAST CANCER CELLS

Rija Awan[1], Chitra Subramanian[2], Mark Cohen[1,2]

[1]Department of Pharmacology and [2]Surgery, University of Michigan Medical School

Breast cancer is the most common cancer found in women worldwide and is the second leading cause of death in women. Triple-Negative Breast Cancer (TNBC) cells are the most aggressive lines of breast cancer and are difficult to treat due to their lack of estrogen receptor (ER), progesterone receptor (PR), and HER2 (Human Epidermal Growth Factor Receptor 2) expression. The absence of these molecular markers makes TNBC less responsive to typical hormone and drug therapies. The lack of an effective targeted drug therapy and poor prognosis warrants the critical need to develop an effective novel therapeutic option for TNBC. HSP90 is a molecular chaperone that regulates the folding and maturation of client proteins involved in the hallmarks of cancer. Therefore, treatment of TNBC cells with HSP90 inhibitors (HSP90i) simultaneously targets several key oncogenic pathways involved in TNBC development. Previous studies with the N-terminal HSP90 inhibitors showed initial preclinical promise, but had issues of decreased solubility, organ impairment, and activation of the heat shock response in preclinical trials. To overcome these, our group has developed a novel strategy to target specific cancer protein pathways through C-terminal HSP90 inhibitors. These novel compounds avoid the cytotoxicity and heat shock response associated with N-terminal inhibitors in several cancers. In the current study, we evaluated the efficacy of two C-terminal HSP90 inhibitors, KU757 and KU758, in targeting two TNBC cell lines: MDA-MB-231 and MDA-MB-468. The cells were treated with varying concentrations of KU757 and KU758, and the viability of the cells were measured using an MTS assay after 72 hours. Half-maximal inhibition concentrations (IC50) calculated using the Prism software indicated that MDA-MB 231 cells and MDA-MB-468 cells have an IC50 value of 3.93 and 2.00 uM, respectively, for KU757 and 1.19 and 3.04 uM, respectively, for KU758. Clonogenic assays after treatment of cells with varying concentrations of HSP90i for 24 and 48 hours showed a dose dependent decrease in viable cells. Migration assays conducted to measure migration of cells after 24- and 48-hour treatment of cells showed a decrease in migration and no wound healing for the drug-treated cells, whereas complete wound closure was observed for control non-treated cells. Western Blot analysis was performed to measure changes in client proteins in cancer cells. To evaluate the changes in exosomal markers, exosomes were isolated. These preclinical studies provide early support for the potential therapeutic effects of C-terminal HSP90 inhibitors on TNBC and will need to be validated in future in vivo studies. Future studies are needed to characterize the exosomes and the pathways targeted by these novel drugs.

LINKING HERG1 MUTATATIONS TO SEIZURE AND EPILEPSY USING STEM CELL DERIVED CARDIOMYOCYTES AND INEURONS

Mia Smith[1], Francisco Sanchez-Conde[2], Dr. Eric Jimenez-Vazquez[2], Abhilasha Jain[2] and Dr. David Jones [2]

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Long QT syndrome (LQTS) is a potentially fatal cardiac disorder characterized by prolongation of the QT interval. LQTS patients often present with comorbid seizure and epilepsy. In individuals with both LQTS and epilepsy, there is a heightened risk of sudden unexpected death in epilepsy (SUDEP), the most common cause of epilepsy-related premature death. *KCNH2* encodes hERG1, the voltage-gated potassium channel responsible for conducting the cardiac repolarizing current I_{Kr}. I_{Kr} loss causes long QT syndrome type 2 (LQTS2) which increases the risk of syncope, cardiac arrhythmia, and sudden cardiac death. Clinical studies show patients with LQTS2 experience seizure and epilepsy at roughly 4 times higher rates than individuals without LQTS and 2 times higher than all other genetic forms of LQTS. *KCNH2* is also expressed in the brain alongside two other hERG orthologues, KCNH6 and KCNH7, that together regulate neuronal excitability. We hypothesize KCNH2 mutations pre-dispose patients to neurological dysfunction. We used CRISPR/Cas9 to generate a hERG1 KO embryonic stem cell (eSC) line, then differentiated the KO eSCs into cardiomyocytes (CMs) and superficial cortical-like iNeurons to study the parallel effects of hERG1 KO on neuronal and cardiac action potential firing. Patch-clamp recordings and immunostaining of differentiated CMs show successful KO of hERG1 immunofluorescence and the absence of IKr. Preliminary patch-clamp recordings and immunostaining in neurons demonstrate our lab's ability to differentiate hERG1 KO eSCs into neurons. Future work involves differentiating patient-derived eSCs into iNeurons to study the effects of LQTS2 mutations on neuronal firing.

SCFV ANTIBODY USED TO MODULATE HERG BEHAVIOR

Israa Zaher, and Dr. David Jones

Department of Pharmacology, University of Michigan Medical School

The human ether-à-go-go-related gene (hERG) encodes the voltage-gated potassium channel that conducts the delayed rectifier potassium current, IKr. Disrupting hERG function causes the cardiac disorder Long QT Syndrome Type 2 (LQTS2), which increases the risk for arrhythmia and sudden cardiac death. We previously demonstrated that selective inhibition of the hERG N-terminal Per-Arnt-Sim (PAS) domain using intracellular perfusion of a PAS-targeting single chain variable fragment antibody, scFV2.10, increased IKr and shortened the action potential duration in human stem cell-derived cardiomyocytes (hiPS-CMs). We hypothesized that targeted modulation of the hERG PAS domain could serve as a therapeutic approach for LQTS and other disorders of cardiac electrical excitability. To test this hypothesis, we generated hiPS-CMs derived from patients with LQTS1, and compared the electrical excitability of cardiomyocytes transduced with the PAS-targeting scFv2.10 to cells transduced with GFP. Our preliminary data suggest that PAS modulation could be leveraged as an antiarrhythmic long-term treatment of LQTS.

MODEL FOR COMBINED BONE-TARGETING AND ACID-MEDIATED RELEASE OF A SMALL MOLECULE THERAPEUTIC

Javier E. Santiago Pérez[1,2], Boya Zhang[2], W. Benton Swanson[3], and Colin F. Greineder[2,4]

[1]Department of Chemistry, University of Puerto Rico-Río Piedras Campus, and [2]Department of Pharmacology, [3]Biologic and Materials Science, and [4]Emergency Medicine, University of Michigan

Rheumatoid arthritis (RA) is a highly-prevalent autoimmune and inflammatory disease which involves degradation of joints and decreased quality of life. Treatment requires systemic immunosuppression, exposing patients to opportunistic infections and other toxicities. No existing drugs are capable of local accumulation or selective activity in the joint space. In response to this need, our group has designed a drug delivery platform which combines affinity for the bone surface with local release within the acidified microenvironment within rheumatoid joints. To better characterize the pH-sensitive release of cargo from the platform molecule, we incorporated a fluorophore, Cy5, as a model drug. The resulting molecule, ALD-Z-Cy5, was compared to an analogue, ALD-Cy5, which lacks the pH-sensitive release mechanism. Both molecules were bound to hydroxyapatite (HA) beads, which mimic the bone surface, and release was quantified by spectrophotometry, which was found to be linear over a wider range of pHs and concentrations than fluorescence. HA beads decorated with ALD-Z-Cy5 released free Cy5 faster and to a greater extent at acidic than neutral pH. As expected, no release was seen from beads decorated with the non-labile ALD-Cy5. Finally, we compared the release from ALD-Z-Cy5 across a range of pHs and demonstrated a gradient of release profiles, with faster and more extensive release seen at more acidic pHs. In conclusion, ALD-Z-Cy5 provides proof of concept for pH-sensitive release of therapeutic cargo in acidified microenvironments, such as those found in RA joints.

THE MOLECULAR SPECIFICITY OF THE INTERACTION BETWEEN THE Gαi G PROTEIN SUB-FAMILY AND A NOVEL EFFECTOR PDZ-RhoGEF

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Heterotrimeric guanine nucleotide-binding proteins (G proteins) are essential for signal transduction of activated G protein-coupled receptors (GPCR) which are involved in numerous cellular and physiological processes. There are four families of G proteins categorized based on their α subunit, $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$ with $G\alpha_{i/o}$ being the most abundant. Recent BioID proximity-labeling experiments coupled with mass spectrometry identified a novel effector of $G\alpha_i$, PDZ-RhoGEF (PRG). Previously, $G_{12/13}$-coupled GPCRs have been linked to activating Rho via regulation of PDZ-RhoGEF (PRG). Rho activation plays a key role in multiple biological functions such as cell migration and adhesion, microtubule rearrangement, and vesicle transport. However, preliminary data shows that Gαi sub-types 1 and 3 activate PDZ-RhoGEF in a nucleotide dependent manner, whereas $G\alpha i_2$ showed no significant activation of PDZ-RhoGEF. $G\alpha_i$ sub-types exhibit a very high sequence conservation yet, only constitutively active $G\alpha_{i1}$ showed full activation of PDZ-RhoGEF and the molecular basis of this interaction is unknown. Therefore, the main goal of this study has been to identify portions of $G\alpha_{i1}$ that are responsible for this novel protein-protein interaction. Initial experiments using Gai1/2 protein chimeras revealed that a portion of the Gai1 helical domain plus a C-terminal portion of the Ras-like nucleotide binding domain are sufficient to fully activate PRG. Thus, we hypothesize that the ability to activate PDZ-RhoGEF is conferred by residues in multiple domains that are common between $G\alpha i_1$ and $G\alpha i_3$, but different in $G\alpha i_2$. To further analyze the specific amino acids implicated in this interaction we performed progressive site-directed mutagenesis on the protein chimera $G\alpha i_{2\text{-}1HD\text{-}1}$ and measured the activation of PDZ-RhoGEF in cells via an SRE luciferase reporter assay in HEK293 cells. Furthermore, in order to verify the expression and function of the Gαi proteins and chimeras, additional assays such as Western blotting and cAMP accumulation assay were performed. Our results here suggest that $G\alpha_{i1}$ D229 plays some important role in this interaction. However, we cannot conclude that this residue acts alone or in conjugation with the αA helix in the HD. Future studies will involve further mutagenesis to narrow down the residues responsible for this interaction as well as direct binding and activation assays to examine the activation of PDZ-RhoGEF by $G\alpha i_1$.

THE ROLE OF SIGNALING MOLECULES IN THE BEHAVIORAL EFFECTS OF SNC80

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Opioid analgesics are drugs clinically used for pain relief, but are also responsible for overdose deaths in recent years. Opioid analgesics are agonists that activate the mu-opioid receptor type; however, there are 3 additional types of opioid receptors (delta-, kappa-, and opioid receptor-like (ORL, NOP)) in humans and animals. Much less is known about the function and activity of these other opioid receptor types. Drugs that activate the delta-opioid receptor (DOR), such as SNC80, produce antinociception, antihyperalgesia, and antidepressants-like effects in animal models. However, SNC80 also produces convulsions and rewarding effects. Previous research has shown that each of these behavioral and physiological effects of SNC80 may be mediated by different signaling molecules downstream of the DOR. In the present study, we evaluated the role of regulator of G protein signaling 4 (RGS4) in the rewarding effects of SNC80 as this signaling molecule has previously been shown to be involved in the antihyperalgesic effects of SNC80. We hypothesized that the elimination of RGS4 would enhance the reward mediated effects of SNC80. To investigate the rewarding effects of SNC80, we used the Conditioned Place Preference (CPP) test. Mice lacking RGS4 were conditioned for five days with 1mg/kg of SNC80 by pairing one environment with SNC80 and a separate environment with saline injections. The amount of time mice spent on the SNC80 paired side of the CPP box was measured and compared to the amount of time spent on that side prior to conditioning. RGS4 knockout mice had no significant difference in time spent on the drug paired side when comparing pre and post-conditioning with SNC80. These results suggest that RGS4 is not involved in the reward mediated effects of SNC80. Further investigation into other signaling molecules downstream of the DOR could provide insight into the development of DOR agonists devoid of adverse effects.

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NOVEL C-TERMINAL HSP90 INHIBITORS, KU757 AND KU758, TARGET NEUROBLASTOMA CELLS AND ARE EFFECTIVE AGAINST CELL PROLIFERATION AND MIGRATION

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Neuroblastoma (NB) is a pediatric neoplasm arising from the adrenal gland. Although the five - year survival rate for neuroblastoma is 81%, the prognosis of advanced NB with N-myc amplification remains very poor, with a mortality rate over 50%. Therefore, novel treatment options that target multiple cancer pathways simultaneously can be impactful and are needed in the field. Heat shock protein 90 is a molecular chaperone responsible for the assembly, folding, and maturation of client proteins that regulate several cellular processes contributing to the hallmarks of cancer. Several HSP90 inhibitors targeting the N-terminal ATP binding site were evaluated in clinical trials and despite some early efficacy, each failed to progress to FDA approval as a monotherapy due to dose limiting toxicities (DLTs) which in part are associated with a induction of the heat shock response and compensatory pro-survival upregulation of HSP70 requiring higher doses of the inhibitor to maintain the response leading to DLTs. To combat these limitations of N-terminal inhibitors, our group and collaborators engineered novel C-terminal HSP90 inhibitors that overcome the toxicity associated with the N-terminal inhibitors and do not induce the compensatory heat shock response. We have previously demonstrated these C-terminal HSP90 inhibitors have shown efficacy in several cancer models. The effects of these novel C-terminal HSP90 inhibitors on neuroblastomas (NB) is not yet known. We hypothesize that inhibition of the heat shock protein 90 (HSP90) with two novel C-terminal inhibitors KU757 and KU758 will lead to the disruption of cell proliferation, providing a new therapeutic strategy for NBs. We first measured the efficacy of KU757 and KU758 against the neuroblastoma cell line, SHEP. Viability of NB cells after treatment with varying concentrations of KU757 and KU758 was measured by thee MTS assay. The half maximal inhibitory concentration for SHEP treated with KU757 and KU758 is 0.962 uM and 7.805 uM, respectively. Clonogenic assay of NB cells after 24 hour treatment with KU757 showed a dose dependent decrease in viability of cells. Migration of cells was measured using wound healing scratch assay. Following treatment of cells with KU757, wound closure and cell migration of the scratched area was significantly inhibited compared to untreated controls. Western blot analysis demonstrated modulation of NB tumorigenic pathway proteins that are HSP90 client proteins inhibited by KU758. This early preclinical in vitro data supports C-terminal inhibitors as a novel therapeutic with anti-cancer activity in NB cells. Further in vivo studies will be needed to validate the role of C-terminal HSP90 inhibitors for clinical translation in neuroblastomas.

IDENTIFYING RACIAL DIFFERENCES IN PLATELET ACTIVATION BETWEEN NATIVE AMERICANS, AFRICAN AMERICANS, AND CAUCASIAN AMERICANS

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The leading cause of death in the Native American population is cardiovascular disease. This is not surprising since they are 20 % more likely to develop coronary heart disease in comparison to the general US population. In this project, we sought to evaluate environmental and biological factors in the Native American population to determine if they are prone to higher levels of platelet aggregation in comparison to African Americans and Caucasian Americans. Previous studies have demonstrated that 84% of the African American population expresses a polymorphism in the thrombin receptor PAR4 which contributes to a decrease in protection from commonly prescribed antiplatelet drugs such as aspirin and clopidogrel. Therefore, we hypothesize a similar polymorphism can be found in the Native American population since they represent a homogenous population that could be enriched for inherent genetic differences contributing to an elevated platelet aggregation response that may be resistant to current antiplatelet therapy. A survey will be provided to the participants of this study to determine confounding environmental factors that could influence their platelet activation such as diet, drug intake, income, access to healthcare, level of education, and socioeconomic status. Following completing of the survey, we will measure platelet activation through an ex vivo platelet aggregation test which will be stimulated with thrombin, collagen, PAR4-AP (PAR4-activating peptide), and PAR1-AP (PAR1-activating peptide). In the future we will recall these subjects and evaluate their platelet reactivity by flow cytometry and correlate all of our findings to their genetic background using PCR and other advanced genetic techniques. Currently, we are in the process of recruiting donors and evaluating their aggregation status in this blinded study.