Speaker Abstracts

Efficacy and Safety of Ceftazidime-Avibactam in Combination with Aztreonam

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Treatment of patients with serious infections due to highly resistant Gram-negative bacteria (GNB) remains problematic and is a major public health concern. Not only is there increased resistance among frequently encountered GNB like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, but there is also a rise in the number of multidrug-resistant strains [1-3]. This change is driven in large part by the increasing prevalence and complexity of a variety of β-lactamases. The most important of these are the extended-spectrum β-lactamases (ESBLs) and carbapenemases, such as *Klebsiella pneumoniae* carbapenemase (KPC). The metallo-β-lactamases (MBLs), which have the ability to hydrolyze all β-lactams except aztreonam (ATM), have emerged recently as problematic carbapenemases. While there has been an impressive drug development response to combat ESBL- and KPC-producing GNB infections, none of the recently approved antibiotics have reliable activity against MBL-producing GNB. Several antibiotics with activity against MBL-producing GNB are being developed, but none are anticipated to be available until at least 2021.

One strategy that may serve as a "bridge" treatment for MBL-producing GNB infections is ceftazidime-avibactam (AVYCAZ®) combined with ATM. In the combination of ATM with AVYCAZ®, AVI inhibits the ESBL and KPC beta-lactamases that are often present in MBL-producing GNB, allowing ATM, which is unaffected by MBLs, to effectively bind to bacterial penicillin binding proteins. Limited data to date suggest that AVYCAZ® combined with ATM is a promising treatment. Although the precise mechanism of improved bacterial killing activity with AVYCAZ® combined with ATM is not completely understood, it is likely attributable to maximal saturation of the diverse penicillin binding proteins present in GNB, flooding of periplasm with β-lactams, and maximal binding of available β-lactamases

Before uniform adoption of this "bridge" treatment, it is critical to identify AVYCAZ® combined with ATM regimens associated with maximal efficacy and well-defined safety due to the potential of cumulative toxicity from "dual-β-lactam". This presentation will review the results of hollow fiber infection model (HFIM) studies that were conducted to identify AVYCAZ® combined with ATM regimens that resulted in maximal bacterial kill and resistance suppression. This presentation will also review the design of an ongoing phase 1 study in healthy adults that will evaluate the safety and PK of the two optimal AVYCAZ® combined with ATM regimens identified in the aforementioned HFIM studies.

Insights into the evolution of *Staphylococcus aureus* daptomycin resistance from an *in vitro* bioreactor model

Meenakshi Malik, D.V.M., Ph.D.
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Department of Basic and Clinical Sciences

The extensive use of daptomycin for treating complex methicillin-resistant *Staphylococcus aureus* infections has led to the emergence of daptomycin-resistant strains. Although genomic studies have identified mutations associated with daptomycin resistance, they have not necessarily provided insight into the evolution and hierarchy of genetic changes that confer resistance, particularly as antibiotic concentrations are increased. Additionally, plate-dependent *in vitro* analyses that passage bacteria in the presence of antibiotics can induce selective pressures unrelated to antibiotic exposure. We established a continuous culture bioreactor model that exposes *S. aureus* strain N315 to increasing concentrations of daptomycin without the confounding effects of nutritional depletion to further understand the evolution of drug resistance and validate the bioreactor as a method that produces clinically relevant results. Samples were collected every 24 hours for a period of 14 days and minimum inhibitory concentrations were determined to monitor the acquisition of daptomycin resistance. The collected samples were then subjected to whole genome sequencing. The development of daptomycin resistance in N315 was associated with previously identified mutations in genes coding for proteins that alter cell membrane charge and composition. Although genes involved in metabolic functions were also targets of mutation, the common route to resistance relied on a combination of mutations at a few key loci. Tracking the frequency of each mutation throughout the experiment revealed that mutations need not arise progressively in response to increasing antibiotic concentrations and that most mutations were present at low levels within populations earlier than would be recorded based on SNP filtering criteria. In contrast, a serial-passaged population showed only one mutation in a gene associated with resistance and provided limited detail on the changes that occur upon exposure to higher drug dosages. To conclude, this study demonstrates the successful *in vitro* modeling of antibiotic resistance in a bioreactor and highlights the evolutionary paths associated with the acquisition of daptomycin non-susceptibility. An understanding of the mechanisms underlying resistance and the evolutionary fates of different mutations within a system that more realistically mimics *in vivo* conditions will facilitate the development of new strategies to counter the problem of antibiotic resistance.

Bio-omics: a team-based approach

Stanley Stevens, Ph.D.
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Department of Pharmaceutical Sciences

Bio-omics encompasses several powerful and unbiased approaches to understand the complex molecular mechanisms underlying fundamental biological processes as well as mechanisms associated with the development and progression of human disease. Our laboratory is actively investigating the epigenetic and proteomic changes that occur in the brain and liver after acute and chronic alcohol using various “omics” approaches. In addition to our own research, we have collaborated with numerous investigators to apply the approaches developed in our lab to unravel the complex pathophysiological mechanisms of several diseases including cancer, diabetes, and neurodegenerative diseases. The interdisciplinary, team-based nature of these projects will be discussed including strategies for team-based approaches when applying for federal funding.

Visual Cycle Modulators for the Potential Treatment of Atropic Age-related Macular Degeneration and Stargardt Disease

Christopher Cioffi, Ph.D.
Assistant Professor, Medicinal Chemistry
Department of Basic and Clinical Sciences/Pharmaceutical Sciences

Accumulation of lipofuscin in the retina is associated with the pathogenesis of atrophic age-related macular degeneration (AMD) and Stargardt disease. Evidence has shown that bisretinoids such N-retinylidene-N-retinylethanolamine mediate lipofuscin toxicity. The synthesis of lipofuscin bisretinoids is a natural outcome of the visual cycle and is dependent upon the influx of retinol from serum to the retina. Thus, agents that modulate the visual cycle by impeding ocular uptake of serum retinol show promise as potential pharmacotherapies by which to stem further neurodegeneration and concomitant vision loss associated with geog. atrophy of the macula. Specifically, we have shown that compounds antagonizing the retinol-dependent interaction of retinol-binding protein 4 (RBP4) with transthyretin (TTR) reduce serum RBP4 and retinol levels and inhibit bisretinoid formation in the retina of Abca4-/- mice. This lecture will highlight work conducted in collaboration with Columbia University and as part of the NIH Blueprint Neurotherapeutics Network whereby novel RBP4 antagonists with exquisite in vitro RBP4 binding affinity and favorable drug-like characteristics were identified. Furthermore, we will show that standout analogs exhibited robust in vivo efficacy by reducing circulating plasma RBP4 levels by greater than 90% in rodents.

Poster Abstracts

Pharmacist Driven Pharmacogenetic Testing Initiative: Implementation to Results

Jacqueline Cleary and Emily Seidl

Purpose: Pharmacogenetic testing is currently an expanding field in which clinicians are able to streamline medication selection based on a patient's genetic makeup. These efforts can be focused on drug binding, drug metabolism, or other various gene expressions. This can avoid possible toxicities or subtherapeutic levels of a medication. The purpose of this study is to demonstrate the value of a clinical pharmacy team facilitating identification, execution, and interpretation of in office pharmacogenetic testing (PGT).

Methods: This year-long study involves patients being identified by a Hometown Health Center (HHC) clinician; this includes psychiatrists, primary care providers, or members of the clinical pharmacy team. Patient subgroups identified include pain, psychiatry, and ADHD patients. The pharmacy team assists with the collection of the sample, completes the ordering and paperwork, and provides a result interpretation and therapy recommendation to the provider. Patients who had a PGT test resulted are candidates for the study. Once the patient's PGT test is resulted, the following data was collected: patient name, date of birth, sex, age, order date, test, provider, the results of the PGT test, and if any intervention was made based on the PGT test results. Also, once the patient gets their results, they will come back to the clinic for the provider to make the necessary changes to their therapy, which may involve personal phone calls to the patient to ensure follow-up. The primary outcome of this study is the amount of adjustments made to patient's therapy as a result of PGT testing. Secondary outcomes include: the number of patients who obtain PGT, PGT test results, and comments from providers utilizing this service. Only descriptive statistics were performed on this dataset. This study is only a yearlong; however, patients will continue to be identified for PGT once this study is complete.

Results: Since this initiative was started, 53 tests have been done, with polymorphisms shown on all tests. There have been 30 drug interactions discovered, with 31 interventions being made.

Conclusion: Overall, this study portrayed the successful utilization of PGT in a clinic setting. A multitude of patients were switched to therapy options best suited for their genetic make-up. This type of testing can also be used for refractory patients who have worsening pain or psychiatric issues. This type of testing will help to streamline how providers prescribe medications and allow the pharmacist to become more integrated in patient-centered care.

Poster 1

Student Perception of the Opioid Epidemic at Albany College of Pharmacy and Health Sciences

Madyson Allard, Nicolas James, Jacqueline Cleary

Background: Over 130 people die daily as a result of an opioid overdose. This rapidly growing public health crisis has put pharmacists on the front line of the epidemic. Pharmacy students must be formally educated and trained to reduce the causes and occurrences of opioid abuse and help fight the opioid epidemic.

Methods: First year (P1) and third year (P3) pharmacy students at ACPHS were asked to complete a survey regarding student perception surrounding the opioid epidemic. The survey was created by students using Google Forms, and reviewed by ACPHS faculty members. The survey was presented in one course per class year, and time was allotted in class to complete the anonymous survey. The survey was structured to assess previous education, personal opinions, responsibility of the epidemic, and confidence of treating those experiencing addiction to opioids. The survey then offered six case-based scenarios to assess how education and bias influence clinical practice decisions, utilizing a Likert Scale for responses.

Results: Exactly 250 participants completed this survey - 106 P3 students and 144 P1 students. The two class years differed in many of the opinion/perception based questions. Regarding support of supervised injection sites, 41.3% of P3 students said yes compared to 29.7% of P1 students. There was also a difference in responsibility of the opioid epidemic. P3 students (45.7%) believed "prescribing physicians" were most responsible, whereas P1 students (43.9%) believed it has developed from "drug abusers." Given several case-based questions, similar distributions along the Likert scale were observed between both groups, with the exception of the supplying needles to a patient with a history of drug abuse; 66.7% of P3 students reported a score of 6-9, indicating they would be more likely to provide needles, whereas 57.2% of P1 students reported this score.

Conclusion: Through the analysis of the surveys, there is a favorable shift in perception and confidence in treating patients addicted to opioids, however the moderate confidence displayed leaves room for improvement. The variance between and among class years when addressing the opioid epidemic in clinical practice is demonstrated in the survey of ACPHS students and can help in developing curriculum and course content. Future direction of this project includes a statistical analysis comparing the responses of students in their didactic years to students after their rotational experiences. Ultimately, the hope is that this project highlights the need for improved pharmacist education surrounding the opioid epidemic.

Poster 23

Mechanisms of ricin-mediated programmed cell death

Cody Kempen, Alexa L. Hodges, William D. McCaig, Yinghui Rong, Jennifer Westfall, Nicholas J. Mantis and Timothy J. LaRocca

Ricin is a protein toxin derived from the castor bean. Ricin causes widespread damage to the lung epithelium in vivo, however, lung epithelial cells are somewhat insensitive to ricin in vitro. We hypothesize that this discrepancy is due to synergistic effects between ricin and cell death stimuli which also function as cytokines (TNF-α, FasL, TRAIL) in vivo. These stimuli may be released from macrophages, prominent in the inflammatory response to ricin, resulting in bystander death of lung epithelial cells. Here we show that ricin causes significant death of human macrophages (U937 cells) while its efficacy is reduced against human lung epithelial cells (A549 cells) in vitro. Cytotoxicity assays of co-cultured A549 and U937 cells resulted in increased sensitization of A549 cells to ricin. Co-administration of cell death stimuli, specifically TRAIL, resulted in increased A549 sensitization to ricin. Furthermore, cell death induced by ricin + TRAIL was substantially reduced when caspases were inhibited suggesting activation of apoptosis. Inhibition of RIP1 or caspase-1 had no effect on cell death ruling out a role for necroptosis and pyroptosis. Levels and activation of caspases increased in lung epithelial cells following treatment with ricin + TRAIL relative to treatment with ricin alone, further indicating activation of apoptosis. Moreover, Nanostring nCounter analysis revealed the upregulation of genes associated with apoptosis in lung epithelial cells following treatment with ricin + TRAIL. This work sheds light on mechanisms of ricin-induced death of lung epithelial cells and indicated that ricin is incapable of inducing apoptosis in these cell in the absence of co-administration of cell death stimuli.

Poster 2

Mitochondrial ROS prime cells for the hyperglycemic shift to necroptosis

William D. McCaig, Matthew A. Deragon, Payal S. Patel, Nicole L. Shakerley, Tori A. Smiraglia, and Timothy J. LaRocca

Programmed cell death (PCD) is most commonly characterized by the non-inflammatory, caspase-dependent apoptosis and the inflammatory, caspase-independent necroptosis. Necroptosis is driven by a complex known as the necrosome, which is comprised of the kinases RIP1, RIP3, and MLKL. Previously, we observed a novel shift from apoptosis to necroptosis in a hyperglycemic environment. We have shown that this PCD shift is relevant to neonatal hypoxia-ischemia (HI) brain injury as it was exacerbated by hyperglycemia in a RIP1-dependent manner. Moreover, during this injury we observed decreases in downstream effectors and components of apoptosis and increases in RIP1 and MLKL. Here we highlight an essential role for reactive oxygen species (ROS) and mitochondrial factors in the hyperglycemic shift of apoptosis to necroptosis. We show that glycolysis and ROS are critical for the hyperglycemic shift to necroptosis and that ROS alone are sufficient to produce the cell death shift. ROS were shown to enhance the oligomerization and activation of RIP1 while they antagonized caspases. Translocation of RIP1, MLKL, Drp1, and Bax to the mitochondria was enhanced in hyperglycemic conditions. Moreover, inhibition of these factors prevents the hyperglycemic shift to necroptosis to varying degrees.

Poster 3

Role(s) of pp(p)Gpp in Oxidative Stress Response and Virulence in Francisella tularensis LVS

Zhuo Ma, Kayla King, Chandra Shekhar Bakshi, Meenakshi Malik

Francisella tularensis (Ft) is an important Gram-negative facultative intracellular human pathogen responsible for causing tularemia. It is also classified as a category A agent by the CDC based on its possible use as a bioterror agent. Although the molecular basis for the high infectivity and virulence of Ft is not well understood, the pathogenicity of Ft is mainly dependent on its ability to persist and replicate in phagocytic cells. To survive and replicate inside the cells, Ft has to resist the attack of host-generated reactive oxygen and nitrogen species. Multiple antioxidant enzymes that are important defense factors against oxidative stress have been identified and implicated in the pathogenesis of Ft in multiple studies. Our previous study has demonstrated that some of these antioxidant enzymes are regulated by a LysR family transcriptional regulator, OxyR. In the present study, we further investigate the roles of pp(p)Gpp, stringent response molecules produced by RelA and SpoT proteins, in the oxidative stress response and virulence of Ft. We firstly generated a series of Ft LVS mutant strains including a relA gene deletion mutant (ΔrelA), a relA/spoT double gene mutant (ΔrelA/spoT) and their corresponding transcomplements. These mutant strains were characterized for their sensitivity towards oxidants by generating growth curves, by disc diffusion and bacterial killing assays; for their ability to survive in macrophages by invasion assay, and for their virulence in mice. Impressively, the ΔrelA/spoT double mutant, which is unable to produce pp(p)Gpp, showed strong sensitivity to the oxidative stress; defect in replication and survival in macrophage and attenuated virulence in mice. In addition, as compared with wild type LVS, the growth of ΔrelA/spoT mutant at high temperatures is also decreased. These results suggest that pp(p)Gpp in Ft play an important role in providing resistance against bacterial stress, intramacrophage survival and virulence in mice. Further studies employing molecular and biochemical approaches including RNA-seq and qRT PCR revealed the global effect of pp(p)Gpp on the expression of genes including part of antioxidant genes such as thioredoxin1; multiple groups of heat shock protein genes; and particularly, genes of intracellular growth locus: iglA,I glB,I glC and iglD. Further studies are under way to elucidate the mechanism of pp(p)Gpp in the regulation of stress responses and virulence in Francisella tularensis.

Poster 4

Genetic Basis for Daptomycin Resistance in Methicillin Resistant Staphylococcus aureus

Smruti Mishra, Erica Lasek-Nesselquist, Jackson Lu, Ryan Schneider, Zhuo Ma, Janice Pata, Kathleen McDonough, Meenakshi Malik

The extensive use of daptomycin for treating complex methicillin-resistant Staphylococcus aureus infections has led to the emergence of daptomycin-resistant Staphylococcus aureus strains. Although genomic studies have identified mutations associated with daptomycin resistance, they have not necessarily provided insight into the evolution and hierarchy of genetic changes that confer resistance. Further, plate-dependent in vitro analyses that passage bacteria in the presence of antibiotics can induce selective pressures unrelated to antibiotic exposure. We established a continuous culture bioreactor model that exposed S. aureus strain N315 to increasing concentrations of daptomycin without the confounding effects of nutritional depletion. Samples were collected every 24 hours for a period of 14 days and minimum inhibitory concentrations (MICs) were determined. Additionally, the collected samples were subjected to whole genome sequencing to monitor the acquisition of daptomycin resistance. The development of daptomycin resistance in N315 was associated with mutations in genes coding for proteins that alter cell membrane charge and composition. Although genes involved in metabolic functions were also targets of mutation, the common route to resistance relied on a combination of mutations at a few key loci with the varying competitive advantages. Furthermore, this resistance to daptomycin was stable in N315 strain and reversion to the susceptible phenotype was not observed till 14 days. To conclude, this study demonstrates the successful in vitro modeling of antibiotic resistance in a bioreactor and highlights the evolutionary paths associated with daptomycin resistance. An understanding of the mechanisms underlying resistance and the evolutionary fates of different mutations within a system that more realistically mimics in vivo conditions will facilitate the development of new strategies to counter the problem of antibiotic resistance.

Poster 5

Developing a Murine Model for the Investigation of Vibrio parahaemolyticus Infections

Chen Shu Dong, Brittany Vojnar, Brittney Maring, Michelle A. Parent

Vibrio parahaemolyticus is a gram-negative curved bacilli that survives and thrives in brackish waters. With warming water temperatures and recreational salt-water exposure, along with the potential problematic processing of seafood, there has been an increase in the numbers of individuals infected by this pathogen. *V. parahaemolyticus* is the most common cause of bacterial, seafood-associated gastroenteritis in the United States. Gastrointestinal infections are typically self-limiting for healthy individuals, as most patients do not seek medical help and fully recover. However, open wound exposure can lead to significant, life-threating septic infections especially in the elderly and in immunocompromised populations. Currently, there is a dearth of literature regarding the host response to infection. Previous murine models to study infection with this emerging pathogen have been problematic, due to an unnatural route of organism delivery or the inability to stimulate the host immune response. At this time, with an increasing number of deaths related to *V. parahaemolyticus* septicemia, we have developed a sepsis model of infection. We are currently developing and establishing a model that allows for investigation of those host innate immune parameters required to eliminate infection with *V. parahaemolyticus*. Toward that end, our goal is to provide clinicians a path to understanding infection related mortality and possible effective treatments.

Poster 6

Vibrio parahaemolyticus Response to Oxidative Stress

Brittany Vojnar, Brittney L. Maring, Chen Shu Dong, Stephanie Waters-Wezalis, Michelle A. Parent

Vibrio parahaemolyticus, a gram-negative curved bacilli, is the most common cause of seafood-associated bacterial gastroenteritis. This pathogen is tolerant of high salt concentrations which are frequently encountered in warm coastal and estuarine waters. *V. parahaemolyticus* is commonly acquired through the consumption of raw seafood, which includes oysters and crustaceans. Additionally, it can cause significant wound infections especially in the elderly and in immunocompromised populations. The CDC estimates that this isolate causes approximately 45,000 illnesses each year in the United States. However, as most infections are self-limiting, and individuals do not seek out medical attention, actual numbers are much higher. In order to understand how *V. parahaemolyticus* combats the host response to infection, we are examining the bacterial genes that are involved in combating oxidative stresses, induced by reactive oxygen species (ROS) produced by the host. Currently, we are examining the ROS response in the host, while also looking at the oxidative stress response through the gene expression from recovered bacteria. By utilizing infected macrophage and varied bacterial culture conditions we are investigating the ROS stress response genes induced in *V. parahaemolyticus* during H_2O_2 exposure. We will demonstrate that *V. parahaemolyticus* expresses differing levels of antioxidant genes in response to H_2O_2 exposure, determining how this bacteria combats the host response to infection.

Poster 7

Co-culture between HIV-1 Infected MT2 Cells and THP1 Cells Induced Cell Death

Lalhming Zaua, Liang Xi, Laura Graf and Binshan Shi

The mechanism responsible for CD4 T cell death in HIV-1 infection has been a long-standing puzzle. Previous studies have suggested that the majority of CD4 T cells in human lymphoid tissue actually die through a form of lytic programmed cell death termed pyroptosis. However, pyroptosis has been mostly identified as an innate immune response found in monocytic lineage and mucosal epithelial cells. Our recent result has suggested that we have established a pyroptosis cell model in differentiated monocyte infected by retrovirus through cell-to-cell transmission. MT2 is a T cell line infected with HTLV1 endogenously. The infection of HTLV1 is mostly through cell-to-cell transmission. MT2 cells are highly permissive to HIV-1 infection. It was found that MT2 cells co-infected with HIV1 and HTLV1 was able to transmit HIV-1 to human epithelial TZM-bl cells by cell-to-cell transmission. Meanwhile this data also suggests that HIV-1 can be pseudotyped by HTLV1 and change its tropism. Interestingly, it was further found that the co-culture between HIV-1 infected MT2 cells and human monocyte THP1 cells induced an aggressive form of cell death accompanied with the increase of LDH release. The increased release of LDH can be inhibited by caspase inhibitor ZVAD. Further understanding of the condition that induces pyroptosis in retrovirus infection and its mechanism will not only increase our knowledge in HIV-1 virology but also will shed light on the understanding of pathogenesis of AIDS disease in future.

Poster 8

Quantification of HIV-1 1-LTR Circle DNA by Using A Nested Real Time PCR

Laura Graf, Katie Mellon and Binshan Shi

HIV-1 episomal HIV-1 DNAs in the form of either 1-LTR circle DNA or 2-LTR circle DNA are created in its early replication phase. Due to a lack of a replication system, the amount of HIV-1 episomal HIV-1 DNA is eventually diminished overtime. There has been an immense interest in using HIV-1 LTR circle DNA measured by qPCR as a clinical diagnostic assay, since their levels would be representative of nascent infection. However, quantification of 1-LTR circles by quantitative PCR has previously been too difficult to carry out successfully. We report here the development of a nested real time PCR with an ability to detect HIV-1 1-LTR circle DNA with improved specificity and sensitivity. The PCR primers were designed to amplify HIV-1 1-LTR circle DNA. PCR conditions, especially extension time and DNA polymerases, were optimized to establish a reaction that exclusively amplifies HIV-1 1-LTR circle DNA template. A nested PCR was developed for HIV-1 1-LTR circle DNA quantification which includes the 1st round of regular PCR that uses Phusion High-Fidelity DNA polymerase and the 2nd round TaqMan real time PCR. This PCR assay was used to quantify HIV-1 1-LTR circle DNA in HIV-1 infected human monocyte cell line THP1 cells in a 24 well plate. Our results showed that the PCR using Phusion High-Fidelity DNA polymerase had the capability to amplify 1-LTR circle DNA template exclusively under optimized condition. A nested PCR was designed to increase the sensitivity and specificity. The use of tailed primer with unique R sequence avoids 2nd round PCR amplification using templates from HIV linear cDNA, integrated DNA and 2-LTR circle DNA templates, which greatly increases assay specificity. It was found that the real time PCR is very successful with the sensitivity of detecting 5 copies of HIV-1 1-LTR templates. Finally, the nested PCR was applied to quantify HIV-1 1-LTR circle DNA in PMA differentiated THP1 cells infected with VSV-G pseudotyped HIV-1 virus in a single round infection experiment. The results showed that the amount of HIV-1 1-LTR circle DNA decreased significantly at 120 hours after infection. Our results have demonstrated the successful development of a quantitative PCR assay to detect HIV-1 1-LTR circle DNA with increased sensitivity and specificity. A biomarker of HIV-1 de novo infection has a great value in clinical diagnostics, guidance of therapy, and control of disease progression.

Poster 9

Contribution of Thioredoxin A to Acinetobacter baumannii Virulence

Catherine Phelps, Patrick M. Ketter, Bernard P. Arulanandam, Nicole L. Shakerley

Acinetobacter baumannii has become an increasingly serious threat to global health due to its ability to rapidly develop antibiotic resistance and persist within the healthcare environment. Both the WHO and CDC have classified *Acinetobacter baumannii* as a critical pathogen for the development of new therapeutics. While it is known that *Acinetobacter* induces host immune responses in attempt to clear the infection, specific mechanisms of bacterial virulence are lacking. Thioredoxin is an enzyme that is essential to bacterial survival due to its diverse range of functions, including roles in gene modulation, oxidative stress alleviation, and enzyme regulation. In this study, we examine the contribution of thioredoxin A to *Acinetobacter* virulence by utilizing a multi-drug resistant clinical respiratory isolate (Ci-79) that was obtained from an Operation Iraqi Freedom service member. Growth curve analysis demonstrated that thioredoxin A deficient *A. baumannii* (ΔTrxA) has a growth disadvantage under normal growth conditions as compared to both Wild-type (Ci-79) and plasmid complemented strains (ΔTrxA + pTrxA). Additionally, ΔTrxA exhibited significant increases in susceptibility to hydrogen peroxide and pyrogallol but exhibited increased resistance to oxidative stress induced by cumene, an organic peroxide. To better characterize the contribution of TrxA to bacterial viability, strains were cultured in the presence of the thiol oxidizing agent diamide. In line with its function, the ΔTrxA mutant exhibited slowed growth and increased susceptibility to low doses of diamide. The ΔTrxA strain also exhibited increased susceptibility to the antibiotic agents chloramphenicol, erythromycin, tetracycline, meropenem, and doripenem. In vitro infection of J774 murine macrophages with ΔTrxA resulted in significantly increased bacterial burdens despite having confirmed equivalent inoculum. The ΔTrxA mutant also demonstrates attenuated virulence in a *Galleria mellonella* larvae model. These data provide evidence that furthering our understanding of *Acinetobacter* thioredoxin A function may shed light on novel pathogenesis and antibiotic resistance mechanisms.

Poster 10

Synthesis of Nicotinamide Riboside Derivatives

Song Zheng, Chinomso Ogbonna, Wilson Ebhohon, Yana Cen

Sirtuins are NAD-dependent protein deacetylases found in a wide range of organisms from archaea to human. They play important roles in apoptosis, metabolism, DNA repair, and genome stability. During the last twenty years, this family of enzymes has been studied intensively as therapeutic targets. However, little is known about one of the mitochondrial sirtuins, SIRT5. Previous studies suggested that SIRT5 regulates ammonia detoxification through deacetylating and activating carbamoyl phosphate synthetase 1 (CPS1), the rate-limiting enzyme in the urea cycle. CPS1 activity was significantly reduced in SIRT5 KO mice. Subsequently, these mice developed severe hyperammonemia. Overexpression of SIRT5, on the other hand, resulted in CPS1 hypoacetylation. As a result, both urea production and CPS1 activity were elevated.

Pharmacological activation of SIRT5 may open new possibilities, not only for treatment of metabolic diseases characterized by mitochondrial dysfunction, but also for disease prevention and health maintenance. But to date, only one set of SIRT5 activators has been reported. Resveratrol and piceatannol, plant-derived polyphenols, were able to stimulate recombinant SIRT5 activity up to 2.5-fold with the EC50 in the micromolar range. However, these compounds failed to activate SIRT5 when physiological substrates were employed. Furthermore, the low solubility of these polyphenols in water also dampened the enthusiasm of pursuing them as therapeutic agents. The need for novel scaffolds that can activate SIRT5 in an isoform-selective manner becomes apparent.

We have discovered a SIRT5-specific small molecule allosteric activator, nicotinamide riboside (NR). The current project focuses on the development of NR derivatives as SIRT5 activators. These new chemical probes will enable us to specifically manipulate SIRT5 activity in the native cellular setting, to evaluate the potential therapeutic efficacy for upregulating SIRT5 for the treatment of disease such as hyperammonemia.

Poster 11

Chemo-enzymatic Synthesis of Isotopically Labeled Nicotinamide Riboside

Ai Tran, Ryota Yokose, Yana Cen

Nicotinamide riboside (NR), a trace nutrient found in milk, is a potent NAD elevating agent. Increasing cellular NAD concentration was thought to provide beneficial effects on healthspan and lifespan extension. Repletion of the intracellular NAD pool using NR, therefore, has been suggested as a novel therapeutic for the treatment of metabolic and age-related diseases. We developed a chemo-enzymatic synthesis of NR with the specific incorporation of isotope labels into the ribose ring and nicotinamide. We took advantage of a pre-existing enzymatic synthesis of NAD and coupled it with enzyme catalyzed "base exchange" and degradation for the production of isotopically labeled NRs. Using these isotopically labeled NRs, we demonstrate that in mammalian cells NR can be converted to NAD independent of decomposition. We also establish the importance of NRK-dependent pathway in maintaining NAD biosynthetic capacity when salvage pathway is compromised, further highlighting the homeostatic mechanism in NAD biosynthesis.

Poster 12

Synthesis of Novel RPE65 Inhibitors for the Treatment of Age-Related Macular Degeneration and Stargardt's Disease

Machayla Donovan, Parthasarathy Muthuraman, Arun Raja, Christopher Cioffi

Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries. There is no treatment for the most prevalent dry (atrophic) form of AMD. Age-dependent accumulation of cytotoxic lipofuscin in the retinal pigment epithelium (RPE) matches the age-dependent increase in dry AMD prevalence and thus is frequently cited as one of potential pathogenic factors contributing to the disease progression. In addition to direct cytotoxicity, lipofuscin seems to induce dysregulation of the complement system in the retina, which may significantly contribute to dry AMD pathology. In addition to dry AMD, dramatic accumulation of lipofuscin is the hallmark of Stargardt's disease (STGD), an untreatable inherited form of juvenile-onset macular degeneration. The major cytotoxic components of RPE lipofuscin are pyridinium bisretinoids (such as A2E) which are formed as by-products of the properly functioning visual cycle. It was suggested that partial inhibition of the visual cycle may reduce the formation of lipofuscin bisretinoids and prolong the RPE and photoreceptor survival in dry AMD and STGD. A critical step in the visual cycle is the conversion of all-trans retinyl ester to 11-cis retinol by the enzyme called isomerohydrolase (IMH). It has been shown that RPE65 represents IMH which produces 11-cis-retinol from all-trans-retinyl ester in the retinal pigment epithelium. The IMH reaction is rate-limiting in the visual cycle function thus making RPE65 an important drug target for visual cycle inhibition. The majority of known RPE65 inhibitors are retinoids which generally predicts broad specificity and multiple off-target activities that may be associated with adverse effects in vivo. Identification and characterization of new classes of non-retinoid RPE65 inhibitors is of high importance. After conducting a screen for the inhibitors of the isomerohydrolase reaction we identified CU239 as a lead compound that requires additional optimization. In addition, we propose to conduct optimization in a second structurally diverse series which is distinct from CU239. The objective of the studies outlined in this grant application is identification and detailed characterization of novel non-retinoid RPE65 antagonists that can be potent and orally bioavailable in vivo proof-of-concept compounds with significantly improved pharmacokinetic characteristics in comparison with current RPE65 inhibitors. The studies outlined in this proposal seek to conduct medicinal chemistry optimization in the lead series using a battery of primary, secondary and counterscreen assays, and to perform evaluation of in vivo efficacy and retinal toxicity for the optimized analogs in mouse models.

Poster 13

Molecular Insights into Di-acetyllysine Histone Recognition by the BRPF1 Bromodomain

Juliet Obi, Mulu Y. Lubula, Chiara M. Evans, Kara McGuire and Karen C. Glass

Bromodomains are often found in chromatin-modifying complexes, whose activity can lead to aberrant expression of genes that drive certain diseases in humans, including cancer, neurological disorders and inflammation. The bromodomain-PHD finger protein 1 (BRPF1) is a part of the MOZ (monocytic leukemic zinc-finger protein) HAT (histone acetyltransferase) complex implicated in chromosomal translocations known to contribute to the development of acute myeloid leukemia (AML). BRPF1 contains a unique combination of post-translational modification (PTM) reader domains, including two plant homeodomain (PHD) fingers, a bromodomain, and a proline-tryptophan-tryptophan-proline (PWWP) domain, through which it is known to modulate the HAT activity of MOZ. We hypothesized that the BRPF1 bromodomain can recognize histone ligands with multiple acetyllysine marks, and that the recognition can be modulated by PTMs adjacent to these marks. We carried out isothermal titration calorimetry (ITC), analytical ultracentrifugation (AU), and site-directed mutagenesis experiments with the purified BRPF1 bromodomain to investigate its binding affinity to different di-acetyllysine histone ligands. Our data reveal the BRPF1 bromodomain is able to bind several histone ligands containing di-acetyllysine, and modifications adjacent to the acetyllysine modifications alter the binding affinity. Notably, our AU data show that the BRPF1 bromodomain binds to mono- and di-acetyllysine ligands as a monomer. Our studies will further elucidate the role of the bromodomain in recruiting BRPF1 to histones, and subsequently assembling and activating the MOZ HAT complex, which has been implicated in acute myeloid leukemia.

Poster 14

Molecular mechanism of di-acetyllysine recognition by the ATAD2B bromodomain

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The ATPase family, AAA+ domain-containing protein 2B (ATAD2B) is a nuclear protein that may play a role in the development of neuronal tissues and tumorigenesis. The ATAD2B protein contains a C-terminal bromodomain that is highly homologous to the ATAD2 bromodomain, with 74.7% sequence identity and 94.4% similarity. The ATAD2 bromodomain is an attractive drug target because overexpression of ATAD2 is positively correlated with the progression of multiple cancer types, and poor patient outcomes. Although ATAD2 and ATAD2B are highly conserved, little is known about the function of ATAD2B, or its role in oncogenesis. We hypothesized that the ATAD2B bromodomain would likely be involved in recognition of di-acetyllysine modifications on the histone tail, similarly to its ATAD2 paralog. We identified the acetylated histone ligands of the ATAD2B bromodomain using a combination of isothermal titration calorimetry and nuclear magnetic resonance techniques. Interestingly, the ATAD2B bromodomain has different substrate specificity than the ATAD2 bromodomain, preferentially selecting for the histone H4K5acK8ac ligand. NMR chemical shift perturbation assays and site-directed mutagenesis were used to map out the acetyllysine binding pocket, enabling characterization of residues involved in coordination of mono- and di-acetylated histone ligands by the ATAD2B bromodomain for the first time. In addition, the X-ray crystal structure of the ATAD2B bromodomain in complex with an ATAD2 bromodomain inhibitor was solved at 2.3 Å resolution. This structure revealed that critical contacts required for bromodomain inhibitor coordination are conserved between the ATAD2/B bromodomains, and many of these residues play a dual role in acetyllysine recognition.

Poster 15

Use of Topical Antioxidants for Inhibition of DNA Modifications Induced by Ultraviolet Radiation

Rebecca Cruz and Martha A. Hass

Exposure of the skin to chronic or repeated acute periods of solar radiation causes direct and indirect DNA damage which can lead to formation of biomarkers such as 8-oxo-deoxyguanosine (8-oxo-dG/8-OHdg) and cyclobutane pyrimidine dimers (CPDs), that are hallmarks of skin cancer. We aim to show that novel antioxidant co-drugs, derived from α-tocopherol (α-TOC) and lipoic acid (LA) can inhibit the formation of these photoproducts in porcine skin exposed to solar radiation. These biomarkers can be quantified using LC-mass spectrometry (LCMS). An Oriel solar simulator is used to irradiate porcine skin and an ILT 1700 Research Radiometer is used to measure the dose of radiation to the skin. Treatment of skin with formulated drugs is done using Franz diffusion cells and penetration of the drugs into the skin layers is monitored by HPLC. DNA from skin samples is isolated with a DNEasy tissue kit. Isolated DNA is quantified using UV analysis. DNA samples isolated from irradiated and non-irradiated skin are digested and analyzed by LCMS to detect and quantify formation of 8-oxo-dG/8-OHdg and CPDs. The radiation dose was measured to be ~26.896 kJ/m^2 after a 40 minute irradiation time. A 40 minute irradiation time is expected to be sufficient to induce formation of 8-oxodG/8-OHdg and CPDs, based on previous experiments conducted in the Hass Lab to induce oxidation of lipids and proteins in the skin. UV analysis was performed on DNA isolated from irradiated and non-irradiated skin. The quantity of DNA isolated from the skin was determined to be ~2.5μg/250mg porcine skin, with an OD260:280 ratio of 2.42. No difference in the quantity of DNA isolated was observed between irradiated and non-irradiated skin. A calibration curve for α-TOC was generated using HPLC analysis and is used to determine concentrations of α-TOC and the co-drug in the skin layers. It is expected that topical treatment of the skin with combinations of α-TOC and LA as well as the α-TOC-LA co-drug will synergistically inhibit formation of in UVR exposed porcine skin which will be shown via LCMS analysis.

Poster 16

Combination of photodynamic and chemotherapeutic agents in the treatment of psoriasis: Assessment of in vitro efficacy of aminolevulinic acid and mycophenolic acid

Manisha Venkatesh, Jeffrey Voigt and Martha A. Hass

Psoriasis is an autoimmune disease of the skin characterized by epidermal hyperplasia, altered keratinocyte differentiation and infiltration of leukocytes. We aim to evaluate the potential therapeutic benefit of combining 5-aminolevulenic acid (ALA), a drug used in photodynamic therapy (PDT), with mycophenolic acid (MPA), in an *in vitro* model using HaCat cells (immortalized human keratinocytes). ALA increases the concentration of the endogenous photosensitizer, PpIX which upon irradiation, modulates hyperproliferation of keratinocytes. MPA, an immunosuppressant, inhibits inosine monophosphate dehydrogenase (IMPDH), and limits recruitment of inflammatory cells to the skin. HaCat cells were grown and passaged, and cell viability was determined by the MTT assay. Calibration curves were generated for PpIX using fluorescence spectroscopy and a fluorescent plate reader. The MTT assay and the hemocytometer showed that the number of cells increased with time and that the cells were viable. As expected, the calibration curves showed an increase in the emission signal at 635nm for PpIX as concentrations of PpIX were increased, verifying that PpIX could be detected and quantified by this method. A dose response curve of 5-ALA induction of PpIX in HaCaT cells was generated. A cell count of 600,000 cells/ml was used with an incubation time with 5-ALA of 4 hours at 37^{o}C. Concentrations of ALA ranging from 0.0011mM- 1.1mM were used to determine the dose-response relationship. A sigmoidal curve was obtained showing that 5-ALA induced PpIX production in a dose-dependent manner across the range of concentrations measured. Experiments to investigate the dose-response relationship of ALA plus MPA on PpIX production in HaCaT cells are underway. An IMPDH inhibition assay to monitor the activity of MPA alone, ALA alone and the combination of both is also underway. We expect that treatment of HaCaT cells with 1:1 molar concentrations of 5-ALA and MPA results in sustained efficacy of each parent compound by demonstrating that MPA does not interfere with elevation of PpIX induced by ALA and that ALA does not interfere with inhibition of IMPDH by MPA.

Poster 17

Cytokines secreted by stromal cells in TNBC microenvironment as potential targets for cancer therapy: experimental and bioinformatics predictions

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In triple negative breast cancer (TNBC), the lack of therapeutic markers and effective targeted therapies result in an incurable metastatic disease associated with a poor prognosis. Crosstalks within the tumor microenvironment (TME) including those between cancer and stromal cells, affect the tumor heterogeneity, growth, and metastasis. Previously, we have demonstrated that IL-6, IL-8, and CCL5 play a significant role in TNBC growth and metastasis. In this study, we performed a systematic analysis of cytokine factors secreted from four stromal components (fibroblasts, macrophages, lymphatic endothelial cells, and blood microvascular endothelial cells) induced by four TNBC cell types. Through bioinformatic analysis, we selected putative candidates of secreted factors from stromal cells, which are involved in EMT activity, cell proliferation, metabolism, and matrisome pathways. Among the candidates, LCN2, GM-CSF, CST3, IL-6, IL-8, and CHI3L1 are ranked highly. Significantly, Lipocalin-2 (LCN2) is upregulated in crosstalk of stromal cells and four different TNBC cells. Taken together, these results propose secreted factors as molecular targets to treat TNBC progression via crosstalk with stromal components.

Poster 18

Structural and Biophysical Characterization of CYP2C9*2 Genetic Variant Bound to an Anti-Hypertensive Drug Losartan

Sonia Parikh, Chiara M. Evans, Juliet O. Obi, Karen C. Glass, and Manish B. Shah

Cytochrome P450 (CYP)-dependent monooxygenases are a superfamily of heme-containing enzymes that play a crucial role in the detoxification and bioactivation of various xenobiotics. The highly polymorphic enzyme, CYP2C9, is an important drug metabolizing enzyme involved in the metabolism of up to 20% of the clinical drugs that include losartan, warfarin, tolbutamide, ibuprofen, and glimepiride. The CYP2C9*2 and *3 are the two most prevalent genetic variants that demonstrate significant reduction of the catalytic activity compared with the wild-type (WT) enzyme for many important CYP2C9 substrates. Despite the abundant information available regarding different CYP enzymes, the structural information about single nucleotide polymorphisms has remained scant. The CYP2C9*2 genetic variant represents an amino acid change from arginine to cysteine at position 144 (R144C), with an allele frequency of around 15-20% in various ethnic populations. To understand how genetic variation in CYP2C9 affects drug binding and oxidation, we solved the crystal structure of CYP2C9*2 in complex with losartan using X-ray crystallography, and further characterized the binding using isothermal titration calorimetry (ITC). The prodrug losartan is an angiotensin II receptor antagonist that is predominantly metabolized by CYP2C9 and 3A4. The structure of the CYP2C9*2 complexed with losartan illustrates remarkable differences compared to the previously determined WT complex. These include altered conformation of important secondary structural elements and residue sidechains responsible for binding interactions with different substrates. Furthermore, the new structure revealed differences in binding of losartan in the active site compared to the previously solved complexes of the CYP2C9 WT, *3 and *30. In addition, isothermal titration calorimetry was used to probe the binding of losartan with CYP2C9 WT and *2 genetic variant. The CYP2C9*2-losartan interaction demonstrated marked reduction in binding affinity and changes in thermodynamic parameters. Together, our findings from multiple techniques illustrate how polymorphism affects drug binding and yield insights into decreased hydroxylation activity of losartan in patients carrying CYP2C9*2 variant allele.

Poster 19

Functional Intereukin-6 (IL-6) Signaling in 419II cells

Brianna Bootier and Jeffrey Voigt

Cytokines such as Interleukin-6 (IL-6) are produced and released by breast cancer cells, as well as other cells, such as adipocytes, that are present in the tumor microenvironment, and have been tied to the regulation of cancer stem cell proliferation and epithelial to mesenchymal transition. IL-6 stimulates STAT3 phosphorylation, resulting in alterations in gene expression. The ability of IL-6 signaling to regulate cancer stem cell growth was investigated in 419II mouse mammary cancer stem cells. IL-6 treatment (5 ng/ml for 20 min) resulted in a significant increase in phosphorylation of STAT3 as assessed by Western blotting. This phosphorylation was blocked by exposure of cells to anti-IL-6 receptor antibody for 24 hours prior to IL-6 treatment, suggesting that a functional IL-6 pathway exists in 419II cancer stem cells. To further investigate the involvement of IL-6/STAT3 signaling in the regulation of cell growth, the effect of BBI608, a STAT3 inhibitor, on the growth of 419II cells was determined. BBI608 treatment (1 μM for 24 hours) significantly decreased the ability of 419II cells to form spheroids. These results suggest that IL-6 treatment, acting through phosphorylation of STAT3, plays a significant role in regulating 419II cell growth. To further investigate this, 419II cells were treated with IL-6 (50 ng/ml for 48 hours and cell number was determined with an MTT assay. IL-6 treatment had no stimulatory effect on cell growth, as expected. The lack of effect of IL-6 on 419II cell growth does not correlate with the ability of STAT3 inhibition to inhibit spheroid formation, since spheroid formation usually provides evidence for self-renewal of stem cells. One possible explanation for this difference is that BBI608 may inhibit cell growth through effects on pathways other than the IL-6 pathway. Further experiments, including confirming the effect of anti-IL-6 receptor antibody on 419II cell growth, will be needed to fully elucidate the role of IL-6 in regulating 419II cancer stem cell growth.

Poster 20

The Role of Endocannabinoids in the Post-Ischemic Survival of Human Brain Microvascular Vein Endothelial Cells

Andrew B. Thurston, Schuyler Pruyn, HaiAn Zheng, Jefferey Voigt, Marcel Musteata

In June 2018, the US FDA approved the first medicinal cannabis-based product. With this the need to understand the nature of the endocannabinoid system (ECS) grows even greater. This study was conducted to measure the expression level of the cannabinoid receptors on a human brain microvascular vein endothelial cell line (hBMVEC), and how ischemic conditions alter their natural expression, as well as to investigate the function of endocannabinoids (eCB, AEA or 2-AG) as neuroprotectives at the level of the blood-brain barrier (BBB). Overall, we hypothesize that the endocannabinoids will have a neuroprotective effect on the membrane stability and vitality of the hBMVEC line. More specifically; [1] The cannabinoid receptors are presented on this hBMVEC line; [2] The expression level of the cannabinoid receptors is impacted by ischemic conditions seen post stroke, mimicked by Oxygen-Glucose Depravation (OGD). [3] The endocannabinoids contain a neuroprotective effect [4] The endocannabinoid's neuroprotective effects are tied to the expression levels of the cannabinoid receptors. In order to test our hypothesis, we [1] Used a commercial human brain microvascular vein endothelial cell line that were grown using traditional cell culture techniques and a commercial medium (called The System from Cell System, WA). [2] The expression levels of the CB1 and CB2 receptors baseline on hBMVEC and after specific time-point in OGD condition were quantified using RT-qPCR. [3] Doses of endocannabinoids are introduced into the hBMVEC environment post OGD to measure it's neuroprotective effect via several stains and assays [4] The optimal dose of the endocannabinoids are introduced into the hBMVEC environment post OGD at timepoints where there is a significant alteration in the expression levels of the cannabinoid receptors. Currently, we have detected and preliminarily quantified the average base expression levels of cannabinoid receptors and their alterations post ischemia by RT-qPCR. Cell culture on Transwell were also established, and TEER data was in line with our expectations regarding media formulations effects on membrane integrity (effects of OGD). The endocannabinoid system and cannabinoids receptors are potential targets for future therapeutics. Upon the results of this study we can use this established model to further study BBB permeability and function as it relates to the endocannabinoid system. Understanding the function of the ECS in various brain conditions is critical to assessing the potential medical benefit of cannabis-based therapies as well as the effects of their long-term use.

Poster 21

Stability and Biodistribution of Endocannabinoids at the Blood-Brain Barrier

Schuyler Pruyn and HaiAn Zheng

The discovery of the endocannabinoid system (ECS) has heightened research interests for broader applications. Revealing characteristics, functions and mechanisms of the ECS is not only essential to evaluate the benefits and risks of Medical Cannabis, but also important for diagnosis, using the endocannabinoids as promising biomarkers for physiological and pathological conditions, such inflammation and neurodegeneration. The ECS is active throughout the central and peripheral nervous systems (CNS, PNS), and the endogenous ligands, the endocannabinoids (eCBs), are produced "on demand" in the CNS for many neurological responses, such as diminishing sensitivity to pain. Recently, we have confirmed that the cannabinoid receptors are expressed on human blood-brain barrier (BBB) endothelial cells and are regulated upon various conditions. Therefore, there is a need to develop simple, yet effective methods to identify and quantify these natural bioactive lipids (e.g. anandamide [AEA] and 2-arachidonoylglycerol [2AG]) from various mediums and bio-matrices of the BBB. Such real-time quantification will enable us to determine their chemical and biological stability, distribution, and metabolism when they circulate throughout the body, cross the BBB, and reach their targets of action. The endocannabinoids AEA and 2AG are relatively stable in water but are degraded more quickly in biological environments. Therefore, as AEA and 2AG are permeable through the blood-brain barrier (BBB), their transportation across the barrier is limited by quick metabolism. The chemical and biological stability and kinetics of these lipids in various mediums are determined and quantified by liquid chromatography-mass spectrometry (LCMS). Solid phase extraction (SPE) using Sep-Pak® C18 cartridges is used to separate compounds of interest from the medium before LCMS analysis. Their stabilities are studied at different temperatures (4-25°C) to mimic common laboratory and experimental procedures. The LCMS method was developed and optimized with selected mass transitions of AEA and 2AG that give good peak resolution in order to detect and quantify both lipids within a short run time. An optimized SPE method was developed successfully to separate the endocannabinoids from biological mediums for LCMS analysis. Stability studies in water, acetonitrile, and cell culture media showed AEA to have greater stability over 2AG, with both having the ability to be detected at concentrations in the pg/mL to ng/mL range from in vitro conditions and models. Our developed methods and preliminary results can be further applied to in vivo models and clinical applications to characterize the biodistribution of endocannabinoids across the BBB and understand their clinical impacts.

Poster 22

Evaluation of the Impact of a Rotavirus Vaccine Program on Pediatric Acute Gastroenteritis Hospitalizations: Estimating the Overall Effect Attributable to the Program as a Whole and as a Per-Unit Change in Rotavirus Vaccine Coverage

Margaret K. Doll, Caroline Quach, David L. Buckeridge

Estimation of the overall effect of a vaccine program is essential, but the effect is typically estimated for a whole program. We estimated the overall effect of the Quebec rotavirus vaccine program, launched in November 2011, and the effect for each 10% increase in rotavirus vaccine coverage on pediatric hospitalizations for all-cause acute gastroenteritis. We implemented negative binomial regressions adjusted for seasonality, long-term trends, and infection dynamics, to estimate the effect of the vaccine program as: 1) a dichotomous variable, representing program presence/absence, and linear term to account for changes in trend in the period after the program began; and 2) a continuous variable, representing rotavirus vaccine coverage. Using exposure 1, the vaccine program was associated with a 51.2% (95% CI: 28.5, 66.7) relative decline in adjusted weekly hospitalization rates for all-cause acute gastroenteritis as of December 28, 2014. Using exposure 2, a 10% increase in rotavirus ≥1-dose coverage was associated with a 7.1% (95% CI: 3.5, 10.5) relative decline in adjusted weekly rates, with maximum coverage of 87.0% associated with a 47.2% (95% CI: 26.9, 61.9) relative decline. Estimation of the overall effect attributable to a change in vaccine coverage might be a useful addition to standard measurement of the overall effect.

Poster 25

Feasibility of a Digital Health Technology to Engage Heart Failure Outpatients and Their Providers to Prevent Avoidable Readmissions

Sean P. Pinney, Jennifer Ullman, Margaret K. Doll, Genna LeDrew, John Donehey, Beth Oliver, Anu Lala

Background: Heart failure (HF) remains one of the leading causes of 30-day hospital readmissions. In this study, we examined the feasibility of incorporating RecoverLINK, a digital health technology which aims to reduce HF readmissions, into the standard of care for recently discharged HF patients at a large, urban teaching hospital. RecoverLINK supplements transitional care programs by providing outpatients with a 30-day app-based education and intervention program, and delivering analytics to alert providers when an early intervention is needed.

Methods: We enrolled a convenience sample of HF outpatients to use the RecoverLINK patient app at home for 30-days following a recent hospital discharge, and collected clinical and operational data from the RecoverLINK case manager system as it was used by participating transitional care providers. Feasibility was measured as a function of patient and provider engagement with the technology. We also examined the association between patient engagement with the RecoverLINK app (defined as high, ≥50% patient-use over 30 program-days, or low, <50% patient-use over 30 program-days) and ≤30-day hospital readmissions.

Results: Among 43 study participants, patients recorded their daily health status in the RecoverLINK app a mean of 59% of program-days (or 18 of 30 program-days), and 64% (95% CI: 49%, 77%) of patients were classified as having high app engagement. Providers addressed 91% (95% CI: 88%, 93%) of patient symptom alerts, and responded within a mean of of 4 (95% CI: 4, 5) business days. Among all participants, the rate of ≤30-day admissions was 16 (95% CI: 3, 28) readmissions per 100 persons. Stratified by engagement level, we observed a rate of 8 (95% CI: 0, 19) readmissions per 100 persons among high engagement patients versus 23 (95% CI: 0, 49) readmissions per 100 persons among low engagement patients. We did not detect any differences between high and low engagement participants on the basis of demographics, disease severity, or other characteristics.

Conclusion: Patients and providers frequently interacted with the RecoverLINK application, supporting the feasibility of its use as a supplement to HF transitional care programs. Further research is necessary to investigate the relationship between RecoverLINK engagement and HF ≤30-day readmissions.

Poster 26

Medicare Part D coverage of phosphate binders

Mariam Gawdat, Wendy Parker, Arthur Johnsen, Tyler Baumeister, Katie Cardone

Purpose: Hyperphosphatemia affects nearly all patients with end-stage kidney disease (ESRD), leading phosphate binders to be mainstays of therapy. Recent clinical practice guidelines for managing hyperphosphatemia recommend limiting exposure to calcium-based phosphate binders due to their association with increased mortality compared with calcium-free binders. Given that patients with ESRD are eligible for Medicare Part D prescription drug plan (PDP) coverage regardless of age, this study examined the formularies of PDPs in four states to identify coverage patterns in calcium and calcium-free phosphate binders.

Methods: Four states from different regions of the US were selected (New York, Texas, Florida, Montana). All stand-alone PDPs in these states were obtained. Premium, deductible, copay/coinsurance, star rating and tier levels for calcium-based and calcium-free binders were identified. Out-of-pocket patient costs were estimated for sevelamer carbonate and calcium acetate both before and after the coverage gap ("donut hole") for plans with sevelamer carbonate tiered below calcium acetate. Chi-square was used for all comparisons. The level of significance was set at 5%.

Results: Twenty, 24, 21, and 23 PDPs were available in NY, TX, FL and MT, respectively. Of the 4 states, only MT had 5-star plans. All PDPs had generic calcium acetate and sevelamer carbonate (brand or generic) on formulary. Ferric citrate was covered by most (87.5%) plans. At least one alternative dosage form (chewable tablet or liquid) phosphate binder was available on each plan. All PDPs covered either brand or generic sevelamer carbonate packets, 28% covered sucroferric oxyhydroxide chewable tablets, 12.5% covered lanthanum carbonate chewable tablets, and 33% covered calcium acetate solution. TX had a lower percentage of PDPs offering sevelamer carbonate at or below the calcium acetate tier at 67%, compared to NY (80%), FL (81%) and MT (78%) ($p<0.05$). Across all PDPs analyzed, four plans had sevelamer carbonate at a lower tier than calcium acetate. Despite being at a lower tier in these four PDPs, estimated out-of-pocket costs were higher for sevelamer carbonate than for calcium acetate.

Conclusions: Variability exists between plans in various states and among PDPs within the same state. Regardless of tier assignment, calcium acetate has the lowest out-of-pocket cost for patients, even in those plans with sevelamer carbonate set at a lower tier than calcium acetate. Despite current treatment recommendations, calcium acetate remains the lowest cost phosphate binder for patients with PDP coverage. Future research should investigate the impact this has on prescribing patterns, patient filling patterns, and adherence.

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