comfortable for use in human subjects. DISCUSSION/SIGNIFICANCE OF IMPACT: This novel technique allows for an inexpensive, noninvasive, and reproducible ocular blood flow imaging platform. By optimizing this technique, we can proceed with our future plan for a pilot study to compare our imaging technique with the current standard, paving the way for future clinical studies.

Optogenetic stimulation of corticotropin-releasing hormone expressing neurons in Barrington’s nucleus recapitulates the social stress voiding phenotype in mice

Jason Van Batavia1, Stephan Butler2, Joanna Fesi2, Rita Valentino3 and John D. Roache, Tara Wright, Donald Dougherty and Martin Javors

1 University of Pennsylvania School of Medicine; 2 Division of Urology, Children’s Hospital of Philadelphia; 3 National Institute on Drug Abuse, National Institutes of Health

OBJECTIVES/SPECIFIC AIMS: Voiding postponement is common cause of LUT dysfunction in which children void infrequently with large volumes. This condition is modeled in mice that are subjected to social stress who show decreased voiding frequency and increased voided volumes along with increases in corticotropin-releasing hormone (CRH) expression in Barrington’s nucleus (BN) (i.e., the pontine micturition center). Optogenetics is a technique to selectively stimulate cells or neurons of interest via light activated channel receptors [i.e., channel-2 rhodopsin (ChR2)]. Here we examined the effects of optogenetic manipulation of CRH BN neurons on the in vivo voiding phenotype and urodynamics in awake mice. We hypothesized that stimulating these neurons at higher frequencies (10–50 Hz) would lead to CRH-dependent alterations in voiding phenotype (i.e., larger voided volumes and longer intermicturition intervals. METHODS/STUDY POPULATION: Double transgenic mice expressing ChR2 in CRH cells were generated using the Cre-lox recombinase system and had fiberoptic probes implanted into BN at 8 weeks of age. The mice also underwent simultaneous catheter placement into the bladder for in vivo cystometry. In vivo cystometry before and during optogenetic stimulation at various frequencies was performed 5–7 days postoperatively. Saline was perfused at 10 µL/min and baseline stable voiding cycles were established. Bladder capacity, threshold pressure, voiding pressure, and voided volume were recorded at baseline and at each optogenetic setting. In some mice, the protocol was repeated in the presence of CRH antagonist (NBI 30775). RESULTS/ANTICIPATED RESULTS: Fiberoptic stimulation (470 nm at 25 and 50 Hz) produced a significant rise in the intermicturition interval, bladder capacities and increased voided volumes. This effect was especially pronounced in females in whom bladder capacity and intermicturition interval more than doubled at 50 Hz stimulation. Fluoroscopic images confirmed complete bladder emptying with each void. The increased bladder capacity at high frequencies (25 and 50 Hz) was CRH-dependent as injection of a CRH antagonist (NBI 30775) blocked the optogenetic effect. Control non-mice double showed no effects from optogenetic stimulation. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results suggest that optogenetic stimulation of CRH-expressing neurons in BN at high frequency (25 and 50 Hz) inhibits micturition and recapitulates the voiding phenotype seen in socially stressed mice (large, infrequent voids). Lower frequencies of optogenetic stimulation (2 and 10 Hz) had no effects on cystometry parameters or voiding phenotype. In addition, females had a greater response to optogenetic stimulation compared with males with larger bladder capacities and longer intermicturition intervals. The changes in voiding phenotype seen were CRH dependent as blockage of the CRH receptor prevented changes in cystometry parameters with optogenetic stimulation. Further elucidation of these and other neural subpopulations in BN are warranted to understand micturition and how it may be manipulated in disease states such as voiding postponement and acute urinary retention.

Personalized models of distal airway epithelial-stromal unit in COPD

Seyed B. Mahjour, Kazunori Gomi1, Busub Lee1, Olivier Elemento2, Scott Randell3 and Renat Shaykhiev1

1 Department of Medicine, Weill Cornell Medical College, New York, NY, USA; 2 Department of Physiology and Biophysics, Weill Cornell Medical College, New York, NY, USA; 3 Marisco Lung Institute/Cystic Fibrosis Center, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

OBJECTIVES/SPECIFIC AIMS: The objective of this study is to develop patient-derived “personalized” organotypic models of human distal airways, in which basal stem cells (BCs) isolated from the pre-terminal conducting airway region are co-cultured with autologous stromal cells from the same region to reproduce patient-specific distal airway epithelial-stromal units and their remodeling in COPD.

METHODS/STUDY POPULATION: We established a protocol to isolate and propagate epithelial BCs, fibroblasts, and endothelial cells from the distal airways of normal and COPD lung donors. Heterogeneous cellular and molecular phenotypes in the human distal airways were characterized using immunofluorescence and single-cell RNA sequencing. Patient-specific distal airway epithelial-stromal units were reconstructed by co-culturing BCs and autologous stromal cells using an air-liquid interface-based airway wall model and a bronchus-mimicking 3D distal airway organoid assay. RESULTS/ANTICIPATED RESULTS: Histologic analysis of derived epithelial-stromal units revealed heterogeneous patient-specific phenotypes characterized by hypo-hyper-metaplastic lesions (hypo-regenerative phenotype, mucous cell hyperplasia, squamous metaplasia, distal-to-proximal repatterning) in the epithelial compartment, accompanied, in some samples, by stromal remodeling. Candidate epithelial-stromal cross-talk mechanisms were identified using quantitative real-time RT-PCR analysis of autologous epithelial and stromal compartments of established patient-specific distal airway unit models. DISCUSSION/SIGNIFICANCE OF IMPACT: Epithelial and stromal cells isolated from distal airways of subjects with and without COPD can be assembled into functional, organ-level tissue which mimics the architecture of human distal airways and, in patients with COPD, reproduces several distal airway remodeling phenotypes. Patient-specific models of distal airway epithelial-stromal cross-talk established in this study can be used to identify candidate pathways that mediate disease-relevant airway remodeling and potentially utilized as pre-clinical platforms for developing personalized therapeutic approaches to suppress the progression of distal airway remodeling in chronic lung diseases, including COPD.

Pharmacokinetics of phosphatidylethanol 16:0/20:4 homolog in human blood after consumption of 0.4 and 0.8 g/kg alcohol in a laboratory clinical study

Marisa Lopez-Cruzan, Nathalie S. Hill-Kapturczak, Jesu S. Sanchez, John D. Roache, Tara Wright, Donald Dougherty and Martin Javors

University of Texas Health Science Center San Antonio

OBJECTIVES/SPECIFIC AIMS: The purpose of this study was to characterize the pharmacokinetics of phosphatidylethanol (PETH) 16:0/20:4 homolog in uncoagulated, human blood samples taken from 18 participants in a clinical laboratory setting after consumption of 2 doses of ethanol. METHODS/STUDY POPULATION: Male and female participants received either 0.4 or 0.8 g/kg oral doses of ethanol during a 15-minute period. Blood samples were collected before and throughout 6 hours immediately after alcohol administration, then after 2, 4, 7, 11, and 14 days of administration day. PETH 16:0/20:4 levels were quantified by liquid mass spectrometry. Breath ethanol concentrations were measured concurrently with each blood collection during the administration day, as well as transdermal ethanol concentrations monitored constantly before, during and after ethanol administration day. RESULTS/ANTICIPATED RESULTS: (1) Single doses of 0.4 and 0.8 g/kg oral produced proportional increases in BrAC and PETH 16:0/20:4 levels; (2) the increase of PETH 16:0/20:4 from base line to Cmax was less than either PETH 16:0/18:1 or PETH 16:0/18:2 during the 6-hour period after alcohol administration; (3) the mean rate of formation of PETH 16:0/20:4 was lower than those of the other 2 homologs; (4) the mean half-life of PETH 16:0/20:4 was 2.18 days, which was shorter than that of either PETH 16:0/18:1 and PETH 16:0/18:2, which were 6.80 and 6.62, respectively. DISCUSSION/SIGNIFICANCE OF IMPACT: The results of this study further confirm that PETH homologs are a sensitive biomarker for ethanol consumption. The measurement of three PETH homologs appears to provide additional information about the level and time frame of drinking.

Predictive cytological topography (PiCT): A radiopathomics approach to mapping prostate cancer

Sean D. McGarry, Sarah L. Hurrell, Kenneth Ickzkowski, Anjshnu Banerjee, Kenneth Jacobsohn, William Hall, Mark Hohenwalter, Peter LaViolette, Amy Kaczmarowski1, Tucker Keuter1, Marja Nevalainen1 and William See1

1 Medical College of Wisconsin

OBJECTIVES/SPECIFIC AIMS: The objective of this study is to use machine learning techniques to generate maps of epithelium and lumen density in MRI

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space. METHODS/STUDY POPULATION: Methods: We prospectively recruited 39 patients undergoing prostatectomy for this institutional review board (IRB) approved study. Patients underwent MP-MRI before prostatectomy on a 3T field strength MRI scanner (General Electric, Waukesha, WI, USA) using an endorectal coil. MP-MRI included field-of-view optimized and constrained undistorted single shot (FOCUS) diffusion weighted imaging with 10 b-values (b = 0, 10, 25, 50, 100, 200, 500, 1000, and 2000), dynamic contrast enhanced imaging, and T2-weighted imaging. T2 weighted images were intensity normalized and apparent diffusion coefficient maps were calculated. The dynamic contrast enhanced data was used to calculate the percent change in signal intensity before and after contrast injection. All images were aligned to the T2 weighted image.

Robotic prostatectomy was performed 2 weeks after image acquisition. Prostate samples were sliced using a 3D printed slicing jig matching the slice profile of the T2 weighted image. Whole mount samples at 10 μm thickness were taken, hematoxylin and eosin stained, digitized, and annotated by a board certified pathologist. A total of 210 slides were included in this study. Lumen and epithelium were automatically segmented using a custom algorithm written in MATLAB. The algorithm was validated by comparing manual to automatic segmentation on 18 samples. Slides were aligned with the T2 weighted image using a nonlinear control point warping technique. Lumen and epithelium density and the expert annotation were subsequently transferred into MRI space. Co-registration was validated by applying a known warp to tumor masks noted by the pathologist and control point warping the whole mount slide to match the transform. Overlap was measured using a DICE coefficient. A learning curve was generated to determine the optimal number of patients to train the algorithm on. A PLS algorithm was trained on 150 random permutations of patients incrementing from 1 to 29 patients. Slides were stratified such that all slides from a single patient were in the same cohort. Three cohorts were generated, with tumor burden balanced across all cohort. A PLS algorithm was trained on 2 independent training sets (cohorts 1 and 2) and applied to cohort 3. The input vector consisted of MRI values and the target variable was lumen and epithelium density. The algorithm was trained lesion-wise. Trained PCT methods were applied to test cohort 3 lesion-wise to generate 2 new image contrasts. Mean lesion values were compared between high grade, low grade, and healthy tissue using an ANOVA. An ROC analysis was performed lesion-wise on the test set. RESULTS/ANTICIPATED RESULTS: Results: The segmentation accuracy validation revealed R = 0.99 and R = 0.72 (p < 0.001) for lumen and epithelium, respectively. The co-registration accuracy revealed a 94.5% overlap. However, analysis of their data showed that the majority of DEGs we identified either do not contain DIs or DI splicing was not affected by targeting PRMT5.

We aimed to develop an assay to measure new protein synthesis after Antisense Oligonucleotide treatment, which we hypothesize will be the earliest biophysical and biological hallmark of RNA-targeting therapy efficacy. METHODS/STUDY POPULATION: We treated 2 transgenic animal models expressing proteins implicated in neurodegenerative disease: human tau protein (hTau) and human superoxide dismutase 1 (hSOD1), with ASO against these mRNA transcripts. Animals received isotope-labeled 13C6-Leucine via drinking water to label newly synthesized proteins. We assayed target protein synthesis and concentration after ASO treatment to determine the earliest identification of ASO target engagement. RESULTS/ANTICIPATED RESULTS: hTau ASO treatment in transgenic mice lowered hTau protein concentration 23 days post-treatment in cortex (95% CI: 0.05%–64.0% reduction). In the same tissue, we observed lowering of hTau protein synthesis as early as 13 days (95% CI: 29.4%–123%). In hSOD1 transgenic rats, we observed lowering of 13C6-leucine-labeled hSOD1 in the cerebrospinal fluid 30 days after ASO treatment compared with inactive ASO control (95% CI: 12.0%–48.4%). DISCUSSION/SIGNIFICANCE OF IMPACT: In progressive neurodegenerative diseases, it is crucial to develop measurements that identify treatment efficacy early to improve patient outcomes. These data support the use of stable isotope labeling of amino acids to measure new protein synthesis as an early pharmacodynamics measurement for therapies that target RNA and inhibit the translation of proteins.

**OBJECTIVES/SPECIFIC AIMS:** Our objective is to determine if decreased adiponectin and increased leptin levels are associated with prostate cancer.