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remodeling is critical. Sonic hedgehog (SHH) treatment by peptide amphiphile (PA) nanofiber hydrogel, suppresses smooth muscle apoptosis and improves erectile function in a rat CN injury ED model. We examine if human corpora cavernosal smooth muscle responds in a similar manner to SHH treatment.

**Objective:** In this study we: 1.) Examine if human corpora cavernosal smooth muscle cells from primary culture of ED patient tissues, respond to Sonic hedgehog (SHH) treatment by increasing growth rate, as in our in vivo animal model. 2.) Determine if human corpora cavernosal cells from patients with differing underlying mechanisms of ED development (prostatectomy, diabetes, hypertension, cardiovascular disease), respond similarly to SHH treatment. 3.) Identify if organ culture conditions (glucose) effect growth of corpora cavernosa smooth muscle and the response to SHH signaling. 4.) Examine the vascular component of ED (hypertension and cardiovascular disease).

**Methods:** Human corpora cavernosal tissue was obtained from prostatectomy, diabetic, hypertension, cardiovascular disease and Peyronie’s (control) patients (n=40) that were under going prosthesis implant to treat ED. Primary cultures (n=17) were established, and corpora cavernosal cells from prostatectomy, diabetes, hypertension, cardiovascular disease patients in a similar manner (43-53%). SHH inhibition decreased smooth muscle growth (24-32%). There was no difference in growth using 25ug and 10ug SHH peptide. A more active (150X) SHH peptide further enhanced growth (20%). SHH protein increased growth more in diabetic smooth muscle under high glucose conditions. SHH inhibition was less effective under high glucose conditions. SHH treatment increased growth less in cells from hypertension and cardiovascular disease patients.

**Conclusions:** Corpora cavernosa smooth muscle from prostatectomy, diabetic, and Peyroinie’s patients increased in response to SHH treatment in a dose-dependent manner. Plasma levels of several signaling molecules known to influence vaginal lubrication, including the steroid hormones estradiol, progesterone, and testosterone, as well as the gaseous vasodilator nitric oxide, increase at specific time points following IV meth in female rats. Accordingly, we were able to decrease the amount of vaginal fluid produced by pre-treating the animals with the nitric oxide synthase inhibitor, L-NAME, prior to meth.

**Objective:** The present work expands the aforementioned findings by measuring plasma levels of vasoactive intestinal polypeptide (VIP), an established mediator of female sexual arousal, and corticosterone, a steroid hormone implicated in drug addiction, after IV injections of meth. We also determined the involvement of estradiol in meth-induced vaginal lubrication using an estrogen receptor antagonist.

**Methods:** We implanted adult female Wistar rats with chronic indwelling jugular catheters and allowed them at least one week to recover from surgery. We pre-treated the rats with fulvestrant, an estrogen receptor antagonist, and then anesthetized them with isoflurane gas before injecting them intravenously with meth via the implanted catheter. A pre-weighed cotton-tipped swab, inserted into the vaginal canal, collected fluid secreted following the administration of meth. The change in the weight of the swab before and after meth indicated the amount of fluid produced. In a separate group of rats, we used the jugular catheter to remove blood at various time points (1-60 min) following the meth injections to measure plasma levels of VIP and corticosterone.

**Results:** Although we have not yet completed these experiments, we hypothesize that the inhibition of estrogen receptors will reduce meth-induced vaginal lubrication similar to the reduction produced by L-NAME. Further, we predict that both VIP and corticosterone levels will increase between 5 and 20 min after the injections of meth, similarly to the other signaling molecules we measured previously.

**Conclusions:** These findings have far-reaching and potentially life-changing implications, as the majority of women will experience vaginal dryness a least once in their lifetimes, if not chronically. The underlying mechanisms of meth-induced vaginal lubrication may provide the necessary pharmacological target to treat vaginal dryness.

**Disclosure:** Any of the authors act as a consultant, employee or shareholder of an industry for: Goeders is on the Scientific Advisory Boards for Embera NeuroTherapeutics and JanOne; however, the work of these companies is unrelated to the submitted abstract.
Methods: We identified and examined six COVID-19 patients who all were confirmed with polymerase chain reaction (PCR), including three COVID-19 (+) men without orchitis (controls) and three COVID (+) men with orchitis (bilateral testicular pain for at least 5 days around the time of testing PCR positive). Of note, among the three men with COVID-19 who had orchitis, two of them were siblings. DNA extraction and whole exome sequencing were performed on blood using the QIAmp blood maxi kit on five of the six patients. Variants were prioritized by being shared between the two siblings with orchitis, absent in controls, and introducing nonsense, frameshift, splicing or non–synonymous amino acid changes and less than 10% in population prevalence. Based on WES findings, DuoSeq® Human ACE2 reagent kit 2 (catalog number: DY933-05) was purchased from R&D Systems, USA, and used to measure the level of soluble ACE2 in the plasma samples.

Results: The average age of the men in the study was 25 years old. The average duration of COVID symptoms (fever, sore throat, cough, body aches) were 7 days. Among the men who developed bilateral testis pain, the symptoms lasted for an average of 22 days. A list of 16 variants was generated that found to be shared between the two siblings with COVID orchitis along with the unrelated subject with COVID orchitis, and not present in the two controls. Among the 16 variants, a nonsynonymous non-frameshift deletion in NACAD variant on chromosome 7 with a frequency of 3.9% prevalence in ExAC was prioritized based on known involvement in the ACE2 pathway, read depth, and genotype quality. Phenotypically, we found that circulating levels of soluble ACE2 was 3.72 ng/ml among men who had COVID orchitis and was lower than men who developed COVID without orchitis. Conclusions: We observed a stop mutation in NACAD in 2 brothers and 1 unrelated man who developed COVID orchitis. Interestingly, we found lower circulating ACE2 serum levels in both brothers with orchitis and the one nonrelated orchitis subject but normal serum levels in all controls. NACAD when involved with cellular ability to shut out ACE2 becomes critical for COVID symptomatology. With decreased transcellular and extracellular transport of ACE2 being possible in subjects with the gene mutation, it can be postulated more ACE2 will be found intracellularly leading to increased cellular entry of SARS CoV-2 and possibility of orchitis sequelae. Disclosure: No

57 PATHWAY ENRICHMENT ANALYSIS OF MICROARRAY DATA FROM HUMAN PENIS OF DIABETIC AND PEYRONIE’S PATIENTS, IN COMPARISON WITH DIABETIC RAT ERECTILE DYSFUNCTION MODELS

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Introduction: Erectile dysfunction (ED) is a debilitating medical condition in which current treatments are minimally effective in diabetic patients due to neuropathy of the cavernous nerve, a peripheral nerve that innervates the penis. Loss of innervation causes apoptosis of penile smooth muscle, remodeling of corpora cavernosa (penile erectile tissue) morphology, and ED. Objective: In this study, microarray and pathway analysis were used to obtain a global understanding of how signaling mechanisms are altered in diabetic patients and animal models as ED develops, in order to identify novel targets for disease management, and points of intervention for clinical therapy development.

Methods: Human corpora cavernosal tissue was obtained from diabetic (n=4) and Peyronie’s (control, n=3) patients that were undergoing prosthesis implant to treat ED. Rat tissue was obtained from BB/WOR diabetic (n=5) and diabetes resistant (n=5) rats. RNA was extracted using TRIzol, DNase treated, and purified by Qiagen mini kit. Microarray was performed using the Human Gene 2.0 ST Array. 1) Alterations in patient and diabetic rat pathway signaling were examined using several analytical tools (ShinyGO, Metascape, WebGestalt, STRING) and databases, 2) Strengths/weaknesses of the different pathway analysis tools were compared, and 3) Comparison of human and rat (BB/WOR and Streptozotocin) pathway analysis was performed. Two technical replicates were performed. P value (FDR) < 0.15 was used as threshold for differential expression. FDR<0.05 was considered significant.

Results: Microarray identified 182 differentially expressed protein-coding genes. Pathway analysis revealed similar enrichments with different analytical tools. Down regulated pathways include development, tubular structure, sprouting, cell death, ischemia, angiogenesis, transcription, second messengers, and stem cell differentiation. ED patients, who have diabetes, incur significant loss of normal regulatory processes required for repair and replacement of injured corpora cavernosal tissue. Combined with loss of apoptotic regulatory mechanisms, this results in significant architectural remodeling of the corpora cavernosa, and loss of regenerative capacity in the penis.

Conclusions: Penis from diabetic ED patients lacks capacity for maintenance of corpora cavernosal architecture and regeneration, which are critical points for intervention for therapy development. The analysis of tissue specific gene expression profiles provides a means of understanding drivers of disease and identifying novel pathways for clinical intervention. This first report of microarray and pathway analysis in human corpora cavernosa, is critical for identification of novel pathways pertinent to ED and for validating animal models. Disclosure: No