exposures to both sunitinib and to increases in afterload will augment sunitinib toxicity in CMTs via the primary outcomes mentioned in Aim 1. Additionally, we hypothesize that decreases in afterload will decrease effective sunitinib toxicity in CMTs via the primary outcomes mentioned in Aim 1. Aim 3: We hypothesize that exposure to an AMP-kinase activator but not carvedilol will decrease the effects of sunitinib toxicity in CMTs via the primary outcomes mentioned in Aim 1. DISCUSSION/SIGNIFICANCE OF IMPACT: The use of small molecule, targeted chemotherapeutic agents is increasingly common. Many of these agents cause cardiotoxic side effects, the mechanisms of which are incompletely understood. Our lab has developed a novel 3D tissue engineering platform capable of supporting durable in vitro cardiac microtissues that experience dynamic alterations in their biomechanical load. By using this platform to examine the cardiotoxic effects of sunitinib, insight into treatment and prevention of this common problem will be developed.

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The effect of common genetic variants in the oxytocin receptor gene on oxytocin response.

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OBJECTIVES/SPECIFIC AIMS: Previous studies suggest that genetic variants in the oxytocin receptor (OXTR) may alter oxytocin dose requirement for labor induction and may increase risk for preterm labor and neurodevelopmental disorders. However, the mechanisms of actions of these variants remain unknown. The goal of this study was to functionally characterize common missense and noncoding variants in OXTR. First, we aimed to determine the effects of missense variants on two major aspects of receptor function: calcium signaling and β-arrestin recruitment. Second, we used allelic expression imbalance assays in an effort to identify regulatory single nucleotide polymorphisms (SNPs) in noncoding regions of OXTR that alter OXTR mRNA expression. METHODS/STUDY POPULATION: We used the Exome Aggregation Consortium database to identify the 12 most prevalent missense single nucleotide variants in OXTR. To determine the functional effects of these variants, we transfected human embryonic kidney cells (a common model system used to study receptor function) with wild type OXTR, variant OXTR, or empty vector control. We used the calcium-sensitive dye Fluo4 to quantify intracellular calcium flux in response to oxytocin treatment, and used bioluminescence resonance energy transfer assays to measure recruitment of the signaling partner β-arrestin to the receptor. To investigate potential effects of noncoding SNPs on OXTR mRNA expression, we quantified allele-specific expression of OXTR in human uterine tissue obtained from participants at the time of Cesarean section. We used next-generation sequencing (Illumina MiSeq) to count alleles of a reporter SNP in OXTR exon 3. RESULTS/ANTICIPATED RESULTS: Of the 12 most prevalent missense single nucleotide variants, four were predicted to be deleterious by PolyPhen variant annotation software. We anticipate that these variants will alter receptor signaling through calcium or β-arrestin pathways. We further observed that a reporter SNP in OXTR exon 3 exhibits significant allelic expression imbalance in a subset of our myometrial tissue samples, indicating that OXTR expression may be regulated by a functional SNP. Our current work focuses on discovering the functional SNPs in OXTR responsible for the pattern of allelic expression imbalance seen in mRNA. In the future, we will seek to explore the effects of these variants on uterine function by using genome editing of uterine smooth muscle cells. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results suggest that both missense and noncoding variants may affect OXTR expression and function. Future studies may suggest that OXTR sequencing, genotyping, or expression analysis would be useful to identify individuals likely to respond or fail to respond to safe doses of oxytocin for labor induction. Personalizing approaches for labor induction in this way would increase the safety of oxytocin and potentially reduce maternal morbidity and mortality.

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The Effects of Aging on the Rectal Mucosal CD4+ T cell Compartment and its Implications for HIV Transmission

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OBJECTIVES/SPECIFIC AIMS: In the first aim, we will evaluate the proportion of highly HIV-susceptible memory CD4+ T cells present in the rectal mucosa, based on the proliferation status and expression of the HIV susceptibility markers, CCR5 and α4β7, between HIV-negative adolescent MSM and adult MSM engaging in RAI. The second aim will assess differences between the two study groups in the ratio of Th17 cells (CD4+ IL17+) to Treg cells (CD4+ FoxP3+ CD25+) in the rectal mucosa as a determinant of mucosal inflammation. Finally, in the third aim, we will utilize ex vivo rectal biopsy explant challenge experiments to examine whether HIV target cell availability and the Th17/Treg ratio influence rectal mucosal HIV susceptibility. METHODS/STUDY POPULATION: Rectal biopsy specimens are being collected from healthy, HIV-negative men that comprise the two study groups: 40 adolescent MSM 18-21 years of age who have engaged in RAI at least once previously in their lifetime and 40 adult MSM ≥35 years of age who have engaged in RAI for the previous 5 consecutive years with a minimum of 12 episodes annually. To identify CD4+ subsets of interest for aims 1 and 2, rectal mucosal mononuclear cells are isolated and phenotyped with CD45, CD3, CD4, CD45RA, CCR7, CD69, CCR5, α4β7, Ki67, FOXP3, and CD25 antibodies. To identify the Th17 cell subtype, the cells are stimulated with PMA/Ionamycin and stained with an antibody specific to IL-17A. Using cross-sectional analyses, we will compare the frequencies of mucosal CD4+ T cells that express certain phenotypic characteristics and evaluate differences in the Th17/Treg ratio between adolescent and adult MSM. For aim 3, rectal biopsy specimens are inoculated with HIV virus and the culture supernatant is assayed for p24 concentration on days 3, 7, 10 14, and 18. Longitudinal analyses will be performed to detect differences in p24 concentration at each time point and assess associations with mucosal target cell availability and with the Th17/Treg ratio. RESULTS/ANTICIPATED RESULTS: We hypothesize that younger age will be associated with enhanced memory CD4+ T cell proliferation and increased expression of HIV susceptibility markers (CCR5 and/or α4β7). In addition, we expect that the rectal mucosa of adolescent MSM will demonstrate a higher Th17/Treg ratio as compared to adult MSM, which could facilitate HIV transmission. It is also anticipated that rectal mucosal immune phenotypes characterized by increased HIV target cell availability and high Th17/Treg ratios

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