Epithelial estrogen receptor-alpha is involved in the development of lower urinary tract dysfunction

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Abstract

The objective of this study was to determine if epithelial ER-α was necessary for the development of LUTD. To determine the specific role of estrogen receptor-alpha (ER-α) in prostatic epithelial tissue we used epithelial specific cre: (B6.Cg-Shh tm1(EGFP/cre)Cit/J) mice crossed to ER-α floxed mice, which deleted prostatic epithelial ER-α. These mice were used in a novel two-hit model exposing *in utero* mice to TCDD and administering hormone in adulthood. To assess voiding behavior, we utilized void spot assays (VSA) each week for four weeks following hormone treatment. Mice were necropsied after the 4th VSA to assess the bladder and prostate. We found the knock of ER-α in the epithelium reduced aberrant voiding patterns in comparison to the control mice.

Background

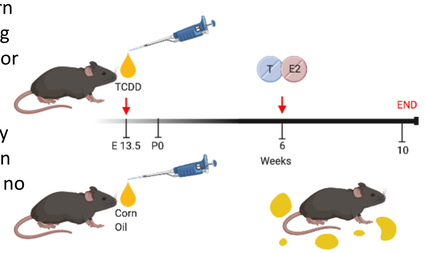
Benign prostatic hyperplasia associated with lower urinary track symptoms (BPH-LUTS) has highly irritative symptoms (urinary frequency, urge incontinence, nocturia, painful urination) and obstructive symptoms (hesitancy, straining weak flow, prolonged voiding and urinary retention). The risk of developing BPH-LUTS increases with age, estimating 90% of men over the age of 80 years to exhibit symptoms. The prevalence of this disease in aging men may be due to a shift in hormone levels in which the estrogen to testosterone ratio increases. We recently determined that *in utero* and lactational exposure to the persistent environmental toxicant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), sensitizes voiding dysfunction in the testosterone (T) and 17β-estradiol (E2) model of LUTD in wildtype mice.[1, 2] Our previous studies found selective estrogen receptor modulators (SERMs) prevented the development of LUTD. It was also demonstrated that estrogen receptor-alpha (ER-α) was necessary for the development of LUTD in C57Bl/6 male mice.[3] ER-α is present in both the stroma and the epithelium of the prostate and BPH-LUTS can exhibit increased cell proliferation in the stroma-epithelium.

Objective and Hypothesis

The objective of this study was to determine if epithelial ER-α was necessary for the development of LUTD using a two-hit model. We hypothesized that a tissue specific knock out of functional ER-α in the epithelium would hinder the development of LUTD.

Materials and Methods

Mouse treatment
Epithelial specific cre: (B6.Cg-Shh tm1(EGFP/cre)Cit/J) mice crossed to ER-α floxed mice were administered a single dose of TCDD or the vehicle, corn oil, at E13.5. At six weeks in adulthood 25mg testosterone (T) and 2.5mg 17β-estradiol (E2) slow-release pellets were subcutaneously implanted or untreated control underwent sham surgery.
Void Spot Assay (VSA)
VSA was conducted same week as hormone implant but prior to surgery and each week following treatment for four weeks. Mice were housed in clean cages lined with 3mm Whatman filter paper for 4h with food and no water. Filter paper was imaged using a Bio-Rad Chemidoc imager and analyzed using Void Whizzard Software.
Lower urinary tract measurements
At 10 weeks a necropsy was performed to collect the lower urinary tract and to measure bladder weight and volume, and the prostate lobes weights.



Results

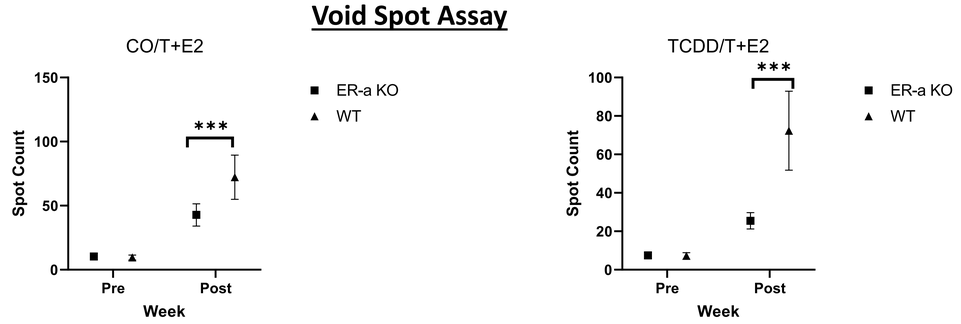


Figure 1. Void spot count prior to hormone implant (Pre) and 4 weeks post treatment (Post). Black squares are tissue specific knockout (Shh cre) and triangles are cre non-expressing controls. student's *t-test* ($p<0.05$) n=12

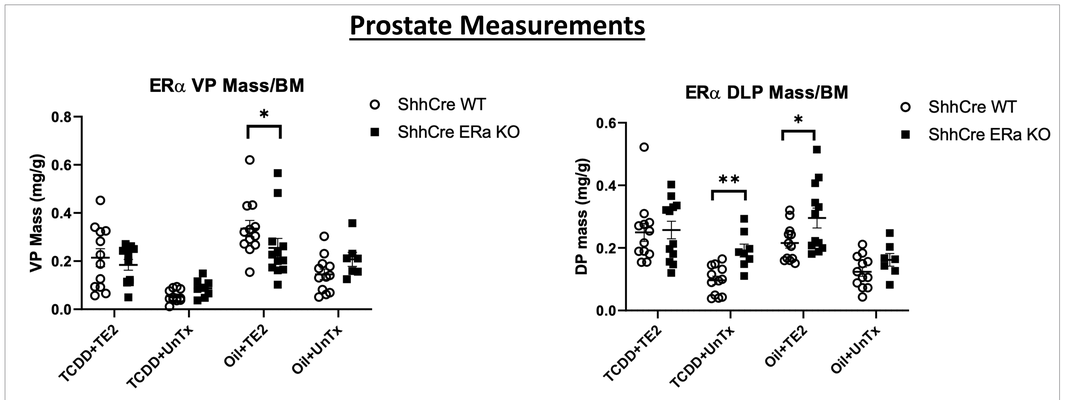


Figure 2. Prostate lobe measurements of the ventral (VP) and dorsolateral (DLP) prostate lobes. Black squares are tissue specific knockout (Shh cre) and open circles are cre non-expressing controls. student's *t-test* ($p<0.05$) n=12

Results (cont.)

- There were no significant differences for the other treatment conditions such as the hormone alone treated group and the untreated group for the Shh cre in comparison to the cre non-expressing control
- The ventral prostate mass increased significantly in the hormone only treated cre non-expressing control in comparison to the Shh cre.
- No differences were observed in the bladder weight or volume for each condition comparing the cre expressing knockout vs the cre non-expressing control.

Discussion

- While our study did not show some of the expected results the VSA phenotype implies the involvement of ER-α in LUTD.
- To ascertain the role of ER-α in other cell types attributing to LUTD, concurrent work uses stromal specific smooth muscle-cre (B6.Cg-Tg(Tgln-cre)1Her/J) mice.
- We will also assess cell proliferation in the harvested urethra and prostate lobes by using KI-67.
- We will also investigate the potential of selective estrogen receptor alpha modulators (SERαMs) to protect mice from the development of LUTD in this two-hit model.

References

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3. Nicholson TM *et.al.* Estrogen receptor-α is a key mediator and therapeutic target for bladder complications of benign prostatic hyperplasia. J Urol. 2015 Feb;193(2):722-9.

Acknowledgements

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