IMPACT OF DIABETES MELLITUS ON BLADDER UROEPITHELIAL CELLS.

Hypothesis / aims of study
Lower urinary tract (LUT) pathologies are highly prevalent complications of Diabetes Mellitus (DM), the most common of which is diabetic cystopathy / diabetic bladder dysfunction (DBD). DBD is characterized by large bladder capacity, diminished bladder sensation, poor contractility and elevated post-void residual volume. Incomplete emptying can lead to chronic urinary retention, recurrent urinary tract infections and deterioration of the upper urinary tract. Treatment modalities for diabetic cystopathy are limited (1).

In addition to functioning as an important barrier between the urine and underlying bladder tissue, the epithelial lining of the urinary bladder (the urothelium; UT) is a dynamic sensory tissue expressing a wide array of receptor types and releasing neuroactive molecules such as adenosine triphosphate (ATP), nitric oxide (NO) and acetylcholine (ACh)(2,3). As such, the urothelium is likely to play an important role in bladder function by actively communicating with bladder nerves, smooth muscle and cells of the immune and inflammatory systems and may contribute to bladder pathophysiology such as DBD. Changes in bladder smooth muscle (detrusor) and innervation have been reported in DM patients. However, the impact of this debilitating disease on urothelial cell physiology is largely unknown.

This study was undertaken to investigate temporal (3-20 weeks; 3-20wk) changes in UT-cell morphology and gene expression in the urinary bladders of rats treated with streptozotocin (STZ) to induce diabetes compared with bladders isolated from age-matched untreated control rats.

Study design, materials and methods
Diabetes mellitus was induced in adult (3 month old) female Sprague Dawley rats by intraperitoneal injection of streptozotocin (STZ; 35mg/kg dissolved in 0.1M citrate buffer, pH 4.5). Blood samples were taken 72 h after streptozotocin injection to confirm diabetes (blood glucose level>350 mg/dL). Blood sampling were continued at regular intervals inclusive of immediately prior to sacrifice to confirm continued state of hyperglycemia. Animals were sacrificed at 3, 9 and 20 weeks following STZ-treatment, along with time-matched control/untreated rats. Bladders were excised and divided in sub-groups (n=3-5 per group) to be processed for molecular studies (reverse-transcription–quantitative polymerase chain reaction; RT-QPCR), and morphological studies using transmission and scanning electron microscopy. In addition, proliferative activity was accessed in vitro in primary cultures of test and control UT cells.

Proliferative activity: Nuclear incorporation of 5-bromo-2-deoxyuridine (BrdU) was quantitatively accessed in primary cultures of test (STZ-DM) and control UT cells.

RT-PCR: Quantitative PCR (QPCR) amplification (normalized to the reference gene hypoxanthine-guanine phosphoribosyltransferase/ HPRT) was used to compare temporal urothelial expression of genes related to structure, function and metabolic pathways in STZ-DM UT and Normal UT (Livak Method, ΔΔCt) and expressed as fold difference DM (3-20 wk) with respect to (wrt) normal UT.

Results
Initial findings from scanning electron microscopy indicate dramatic morphological changes in surface UT cells in STZ-diabetic bladders indicating breaches in barrier function, most pronounced at the 9-week stage. In addition, poor proliferative activity was noted in cultured in STZ-UT compared with control UT. Molecular data (STZ vs Control bladder urothelium; 3, 9, and 20wk) indicate distinct temporal variation in expression profiles for genes related to: (a) Cell structure: zona occludens -1 (ZO-1); uroplakin3alpha (UP3a); (b) Cell function/signaling: muscarinic acetylcholine receptors; mammalian target of rapamycin (mTOR) - a protein kinase that regulates cell growth, cell proliferation and cell survival. (c) Cellular metabolism: aldose reductase (AR); sorbitol dehydrogenase (SDH).

Interpretation of results
Pathological changes in urothelial cell physiology with diabetes mellitus could result in alteration in barrier as well as sensory and signalling function. This in turn could impact on activity in underlying excitable tissue: interstitial cells of Cajal; detrusor smooth muscle and nerve endings.

Concluding message
Taken together, plasticity in urinary bladder urothelium in diabetes mellitus may contribute to the pathogenesis of diabetes-induced bladder dysfunction.

References

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What were the subjects in the study? ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained? Yes
Name of ethics committee Case Western Reserve University, Cleveland, OH, USA
Institutional Animal Care and Use Committee (IACUC)and...