254

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RADIATION CYSTITIS-INDUCED FIBROSIS ALTERS BLADDER WALL COMPLIANCE AND STRETCH-INDUCED ATP RELEASE – POTENTIAL IMPLICATIONS FOR UNDERACTIVE BLADDER

Hypothesis / aims of study

Urinary bladder fibrosis is a consequence of various chronic inflammatory conditions (*e.g.* ketamine, radiation and interstitial cystitis) as well as aging. Increased fibrosis is an underappreciated factor in the aetiology of underactive bladder (UAB) as it will decrease contractile performance and voiding efficiency, as well as decrease bladder compliance and bladder capacity. Concurrently, the increased passive bladder wall tension during filling, due to reduced compliance, may potentiate stretch-evoked ATP release which can increase sensory nerve outflow. The combination of these effects could explain why patients with UAB also experience symptoms associated with bladder overactivity (OAB). Our aim was to utilise a mouse model of chronic radiation cystitis as a surrogate for fibrosis-induced UAB and determine its effects on bladder wall compliance and stretch-evoked ATP release.

Study design, materials and methods

Selective bladder irradiation: Adult female C57Bl/6 mice (six weeks of age) were used for this study and subjected to selective bladder irradiation. Briefly, mice were anesthetised with avertin (2,2,2-tribromoethanol, 300 mg/kg, IP) and a small incision was cut into their lower abdominal wall to expose the bladder. A suture was tied to the urachus after which the mice were placed sideways on a Lexan platform, allowing the organ to be held outside the abdominal cavity by the suture during irradiation. The mice on the platform were placed in an X-RAD 320 biological irradiator (Precision X-Ray) and the collimator and table height adjusted to focus the irradiation beam on the exposed organ. An irradiation dose of 10 Gray (1 Gy = 100 rads) was delivered, the organ returned to the abdominal cavity and the incision was sutured closed. Mice were used for experiments 12 weeks following irradiation. Age-matched non-irradiated animals were used as controls.

Isolated bladder sheet tension recordings and ATP measurements: Mice were humanely sacrificed and the bladders isolated. Bladders were cut along the ventral midline to form sheets approximately 8 mm long and 3 mm wide. Preparations were mounted in a temperature controlled recording chamber and connected to a tension transducer in line with a computer controlled stepper motor to implement stretch protocols. Bladders were superfused with a modified Tyrode's solution gassed with O_2/CO_2 (95%/5%) and maintained at 36 ± 0.5°C. Tissues were stretched by 500 to 1500 µm at a rate of 10-100 µm/s and the stretch was held for five minutes before returning to baseline. During maintained stretch, 100 µl superfusate samples were taken from the proximity of the mucosal surface and ATP concentrations were measured in a luminometer (Promega) using a luciferin-luciferase based assay. The biomechanical properties of the mouse bladder were determined as magnitudes of steady-state tension (E1), transient tension (E2) and τ (tau), the time constant for the relaxation phase of E2 (Figure 1A); tau was measured from an exponential fit to the transient section of the tension recording after stretch. These parameters were determined for each stretch protocol. Data are mean±SD, *n*=number of mice. Differences between data sets were tested by Student's *t*-test, the null hypothesis was rejected at $p<0.05$.

Results

Chronic radiation cystitis resulted in stiffer bladders as shown by the increase of steady-state passive tension (E1) on stretch (Figure 1B). The magnitude of the transient component (E2), equivalent to stress-relaxation, was unchanged in the irradiated bladders. However, the rate of decline of stress-relaxation was significantly slowed, as shown by a greater value of the relaxation time-constant, tau (Figure 1C). Overall, radiation cystitis generated a less compliant bladder wall that also relaxed more slowly after a stretch. The change of biomechanical properties with bladder irradiation was mirrored by a greater release of ATP during the stretch protocol (figure 1D).

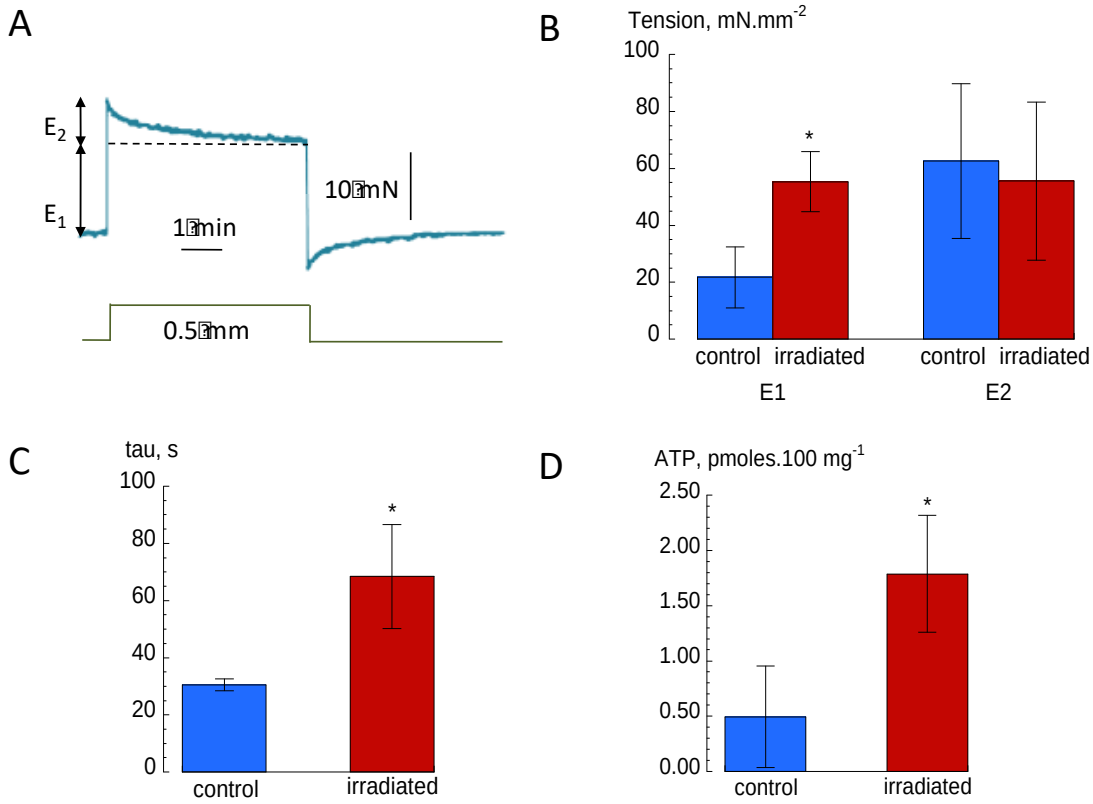

Figure 1. Fibrosis-induced alterations of biomechanical properties and stretch-evoked ATP release in the mouse bladder. A, Example tension trace of stretch protocol to show the elastic values of steady-state tension (E1), transient tension (E2) and relaxation time constant for E2 (tau). B, bar chart of E1 and E2 values from control (blue bars) and irradiated (red bars) mouse bladders showing E1 value is increased in irradiated bladders. C, bar chart of tau showing a significant increase following radiation exposure. D, bar chart of the superfusate ATP concentration during stretch of control and irradiated bladders demonstrating significantly higher levels in irradiated bladders.

Interpretation of results

Chronic radiation cystitis results in decreased bladder compliance and poor contractile function [1] with excessive deposition of extracellular matrix. These changes are reflected in the altered biomechanical properties of greater stiffness and slowed stress relaxation. Furthermore, the changes to passive stiffness were accompanied by greater stretch-induced ATP release. Such ATP release is postulated to underlie activation of afferent nerves in the bladder wall [2] and such enhanced release would result in greater sensations of urge, and even urgency on filling.

Concluding message

Chronic inflammation within the lower urinary tract can result in tissue fibrosis which adversely affects bladder storage and voiding functions. Furthermore, increased bladder wall tension enhances stretch-evoked ATP release and potentially sensory nerve activity. Bladder fibrosis and the consequent increase in basal ATP release may be a contributing factor to the OAB-like symptoms in UAB.

References

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Disclosures

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