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THE HORMONE RELAXIN REVERSES FIBROSIS AND INCREASES DETRUSOR FORCE GENERATION TO RESCUE FIBROTIC BLADDERS DUE TO CHRONIC RADIATION CYSTITIS

Hypothesis / aims of study
Fibrosis is implicated as central in many lower urinary tract pathologies, including chronic radiation cystitis, that exhibit decreased bladder compliance and contractile failure leading to urinary retention. Patients may need to use intermittent self-catheterisation or ultimately undergo a cystectomy, as there are no effective therapies to reverse fibrosis. Human relaxin-2 (hRLX2) is a pleiotropic insulin-like hormone, primarily detected in the blood-stream during pregnancy, to relax the uterus and allow the cervix and symphysis pubis to expand during delivery. It acts on G-protein coupled relaxin receptors 1 & 2, causing transient elevations of cAMP and cGMP and activation of transcription factors that lead to anti-inflammatory, vasodilatory and anti-oxidative properties, and reversal of fibrosis. We have established a mouse model of radiation cystitis that develops severe fibrosis within seven weeks and tested a hypothesis that hRLX2 can reverse fibrosis to re-establish normal bladder function.

Study design, materials and methods
Adult female C57BL/6 mice were anesthetised (avertin, 300 mg/kg, IP), a lower midline incision made and the urinary bladder externalised for selective irradiation (10 Gray=1000 rad; X-RAD 320 KV, Fig. 1A). Seven weeks later, subcutaneous osmotic pumps were implanted in the lower back to deliver saline or hRLX2 (400 µg/kg/day over 14 days; blood levels = 17.5 ng/ml at steady-state). Daily analysis of urine spots was performed to monitor changes in voiding function. After treatment, voiding function was evaluated by anesthesia-free decerebrate cystometry (CMG) and external urethral sphincter (EUS) electromyograms (EMG). *In vitro*, bladder active and passive contractile function was measured in superfused preparations; Cav1.2 (L-type Ca^{2+} channel subunit) and relaxin receptor 1 & 2 expression by immunofluorescence; and bladder wall architecture (urothelial integrity, connective tissue and detrusor muscle content) by H&E staining. Data are means±SD with differences between sets tested using Student's *t*-test, the null hypothesis was rejected at $p<0.05$.

Results
Urine spot analysis showed that non-irradiated mice were continent by voiding in one area of the cage; irradiated animals had leak incontinence by two weeks and retention 12 weeks after irradiation. hRLX2-treated irradiated mice had voiding patterns as in controls (not shown). Irradiated, untreated animals had overflow incontinence, associated with decreased compliance and pressure generation, with a prolonged EUS guarding reflex (Fig. 1B, C). hRLX2-treated mice, exhibited more efficient voiding, longer intercontractile intervals (ICI), higher bladder compliance and EUS activity as in controls (Fig. 1D). At nine weeks post-injury, bladder histology of irradiated mice showed urothelium loss, increased fibrosis and muscle layer disorganisation. hRLX2-treated mice had an intact urothelium, normal smooth muscle architecture and a smaller connective tissue:smooth muscle ratio (Fig 2A). Contractile responses to oxotremorine (10 µM), ABMA (10 µM) and high-K (120 mM KCl) were reduced significantly in muscle strips from irradiated bladders, but restored in hRLX2-treated mice (Fig 2B, $n=5$). Passive length-tension curves (Fig 2C, left) showed increased tension in irradiated bladders, reversed in hRLX2-treated mice. By contrast, active force was decreased in irradiated mice and restored by hRLX2-treatment (Fig 2C, right). Finally, Cav1.2 expression was greatly enhanced in irradiated bladders from hRLX2-treated mice (Fig 2D).

Interpretation of results
Responses to bladder radiation exposure include decreased bladder compliance and force generation and greater fibrosis. This is associated with inflammation from urothelial apoptosis, urine infiltration, and damage to the vascular endothelium. Treatment with hRLX2 reversed fibrosis by reducing collagen synthesis or enhancing its degradation. Contractile function also recovered, with responses to some agonists even augmented, associated with increased Cav1.2 expression that would enhance Ca^{2+} influx into detrusor myocytes. hRLX2 was also anti-inflammatory, inhibiting damage to the urothelium and bladder wall.

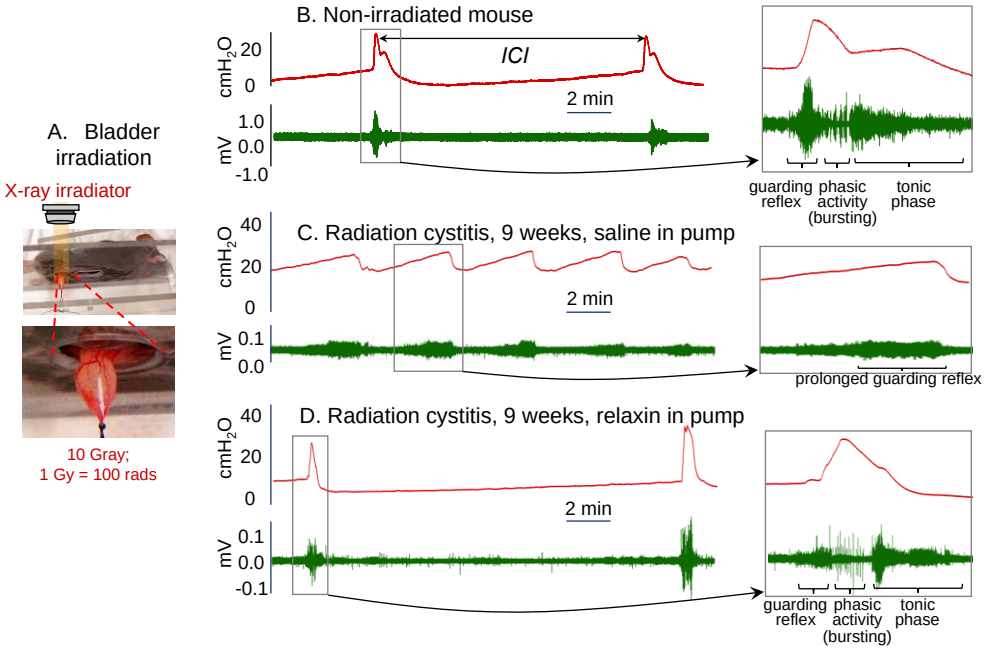

Figure 1. Bladder function of irradiated mice with or without hRLX2 treatment. A: Method for selective irradiation of the bladder. B-D: CMGs/EUS-EMGs in decerebrate mice. B: Control, non-irradiated mouse. C: Irradiated mouse with saline infusion for two weeks. D: Irradiated mouse with hRLX2 infusion (400 μg/kg/day) for two weeks.

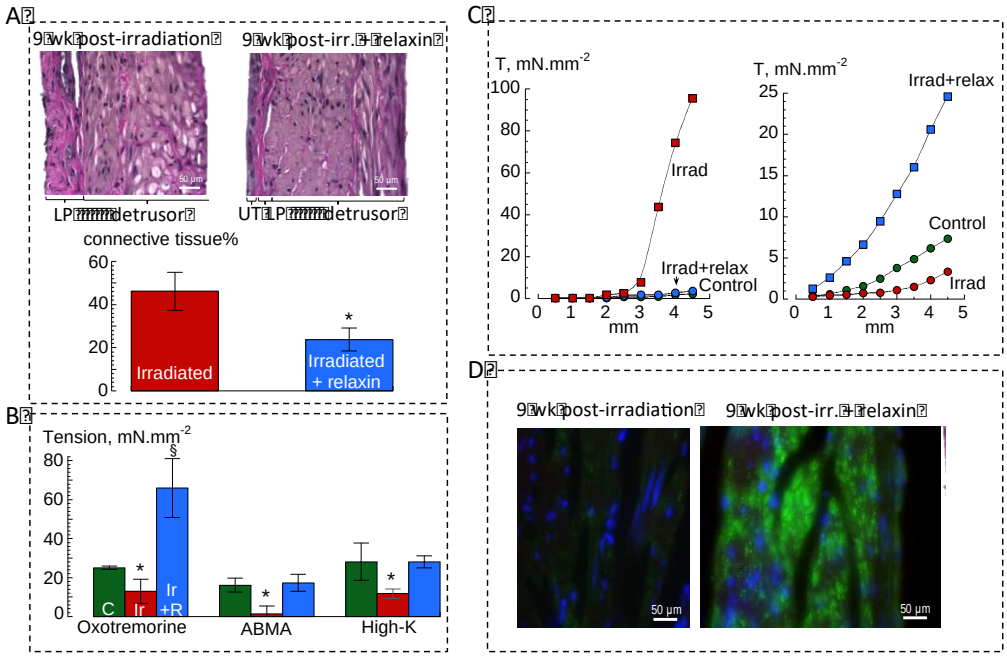

Figure 2 hRLX2 treatment, bladder morphology and function post-irradiation (Irr). A: H&E staining and % connective tissue in irradiated bladders±relaxin. B: Contractile responses to oxotremorine, ABMA and high-KCl solutions. C: Passive (left) and active (right) contraction force as a function of muscle length (SD error bars omitted for clarity, always <20% of mean). D: Detrusor Cav1.2 expression from irradiated bladders±relaxin. LP: lamina propria; UT: urothelium. * and § $p<0.05$ *vs.* control.

Concluding message

These studies are the first to demonstrate that treatment with hRLX2 reversed fibrosis and improved lower urinary tract function in chronic radiation cystitis. These improvements included cystometric and external sphincter functions, urothelial and muscle integrity, and detrusor contractility. The dose range used in these studies, is somewhat greater than those used in some clinical trials for other systemic conditions [1] and further studies will be needed to establish a working range to best manage lower urinary tract conditions.

References

1. Snowdon VK et al. PLOS Medicine, 14:1, 2017

Disclosures

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