314: *Soluble Guanylate Cyclase Activator, BAY 58-2667, Decreases Neurogenic Detrusor Overactivity and Reverses Bladder Fibrosis in Mice with Radiation Cystitis*

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Introduction

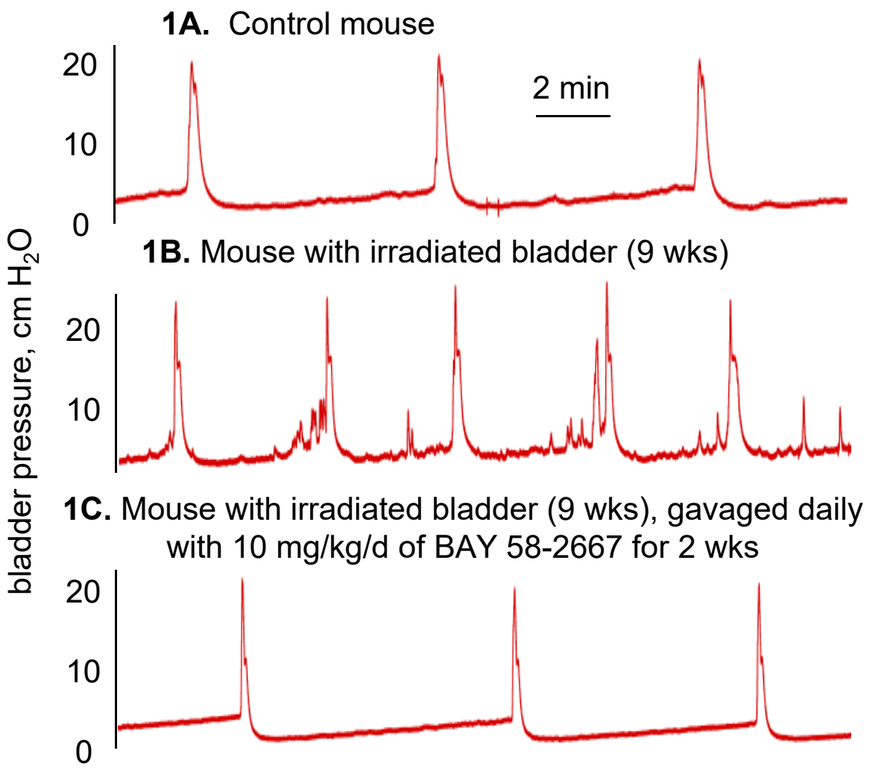
The role of nitric oxide ($NO^{\bullet}$) signalling in the urinary bladder is incompletely understood. Early studies demonstrated $NO^{\bullet}$-mediated relaxation in the bladder neck and urethra, while detrusor relaxation is generally thought to be solely through β-adrenergic receptor stimulation. However, there is robust expression of soluble guanylate cyclase (sGC) and $NO^{\bullet}$-induced cyclic guanosine monophosphate (cGMP) in the urothelium, vascular smooth muscle and interstitial cells of the bladder wall. $NO^{\bullet}$ activates sGC by binding to its β-subunit, inducing a conformational change that converts GTP to cGMP. A prerequisite for $NO^{\bullet}$-induced sGC activation is a reduced heme iron (Fe^{2+}), as $NO^{\bullet}$ cannot bind to the oxidized form (Fe^{3+}). sGC activators do not require $NO^{\bullet}$, which can be low or absent in conditions of oxidative stress and nitrergic nerve damage where cGMP signaling is attenuated and PDE-5 inhibitors (*e.g.*, sildenafil/Viagra) are ineffective. Activators displace oxidized heme binding to sGC at the same site to promote cGMP generation. In pathology, $NO^{\bullet}$-cGMP modulators can dampen afferent nerve firing to suppress bladder overactivity [1] and inhibit TGF-β1 expression and collagen deposition leading to fibrosis in other tissues [2]. Accordingly, the aims of this study were to investigate if sGC activators can reduce fibrosis and re-establish normal voiding function in mice with chronic radiation cystitis.

Methods

Adult female C57BL/6 mice were anesthetized with avertin, 300 mg/kg, and had their urinary bladders externalized and selectively irradiated (10 Gy; 320 KV X-ray irradiator) without affecting other pelvic structures. We have previously demonstrated that this procedure causes development of fibrosis four to six weeks later. After seven weeks following irradiation, mice were gavaged for two weeks daily with BAY 58-2667 (10 mg/kg/day) or vehicle (0.5% methylcellulose and 10% DMSO). Following treatment, bladder function was evaluated *in vivo* using decerebrate cystometrograms (CMGs) and *in vitro* using length-tension recordings from bladder strips. Histological staining was used for bladder wall collagen content quantification. Experiments were carried out on n ≥ 4 mice. Unpaired student *t*-test determined differences between irradiated *versus* control groups or parameters with and without treatment.

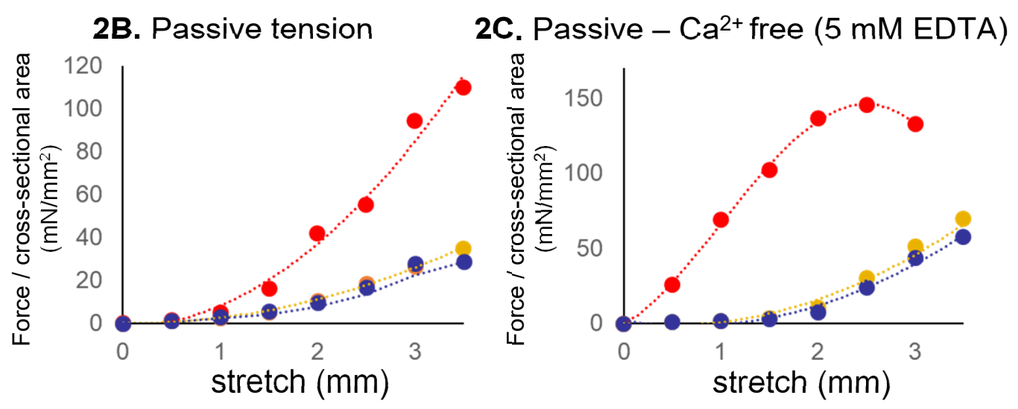
Results

Mice with chronic radiation cystitis developed neurogenic detrusor overactivity (NDO) responsible for significant decreases in intercontractile intervals, non-voiding contractions and compliance in CMG recordings (Figure 1B). Daily gavage with BAY 58-2667 normalized the CMG profiles (Figure 1C).

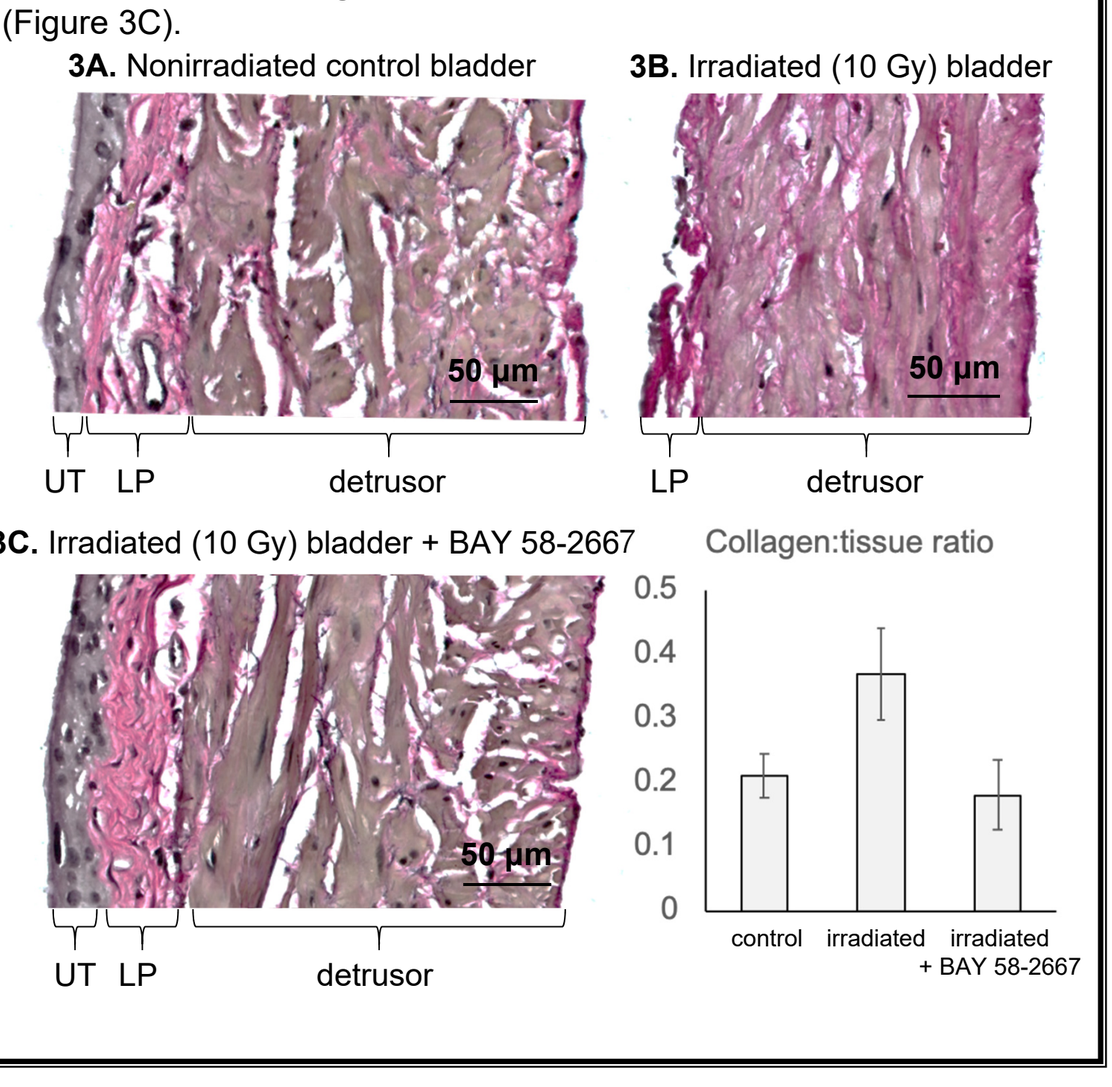


Length-tension measurements from isolated bladder strips showed a significant decline in active force generation (Figure 2A) and increased passive tension/tissue stiffness (Figures 2B and 2C) in chronically irradiated mice compared to controls. BAY 58-2667 treatment increased force generation and reversed fibrosis normalizing bladder compliance.

Results



Modified Verhoeff Van Gieson staining showed significant collagen deposition (pink, Figure 3B) and increased collagen:tissue ratio as well as damaged urothelial layer suggestive of re-occurring inflammation. Irradiated mice treated with BAY 58-2667 exhibited an intact urothelium and decreased collagen content similar to non-irradiated controls (Figure 3C).



Interpretation of Results

Our studies demonstrate that BAY 58-2667 can decrease NDO and reverse fibrosis in mice with chronic radiation cystitis. BAY 58-2667 treatment decreased passive and increased active tension profiles in isolated bladder strips demonstrating improved bladder compliance and force generation. This treatment also inhibited re-occurring inflammation permitting the urothelium to recover and re-establish barrier function. These results support a role for sGC-cGMP signalling in dampening irradiation-induced NDO and reversing fibrosis to improve bladder function.

Conclusions

BAY 58-2667, and other sGC activators, have been developed as potential treatments for fibrosis in a number of non-bladder related pathologies including pulmonary hypertension, acting through suppression of TGF-β1, upregulation of matrix metalloproteinases and downregulation of tissue inhibitors of metalloproteinase. There is also evidence that these compounds ameliorate cyclophosphamide-induced NDO, which correlated with downregulation of bladder sGC expression [3]. We have demonstrated that BAY 58-2667 can reverse fibrosis and restore normal bladder function in mice with chronic radiation cystitis. As fibrosis is also implicated in a number of other bladder pathologies, this study has considerable translational potential.

References

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Disclosures

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