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INHIBITION OF UROTHELIAL TRPA1 CHANNELS PROTECTS THE BLADDER AGAINST RADIATION CYSTITIS.

Hypothesis / aims of study

We have previously demonstrated that irradiation of the bladder activates a Ca^{2+}-dependent nitric oxide (NO•) synthase (NOS) in urothelial cells. NO• binds to cytochrome oxidase, resulting in superoxide ($•O_2^-$) production and peroxynitrite (ONO_2^-) formation which inhibits respiration. This results in mitochondrial cytochrome *c* release and apoptosis of urothelial cells, leading to disruption of barrier function, mast cell activation, afferent sensitization and cystitis. A remaining unknown are the channels responsible for increased intracellular Ca^{2+} that activates NOS during irradiation. Irradiation produces electrophilic fatty acid derivatives (EFAD) and lipid peroxidation products including acrolein, a known activator of TRPA1 channels. As these Ca^{2+} conducting channels are highly expressed in the urothelium, and urothelial cells are particularly radiosensitive, we hypothesize that irradiation-induced bladder damage is a consequence of TRPA1 channel activation. We further hypothesize that TRPA1 channel blockers or nitro-oleic fatty acid (NO_2-OA) can be radioprotective. NO_2-OA can covalently modify cysteine residues of TRPA1 channels, predominantly in the ankyrin-like N-terminal region, activating and then desensitizing these channels. Accordingly, our aim was to determine if TRPA1 channels are involved in irradiation-induced cystitis by investigating the radioprotective benefits of the TRPA1 channel blocker (HC030031) and NO_2-OA on irradiated bladders and cultured urothelial cells.

Study design, materials and methods

Mice were anesthetized using sodium pentobarbital (75 mg/kg, IP) and a small incision was made into the abdominal cavity. Bladders were emptied *via* a PE-10 transurethral catheter and HC030031 (30 μM; in HBSS and 0.1% DMSO) instilled. The animals were placed sideways on a Lexan platform, which allowed the bladders to be held outside the cavity during irradiation, using a suture tied to the urachus (Fig. 1). The organs were irradiated using a 6MeV Varian linear accelerator at a dose of 10 Gy (1 Gy = 100 rads). The irradiation area was reduced to a 20 mm wide beam and only the exposed bladder was irradiated. Following irradiation, the organs were returned to the abdominal cavity, the incisions sutured closed and the animals allowed to recover. One week after irradiation, supracollicular decerebration was carried out and cystometrograms were performed (Fig. 2). All experiments were carried out on n ≥ 3 mice.

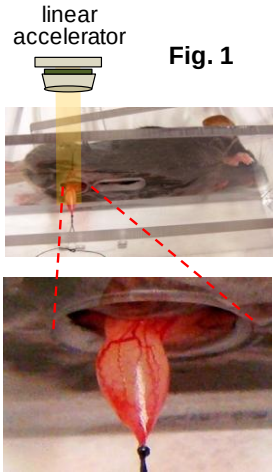


Fig. 1

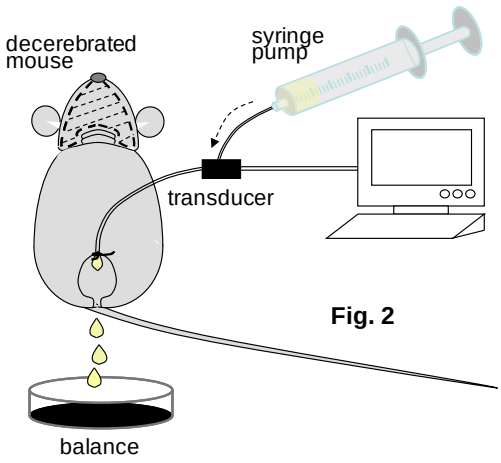
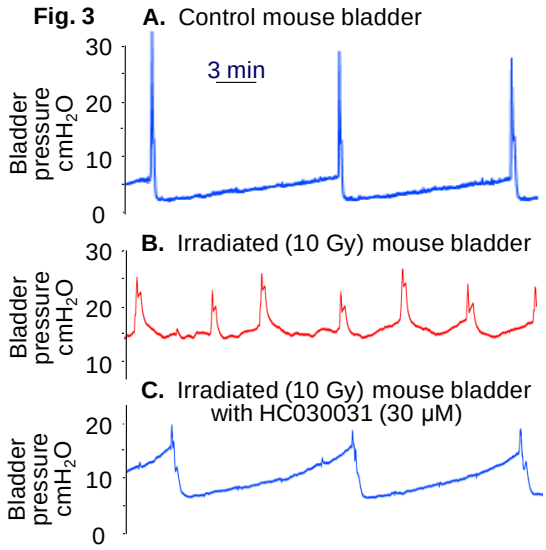


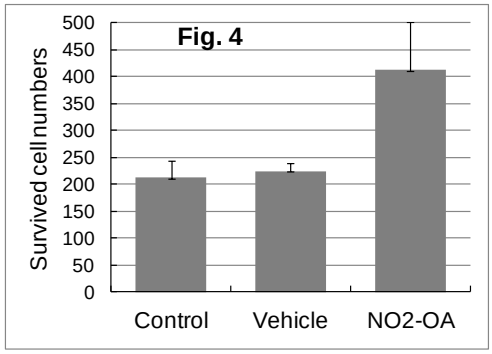
Fig. 2

To demonstrate the involvement of urothelial TRPA1 channels, mouse urothelial cells were obtained by pinning bladder sheets open on a dissection dish and treating them with DMEM and dispase overnight at 4°C. The mucosal linings were scraped gently using a spatula and the cells were treated with 0.25% trypsin EDTA and resuspended in bladder epithelial cell media (CNT-16). Following irradiation (10 Gy), urothelial cells (~10^6/bladder) were plated at 5000 cells per well, incubated and scored after 7 days.

Results

After irradiation, bladders exhibited intercontraction interval shortening (2.8 ± 0.7 min *versus* 12.0 ± 3.0 min in controls, p ≤ 0.01, Fig. 3B) and decreased bladder compliances calculated as the saline volume infused between two micturition pressure thresholds (5.8 ± 2.0 μl/cmH_2O *versus* 12.0 ± 6 μl/cmH_2O in controls). Cystometries of the bladders treated with intravesical TRPA1 channel blocker exhibited parameters similar to control: intercontraction intervals 9.8 ± 4.4 min and bladder compliances 10.7 ± 4.0 μl/cmH_2O (Fig. 3C). Irradiated bladders treated with vehicle showed patterns and parameters of irradiated untreated bladders (not shown).





We used a high dose of irradiation for these initial studies and as a consequence less than 5% of cells survived a week after irradiation. However, cells treated with NO_2-OA (10 μM) during irradiation exhibit twice the viability where 10% of the cells survived ($n \geq 4$, Fig. 4). Vehicle treatment (HBSS + 0.2% EtOH) did not have any effect.

Interpretation of results

Our irradiation cystitis model demonstrated shortened intercontraction intervals which are a consequence of bladder afferent sensitization and decreased bladder compliance which is due, in part, to collagen deposition. However, bladders treated with the TRPA1 channel blocker were partially protected from irradiation damage as they did not show changes in intercontraction intervals and only modest compliance changes compared to controls. Isolated urothelial cells treated with NO_2-OA at the time of irradiation had doubled survival rates compared to irradiated untreated cells and irradiated cells treated with vehicle.

Concluding message

TRPA1 channels are highly expressed in urothelial cells and are known to be activated by acrolein, a by-product of fatty acid lipid peroxidation due to reactive nitrogen and oxygen species (*e.g.*, NO• and $•O_2^-$) generated in cells by ionizing irradiation. Activation of TRPA1 channels by exogenously instilled acrolein or systemically administered cyclophosphamide (the breakdown product is acrolein) results in chemical cystitis with symptomology similar to radiation cystitis. These channels are also highly expressed in sensory nerves innervating the bladder, colon and other pelvic organs suggesting their involvement in irradiation-induced afferent sensitization. In this study, we have demonstrated that intravesical administration of the TRPA1 channel blocker is radioprotective. Moreover, the application of NO_2-OA to cultured urothelial cells was also radioprotective and doubled their survival rate. We hypothesize that NO_2-OA is therapeutically beneficial by first activating and then desensitizing TRPA1 channels.

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Is this a clinical trial?	**No**
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	**Institutional Animal Care and Use Committee of University of Pittsburgh**