267

Zabbarova I[1], Birder L[1], Roppolo J[1], DeGroat W[1], Kanai A[1]
1. *University of Pittsburgh*

BLADDER-COLON CROSS-SENSITIZATION INDUCED BLADDER OVERACTIVITY

Hypothesis / aims of study

Chronic pelvic pain is a debilitating condition of unclear etiology where clinical studies suggest a high concurrence of gastrointestinal and lower urinary tract dysfunctions.

A potential mechanism for this concurrence is afferent cross-sensitization of pelvic organs and the release of inflammatory neurotransmitters through co-innervating neurons. Cross-sensitization between pelvic organs promotes the transmission of noxious stimuli from an irritated organ to an adjacent structure sensitizing the neurons that innervate both affected and unaffected organs.

We have developed a mouse model for studying the mechanism and consequence of cross-sensitization of the urinary bladder and colon. The descending colon is briefly withdrawn from the abdomen and selectively irradiated without affecting surrounding organs. Our studies suggest that selective irradiation of the colon results in the development of colonic inflammation and bladder overactivity within 7 days. Accordingly, our objective was to characterize the bladder overactivity and, ultimately, the mechanism of bladder-colon cross-sensitization.

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. The descending colon was carefully pulled out using a suture. The mouse was placed sideways on a Lexan platform (Figure 1, *permission for displaying this figure obtained*), which allowed us to hold the colon outside the cavity during irradiation.

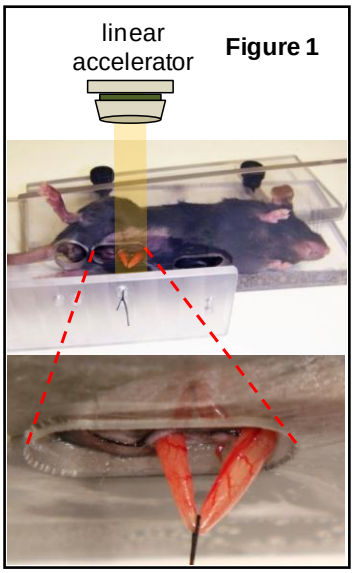
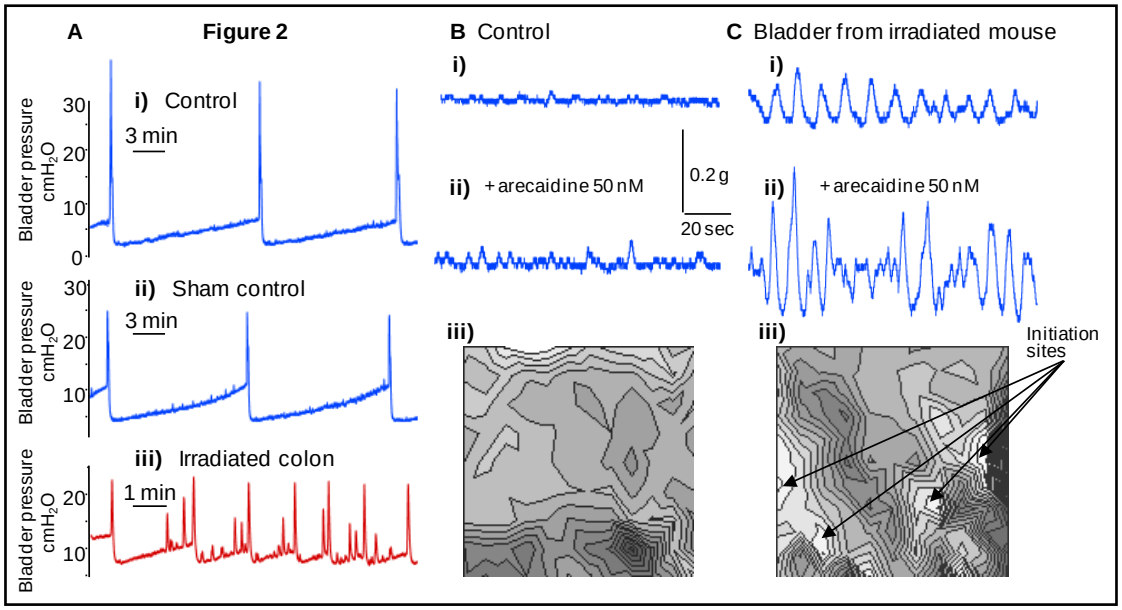


Figure 1

The organs were irradiated using a 6MeV Varian linear accelerator at a dose of 10 Gy (1 Gy = 100 rads). The irradiation was reduced to a 20 mm wide beam to insure that only the area encompassing the exposed colon was irradiated. Following irradiation the colon was returned to the abdominal cavity, the incision sutured closed and the animals allowed to recover. One to two weeks after irradiation, supracollicular decerebration was carried out and recordings of cystometrograms (CMG) were performed followed by excision of the bladder. The excised bladders were cut from base to dome along the dorsal aspect to form a sheet. The sheet preparations were stained with Ca^{2+} sensitive dye (5 μM Rhod-2AM). After staining, the outlets of the bladders were pinned to the fixed platform in the recording chamber with the dome end connected to a tension transducer. The tissues were perfused with Tyrode's solution (95% O2 and 5% CO2, pH 7.35) at 37ºC, stretched to resting tension, and imaged from the mucosal surface. Isochronal maps were generated from the local activation time-points for up to 256 optical action potentials and intracellular Ca^{2+} transients using cross-correlation analysis. All experiments were carried out on $n \geq 5$ mice.

Results

At 1 to 2 weeks following irradiation of the colon, cystometric studies on decerebrated mice demonstrated non-voiding contractions (3 to 6 per contractile interval) and decreased intercontraction intervals (from 12 ± 3 min in controls to 1 ± 0.5 min in irradiated mice) characteristic of bladder overactivity (Figure 2Aiii). Sham control surgery, when the colon was pulled out the body without irradiation, as well as control irradiation, when mice were placed next to the irradiation beam, did not result in bladder overactivity (Figure 2Aii).



Optical mapping studies with control mice demonstrated that there is almost no spontaneous activity in control bladders (Figure 2Bi). Alternatively, the spontaneous activity of bladders from mice with irradiated colons, one week after irradiation, was significantly higher (Figure 2Ci). Stretch or low-dose muscarinic agonist arecaidine (50 nM) enhanced the amplitude of spontaneous contractions in bladders from colonic irradiated mice (Figure 2Cii) but not significantly – in control bladders. Ca^{2+} maps from control mouse bladders showed almost no activity, whereas irradiation of colon resulted in contractions originated from multiple initiation sites (Figures 2Biii and 2Ciii).

Interpretation of results

Our irradiation-induced colitis model allows us to induce colonic inflammation without instilling chemical agents into the colon that may leak out and enter the circulation thereby directly affecting the bladder or its sphincter. Using this model we have demonstrated the development of bladder overactivity following colonic irritation. Since present in the excised bladder sheets, this overactivity is intrinsic to the detrusor.

Concluding message

There are several theories regarding the development of pelvic organ cross-sensitization, but the precise mechanism is unclear. We believe that our model will help elucidate the mechanism of pelvic organ cross-sensitization and, ultimately, to identify the therapeutic targets for the treatment of concurrent bladder dysfunction.

Specify source of funding or grant	**This research is funded by NIH grants to A. Kanai (DK071085) and L. Birder (DK54824) and Pfizer grant to I. Zabbarova.**
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**