268

Zabbarova I[1], Wipf P[1], Birder L[1], Epperly M[1], DeGroat W[1], Kanai A[1]
1. *University of Pittsburgh*

MOUSE MODEL FOR CHARACTERIZATION OF RADIATION CYSTITIS AND ITS PREVENTION USING A NEW CLASS OF RADIOPROTECTORS

Hypothesis / aims of study

Each year a large number of patients undergo radiation treatment for various pelvic malignancies. Irradiation of the pelvic region results in bladder inflammation and dysfunction. Cystitis or its likelihood develops increasing the incidence of bladder cancer, restricting radiation treatment for bladder tumors and limiting the allowable radiation dose for treating other pelvic malignancies.

Radiation damage is mainly mediated by reactive nitrogen and oxygen species (RNS and ROS). Hence, scavengers of free radicals form the principal group of radioprotective agents. Recent findings by our group suggest that the principal site of radiation-induced free radical generation is in the mitochondria and that nitric oxide synthase (NOS) antagonists acting there are radioprotective [1, 2].

The ultimate aim of our studies is to develop the new class of potent and selective radioprotectors based on NOS antagonists. We recently synthesized a number of new NOS inhibitors. To test their potency *in vivo* we used a mouse model, where the pelvic region is irradiated. However, our findings suggest that bladder dysfunction can be caused not only by direct irradiation, but also through cross-sensitization by other irritated pelvic organs (*e.g.*, colon). Accordingly, it was necessary to develop a new model for *in vivo* characterization of radiation cystitis and its prevention by radioprotective compounds.

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

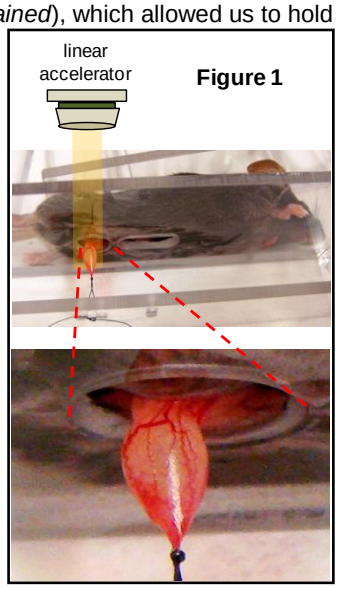
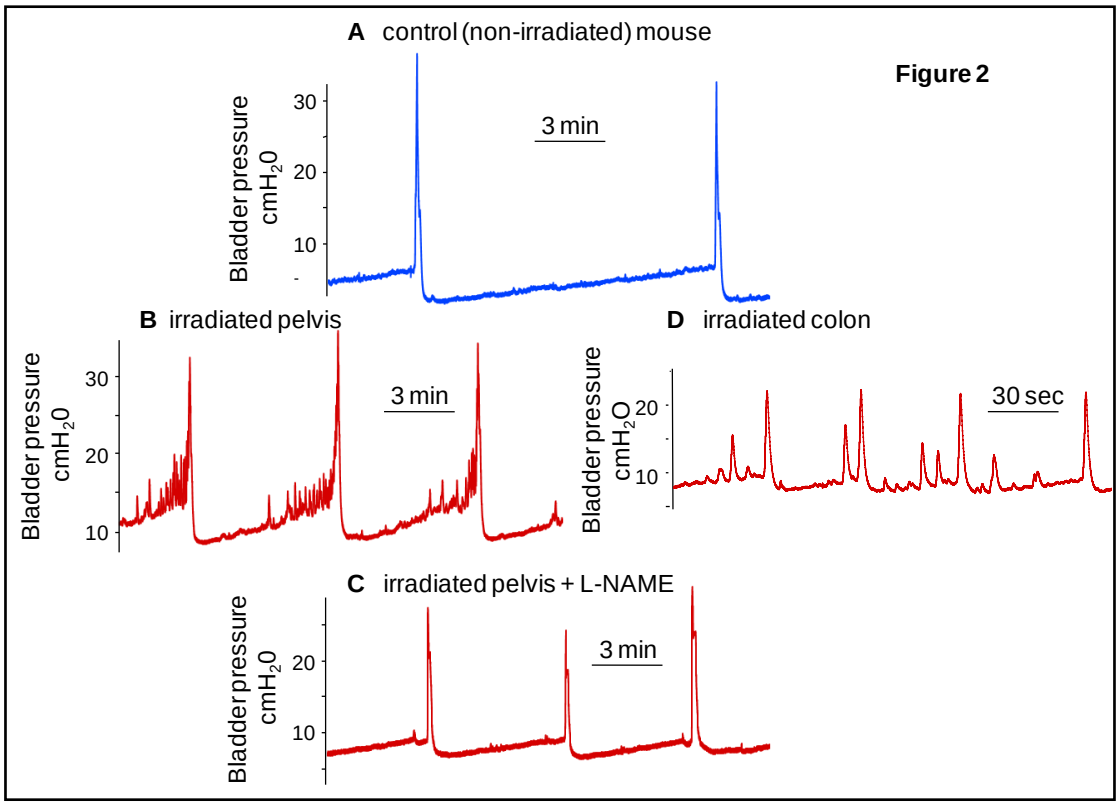


Figure 1

The organs were irradiated using a 6MeV Varian linear accelerator at a dose of 2 Gy (1 Gy = 100 rads). The irradiation was limited to the area encompassing only the exposed bladder. Following irradiation the bladder was returned to the abdominal cavity, the incision sutured closed and the animals allowed to recover.

Results

Figure 2 shows cystometrograms recorded from a control mouse (A), a mouse after pelvic irradiation with saline in the bladder (B), a mouse with selectively irradiated colon (D), and a mouse after pelvic irradiation with a NOS antagonist (L-NAME, 200 μM) in the bladder (C). These findings demonstrate that 1 week following irradiation, bladders from mice with irradiated pelvises as well as selectively irradiated colons exhibit nonvoiding contractions and decreased intercontraction intervals. In organs of animals irradiated with L-NAME in the bladder nonvoiding contractions disappear, but intercontraction intervals remain decreased.



Interpretation of results

These findings suggest that intravesical nitric oxide synthase (NOS) antagonists are only partially protective because damage to the bladder can occur not only through direct irradiation but also through cross-sensitization by other irradiated pelvic organs (*e.g.*, colon). Our new approach of selective bladder irradiation allows us to avoid these complications and more accurately characterize radiation cystitis and new radioprotective agents.

Concluding message

The development of safe, long-acting, orally administrable radioprotective agents is the subject of intense research due to their potential benefit in radiation therapy and for occupational and terrorist initiated radiation exposure. We synthesized the number of new potential radioprotectors and developed the new animal model for their effective *in vivo* testing. In ongoing studies, we are tagging radioprotectors with peptide isosteres (from gramicidin S) to selectively target these compounds to the mitochondria to permit safe oral administration.

References

1. Kanai A, Zabbarova I, et al. Org Biomol Chem. 5:307-309, 2007
2. Zabbarova I and Kanai A. Mol Intervention. 8:294-302, 2008

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