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INHIBITION OF MITOCHONDRIAL NITRIC OXIDE SYNTHASE DURING PELVIC IRRADIATION PREVENTS UROTHELIAL DAMAGE AND PROTECTS THE BLADDER AGAINST RADIATION CYSTITIS

Hypothesis / aims of study

Radiation therapy for pelvic malignancies can cause bladder cystitis resulting in decreased compliance, reduced capacity and the symptoms of frequency, urgency, and dysuria. Furthermore, the potential to develop radiation cystitis prevents the use of radiation therapy to treat bladder cancer and limits the radiation dose that can be used to treat other pelvic malignancies. The mechanism of radiation cystitis has not yet been described, but our findings suggest that it may involve radiation-induced activation of urothelial mitochondrial nitric oxide synthase (mtNOS), disruption of the urothelial permeability barrier, and exposure of the underlying tissues to urine. We propose that radiation-induced activation of mtNOS results in prolonged mitochondrial NO production which inhibits the respiratory chain and thereby increases superoxide (O_2^-) generation. Superoxide, in turn, reacts with manganese superoxide dismutase (MnSOD) to form hydrogen peroxide (H_2O_2) and/or with NO to form peroxynitrite (ONO_2^-), both of which can damage the urothelium leading to necrotic and apoptotic cell death and disrupt the permeability barrier (Fig 1). Accordingly, our aim was to inhibit mtNOS during irradiation and determine if we could protect the bladder urothelium and prevent the development of radiation cystitis.

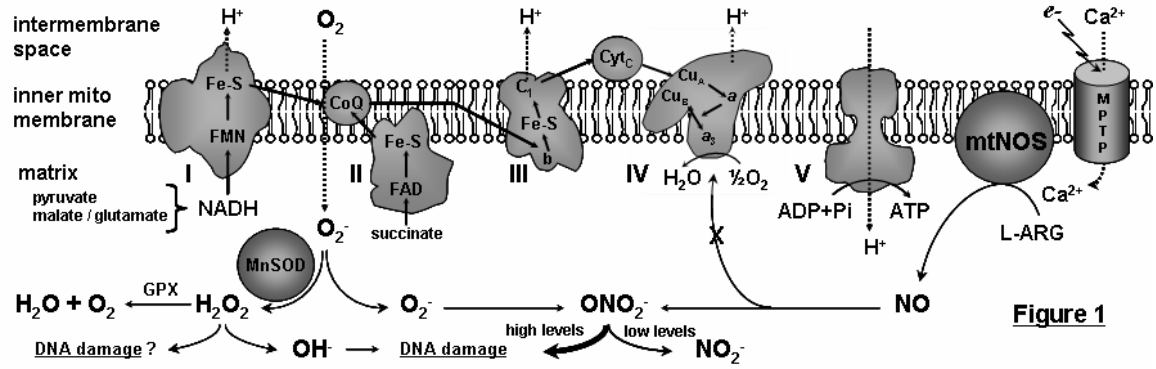

Figure 1

Study design, materials and methods

We have developed a mouse model of radiation cystitis where single- or fractionated-dose irradiation results in decreased transepithelial resistance and increased urea and water permeabilities within 24 hrs following final radiation exposure. At six months, bladders exhibit non-voiding contractions, increased baseline pressures and decreased bladder compliance indicative of radiation cystitis in humans. Mice were irradiated with 10 or 50 Gray (Gy; 1Gy = 1 J/kg = 100 rads) fractions to the bladder to a total of 50 Gy. The following preparations were used: 1) Wild-type (WT) mice; 2) WT mice whose bladders were transfected with the transgene for human manganese superoxide dismutate (MnSOD; a mitochondrial scavenger of O_2^-), 3) WT mice with a nitric oxide synthase (NOS) antagonist (L-NMA; 500 μM) in the bladder lumen during irradiation; and 4-5) nNOSα$^{-/-}$ (the isoform of mtNOS) or eNOS$^{-/-}$ mice. Twenty-four hours after the end of the radiation regimen, bladders were excised and placed in an Ussing chamber for measurements of transepithelial resistance (TER) and water and urea permeabilities, or placed in a muscle bath for simultaneous microsensor measurements of NO and ONO_2^- production in the urothelium.

Results

Irradiation of the bladder resulted in decreased TER (Fig 2a) and increased urea (2b) and water (2c) permeabilities. Prior transfection with human MnSOD partially protected the urothelium resulting in a smaller decrease in TER (2a) and smaller increases in urea (2b) and water (2c) permeabilities. However, the presence of L-NMA in the bladder lumen during irradiation resulted in almost complete protection of the bladder urothelium (2a-c). The involvement of the urothelium in radiation-induced damage was demonstrated in scanning electron micrographs (SEMs) where irradiated bladders show selective destruction of the

umbrella cell layer (2e) compared to normal urothelium (2d). However, in MnSOD transfected bladders (2f) the disruption of the urothelium is markedly decreased and in an L-NMA protected bladder, the urothelium looks like the control (not shown).

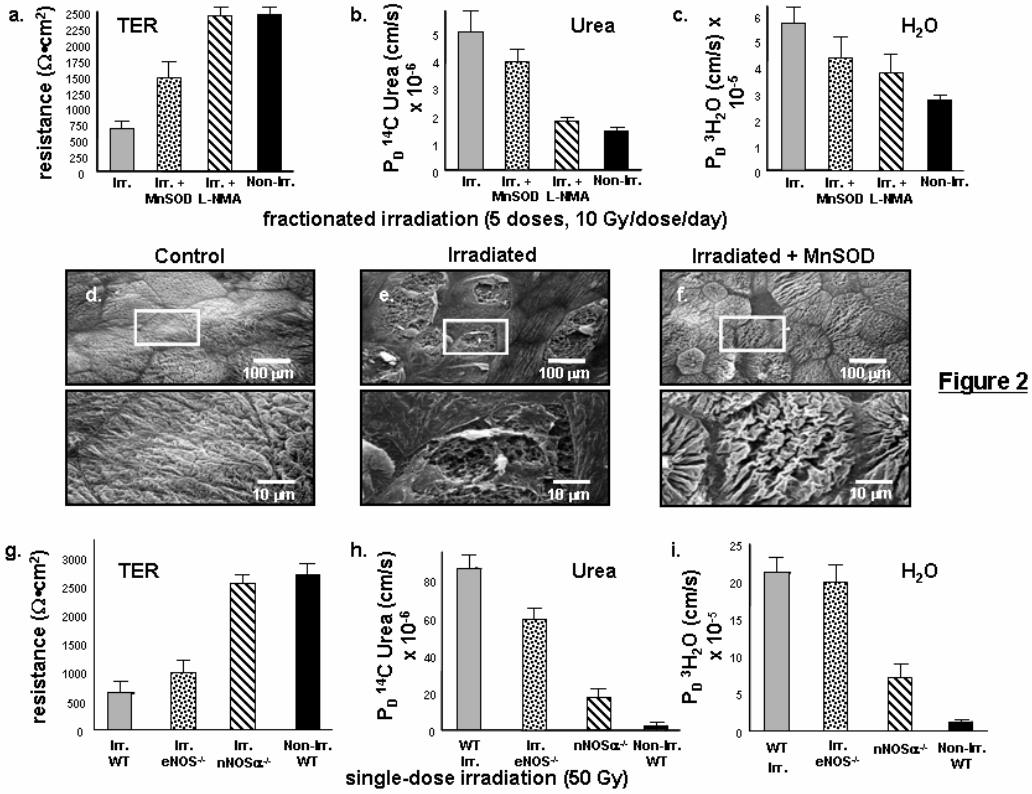

Figure 2

To further test the hypothesis that ionizing radiation activates mtNOS, we irradiated the bladders of nNOSα$^{-/-}$ and eNOS$^{-/-}$ mice. While the decrease in TER (2g) and increases in urea (2h) and water (2i) permeabilities of eNOS$^{-/-}$ were comparable to those seen in irradiated wild-type mice, the bladders of nNOSα$^{-/-}$ mice were radioprotected with barrier properties similar to non-irradiated WT mice (2g-i). As further support, direct microsensor measurements from the urothelial surface of excised bladders revealed that NO production (evoked with capsaicin; a normal response) resulted in a concomitant production of ONO_2^- (an abnormal response indicating excessive O_2^- levels) in unprotected irradiated bladders. The NO evoked formation of ONO_2^- was not seen in non-irradiated bladders or irradiated ones overexpressing MnSOD or protected with L-NMA (not shown). All experiments were carried out in n≥6 bladders.

Interpretation of results

SEMs demonstrate that ionizing radiation selectively disrupts the urothelium. Mitochondria are implicated since MnSOD is localized there and is partially protective. MnSOD is partially protective because it steals O_2^- from NO preventing the formation of highly reactive ONO_2^- and increasing the formation of the less reactive but still potentially damaging H_2O_2. Inhibition of mtNOS on the other hand prevents the formation of both ONO_2^- and H_2O_2 (see Fig 1). This theory is further supported by the formation of ONO_2^- in irradiated bladders, but not in irradiated bladders protected with MnSOD or L-NMA.

Concluding message

These data suggest that ionizing radiation activates the mtNOS in the urothelial cells lining the bladder lumen. This results in the disruption of the urothelial permeability barrier leading to the development of radiation cystitis. Thus, the transfection of the urothelium with the human MnSOD transgene prior to irradiation, or better still, the presence of a NOS antagonist in the lumen during irradiation, protects the bladder against radiation cystitis and offers a potentially clinically relevant treatment for the prevention of radiation cystitis.

FUNDING: NIH - NIDDK