322: Mitochondrial-targeted Free Radical Scavenger, XJB-5-131, Offers Prolonged Protection Against Radiation Cystitis

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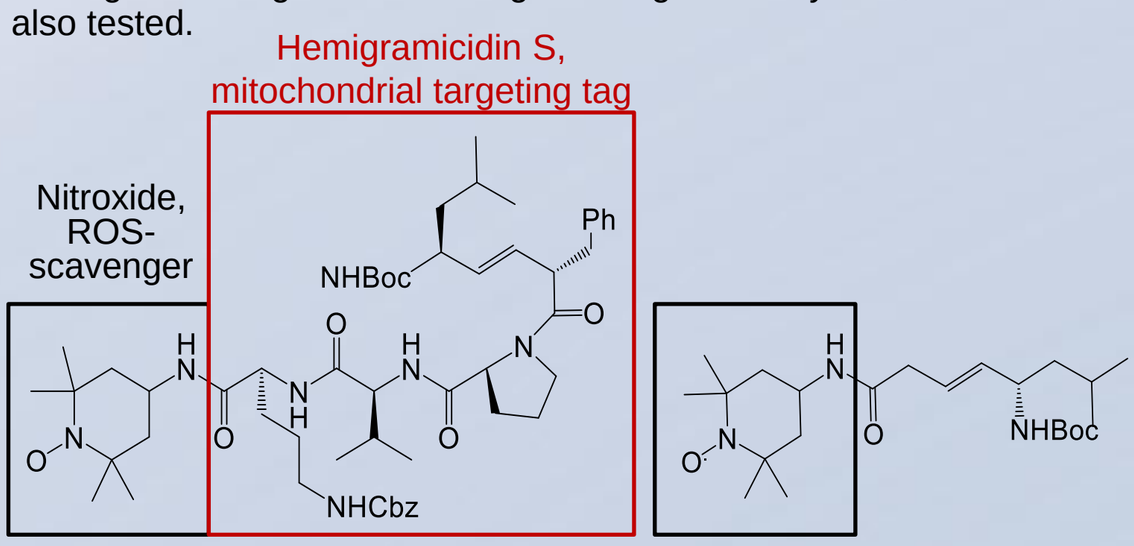
ABSTRACT

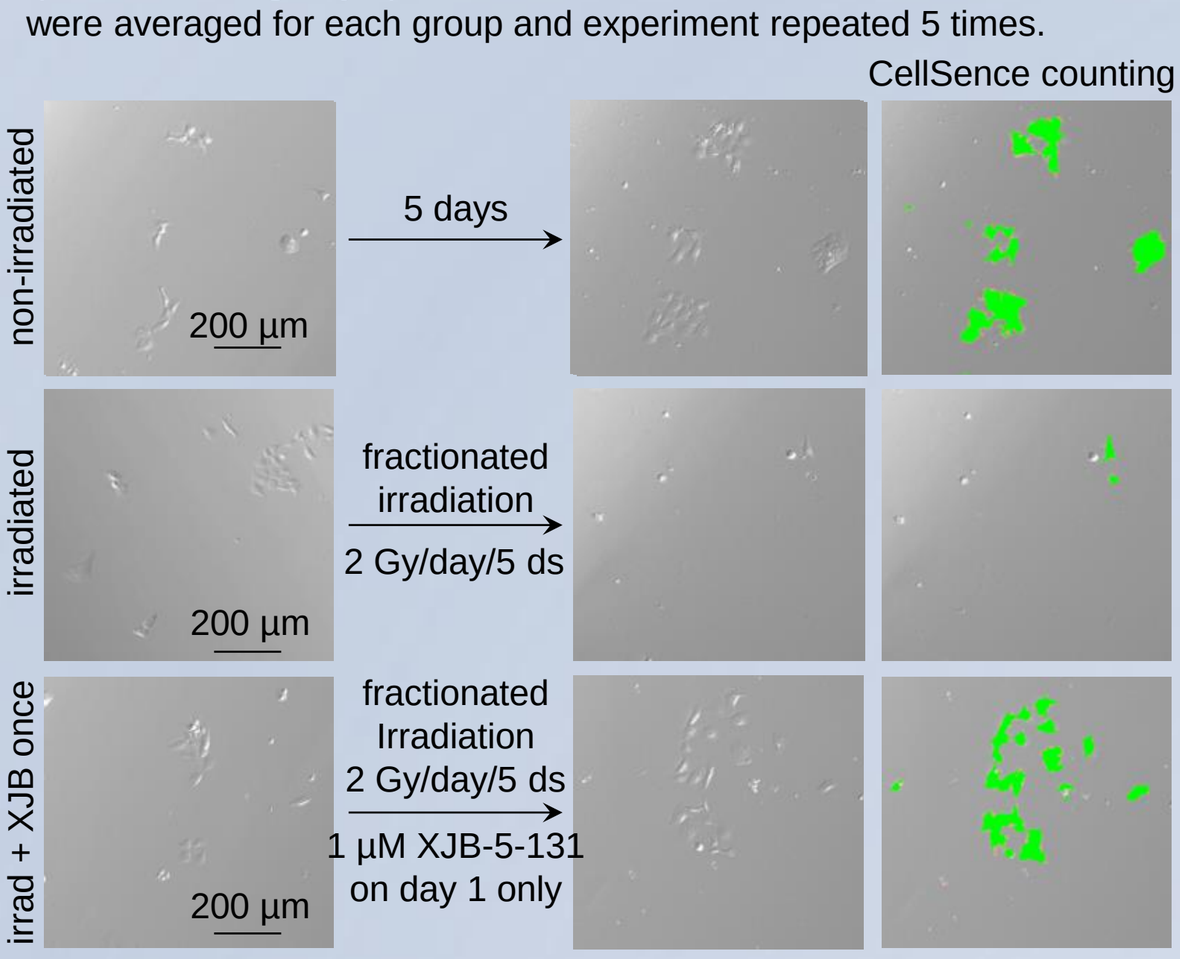
Radiation cystitis results from radiation therapy for pelvic tumors and accounts for up to 7% of emergency urology admissions. It can be classified into acute and chronic stages where the former is characterized by urothelial layer disruption and inflammation and the latter associated with neurogenic detrusor overactivity, chronic fibrosis and potentially fatal hemorrhagic cystitis [1]. Current therapies are limited, invasive and often fail to demonstrate optimal efficacy. Radiation damage is mediated by reactive oxygen and nitrogen species. Our findings suggest that the principal site of damage in the bladder is urothelial mitochondria and mitochondrial-targeted free radical scavengers are radioprotective when instilled into the bladder for delivery to urothelial cells [2, 3]. Since radiotherapy is usually delivered over a treatment course by means of multiple fractions, frequent catheterizations can increase the risks for urinary tract infections and irritation. Therefore, we examined the duration of the protective effect of a single instillation of XJB-5-131 against urothelial damage during five days of fractionated irradiation and if this affected the radiotherapy outcome for pelvic tumors. A similar compound lacking the hemigramicidin S tag and targeted to cytosol, JP4-039, was also tested.



XJB-5-131 ***JP4-039***

METHODS

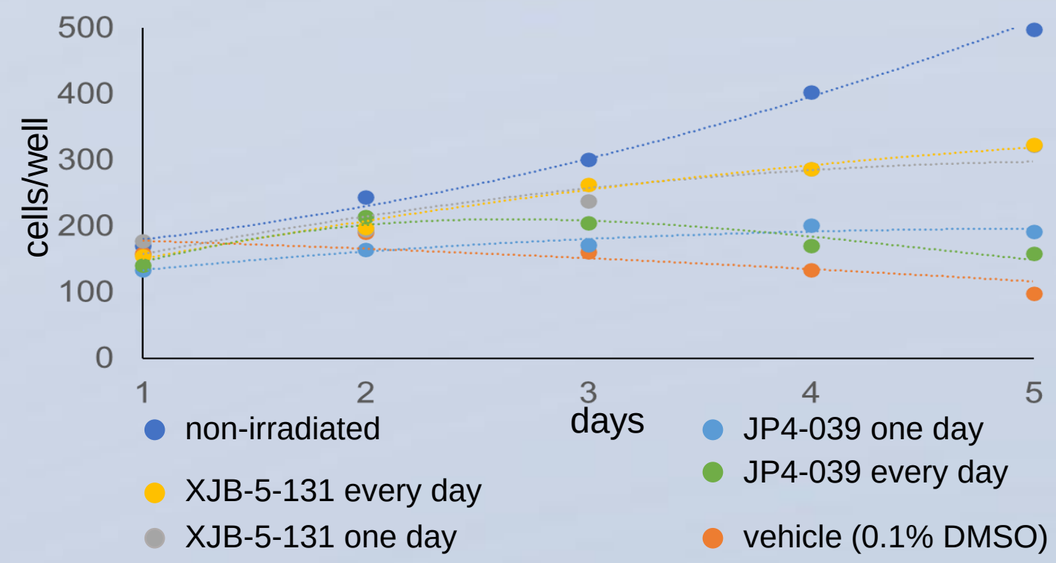
To determine the duration of the protective effect of XJB-5-131 and JP4-039 against urothelial damage, we used human immortalized uroepithelial cells (UROtsa). Cells cultured to 10-15 passage were plated in 48 well plates at 150 cells/well and subjected to fractionated irradiation (2 Gy/day/5 days). Cells were treated with 1 μM XJB-5-131, 1 μM JP4-039 or vehicle (0.01% DMSO) once prior to the first irradiation dose or every day. Following each irradiation, cells at the center of each well (≈0.3 cm^2 area) were imaged microscopically and quantified using Olympus CellSens software. Results from 8 wells were averaged for each group and experiment repeated 5 times.

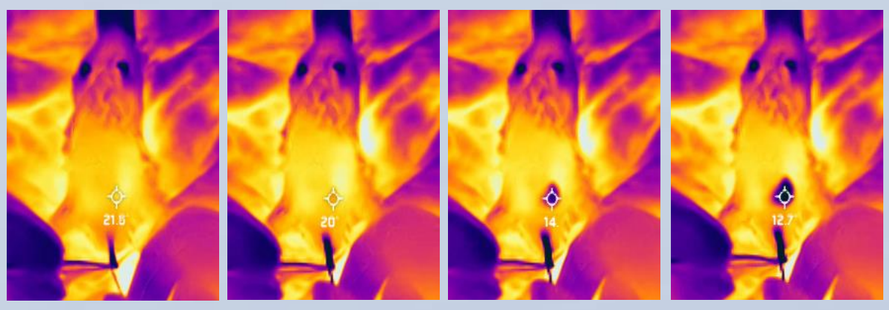


To determine if XJB-5-131 pretreatment can adversely affect the outcomes of radiotherapy, male C57Bl/6 mice with orthotopic tumors were used. TRAMP-C1 cells (6 x 10^6 cells/50 μl) were injected into the ventral prostate lobes. After 5 weeks, mice received fractionated pelvic irradiation treatment (2 Gy/day/5 days). Prior to the first irradiation, 1 μM XJB-5-131 or vehicle (0.01 % DMSO) was instilled into the bladders. To ensure the delivery to the bladder and not the prostate, the solutions were chilled, and an infrared camera used to visualize instillations. Four days post irradiation, bladder function was assessed by spot tests, and then lower urinary tracts, including the bladder, prostate and prostatic urethra were isolated and analyzed.

RESULTS

Our data demonstrate a dose-dependent cell death over the course of irradiation treatment to ~40% of cells treated with vehicle surviving by the end of 5 days. Non-irradiated cells grew to ~300% of the original number. Both groups of XJB-5-131 treated irradiated cells (those treated once or every day) grew to ~200% cells over the first 5 days. In both groups treated with JP4-039, the growth slowed down after day two of irradiation treatment and cell death prevailed thereafter.

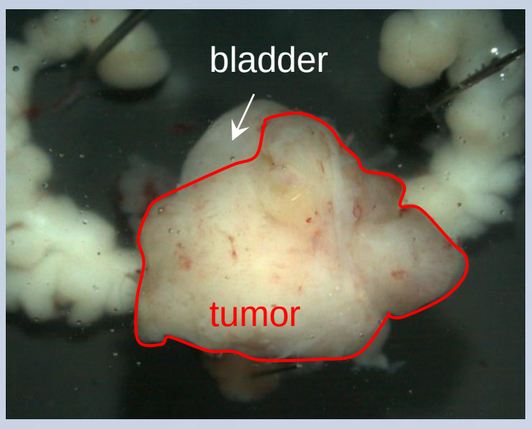




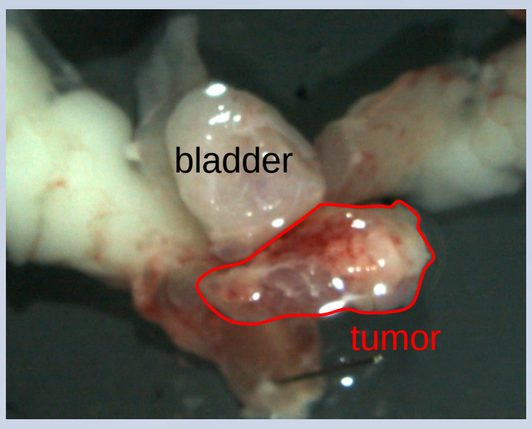
Infrared images verify instillation of cold XJB-5-131 into the bladder, not the prostate.

All mice survived tumor implantation and radiation treatment up to the endpoint. There was significant shrinkage of the irradiated tumors compared to non-irradiated ones with the tissue weight decreasing dramatically in vehicle and, especially, XJB-5-131 instilled groups.

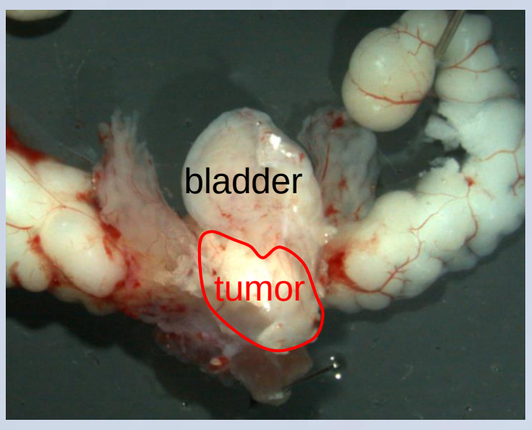
non-irradiated

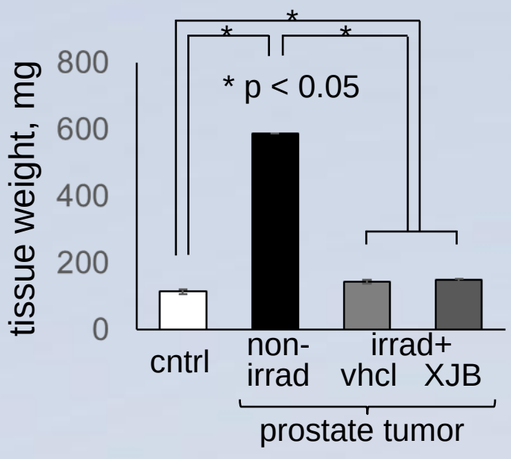


irradiated + vehicle



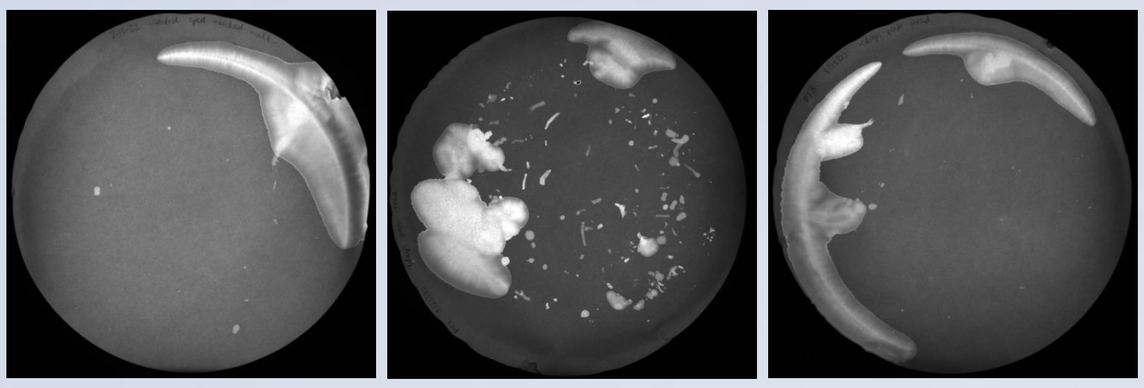
irradiated + XJB-5-131





Vehicle instilled mice showed increased urinary frequency and smaller void spots 4 days after irradiation compared to controls. Instillation of XJB-5-131 prevented development of bladder overactivity/frequent voiding as there was little to no change from controls in voiding spot profiles.

non-irradiated | irradiated + vehicle | irradiated + XJB-5-131



CONCLUSIONS

Mitochondrial-targeted free radical scavenger, XJB-5-131, is protective against radiation cystitis when given on the first day of weekly fractionated radiation therapy without protecting the tumors.

REFERENCES

[1] Smit SG, Heyns CF. Management of radiation cystitis. Nat Rev Urol. 7:206, 2010, PMID: 20212517
[2] Zabbarova I, Wipf P, et al. Mitochondrially-targeted drugs for the prevention of radiation cystitis. *Neurourol Urodyn.* 26(5): 668, 2007.
[3] Zabbarova I, Kanai A. Targeted delivery of radioprotective agents to mitochondria. Mol Interv. 8(6): 294, 2008, PMID: 19144902

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