Role of Oxidative Stress in a Rat Model of Radiation-Induced Erectile Dysfunction

Masaki Kimura, MD, PhD,* Zahid N. Rabbani, MD,† Andrew R. Zodda, MSc,† Hui Yan, PhD,† Isabel L. Jackson, BSc,† Thomas J. Polascik, MD,* Craig F. Donatucci, MD,* Judd W. Moul, MD,* Zeljko Vujaskovic, MD, PhD,† and Bridget F. Koontz, MD†

*Division of Urologic Surgery, Department of Surgery and Duke Prostate Center, Duke University Medical Center, Durham, NC, USA; †Department of Radiation Oncology and Duke Prostate Center, Duke University Medical Center, Durham, NC, USA

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ABSTRACT

Introduction. Chronic oxidative stress is one of the major factors playing an important role in radiation-induced normal tissue injury. However, the role of oxidative stress in radiation-induced erectile dysfunction (ED) has not been fully investigated.

Aims. To investigate role of oxidative stress after prostate-confined irradiation in a rat model of radiation-induced ED.

Methods. Fifty-four young adult male rats (10–12 weeks of age) were divided into age-matched sham radiotherapy (RT) and RT groups. Irradiated animals received prostate-confined radiation in a single 20 Gy fraction.

Main Outcome Measures. Intracavernous pressure (ICP) measurements with cavernous nerve electrical stimulation were conducted at 2, 4, and 9 weeks following RT. The protein expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits (Nox4 and gp91phox), markers of oxidative DNA damage (8-hydroxy-2-deoxyguanosine [8-OHdG]), lipid peroxidation (4-hydroxynonenal [4HNE]), and inflammatory response including inducible nitric oxide synthase, macrophage activation (ED-1), and nitrotyrosine, and endogenous antioxidant defense by nuclear factor erythroid 2-related factor (Nrf2) were evaluated in irradiated prostate tissue and corpora cavernosa (CC). In addition, we investigated the relationships between results of ICP/mean arterial pressure (MAP) ratios and expression level of oxidative stress markers.

Results. In the RT group, hemodynamic functional studies demonstrated a significant time-dependent decrease in ICP. Increased expression of Nox4, gp91phox, 8-OHdG, and 4HNE were observed in the prostate and CC after RT. Similarly, expressions of inflammatory markers were significantly increased. There was a trend for increased Nrf2 after 4 weeks. ICP/MAP ratio negatively correlated with higher expression level of oxidative markers.

Conclusion. NADPH oxidase activation and chronic oxidative stress were observed in irradiated prostate tissue and CC, which correlated with lower ICP/MAP ratio. Persistent inflammatory responses were also found in both tissues after RT. These findings suggest that oxidative stress plays a crucial role in the development of radiation-induced ED.


Key Words. Erectile Dysfunction; Oxidative Stress; Radiotherapy; Radiation-Induced Erectile Dysfunction
Although the technology for delivering RT is rapidly improving, recent studies have shown that the most advanced current RT technique, intensity-modulated radiotherapy, results in more new diagnoses of ED than that of 3DCRT [4]. The decline in sexual HRQoL following RT for prostate cancer is recognized as a point of concern for both clinician and patients. However, preclinical research investigating the molecular mechanisms of radiation-induced ED is limited due to the lack of a well-established and appropriate animal model of ED following RT [5–7].

In this context, we have established a rat model of radiation-induced ED. In this model, RT is delivered using image-guided prostate-confined RT, thereby mimicking prostate irradiation in the clinical setting. Our previous studies have confirmed the feasibility of this model with extremely low rodent toxicity [8,9]. Those studies showed time-dependent development of ED using a bioassay and intracavernous pressure (ICP) measurements with electrical cavernous nerve (CN) stimulation after RT. Moreover, we found that radiation-induced ED was significantly associated with CN injury, followed by subsequent reduction of smooth muscle content and decreased perfusion in penile corpora cavernosa (CC) [9].

A major cause of direct radiation-induced injury to the cell is DNA oxidation. The ionization by radiation leads to chromosome aberrations and gene mutations which lead certain cells toward apoptosis or necrosis among those that cannot repair the DNA damage [10]. Oxidative stress is also an indirect cause of radiation-induced cell injury resulting from ionization of water molecules, which has been described in several articles in radiation-induced kidney, lung, skin, and central nervous system injuries [11]. In particular, reactive oxygen species (ROS) derived from upregulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase are well known as a main source of oxidative stress following radiation-induced normal tissue injuries [12–15]. Oxidative stress is the result of an imbalance between ROS generation and cellular antioxidant defenses [16]. A number of articles have reported ROS to play a key role in cell signaling, particularly among macrophages and neutrophils, as well as regulation of certain cytokines and transforming growth factor (TGF)-β-induced apoptosis and fibrosis [17–19].

There is little evidence investigating a possible relationship between radiation-induced oxidative stress and impairment of erectile function (EF) in radiation-induced ED setting. Considering the important role of oxidative stress in radiation injury, investigation of oxidative stress in an animal model of radiation-induced ED is warranted. Improved knowledge of the mechanisms underlying ED will lead to more effective prevention strategies and development of new therapeutic interventions. In the present study, we hypothesized that oxidative stress by NADPH oxidase-derived ROS could lead to chronic inflammation in prostate and penile tissue, which might be associated with radiation-induced ED. We investigated the association between radiation-induced ED and oxidative stress by assessing physiologic EF and molecular changes in irradiated prostate and penile tissues.

### Methods

#### Animals

Fifty-four young adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA, USA) with an initial age of 10 to 12 weeks old were divided into age-matched sham irradiation and RT groups. Each group was analyzed by ICP measurement in three subgroups at 2, 4, or 9 weeks after RT or sham treatment. All animal handlings and procedures were approved by the Duke University Medical Center Institutional Animal Care and Use Committee.

#### Treatment Planning and Delivery

Treatment planning and irradiation were performed as previously described [8]. In brief, during computed tomography (CT) scanning, each animal was anesthetized using ketamine/xylazine (60 mg/kg, 5.3 mg/kg) intraperitoneal injection. The animals were secured in the prone position on a styrofoam block. The CT isocenter was placed mid-pelvis. For the purpose of 3D images for treatment planning, a diagnostic quality CT scan was performed by a GE AdvantageSim to include the entire pelvis from the iliac crest to the tail base.

The planning CT was imported into Eclipse planning software (Varian Medical Systems, Palo Alto, CA, USA). The prostate, bladder, penile shaft, penile bulb, and rectum were contoured, and then a “rectum + 1 mm” volume was generated. A planning target volume (PTV) was generated by expanding the prostate volume by 1 mm and then subtracting the “rectum + 1 mm” volume. Using dosimetric data from our NovalisTX with multileaf collimation (2.5 mm), a 6 MV single dynamic conformal arc with 360-degree gantry...
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were then placed in a pH 6.0 of citrate buffer
3\% hydrogen peroxide for 10 minutes. The slides
Endogenous peroxidase activity was blocked with
alcohol concentrations between 80\% and 100\%.

deparaffinized and rehydrated with xylene and
previously [12,20]. Briefly, tissue sections were
embedded tissue was performed as described pre-
viously with ED-1, and oxidative stress with 8-OHdG.

Nuclei were counterstained using Hoechst 33342

Immunohistochemistry (IHC) for paraffin-
embedded tissue was performed as described pre-
viously [12,20]. Briefly, tissue sections were
deparaffinized and rehydrated with xylene and
alcohol concentrations between 80\% and 100\%.
Endogenous peroxidase activity was blocked with
3\% hydrogen peroxide for 10 minutes. The slides
were then placed in a pH 6.0 of citrate buffer
(Diagnostic BioSystems, Pleasanton, CA, USA),
and heated in a microwave oven or water bath for
10–20 minutes for antigen retrieval. Tissue sec-
tions were rinsed with phosphate-buffered saline
(PBS) and incubated with primary antibodies to
8-OHdG (1:2,000, JaICA, Shizuoka, Japan),
4HNE (1:100, JaICA), ED-1 (1:200, Serotec,
Oxford, UK), and Nox4 (1:100, Santa Cruz Biotech-
nology, Santa Cruz, CA, USA) overnight at
4°C. Slides were then washed three times in PBS
for 5 minutes, followed by incubation with the
appropriate secondary antibody (1:1,000–2,000,
Jackson Immuno-Research, West Grove, PA,
USA) for 30 minutes at room temperature. Slides
were again washed three times in PBS for 5
minutes, followed by incubation with ABC-Elite
(Vector Laboratories, Burlingame, CA, USA) for
30 minutes at room temperature, and developed
using a 3,3′-diaminobenzidine working solution
(Vector Laboratories, Burlingame, CA, USA) for
30 minutes at room temperature. The sections
were counterstained with Harris’ hematoxy-
lin (Fisher Scientific, Pittsburgh, PA, USA) in
appropriate sample and mounted with coverslips.

In the frozen tissue, expression of inducible
nitric oxide synthase (iNOS) in CC was assessed for
analyzing an inflammatory response. For IHC with
frozen tissue, the skin-denuded penile shaft and
prostate lobe were harvested and stored in optimal
cutting temperature compound at –80°C. Using
the proximal portion of the penile shaft, consecu-
tive 10-\micron sections were cut using a cryomicro-
tome (LEICA CM 1850, Meyer Instruments Inc,
Houston, TX, USA). The sections were fixed in
acetone for 15 minutes. After drying, sections were
washed with PBS twice for 5 minutes each and then
incubated with a blocking solution of 10\% donkey
serum for 30 minutes. Sections were then incubated
with iNOS polyclonal antibody (1:500, Calbio-
chem, San Diego, CA, USA) for overnight at 4°C.
Nuclei were counterstained using Hoechst 33342
(20 \microgram/mL diluted with PBS, Sigma-Aldrich,
St.
Louis, MO, USA) for 5 minutes at room tempera-

ture. Primary antibody signal expressions were
visualized and analyzed through Alexa Fluor 594-
conjugated donkey anti rabbit (1:1,000, Invitrogen,
Carlsbad, CA, USA). Negative controls were
defined as the omission of the primary antibody in
the same microslides section.

Image Analysis

For frozen sections, the whole sample stained by
iNOS antibodies and Hoechst 33342 were
captured with 0.025 \micro liter per step size using a
monochromatic camera under a fluorescence

microscope (Axioskop 2 plus, Carl Zeiss Inc., Oberkochen, Germany) through tetramethylrhodamine isothiocyanate and 4',6-diamidino-2-phenylindole filters. For paraffin sections, four to five randomly selected digital images in the area of CC were acquired from each slide using a ×20–40 objective under an optical microscope (Axioskop 2 plus, Carl Zeiss Inc.). All images underwent semi-quantitative analyses using ImageJ software (Rasband, WS, ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA) by three independent investigators (M.K., A.Z., and Z.R.). To calculate the mean percent areas of positive expression for the markers including iNOS and 4HNE in CC, peripheral components such as urethra, dorsal vessels, or nerves were excluded from measurements. Positive cell counts of 8-OHdG and Nox4 were conducted in randomly selected four to five areas by an automatic nucleus counter plug-in software for ImageJ. Similarly, positive cell count of ED-1 was manually counted using a cell counter plug-in for ImageJ and calculated mean value in four to five sections in the same specimen.

Western Blot Analysis
The snap-frozen penile tissues were homogenized using a bead-beater for 60 seconds three times at 4°C. Homogenized tissues were centrifuged at 13,000 rpm for 5 minutes at 4°C in 700 µL ice-cold buffer containing 1% sodium dodecyl sulfate, 5 mM Tris–HCl (pH 7.4), 2 mM ethylenediamine tetraacetic acid, and 10 µg/mL aprotinin supplemented with 0.5 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA). Protein concentrations were determined using the bicinchonic acid protein assay (Pierce Biotechnology, Rockford, IL, USA) and quantified using a 96-well microplate reader (model680, Bio-Rad, Hercules, CA, USA). Protein concentrations were determined using the bicinchonic acid protein assay (Pierce Biotechnology, Rockford, IL, USA) and quantified using a 96-well microplate reader (model680, Bio-Rad, Hercules, CA, USA). Equal amounts of protein were loaded and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using precast 4–15% polyacrylamide gradient gels (Bio-Rad). Proteins were transferred to polyvinylidene difluoride membranes using a semidry transfer method. Protein transferred membranes were blocked with 5% milk in PBS containing 0.1% Tween 20 and incubated 1 hour at room temperature with antibodies against gp91phox (1:1,000, Santa Cruz Biotechnology), nitrotyrosine (NT; 1:4,000, Abcam, San Diego, CA, USA), and nuclear factor erythroid 2-related factor (Nrf2) (1:2,000, Abcam). Membranes were then washed three times with 0.1% Tween 20 in PBS and incubated with appropriate horseradish peroxidase-conjugated secondary antibodies for 1 hour at room temperature. To control for loading efficiency, blots were reprobed for α-tubulin (Sigma-Aldrich), and all protein levels were normalized with protein loading control for quantitative assessment.

Figure 1 ICP/MAP ratios with 2, 4, 6, and 8 V at 2, 4, and 9 weeks after RT and sham RT, respectively. ICP/MAP ratio was significantly reduced in time-dependent manner in RT group compared to age-matched sham group (N = 9 per group). *P < 0.05 vs. age-matched sham. ICP = intracavernous pressure; MAP = mean arterial pressure; RT = radiotherapy.
Figure 2 Expressions of nicotinamide adenine dinucleotide phosphate oxidase subunits in prostate and penile shaft. (A) Representative histological images of Nox4 staining in the prostate tissue at 2, 4, and 9 weeks (original magnification 200×). Protein expression for Nox4 (black arrow) significantly increased at 9 weeks after RT (N = 8 per group). (B) Representative Western blot image of gp91phox protein in the penile tissue. Relative expression of gp91phox was significant increased in RT comparing to sham at 4 weeks (N = 8 per group). *P < 0.05 vs. age-matched sham. RT = radiotherapy.
Statistical Analysis

Data of physiologic erection response, IHC, and Western blot were analyzed by the Mann–Whitney rank sum test to compare between RT group and age-matched sham RT group at 2, 4, and 9 weeks. To determine the correlation between ICP/MAP ratio and the markers of oxidative stress expressed in CC, scatter plots were illustrated with the Spearman Rank Test to calculate Spearman’s rho ($\rho$). In all box plots, the boxes and error bars represent a mean and standard error of mean, respectively. Statistical significance was considered if the $P$ value is under 0.05. All statistical analyses were conducted using STATA software version 11.0 (Stata Corp, College Station, TX, USA).

Results

ICP Measurement

ICP measurements were conducted in order to analyze nerve-mediated physiological EF. Figure 1 represents the results of ICP/MAP ratio utilizing electrical CN stimulation (2, 4, 6, and 8 V) at 2, 4, and 9 weeks after RT. At 2 weeks, no differences of
ICP/MAP ratio were seen between sham and RT group ($P = 0.397$, $0.733$, $0.984$, and $0.375$). At 4 weeks, there was significant decrease in ICP/MAP ratio with 8 V compared to age-matched sham animals ($P = 0.462$, $0.401$, $0.058$, and $0.019$). At 9 weeks, all ICP/MAP ratios were significantly decreased in all voltages compared to age-matched sham animals ($P = 0.001$, $0.002$, $0.009$, and $0.002$).

**NADPH Oxidase Subunits Expression in Prostate and CC**

To determine NADPH oxidase subunit expression in irradiated prostate and penile tissue, protein expression levels of Nox4 in prostate and gp91phox in CC were investigated (Figure 2). The positive cell count for Nox4 in irradiated prostate was statistically significant compared to sham at 9 weeks ($P = 0.146$, $0.674$, and $0.015$). In Western blot analysis, the relative expression level of gp91phox in CC significantly increased in RT group compared to sham group at 4 weeks.

**DNA Oxidation in Prostate and CC**

8-OHdG expression was evaluated in CC and prostate for determining DNA oxidative stress (Figure 3). The increase of positive 8-OHdG cell in irradiated prostate was obvious from 4 weeks and was statistically significant at 4 and 9 weeks compared to age-matched sham group ($P = 0.674$, $<0.001$, and $0.001$). Increments of positive 8-OHdG cell in CC was also found in RT animals in a time-dependent fashion, which reached statistical significance at 9 weeks compared to age-matched sham RT animals ($P = 0.301$, $0.253$, and $0.037$).

**Lipid Peroxidation in CC**

To investigate lipid peroxidation, IHC for 4HNE in CC were evaluated (Figure 3). The expression
areas of 4HNE consistently increased from 2 weeks following RT, which was statistically significant at 4 weeks compared to age-matched sham ($P = 0.071, 0.027, \text{ and } 0.087$).

**Inflammatory Responses in Prostate and CC**

For investigating inflammation responses, protein expressions of ED-1 (prostate tissue), iNOS (penile shaft), and NT (penile shaft) were evaluated by IHC or Western blot analysis (Figure 4). In irradiated prostate tissue, the expression levels of ED-1 dramatically increased in prostate from 2 weeks compared to age-matched sham treatment group and was statistically significant at all time points ($P = 0.009, <0.001, \text{ and } 0.012$). The expression of iNOS protein in CC significantly increased from 2 weeks after RT and a trend for increased levels throughout the observation period compared to sham group ($P = 0.049, 0.038, \text{ and } 0.249$). The relative protein expression of NT in CC was significantly increased at 9 weeks after RT ($P = 0.105, 0.577, \text{ and } 0.045$).

**Antioxidant Responses in CC**

Nrf2 protein expression was evaluated for antioxidant response in CC (Figure 5). Western blotting analysis showed increased Nrf2 protein expression from 4 weeks in RT group compared to age-matched sham RT group but without reaching statistical significance ($P = 0.767, 0.175, \text{ and } 0.269$).

**Correlations between ICP/MAP Ratio and Oxidative Stress Markers in CC**

The scatter plots illustrate the association between ICP/MAP ratio in 8 V and the results of semi-quantitative analyses for oxidative stress markers in CC (Figure 6). Figure 6A indicated the negative association between ICP/MAP ratio and expression level for 8-OHdG, demonstrating that rat
with lower ICP/MAP ratio are more likely to have DNA oxidative stress in CC ($r = -0.355$, $P = 0.043$). Similarly, Figure 6B demonstrated that rats with impaired EF are more likely to have lipid peroxidation in CC ($r = -0.403$, $P = 0.018$).

**Discussion**

Previous study demonstrated that our prostate-targeted radiation technique impaired EF with extremely low toxicity [9]. There were no general condition changes such as weight loss and decreased water intake between sham and RT groups. Also, average MAP in ICP measurements and serum testosterone were of the same levels in both groups at all time points, which reflect that current RT technique has little systemic effects.

We also found that radiation-induced ED was associated with CN injury prior to a reduction of smooth muscle content in the CC [9]. The present study provides evidence that oxidative stress plays a pivotal role in radiation-induced ED, demonstrating a remarkable increase in chronic oxidative stress measured by 8-OHdG and 4HNE accompanied with upregulation of NADPH oxidase (Figure 7). In addition, we demonstrated that higher expression of oxidative stress markers in penile tissues was associated with physiological ED evaluated by ICP measurements with electrical CN stimulation (Figure 6).

Impairment of EF after prostate cancer treatments is a major concern of patients and clinicians. Development of ED impacts not only sexual health, but also influences overall HRQoL.
**Figure 4** Continued.

- **Panel B**: Images showing the inducible NOS expression in CC over 2 weeks, 4 weeks, and 9 weeks for Sham and RT groups.
- **Panel C**: Bar graphs comparing nitrotyrosine and α-tubulin expression levels across different weeks for Sham and RT groups.
in prostate cancer patients after definitive treatment [21,22]. Even with 3DCRT, approximately 40% of men were diagnosed with ED within 3 years following treatment [23]. Considering that phosphodiesterase type 5 inhibitors are not fully able to ameliorate radiation-induced ED, evidenced by the existence of “non-responders,” a novel prevention and therapeutic strategy is needed based upon insights and findings from basic research on radiation-induced ED [24].

To the best of our knowledge, this is first study investigating oxidative stress in a rat model of radiation-induced ED.

Oxidative stress is well recognized as a pathogenesis of ED in atherosclerosis, hypertension, diabetic mellitus, hypercholesterolemia, cigarette smoking, and CN injury [25–32]. In fact, peroxynitrite (ONOO−) converted from NADPH oxidase-generated superoxide free radicals (O2•−) causes a decrease in nitric oxide (NO) bioavailability and ineffective smooth muscle relaxation [33]; we hypothesize this as a potential mechanism contributing to radiation-induced ED development. Although we could not measure NO concentration in the present study, considering the increased expression of NADPH subunits in prostate and penile shaft after RT, oxidative stress seems to play an important role in the current animal model of radiation-induced ED.

Chronic inflammation was found in prostate and penile tissue after prostate irradiation. In general, acute inflammatory responses occur following irradiation, such as activation of stress-sensitive kinases, transcription factors, and increased production of inflammatory cytokines [19]. Consequently, the antioxidant response could occur against acute inflammatory responses. We investigated Nrf2 protein, one of the major endogenous cellular antioxidant response systems; the expression of Nrf2 was increased from 4 weeks after RT. Although the data are not shown in this study, increased fibrotic changes were observed in irradiated prostate tissue by Masson’s Trichrome staining. This suggests that these inflammatory reactions may overwhelm the healing process throughout the observation period after irradiation. Thus, our observations suggest that chronic upregulation of inflammatory responses is associated with fibrotic changes in irradiated prostate tissue. Interestingly, the inflammatory response was also observed within CC, downstream of the irradiated organ. Based on the data obtained in this study, we hypothesize that the inflammatory responses in CC might be associated with...
decreased smooth muscle content in CC after RT, which was found in our previous study [9].

Associations between ICP/MAP ratio and expression level of 8-OHdG and 4HNE were somewhat intriguing. Based on our results, higher oxidative stress in penile tissue was more likely to have lower physiological EF following RT, which was statistically significant (Figure 6). These findings imply that the markers of oxidative stress might be one of feasible biomarkers for EF after prostate irradiation. In a clinical study, Yasuda et al. evaluated the association of salivary 8-OHdG

Figure 6 Associations between ICP/MAP ratio and expression levels of oxidative stress markers in CC. (A) Scatter plot with linear prediction plot regarding relationship between ICP/MAP ratio and protein expression level of 8-OHdG in CC ($r = -0.355$, $P = 0.043$) ($N = 33$). (B) Scatter plot with linear prediction plot regarding relationship between ICP/MAP ratio and protein expression level of 4HNE in CC ($r = -0.403$, $P = 0.018$) ($N = 34$). ICP = intracavernous pressure; MAP = mean arterial pressure; CC = corpora cavernosa; 8-OHdG = 8-hydroxy-2′-deoxyguanosine; 4HNE = 4-hydroxynonenal

to EF using validated questionnaires in 128 middle-aged male volunteers, revealing that 8-OHdG was significantly associated with the severity of ED in healthy volunteers [34]. Their conclusions suggest that systemic levels of oxidative stress correlate to ED, which partially supports our findings. Based on our results, we consider antioxidant therapy to be a potential therapeutic option for ED after prostate RT.

There are several limitations to this work. Although we confined irradiation to only the targeted organ (prostate) using image-guided current technology, we could not eliminate a potential influence of low-dose radiation scatter from the target organ while RT was being administered. Similarly, some areas of penile shaft, especially the distal area which was not analyzed, could be indirectly irradiated because of quite close distance to the target. Reviewing our animal treatment plans, the calculated average dose in penile bulb was 0.16 Gy. Second, we did not investigate oxidative stress and inflammatory responses in CN. Since the CN was included within the target RT volume and we reported degeneration of CN at 4 weeks after RT in previous study, it is possible that CNs were affected by direct irradiation damage as well as oxidative stress that we found in the prostate. Albeit current findings, further study is needed to form a comprehensive understanding for radiation-induced ED.

In summary, the current data show that time-dependent physiological EF impairment occurs following prostate-confined stereotactic RT with extremely low toxicity. The increase in NADPH oxidase, persistent oxidative stress, and chronic inflammatory responses were investigated in irradiated prostate tissue as well as in penile tissue, even though CC was not directly targeted (Figure 7). Additionally, the functional parameter of ICP/MAP ratio and the expression of oxidative stress markers in CC were strongly correlated, suggesting that oxidative stress plays a crucial role in the development of radiation-induced ED. We assume that radiation-induced ED is multifactorial, but this initial exploratory analysis suggests that oxidative stress within the cavernosa, even though not directly irradiated, does play some role and warrants more detailed investigations. Also, our findings indicate that the application of antioxidant therapy for radiation-induced ED is likely to be a promising therapeutic option, as it may ameliorate oxidative stress not only in irradiated area such as prostate tissue and CNs but also penile tissue, which might protect HRQoL for prostate cancer patients who undergo RT.

Figure 7 Radiation-induced upregulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits such as gp91phox and Nox4 in prostate and penile shaft tissues generates reactive oxygen species (ROS). Imbalance between ROS and antioxidants (Nrf2) leads to persistent oxidative stress resulting in chronic inflammatory responses in those tissues after RT. This provides further support for the hypothesis that oxidative stress might play a key role in radiation-induced erectile dysfunction with regard to fibrotic changes in cavernous tissue, loss of smooth muscle, and endothelium dysfunction. CN = cavernous nerve; 8-OHdG = 8-hydroxy-2’-deoxyguanosine; 4HNE = 4-hydroxynonenal; Nrf2 = nuclear factor erythroid 2-related factor; iNOS = inducible nitric oxide synthase; NT = nitrotyrosine

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Corresponding Author: Masaki Kimura, MD, PhD, 207 Research Dr, 152 Jones Building, Duke University Medical Center, Durham, NC 27710, USA. Tel: (1) 919-681-2632; Fax: (1) 919-681-2651; E-mail: masaki.kimura228@gmail.com

Conflict of Interest: None.

Statement of Authorship

Category 1
(a) Conception and Design
Masaki Kimura; Zeljko Vujaskovic
(b) Acquisition of Data
Masaki Kimura; Andrew R. Zodda; Hui Yan
(c) Analysis and Interpretation of Data
Masaki Kimura; Zahid N. Rabbani

Category 2
(a) Drafting the Article
Masaki Kimura
(b) Revising It for Intellectual Content
Isabel L. Jackson; Thomas J Polascik; Craig F. Donatucci; Judd W. Moul

Category 3
(a) Final Approval of the Completed Article
Bridget F. Koontz; Zeljko Vujaskovic

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