Free Radical Biology and Medicine 103 (2017) 248–255

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Hyperbaric oxygen treatment reverses radiation induced pro-fibrotic and oxidative stress responses in a rat model

N. Oscarsson[a,*], L. Ny[b], J. Mölne[c], F. Lind[d], S.-E. Ricksten[a], H. Seeman-Lodding[a], D. Giglio[b,e]

[a] Department of Anesthesiology and Intensive Care Medicine, Institute of Clinical Sciences, the Sahlgrenska Academy, University of Gothenburg, Sweden
[b] Department of Oncology, Institute of Clinical Sciences, the Sahlgrenska Academy, University of Gothenburg, Sweden
[c] Department of Pathology, Institute of Biomedicine, the Sahlgrenska Academy, University of Gothenburg, Sweden
[d] Section of Anesthesiology and Intensive Care, Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden
[e] Department of Pharmacology, Institute of Neuroscience and Physiology, the Sahlgrenska Academy, University of Gothenburg, Sweden

ARTICLE INFO

Keywords:
Radiation induced injuries
Hyperbaric oxygen
Oxidative stress
Fibrosis
Cystitis

ABSTRACT

Purpose: Radiotherapy is effective in the treatment of tumors in the pelvic area but is associated with side effects such as cystitis and proctitis. Hyperbaric Oxygen Therapy (HBOT) has emerged as a treatment modality for radiation-induced side effects. In a rat model for radiation cystitis, we studied the effects of HBOT on oxidative stress and pro-fibrotic factors.

Materials and methods: Sedated Sprague-Dawley rats underwent bladder irradiation of 20 Gy with and without 20 sessions of HBOT during a fortnight. Control animals were treated with and without HBOT. All four groups of animals were euthanized 28 days later. Histopathological examinations, immunohistochemistry and quantitative polymerase chain reaction (qPCR) were used to analyze changes in oxidative stress (8-OHdG), anti-oxidative responses (SOD-1, SOD2, HO-1 and NRFα) and a panel of Th1-type and Th2-type cytokines (IL-1β, IL-4, IL-5, IL-6, IL-10, IL-13, TNF, TGF-β, IFN-γ) in the urinary bladder.

Results: Bladder irradiation increased the expression of 8-OHdG, SOD2, HO-1, NRFα, IL-10, TNF and tended to increase TGF-β. These changes were completely reversed by HBOT while HBOT in control animals had no effects on the studied markers for oxidative stress, anti-oxidative responses and Th1-type and Th2-type cytokines.

Conclusions: Radiation induced a significant elevation of oxidative stress, antioxidants and pro-fibrotic factors in our animal model for radiation cystitis that were completely reversed and normalized by HBOT. Our findings indicate that HBOT may prevent radiation-induced changes by affecting oxidative stress and inflammatory cascades induced by radiation.

Summary: Radiotherapy may cause the development of chronic inflammation and fibrosis, significantly impairing organ function. We hypothesized that bladder irradiation induces an oxidative stress reaction, thereby triggering the redox system and thus initiating an inflammatory and pro-fibrotic response. We aimed to assess whether these changes would be reversed by hyperbaric oxygen using an animal model for radiation cystitis. Our study show that hyperbaric oxygen may reverse oxidative stress and pro-inflammatory factors induced by radiation.

1. Introduction

Radiotherapy (RT) is an important cancer treating modality. However, adjacent organs may be affected by radiation-induced side effects. These side effects are critical dose-limiting factors and may prove both an acute or chronic nature. Clinical side effects such as increased micturition frequency, urgency, hematuria and pain affect 3–18% of patients undergoing RT for tumors in the pelvic area. These side effects may have severe impact on quality of life and may increase morbidity and mortality [1–3].

Patients with severe symptoms of radiation-induced side effects are mainly offered symptomatic treatment where long-term efficacy is poor [4]. Hyperbaric oxygen treatment (HBOT) ameliorates symptoms of late radiation-induced side effects and relieves patient-perceived symptoms following cancer treatment [5–7]. The exact mechanisms through which HBOT exerts its beneficial effects are only partially understood.

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; HBOT, Hyperbaric Oxygen Therapy; HO-1, Heme oxygenase 1; IFN-γ, Interferon gamma; IL-x, Interleukin; qPCR, quantitative Polymerase Chain Reaction; ROS, Reactive Oxygen Species; RT, Radiotherapy; SOD-x, Superoxid dismutase; TGF-β, Transforming growth factor beta; TNF, Tumor necrosis factor

* Corresponding author.
E-mail addresses: nicklas.oscarsson@vgregion.se, Nicklas.oscarsson@gmail.com (N. Oscarsson).

http://dx.doi.org/10.1016/j.freeradbiomed.2016.12.036
Received 30 June 2016; Received in revised form 10 December 2016; Accepted 24 December 2016
Available online 27 December 2016

Studies suggest that HBOT may stimulate the migration and differentiation of stem cells, by inducing growth of new blood vessels and reducing the development of fibrosis [8–10]. It seems that HBOT is not only able to reduce and revert some of the late radiation-induced side effects [5], but may also prevent late side effects of radiotherapy if given early [8]. However, knowledge is scarce on the effects of HBOT administered in adjunction to RT, especially at an early stage, and randomized controlled trials (RCTs) are lacking.

RT causes the production of reactive oxygen species (ROS) leading to an increased oxidative stress in the tissue [11]. An imbalance between the oxidative stress and the redox system triggers a downstream cascade of events, with the accumulation of extracellular matrix and the development of fibrosis, which may lead to late radiation-induced side effects [12]. An important factor driving the development of fibrosis is transforming growth factor beta (TGF-β) [13]. Radiation also triggers an innate immune response in the affected tissue, resulting in the release of cytokines and inflammation [3,11]. The Th1 immunological response is characterized by the release of cytokines such as IL-1, IL-6 and IFN-γ, while the Th2 immunological response is characterized by IL-4, IL-10 and IL-13 [14]. Th2-type cytokines may inhibit the Th1 immunological response [14]. In a previous study we demonstrated that 14 days after bladder irradiation, IL-6 and nitrergic pathways were suppressed while IL-10 was up-regulated in the urinary bladder [15]. Hence bladder irradiation seemed to trigger an anti-inflammatory response.

The lack of RCTs and limited knowledge on the mechanisms behind the effectiveness of HBOT on radiation-induced side effects, leads to difficulties in defining strategies for appropriate interventions and timing. Relevant animal models are therefore valuable to study mechanisms involved, as well as for evaluation of therapeutic interventions before future clinical studies. In the present study, we hypothesized that bladder irradiation induces an oxidative stress reaction, thereby triggering the redox system and thus initiating an inflammatory and pro-fibrotic response. We aimed to assess whether these changes would be reversed by HBOT.

2. Materials and methods

Study protocol was approved by the Animal Ethics Committee at the University of Gothenburg. All animal work was performed according to the NIH guidelines for the use of experimental animals. The manuscript was prepared in accordance with the ARRIVE guidelines.

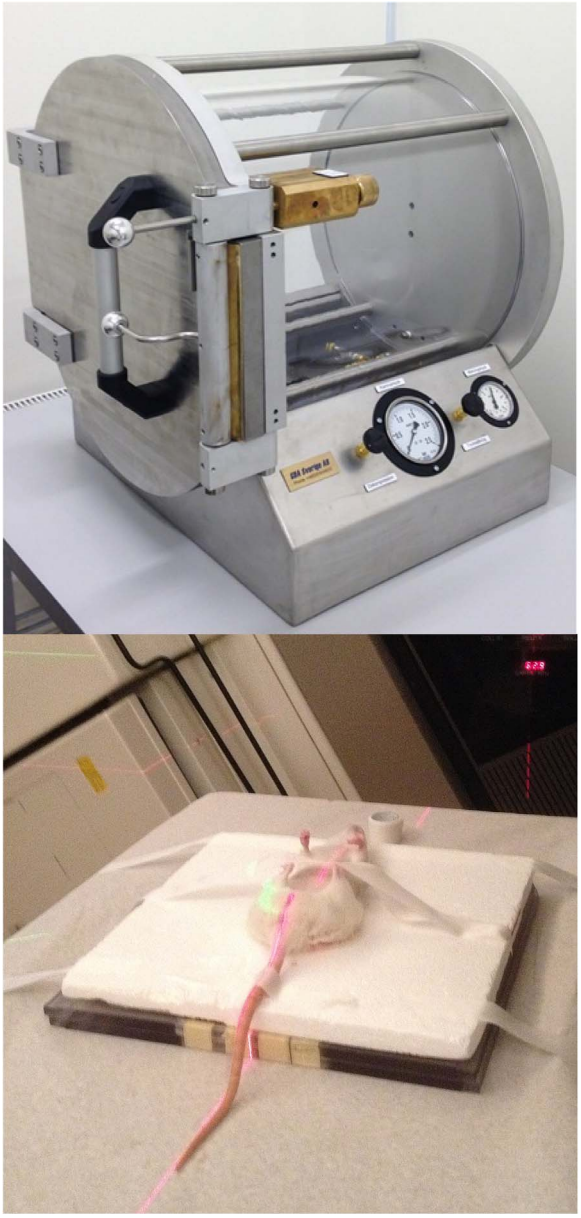
Sprague-Dawley female rats (250–300 g; n = 39; CD® IGS rat, Charles River, Germany) were housed in a temperature-controlled facility with a day and night cycle, and had free access to food and water during the entire study period. Welfare-related assessment was carried out daily during the duration of the study.

2.1. Urinary bladder irradiation

Rats were sedated with pentobarbitone (50 mg/kg) and medetomidine (10 μg/kg) and placed in the supine position with their legs on their abdomens (Picture 1). The urinary bladder was exposed to one fraction of 20 Gy using a linear accelerator with 6 MV nominal photon energy (Varian Medical Systems Inc., Palo Alto, CA, USA). Radiation was administered employing two side-fields to avoid exposure of the spinal cord.

2.2. Hyperbaric oxygen therapy (HBOT)

HBOT was administered in a custom-built hyperbaric chamber (GDA Sverige AB, Gothenburg, Sweden; Picture 1). During each session, five animals were placed in the chamber, which was filled with 100% oxygen and pressurized to 200 kPa for 90 min. The oxygen level in the chamber was monitored every 15 min during the treatment. The flow of fresh oxygen was adjusted so that the level of oxygen in the chamber stayed over 95% at all times during the treatment sessions of HBOT. The rats were treated twice daily (Monday to Friday) with at least four hours between the treatments over a period of two weeks, rendering a total of 20 treatments.



Picture 1. The custom built hyperbaric chamber used (upper photo). For bladder exposure to one fraction of 20 Gy, rats were placed in the supine position. Radiation was administered employing two side-fields to avoid exposure of the spinal cord.

2.3. Experimental setup

Rats were allocated to one of four study arms. A: Control rats (no RT, no HBOT) were sedated on day 1 and then kept without interventions in their cages for 28 days. B: HBO control rats (no RT, HBOT) were sedated and then kept in their cages for 14 days before receiving 20 sessions of HBOT over a period of 14 days. C: Radiation rats (RT, no HBOT) underwent sedation and bladder irradiation on day 1 and were subsequently held in their cages for 28 days. D: HBO radiation rats (RT, HBOT) underwent sedation and bladder irradiation on day 1. They were then kept in their cages for 14 days before receiving 20 sessions of HBOT over a period of 14 days (Fig. 1). All rats were euthanized at day 29 with a bolus dose of pentobarbitone. The urinary bladder was

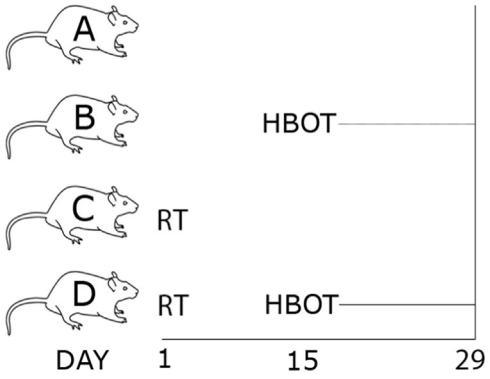


Fig. 1. Experimental set-up. Radiation was administered on day 1 for the radiation group (RT, no HBOT (C)) and for the HBO radiation group (RT, HBOT (D)), while the control group (no RT, no HBOT (A)) and the HBO control groups (no RT, HBOT (B)) were only sedated on day 1. On day 14 the HBO radiation and the HBO control groups were administered HBOT twice daily during 14 consecutive days. All animals were euthanized on day 29.

excised, visually inspected, weighed and cut into two sagittal parts. One part was frozen to −70 °C for later mRNA extraction and one part was put in formalin for paraffin embedding.

2.4. Histological analysis and immunohistochemistry

Immunohistochemistry was performed on 6 μm-sections of paraffin-embedded specimens of the urinary bladders. Four to five slides from each group were randomly selected for each antibody.

After deparaffination in xylene and ethanol, sections were hydrated in tap water and washed in phosphate-buffer saline (PBS). Antigen retrieval was done by cooking the sections for 60 min in citrate buffer, followed by washing in PBS, and incubation with copper sulfate solution for 2 h to quench any auto fluorescence. Further, sections were washed in PBS followed by blocking unspecific binding with either goat serum (5%; Sigma-Aldrich (SA), St. Louis, MO, USA) or horse serum (5%; SA) depending on the primary antibody used. This was followed by incubating the sections with the primary antibody (see list below) overnight at 4 °C. The next day, sections were washed in PBS and incubated with the secondary antibody (see list below) for 1 h. This was followed by washes in PBS and dehydration in ethanol (95–99%). ProLong® Gold Antifade Reagent with DAPI (Life technologies Ltd, Paisley, UK) was then put on the sections and a cover glass was mounted on top of the section on the glass slide. Bladder sections were also stained by Van Gieson and Hematoxylin-eosin staining and histological assessments were made by an assessor blinded to group allocation. Adobe Photoshop CS6 (Version 13.0.1 × 64) was used to measure the degree of staining of 8-hydroxy-deoxyguanosine (8-OHdG) in bladder sections. The mean intensity value from three representative areas from each slide was calculated to measure the intensity of staining. The pixel intensity value for the measured RGB-channel was converted to a percentage of the maximum value of 255.

The following antibodies were used for immunohistochemistry (concentration): Mouse anti-8 hydroxyguanosine (1:500; Abcam, Cambridge, MA, USA; ab62623), mouse anti-heme oxygenase 1 (1:100; Santa-Cruz Biotechnology; SCBT; sc-136960), mouse anti-SOD2 (1:50; SCBT; sc-137254) and rabbit anti-SOD1 (1:1000; SCBT; sc-11407).

2.5. Quantitative polymerase chain reaction (q-PCR)

Bladder tissues were homogenized and RNA extracted according to the protocol of Qiagen RNeasy (Qiagen Ltd, Manchester, UK). Bladder tissues were homogenized and RNA extracted according to the protocol of Qiagen RNeasy (Qiagen Ltd, Manchester, UK). The quality of the RNA samples was assessed with the Tapestation 2200 (Agilent Technologies, Santa Clara, CA, USA). The DeNovix DS-11 spectrophotometer was used to assess the concentration and purity of RNA. c-DNA was synthesized and gene expression was analyzed using the TaqMan gene expression assays. The qPCR was performed using the TaqMan® Gene Expression Master Mix (Part. No. 4369016, Life Technologies, Carlsbad, CA, USA) and NTC for the qPCR was included and performed on the QuantStudio 12 K Flex (Life Technologies, Waltham, MA USA). The PCR reaction mix consisted of TaqMan® Gene Expression Master Mix (5 μl; 2X), NFW (2.5 μl), TaqMan® gene expression assay (0.5 μl; 20X) and cDNS template (2 μl). The Uracil N-Glycosylase protocol was performed for 2 min (95 °C), activation for 10 min (95 °C), denaturation for 15 s (95 °C) and annealing and elongation for 1 min 40–45 s (60 °C; 40–45 cycles). The following TaqMan® assays were used (all primers were purchased from ThermoFisher Scientific): Heme oxygenase (Rn01536933_m1), IL-1β (Rn00580432_m1), IL-4 (Rn01456865_m1), IL-5 (Rn01459975_m1), IL-6 (Rn99999011_m1), IL-10 (Rn00563409_m1), IL-13 (Rn005876-15_m1), INFgamma (Rn00594078_m1), NRF2α (Rn01767215_m1), SOD-1 (Rn00566938_m1), SOD-2 (Rn00690588_g1), TNF-α (Rn99999017_m1), TGF-β (Rn00572010_m1) and GADPH (NM_017008.4).

2.6. Statistics

Analysis of variance (ANOVA) was used to determine significant differences between mean values. Tukey HSD post-hoc analysis was used for multiple comparisons between groups. Values from luminosity measurements were analyzed for normal distribution using the Shapiro-Wilk test and for homogeneity using the Leven´s test. The mean Threshold Cycle (C_T) value of the two replicates from qPCR was calculated. In the cases where one C_T-value was undetermined, the determined value was used. In the cases where both replicates had undetermined values, the highest C_T-value for the group and analysis +1 was used. Gene expressions were related to the expression of GADPH. Mean value for each qPCR analysis for the control group (no RT, no HBO) was considered baseline and adjusted to 1. Results from all other groups were calculated as fold change from baseline.

The level of statistical significance was set at $P < 0.05$. All statistical analyses were made using IBM SPSS Version 23. All graphs were rendered in GraphPad Prism Version 6.07.

3. Results

3.1. Animals and morphology

The average body weight of the rats was 302 ± 39 g (n = 39) at the time of euthanasia. No body or bladder weight differences were observed between the different treatment groups. No significant macro- or microscopically changes in the urinary bladder could be observed by bladder irradiation or HBOT 28 days following bladder irradiation compared with controls (Fig. 2).

3.2. Oxidative stress and anti-oxidative response

Bladder irradiation induced a pronounced up-regulation of the oxidative stress marker 8-hydroxy-deoxyguanosine (8-OHdG) particularly in the urothelium (Fig. 3). In the urothelium of the radiation group, the mean intensity value of 8-OHdG expression was 34 ± 14% (n = 4) compared with 5 ± 2% in the control group (n = 4; $p < 0.001$). In the HBO radiation group, the mean intensity value of the expression of 8-OHdG was 11 ± 5% in the urothelium and hence significantly attenuated compared with the radiation group (n = 4; $p < 0.001$). To note, 8-OHdG was not only expressed in the nuclei but also in the cytoplasm of urothelial cells in response to bladder irradiation. In control animals, expression of 8-OHdG was low and not affected by HBOT (Fig. 3).

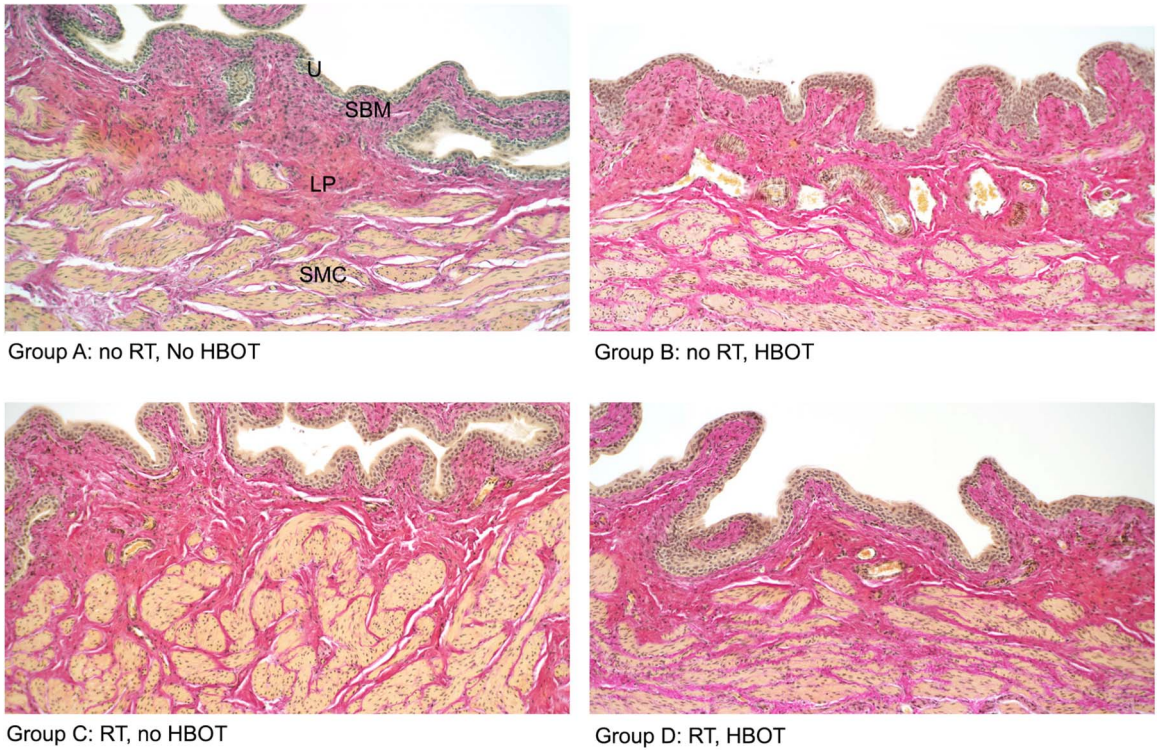


Fig. 2. Histological microphotographs on urinary bladder sections stained with van Gieson from the four different groups. No significant differences were observed between the groups. U = Urothelium, SBM = Submucosa, LP = Lamina Propria, SMC = Smooth Muscle Cell layer.

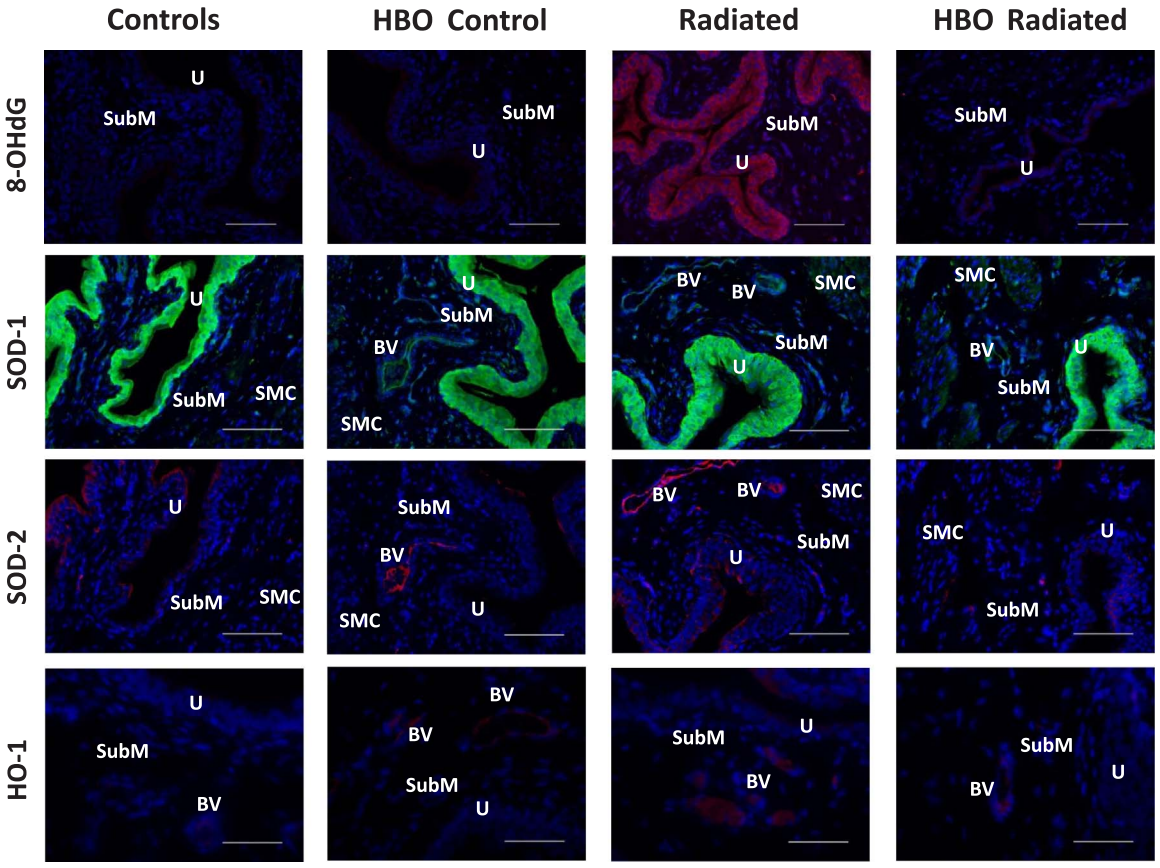


Fig. 3. Microphotographs of urinary bladder specimens immunostained for 8-OHdG, SOD-1, SOD-2 and HO-1 in controls, HBO control group, radiated group and HBO radiated group, respectively. Upregulation of 8-OHdG was observed in the urothelium in the radiation group which was reversed by HBOT (first row). SOD-2 was primarily upregulated in blood vessels and in the urothelium by bladder irradiation (third row). BV = blood vessel(s), SMC = smooth muscle cells, SubM = Submucosa and U = Urothelium. Horizontal bars indicate 100 μm.

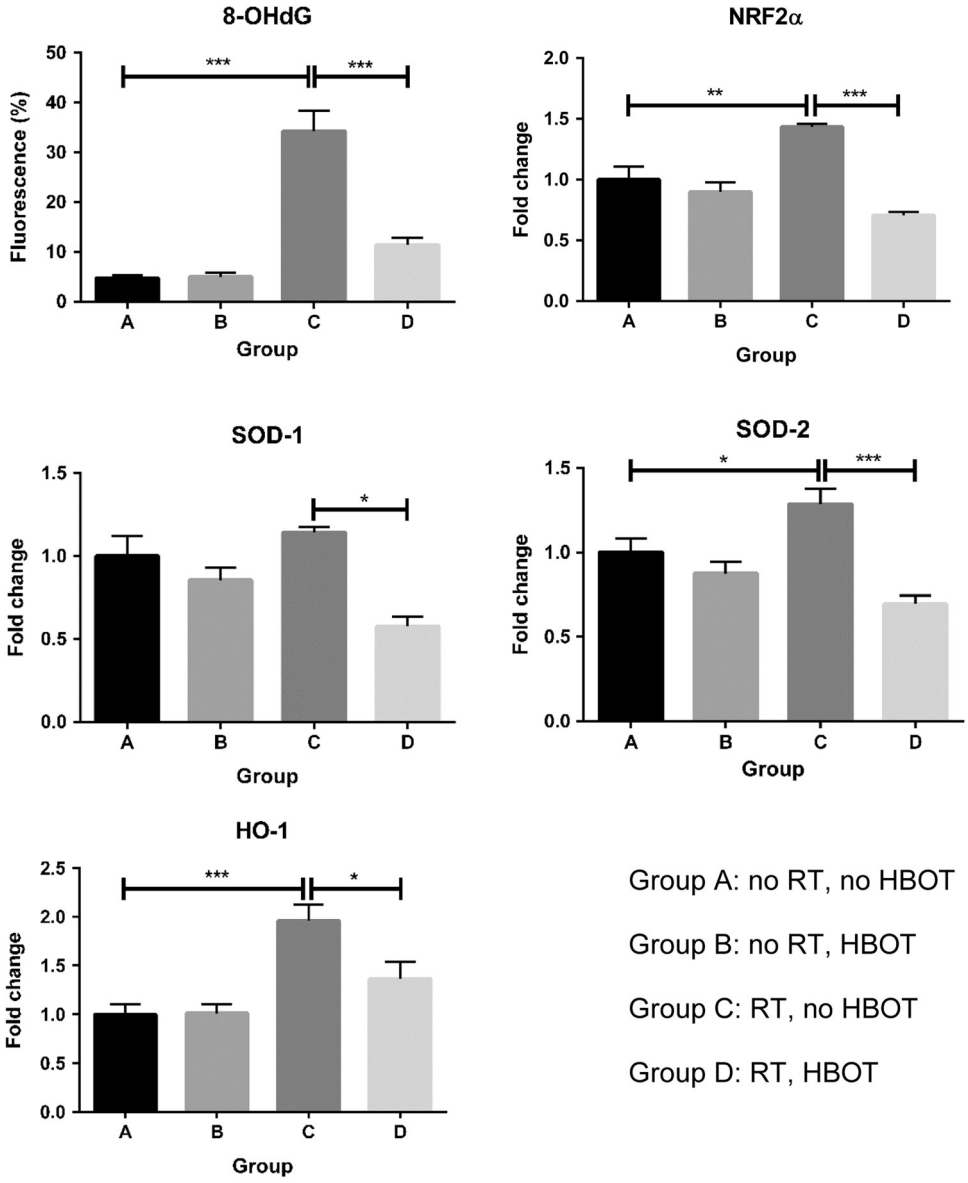


Fig. 4. Expression of 8-OHdG in the urothelium and urinary bladder expressions of SOD-1, SOD-2, NRF2α and HO-1 mRNAs in controls (no RT, no HBOT), HBO control group (no RT, HBOT), radiated group (RT, no HBOT) and HBO radiated group (RT, HBOT), respectively (n = 8–10). * indicates $p < 0.05$, ** indicates $p < 0.01$ and *** indicates $p < 0.001$. Vertical bars represent S.E.M.

Bladder irradiation induced up-regulations of nuclear respiratory factor 2α (NRF2α), superoxide dismutase 2 (SOD-2) and hemeoxygenase-1 (HO-1) in the bladder and these responses were significantly attenuated by HBOT ($p < 0.001$–0.05; n = 8–10; Fig. 4). While the expression of SOD-1 was not statistically changed by bladder irradiation, the expression in irradiated bladders was significantly reduced by HBOT ($p < 0.05$; n = 9–10). In contrast, HBOT did not affect the expression of NRF2α, SOD-1, SOD-2 and HO-1 in control animals. Immuno-histochemical analyses showed that SOD-1 and SOD-2 were expressed in the urothelium of control urinary bladders. SOD-2 and HO-1 were also expressed in blood vessels and the expressions were increased upon bladder irradiation. HBOT attenuated irradiation-induced increases of HO-1 and SOD-2 in blood vessels and the increase of SOD-2 in the urothelium (Fig. 3).

3.3. Inflammatory response to irradiation

A panel of cytokines representing Th1 and Th2 immunological responses was analyzed with qPCR in bladder tissue from the different treatment groups (Fig. 5). Bladder irradiation increased the expression of IL-10 and TNF ($p < 0.01$; n = 7 and 8) and tended to increase the expression of TFG-β in the urinary bladder. HBOT attenuated the expressions of IL-10 and TFG-β ($p < 0.05$; n = 7) and tended to decrease the expression of TNF in the irradiated bladder. In contrast, HBOT did not affect the expressions of IL-10, TFG-β and TNF in control animals. The expressions of IL-1β, IL-4, IL-5, IL-6, IL-13 and IFN-γ were neither affected by irradiation nor by HBOT (Fig. 5).

4. Discussion

This study aimed to test the hypothesis that HBOT would reverse radiation-induced responses in oxidative stress and immune activation in the urinary bladder. Radiation induced a significant elevation of oxidative stress, anti-oxidants and pro-fibrotic factors. HBOT was able to reverse these changes, whereas HBOT in controls had no effect on any of the responses investigated. Tentatively our results may explain some of the beneficial effects of HBOT. Evidence for treatment recommendations is limited and there is a need for RCTs to support

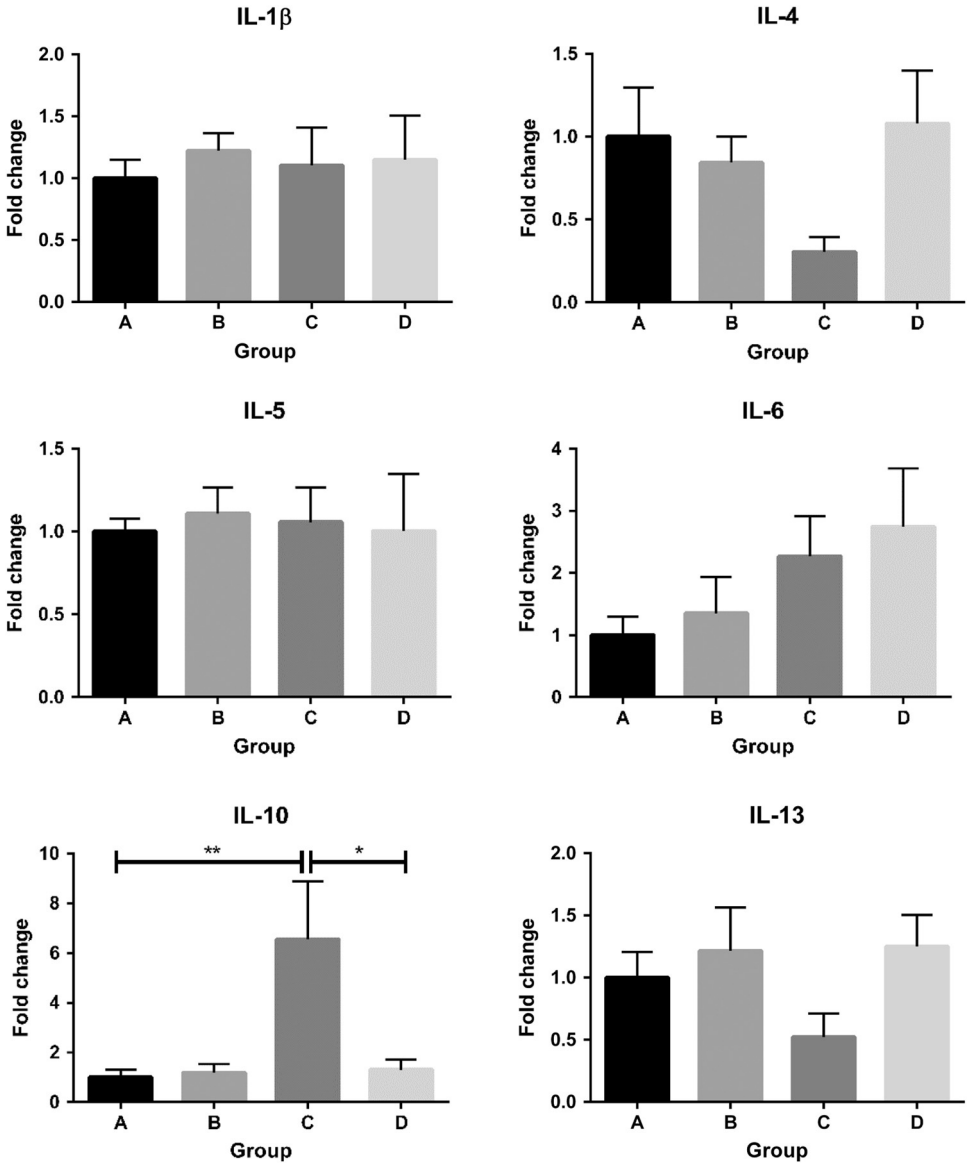


Fig. 5. Urinary bladder expressions of IL-1β, IL-4, IL-5, IL-6, IL-10, IL-13, IFNγ, TNFα and TGF-β mRNAs in controls (no RT, no HBOT), HBO control group (no RT, HBOT), radiated group (RT, no HBOT) and HBO radiated group (RT, HBOT), respectively (n = 8–10). * indicates $p < 0.05$ and ** indicates $p < 0.01$. Vertical bars represent S.E.M.

clinical decision making. The present study was conducted to provide an experimental foundation for such future clinical trials.

We analyzed oxidative stress by studying the expression of 8-OHdG [16], which was particularly up-regulated in the urothelium. 8-OHdG appeared not only to be up-regulated in the nuclei but also in the urothelial cytoplasm, which suggests that it was also generated in mitochondria. Increased oxidative stress typically triggers an anti-oxidative response. We analyzed factors involved in the endogenous oxidative stress defense system. Bladder irradiation significantly increased Nrf2α and the antioxidants HO-1 and SOD-2, while no significant change in SOD-1 was observed. Nrf2α controls the expression of many genes involved in the detoxification of ROS through conjugate reactions and by enhancing the cellular antioxidant capacity including HO-1 [17,18]. SOD-2 is almost exclusively active in the mitochondria and seems to be less affected by an increased level of Nrf2α [19]. We suggest that mitochondria play an important role for oxidative stress in the urinary bladder in light of the fact that SOD-2 was affected by bladder irradiation, SOD-1 affected to a lesser extent and that 8-OHdG also was up-regulated in the cytoplasm. Oxidative stress can lead to dysfunction of the mitochondria, leading to a higher load of ROS, which might trigger apoptosis [21]. Further, TGF-β can enhance ROS production and suppress the redox system, creating an augmented level of oxidative stress [20].

Ultraviolet (UV) light and gamma-radiation exposition may lead to changes in the balance between Th1 and Th2 immune responses. UV light may trigger up-regulation of IL-10 in T-lymphocytes [21,22]. Gamma radiation may trigger IL-10 release and tilt the Th1/Th2 balance to a Th2 immune response in the spleen, [23]. IL-10 has been shown to induce a Th2 immune response and to reverse the development of fibrosis in the lung [24], in the liver [25] and the heart [26]. IL-10 may regulate the expression of TGF-β and TGF-β is tightly connected to oxidative stress and a mediator of fibrosis [27–29]. In our previous study [15], we observed an up-regulation of IL-10 in the bladder submucosa 14 days after bladder gamma irradiation. At 14 days following bladder irradiation, the overall bladder immune response favored a Th2 immune response rather than a Th1 immune response. The present findings show that IL-10 is up-regulated still 28 days after bladder irradiation. In addition to IL-10, we observed an up-regulation of TNF and tendencies to an up-regulation of TGF-β in the urinary bladder in response to bladder irradiation. In line with our findings, it has been reported that up-regulation of TGF-β due to bladder irradiation may lead to changes in collagen content of the bladder wall and

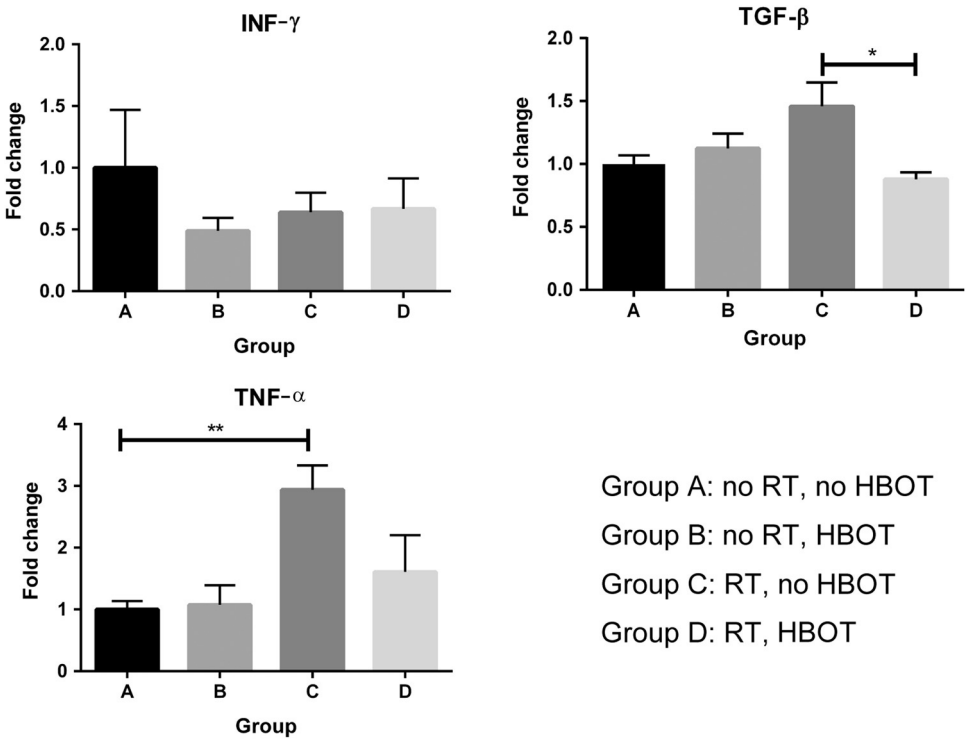


Fig. 5. (*continued*)

bladder dysfunction [27]. Bladder irradiation seems to induce a long-lasting change in the Th1/Th2 balance, however, the character of the immune response may change over time.

We could not observe any fibrosis in the bladder wall at four weeks following bladder irradiation. Our results indicate, however, that IL-10 suppresses a TGF-β-driven urinary bladder fibrosis. Tentatively, HBOT reduced radiation-induced oxidative stress and TGF-β and consequently lowered levels of IL-10 and antioxidants.

Finding the optimal dose of HBOT can be challenging; it is common to administer 30–40 sessions of HBOT to humans suffering from late radiation-induced side effects [4]. Since the rat has a higher cellular turnover rate, we chose to give 20 sessions of HBOT. A higher number of treatments might have increased the effect of HBOT, but it was hypothesized that the total dose of HBOT was appropriate for the scope of this study.

Since HBOT is often used in patients who have undergone radiotherapy due to cancer, concerns have been raised as to whether exposing tissues to high levels of oxygen may increase the risk of tumor generation or recurrence. It is therefore important to note that clinical studies have not shown that HBOT promotes development of cancer [30,31]. The present study also supports that HBOT is inert in normoxic tissue, since HBOT did not have any effects on the expression of the studied factors involved in oxidative stress, anti-oxidation and inflammation in normal bladder tissue.

In conclusion, the findings in our study indicate that HBOT may be used to prevent development of late radiation-induced side effects by affecting oxidative stress and the immune system of the urinary bladder. Future studies analyzing how bladder fibrosis is induced by irradiation and how symptoms associated with radiation cystitis are alleviated by HBOT are warranted.

Conflict of interest

None.

Funding

This work was supported by the King Gustav V Jubilee Clinical Cancer Research Foundation, Gothenburg, Sweden; the Western County Council Clinical Research Foundation [VGFOUREG-374501]; The Sahlgrenska Academy, Gothenburg, Sweden.

Acknowledgement

The authors are thankful to radiation oncology nurse Mohammadreza Abdolalian, Sahlgrenska University hospital, Göteborg, Sweden, for assistance during irradiation of the rats. The authors are also thankful to Histo-Center, Västra Frölunda, Sweden, for help with bladder tissue preparations and to Genomics Core Facility and TATAA Biocenter, Gothenburg, Sweden for qPCR analysis.

References

[1] P.J. Eifel, C. Levenback, J.T. Wharton, M.J. Oswald, Time course and incidence of late complications in patients treated with radiation therapy for FIGO stage IB carcinoma of the uterine cervix, Int. J. Radiat. Oncol. Biol. Phys. 32 (5) (1995) 1289–1300.

[2] J.J. Feldmeier, N.B. Hampson, A systematic review of the literature reporting the application of hyperbaric oxygen prevention and treatment of delayed radiation injuries: an evidence based approach, Undersea Hyperb. Med. 29 (1) (2002) 4–30.

[3] P. Rubin, L.S. Constine, L.B. Marks, Springer Link (Online service), ALERT *(QA (BØ Adverse late effects of cancer treatment volume 2: normal tissue specific sites and systems, Med. Radiol., pp. XX, 700 p. 196 illus.

[4] M.H. Bennett, J. Feldmeier, N. Hampson, R. Smee, C. Milross, Hyperbaric oxygen therapy for late radiation tissue injury, Cochrane Database Syst. Rev. 5 (2012) (CD005005).

[5] N. Oscarsson, P. Arnell, P. Lodding, S.E. Ricksten, H. Seeman-Lodding, Hyperbaric oxygen treatment in radiation-induced cystitis and proctitis: a prospective cohort study on patient-perceived quality of recovery, Int. J. Radiat. Oncol. Biol. Phys. 87 (4) (2013) 670–675.

[6] N.B. Hampson, J.R. Holm, C.E. Wreford-Brown, J. Feldmeier, Prospective assessment of outcomes in 411 patients treated with hyperbaric oxygen for chronic radiation tissue injury, Cancer 118 (15) (2012) 3860–3868.

[7] A.R. Tahir, J. Westhuyzen, J. Dass, M.K. Collins, R. Webb, S. Hewitt, P. Fon, M. McKay, Hyperbaric oxygen therapy for chronic radiation-induced tissue injuries: Australasia's largest study, Asia-Pac. J. Clin. Oncol. 11 (1) (2015) 68–77.

[8] J.J. Feldmeier, D.A. Davolt, W.S. Court, J.M. Onoda, R. Alecu, Histologic morphometry confirms a prophylactic effect for hyperbaric oxygen in the prevention of delayed radiation enteropathy, Undersea Hyperb. Med.: J. Undersea Hyperb. Med. Soc. Inc 25 (2) (1998) 93–97.
[9] S.R. Thom, V.M. Bhopale, O.C. Velazquez, L.J. Goldstein, L.H. Thom, D.G. Buerk, Stem cell mobilization by hyperbaric oxygen, Am. J. Physiol. Heart Circ. Physiol. 290 (4) (2006) H1378–H1386.
[10] S.R. Thom, T.N. Milovanova, M. Yang, V.M. Bhopale, E.M. Sorokina, G. Uzun, D.S. Malay, M.A. Troiano, K.R. Hardy, D.S. Lambert, C.J. Logue, D.J. Margolis, Vasculogenic stem cell mobilization and wound recruitment in diabetic patients: increased cell number and intracellular regulatory protein content associated with hyperbaric oxygen therapy, Wound Repair Regen.: Off. Publ. Wound Heal. Soc. Eur. Tissue Repair Soc. 19 (2) (2011) 149–161.
[11] F.M. Di Maggio, L. Minafra, G.I. Forte, F.P. Cammarata, D. Lio, C. Messa, M.C. Gilardi, V. Bravata, Portrait of inflammatory response to ionizing radiation treatment, J. Inflamm. 12 (2015) 14.
[12] S.M. Bentzen, Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology, Nat. Rev. Cancer 6 (9) (2006) 702–713.
[13] A. Leask, D.J. Abraham, TGF-beta signaling and the fibrotic response, FASEB J. 18 (7) (2004) 816–827.
[14] A. Taylor, J. Verhagen, K. Blaser, M. Akdis, C.A. Akdis, Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells, Immunology 117 (4) (2006) 433–442.
[15] D. Giglio, C. Wasen, J. Molne, D. Suchy, J. Swanpalmer, J. Jabonero Valbuena, G. Tobin, L. Ny, Downregulation of toll-like receptor 4 and IL-6 following irradiation of the rat urinary bladder, Clin. Exp. Pharm. Physiol. 43 (7) (2016) 698–705.
[16] A. Valavanidis, T. Vlachogianni, C. Fiotakis, 8-hydroxy-2′ -deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis, J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 27 (2) (2009) 120–139.
[17] T. Nguyen, P. Nioi, C.B. Pickett, The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress, J. Biol. Chem. 284 (20) (2009) 13291–13295.
[18] B.M. Hybertson, B. Gao, S.K. Bose, J.M. McCord, Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation, Mol. Asp. Med. 32 (4–6) (2011) 234–246.
[19] I.N. Zelko, T.J. Mariani, R.J. Folz, Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression, Free Radic. Biol. Med. 33 (3) (2002) 337–349.
[20] J. Krstic, D. Trivanovic, S. Mojsilovic, J.F. Santibanez, Transforming growth factor-beta and oxidative stress interplay: implications in tumorigenesis and cancer progression, Oxid. Med. Cell Longev. 2015 (2015) 654594.
[21] S.Y. Abo Elnazar, A.A. Ghazy, H.E. Ghoneim, A.R. Taha, A.M. Abouelella, Effect of ultra violet irradiation on the interplay between Th1 and Th2 lymphocytes, Front. Pharmacol. 6 (2015) 56.
[22] M. Toda, L. Wang, S. Ogura, M. Torii, M. Kurachi, K. Kakimi, H. Nishikawa, K. Matsushima, H. Shiku, K. Kuribayashi, T. Kato, UV irradiation of immunized mice induces type 1 regulatory T cells that suppress tumor antigen specific cytotoxic T lymphocyte responses, Int. J. Cancer 129 (5) (2011) 1126–1136.
[23] A.A. Ghazy, S.Y. Abu El-Nazar, H.E. Ghoneim, A.R. Taha, A.M. Abouelella, Effect of murine exposure to gamma rays on the interplay between Th1 and Th2 lymphocytes, Front. Pharmacol. 6 (2015) 74.
[24] A.S. Andrade-Sousa, P. Rogerio Pereira, B. MacKenzie, M.C. Oliveira-Junior, E. Assumpcao-Neto, M.A. Brandao-Rangel, N.R. Damaceno-Rodrigues, E. Garcia Caldini, A.P. Velosa, W.R. Teodoro, A.P. Ligeiro de Oliveira, M. Dolhnikoff, O. Eickelberg, R.P. Vieira, Aerobic exercise attenuated bleomycin-induced lung fibrosis in Th2-dominant mice, PLoS One 11 (9) (2016) e0163420.
[25] L. Hammerich, F. Tacke, Interleukins in chronic liver disease: lessons learned from experimental mouse models, Clin. Exp. Gastroenterol. 7 (2014) 297–306.
[26] P.M. Guedes, V.M. Veloso, L.C. Afonso, M.V. Caliari, C.M. Carneiro, L.F. Diniz, E.A. Marques-da-Silva, I.S. Caldas, M.A. Do Valle Matta, S.M. Souza, M. Lana, E. Chiari, L.M. Galvao, M.T. Bahia, Development of chronic cardiomyopathy in canine Chagas disease correlates with high IFN-gamma, TNF-alpha, and low IL-10 production during the acute infection phase, Vet. Immunol. Immunopathol. 130 (1–2) (2009) 43–52.
[27] M. Kraft, Y. Oussoren, F.A. Stewart, W. Dorr, S. Schultz-Hector, Radiation-induced changes in transforming growth factor beta and collagen expression in the murine bladder wall and its correlation with bladder function, Radiat. Res. 146 (6) (1996) 619–627.
[28] I. Montorfano, A. Becerra, R. Cerro, C. Echeverria, E. Saez, M.G. Morales, R. Fernandez, C. Cabello-Verrugio, F. Simon, Oxidative stress mediates the conversion of endothelial cells into myofibroblasts via a TGF-beta1 and TGF-beta2-dependent pathway, Lab. Investig. 94 (10) (2014) 1068–1082.
[29] P.A. Ruiz, A. Shkoda, S.C. Kim, R.B. Sartor, D. Haller, IL-10 gene-deficient mice lack TGF-beta/Smad-mediated TLR2 degradation and fail to inhibit proinflammatory gene expression in intestinal epithelial cells under conditions of chronic inflammation, Ann. N. Y. Acad. Sci. 1072 (2006) 389–394.
[30] I. Moen, L.E. Stuhr, Hyperbaric oxygen therapy and cancer–a review, Target Oncol. 7 (4) (2012) 233–242.
[31] J. Feldmeier, U. Carl, K. Hartmann, P. Sminia, Hyperbaric oxygen: does it promote growth or recurrence of malignancy?, Undersea Hyperb. Med.: J. Undersea Hyperb. Med. Soc. Inc 30 (1) (2003) 1–18.