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ORIGINAL ARTICLE

Radiant defense: Harnessing boron-based gel for shielding against radiation-induced dermatitis in rat models

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Abstract

Objective: Radiation therapy commonly induces dermatitis as a side effect. This study aims to assess the efficacy of a boron-based gel in alleviating radiation-induced dermatitis and explore its potential molecular mechanisms.

Methods: Thirty-two rats were divided into four equal groups: control, boron alone, irradiation alone, and irradiation with boron groups. The boron-based gel was applied to the skin area receiving 30 Gy of irradiation, 30 minutes before exposure. The evaluation of radiation-induced dermatitis was conducted using a skin scoring system, alongside the analysis of tissue expression levels of Bax, Bcl-2, and Bcl-xl proteins.

Results: In the irradiation with boron group, both the skin scores for radiation-induced dermatitis and the levels of Bax protein were significantly lower compared to the radiotherapy-only group ($p<0.001$, $p<0.05$ respectively). Bcl-2 expression was reduced in both the irradiation alone and irradiation with boron groups compared to the control group ($p<0.05$). Additionally, Bcl-xl expression was lower in the irradiation with boron group compared to the other groups ($p<0.05$).

Conclusion: The application of boron-based gel demonstrates a preferential impact on Bax rather than Bcl-2. Moreover, the use of boron-based gel on the skin effectively reduces radiation-induced dermatitis in rats through a Bax-dependent mechanism.

Keywords: Apoptosis; bax; radiation; rats

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Introduction

Radiation-induced dermatitis (RID) commonly follows radiation therapy (RT). Literature suggests that approximately 85% of patients experience dermatitis, with up to 95% developing moderate to severe skin reactions [1]. Radiation-induced skin reactions manifest as acute (within 90 days of treatment) or late effects (months to years after RT). More common in head and neck, breast, or skin cancer patients due to higher skin radiation doses, risk factors include dose parameters, anatomical sensitivity, breast size/reconstruction, and lifestyle habits like obesity and smoking [2,3]. Skin microbiome, especially Staphylococcus aureus colonization, may exacerbate reactions [4]. Genetic disorders impairing DNA repair and concurrent chemotherapy, or targeted therapy increase susceptibility to severe reactions [5]. The energetic radiation emitted in radiotherapy induces both direct and indirect ionization, leading to cellular macromolecule impairment, mainly through radiation-triggered DNA damage. This DNA-damaging process affects nearly all cellular elements of the skin, with a notable impact on epidermal keratinocytes, encompassing their stem and progenitor cells [6].

Initial treatment typically involves non-pharmacological approaches such as moisturizers and gentle bath therapies like neutral soaps and emollients. For moderate to severe cases, pharmaceutical interventions become necessary [7]. However, cosmetics can play a supportive role in subsequent therapeutic stages by aiding moisturization and alleviating irritation and itching [8]. But a definitive solution has not been found yet.

Boron is a very stable element. The physiological and metabolic mechanisms of boron's action in organisms are not yet fully understood, despite its wide-ranging effects. Boron influences cell-membrane functions, affecting responses to hormonal actions, trans-membrane signaling, and the movement of regulatory ions [9]. Additionally, boron serves as a metabolic regulator in various enzymatic systems [10]. Oxygen free radicals are highly reactive and can cause cellular and tissue damage by interacting with cell membranes and organelles. Boron helps limit oxidative damage by boosting the body's glutathione reserves and its derivatives, or by inducing other agents that neutralize reactive oxygen species [11-13].

While the wound-healing effects of boron compounds have been demonstrated in previous in vivo and in vitro studies using various wound models [14-17], its effects on radiation therapy-induced skin damage have not been evaluated before. In this study, we investigated the effects of a boron-based compound on an in vivo model of radiation-induced dermatitis.

Methods

This study was performed after the approval of the local Animals Ethics Committee. All protocols were in accordance with the regulations governing the care and use of laboratory animals in the Declaration of Helsinki. Before the research protocol started boron-based gel was prepared described as below.

Thirty-two male Sprague-Dawley rats (mean weight 275g; mean age 4 months) were randomly divided into four equal groups. There was no intervention to the control group. RT group rats were anesthetized under aseptic conditions with intramuscular injection of ketamine-xylazine mixture (ketamine, 90mg/kg; xylazine, 10 mg/kg) and the back regions were shaved before RT procedure. Rats received a single dose of 25 Gy to the lower left side of back using bolus and 4x4 cm standard blocking accessories by 6MV photons to the body surface area (SSD 100) in 28 days. RT applications were generated with the Eclipse (Varian, Palo Alto, CA) and Varian Clinac® DHX 2100 (2.2011, USA).

In boron group 1 g/day boron-based gel were applied

lower left side of the back of the rats in 28 days. In boron+ RT group RT procedure applied described as RT group but before 30 minutes of per RT applications procedure 1g/day boron-based gel was applied to the same area.

RID was evaluated daily by a person blinded by the groups according to skin scoring system (table 1) which is adapted from Randall and Coggle's study [18]. All rats were sacrificed on the 28th day. RT areas were excised for Bax, Bcl-2 and Bcl-xl protein analyses with Western blot technique.

Gel Preparation

Sodium pentaborate pentahydrate was kindly provided by National Boron Research Institute-BOREN (Ankara, Turkey). Pluronic F68 and F127 were purchased from BASF® Corporation (Badische Anilin und Soda-Fabrik, Ludwigshafen-am-Rhein, Germany). Hydrogel formulations were prepared by dispersing 1%(w/v) carbopol polymer (Carbopol Ultrez-21, Lubrizol, USA) in distilled water. The neutralization buffer (1.6g of 1M sodium hydroxide solution for 1L polymer-water suspension) was used for the gelation of the polymer. Sodium pentaborate pentahydrate (3% w/v), F68 (2% w/v) and F127 (2% w/v) were mixed into the blank hydrogel and stored at 4 °C until it completely dissolved (approximately 24 h). pH of the hydrogel formulation was set to 6.5-7.0 using 1M sodium hydroxide.

Western blot analysis

Skin tissue was harvested from the lower right back of the rats, immediately frozen in liquid nitrogen, and stored at −80°C. The frozen samples were pulverized into powder, homogenized, and lysed with lysis buffer (1 M Tris-HCl, 5 M NaCl, Triton-X-100, 0,5 M EDTA, protease inhibitor coctail; #20-201, Millipore). Protein content was assessed using a protein assay kit, then quantified with a Qubit Fluorometer 2.0 after (#Q32866; Invitrogen) after staining with Qubit® kit (Q33211; Invitrogen). The lysate was mixed with LDS buffer and Reducing agent, heated, and applied to a Bis-Tris gel (#NP0321BOX; Invitrogen) for electrophoresis at 200 V for 60 min. Proteins were transferred to PVDF membranes (#IB4010-01: Invitrogen), blocked with skimmed milk powder (#sc-2325; ChemCruz), and probed with with polyclonal rabbit anti-Bax (#NB120-777; Novus), polyclonal rabbit anti-Bcl-2 (#2870S; Cell Signaling) and monoclonal rabbit anti-Bcl-XL (#2764; Cell Signaling) primary antibodies overnight at 4°C. After rinsing, membranes were incubated with secondary antibody, assayed with ECL detection solution (ECL prime western blot detection kit, # RPN2232; Amersham), and visualized with a Fusion FX7 (Vilber Lourmat) imaging system. Sample loading was confirmed by β-actin antibody (#sc47778; Santa Cruz) re-probing after stripping with strip buffer solution (10% SDS, 0,5 M Tris HCl, 100 mM β-mercaptoethanol) the blots.

Results

Before 14th day RID skin scores of all groups were 0. RID skin scores were started to elevate on the 14th day in RT group only. In the RT with boron group RID skin score started elevation in 20th day and total score was statistically significant lower compared to RT group ($p<0.001$, table 2, figure 1).

The concentration of Bax protein was statistically significant lower in boron+ RT group compared to other groups ($p<0.05$). Bax protein concentration was statistically significant lower in boron group compared to control group ($p<0.05$, figure 2).

Relative amounts of Bcl-2 protein in the skin were statistically significant lower in RT and boron+ RT groups compared to control group ($p<0.05$, figure 3). But when we evaluated to skin levels of Bcl-xl protein we revealed the boron+ RT group was significantly lower than the other groups ($p<0.05$, figure 4).

Table 1: The criterias for radiodermatitis in dermis

Score	Definition
1	No reaction
2	Mild eryhthema
3	Depigmentation with 25% hair loss
4	Dry desquamation
5	Mild moist desquamation
6	Aggressive moist desquamation
7	Necrosis

Table 2: Mean RID skin scores of the groups after 14th day

Days	Control	RT	Boron	RT + Boron
14	0	9	0	0
16	0	15	0	0
18	0	18	0	0
20	0	21	0	7
22	0	24	0	10
24	0	28	0	12
26	0	31	0	12
28	0	34	0	14
Mean	0	22,5	0	6,8

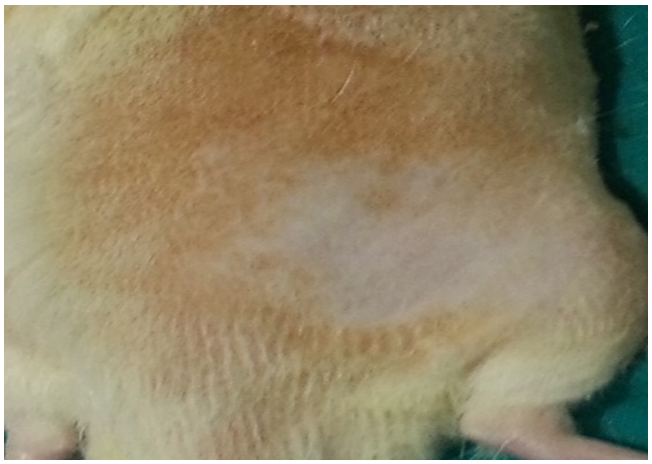

Figure 1: Grade four (moist desquamation) RID (sampling from RT group)

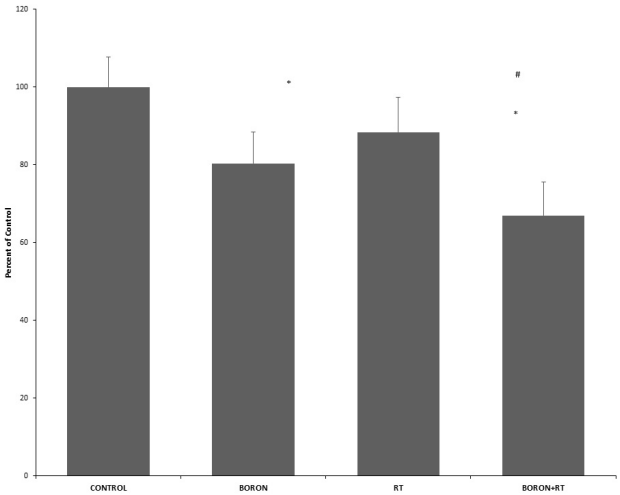

Figure 2: Results of relative amounts of Bax protein in the skin of rats with Western blotting test. (*Significant difference compared to control group, # significant difference compared to RT group)

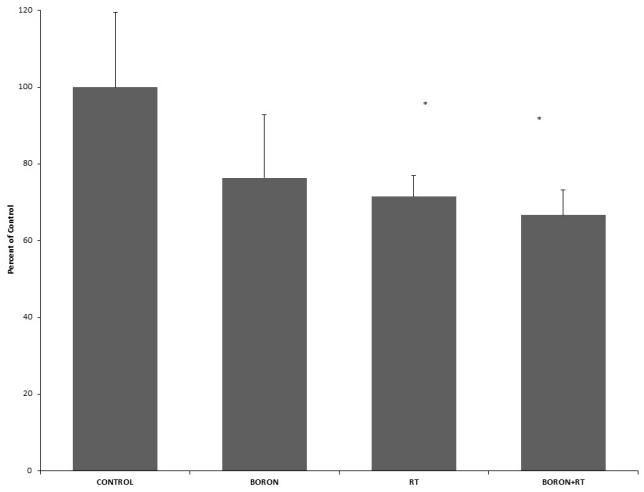

Figure 3: Results of relative amounts of Bcl-2 (compared with actin) in the skin of rats with Western blotting test. (*Significant difference compared to control group, # significant difference compared to RT group)

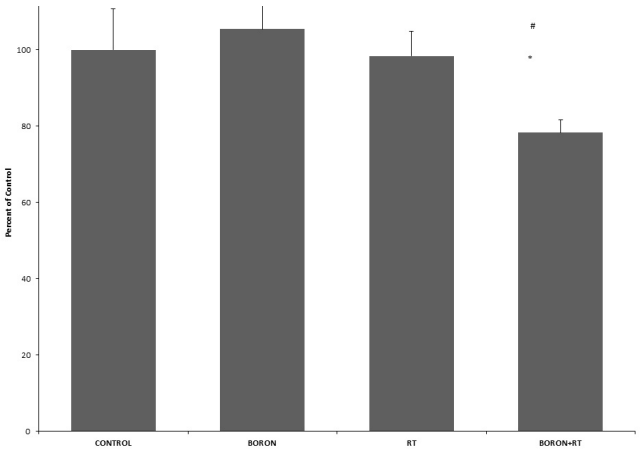

Figure 4: Results of relative amounts of Bcl-xl protein in the skin of rats with Western blotting test. (*Significant difference compared to control group, # significant difference compared to RT group)

Discussion

Radiation exposure leads to tissue damage, resulting in various manifestations such as acute ulcer formation, muscle desquamation, erythema, and pigmentation changes. Additionally, it disrupts metabolic processes by reducing the expression levels of certain growth and survival factors and their receptors. Clinics offer numerous topical agents like corticosteroids, vitamins, minerals, antibiotics, and disinfectants for the protection or treatment of RID [19].

Boron is a bioactive mineral that has been linked to several metabolic processes, including the metabolism of calcium, potassium, vitamin D, insulin, estrogen, glucose, and reactive oxygen species [20]. The only study regarding the use of boron in RID in the literature was conducted by our group, and although the mechanism was not clearly determined in this study, it was found that boron gel reduced RDI compared to the control group [21]. To examine the molecular impact of boron-based gel on RID, we concentrated on the Bcl-2 family proteins. These proteins are crucial in apoptosis regulation due to their involvement in the cell cycle. The pro-apoptotic protein Bax plays a central role in the mitochondria-dependent apoptotic pathway. Conversely, Bcl-2 and Bcl-xl, which inhibit Bax, are anti-apoptotic and promote cell survival. They can be activated by signals that either support survival or inhibit cell death [22-24].

Our findings indicate that the application of boron-based gel reduces the levels of Bax protein, both when applied alone and in conjunction with RT. Boron appears to influence Bax protein levels even without the presence of any inducing agent. The pathogenesis of RID is closely linked to the intrinsic (mitochondria-dependent) apoptotic pathway. Previous reports have shown that irradiation elevates the levels of Bax, a pro-apoptotic Bcl-2 family protein that operates within the mitochondrial pathway [25].

According to the data obtained boron-based gel has a remarkable reductive action on Bax level even after the application of RT which is a triggering factor for Bax production. RT induces DNA damage and incorporates p53 into the process to initiate apoptosis pathway [26]. It was emphasized by variety of studies how p53 has a crucial action in the regulation of apoptosis which is stimulated by radiation [27]. Thus, Bax protein is

expected to increase with the promotion of RT.

The effect of boron-based gel on Bax protein may be associated with both tumor suppressor p53 and pro-survival NFκB transcription factor. NFκB pathway is one of the ways that tumor cells choose to maintain survival, proliferation, protection against apoptosis and metastasis. It is a transcription factor and involved in regulation of many processes such as immune and inflammatory responses, developmental events, cellular survival, and death. NFκB has an inhibitory role in apoptosis as it initiates transcription of anti-apoptotic protein Bcl-xl [28]. The p53 tumor suppressor protein becomes activated in response to various stressors such as excessive oncogenic activity or DNA damage [13]. Upon activation, p53 triggers the expression of numerous genes, including pro-apoptotic factors like Bax, Puma, and Noxa, while suppressing anti-apoptotic factors like Bcl-2 and Bcl-XL, through its sequence-specific transcriptional activity. p53 and NFκB often exert opposing roles in cellular fate determination, either directly or indirectly [29,30]. Considering this, we further investigated Bcl-2 and Bcl-XL, additional members of the Bcl-2 family of proteins involved in the apoptosis pathway as inhibitors. Bcl-2 directly interacts with Bax and plays a crucial role in determining cell survival or death during the cell cycle [22].

With respect to western blot results consistent with literature Bcl-2 protein falls after RT [31]. However, it is not clear to say how boron-based gel application affects Bcl-2 after RT. It is just seen Bcl-2 amount in the group applied with boron-based gel along with RT application is at the similar level with the solely RT applied group relative to the control group.

Conclusion

Based on these results, we can conclude that boron-based gel acts on Bax rather than Bcl-2. This data may eliminate the option of NFκB directed activity of boron. For the further support we looked at the changes in another pro-survival protein Bcl-xl and observed that Bcl-xl amount declines either with the effect of RT or boron. We revealed that boron-based gel application on the skin ensure RID protection via Bax dependent activity.

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Authors' contribution: Surgical and Medical Practices: E.A, U.O.I, Concept: E.A, Design: E.A, Data Collection or Processing: E.A, U.O.I, F.S, Analysis or Interpretation: E.A, U.O.I, F.S, Literature Search: E.A, U.O.I, F.S, Writing: E.A, U.O.I, F.S.

Ethical Declaration: This study was performed after the approval of the local Animals Ethics Committee.

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