BioMed Research Journal
BMRJ, 1(1): 1-5
www.scitcentral.com

Original Research: Open Access

Effects of Therapeutic Ultrasound on Radiation-Induced Skin Damage and Restriction of joint Mobility in Rats

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Received November 24, 2017; Accepted November 28, 2017; Published April 15, 2018

ABSTRACT

This study was designed to evaluate therapeutic ultrasound (TUS) in improvement of radiation-induced skin damages and restriction of joint mobility. Eighteen adult male rats were divided into control (Con-G), 30-Gy x-ray irradiation (30 Gy-G), and 30-Gy x-ray irradiation with TUS treatment group (US-G), and then 30 Gy-G and US-G were 30 Gy irradiated to their right hind limb. US-G underwent pulsed TUS (3 MHz, 0.5 W/cm^2, 10 min/d, 5 d/wk) to irradiated skin. The main outcome included difference in skin reaction score, ankle joint range of motion (ROM) and histological examination of skin. In our results, radiation-induced disorders were significantly severe from 14 days after x-ray irradiation, and TUS significantly improved the skin damages 28 days after x-ray irradiation and reduced the early restriction of ROM. Then, the present findings indicate the possibility of TUS as an adjuvant treatment for radiation-induced skin disorder, though further examinations for the morphological and molecular changes in the x-ray irradiated skin mediated by TUS are required.

Keywords: Therapeutic ultrasound, Radiation injury, Skin, Wound healing, Joint mobility

Abbreviations: Con-G: Control Group; H.E: Hematoxylin-Eosin; IQR: Interquartile Range; ROM: Range of Motion; TUS: Therapeutic Ultrasound; US-G: 30 Gy x-ray Irradiation with TUS Treatment Group; 30 Gy-G: 30 Gy x-ray Irradiation Group

INTRODUCTION

The radiotherapy has been widely accepted as an effective treatment for various type of cancer, but many patients have also suffered from its side effects because of additional damage to the surrounding healthy tissues [1,2]. The radiation skin injury, which occurred in approximately 95% of patients receiving radiotherapy for malignant neoplasms, remains a critical problem in spite of the advances of medical technologies [3]. The harmful severity ranges from mild erythema to moist desquamation, ulceration and soft tissue fibrosis, and those lead to impaired wound healing and joint range of motion (ROM) restriction in severe cases [4,5]. Therefore, it is important for preservation of the patients' quality of life to mitigate radiation damage to skin around target cancer area.

Therapeutic ultrasound (TUS) has been clinically used for various disorders such as bone fracture [6,7] and tendon injury [8,9], and recently reported to be effective in promoting the healing of refractory wound in Escherichia coli-infected [10] or diabetic murine skin [11]. Previous studies have also revealed the positive effect of TUS on the improvement of ROM in rat joint contracture model through the alteration of collagen fibril alignment within its soft tissues [12,13]. TUS, therefore, may be expected to alleviate radiation-induced wounds and subsequent reduction of joint mobility, but there are few reports which investigate the effect of TUS on radiation-induced disorders.

The present study aims to demonstrate the effect of

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Citation: Narita H, Koeda S, Takahashi K & Shimoda H. (2018) Effects of Therapeutic Ultrasound on Radiation-Induced Skin Damage and Restriction of joint Mobility in Rats. Biomed Res J, 1(1): 1-5.

TUS on the wound healing and ROM, using our original rat model with x-ray irradiated hind limb.

MATERIALS AND METHODS

All procedures were carried out in accordance with the Guidelines for Animal Experimentation of our institution.

Eight weeks old 18 male wistar rats (Clea, Tokyo, Japan), given ad libitum access to standard laboratory diet and water under a 12 h light/dark cycle, were randomly divided into the following groups: a control group (Con-G, n=6), a 30 Gy x-ray irradiation group (30 Gy-G, n=6), and a 30 Gy x-ray irradiation with TUS treatment group (US-G, n=6). At 9 weeks of age, the rats in the 30 Gy-G and US-G were anesthetized with an intraperitoneal injection of mixture of medetomidine hydrochloride (0.15 mg/kg), midazolam (2 mg/kg) and butorphanol tartrate (2.5 mg/kg), and their right hind limb (except toes and metatarsal regions) were irradiated with a single dose of 30 Gy using a MBR-1520R x-ray generator (150 kV and 20 mA, Hitachi Medical Corporation, Tokyo, Japan). The rest of the body, including the right toe and metatarsal region, were completely shielded with 4 mm thick lead during x-ray irradiation (**Figure 1).** The Con-G was subjected to a treatment similar to 30 Gy-G and US-G, except x-ray irradiation. The US-G received pulsed TUS treatment (insonification condition: frequency of 3 MHz, intensity of 0.5 W/cm^2 and duty cycle of 20%) to the x-ray irradiated area for 10 minutes per day for 5 days per week until 4 weeks after x-ray irradiation by use of an ultrasonic device with 3.5 cm diameter transducer (US-770; ITO Physiotherapy and Rehabilitation, Tokyo Japan).

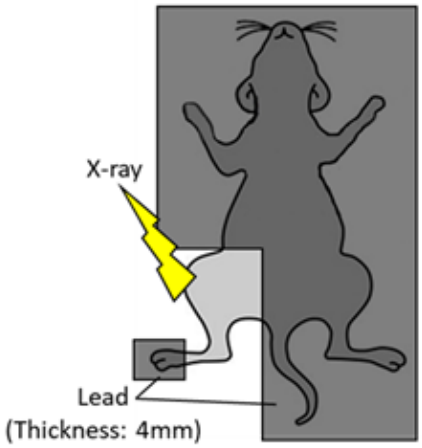

Figure 1. Schematic drawing of shielding technique during x-ray irradiation to rat.
The anesthetized rats are fixed in supine position, shielded using 4 mm thickness lead and then x-ray irradiated.

The radiation-induced skin reaction and ankle joint dorsiflexion ROM of all rat's right hind limbs were evaluated the day before irradiation, and at 3, 7, 14, 21 and 28 days after irradiation. The skin reaction scoring [3,14] (**Table 1**) and ROM measurement [15,16] were performed according to the previous reports. To briefly explain ROM measurement, the ankle joint was passively dorsiflexed maximally with the hip and knee joints 90 degrees under anesthesia by blinded investigator. The angle formed by a line connecting the lateral malleolus and the centre of the knee joint to a line parallel to the bottom of the heel were measured from vertical position at 5 degree intervals using an angle meter.

Table 1. Radiation-induced skin reaction score

Score	Observation
0	Normal
0.5	Very slight reddening
1	Definite abnormality with reddening
1.5	Moist breakdown in one very small area with scaly or crusty appearance
2	Breakdown of large areas of skin, possibly moist in places
2.5	Breakdown of large areas of skin with definite moist exudate
3	Breakdown of most of skin with moist exudate
3.5	Complete moist breakdown of irradiated area, necrosis

At the end of the experimental period, all rats were sacrificed under deep anesthesia and their skins of irradiated area were extracted. Extracted skins were fixed in phosphate buffered 4% paraformaldehyde and embedded in paraffin. Sections (5 mm thickness) were cut and stained with hematoxylin-eosin (H.E.).

The data of skin reaction score and ROM were presented as median with interquartile range (IQR) and means ± standard

deviations, respectively. Both inter- and intra-group comparisons of skin reaction score and ROM were performed by Steel-Dwass and Tukey's test, respectively, at a significance level of 0.05 using Statcel 3 software (OMS Publishing Inc., Saitama, Japan).

RESULTS

In the 30 Gy-G and US-G, radiation-induced skin reddening slightly appeared 3 days following x-ray irradiation, and became significantly such severe conditions as hair loss and effusions of the right hind limb from 14 days post-irradiation onward **(Figure 2).** Twenty eight days after x-ray irradiation, the US-G showed a significant improvement in skin injury, compared with the 30 Gy-G (**Figure 2).**

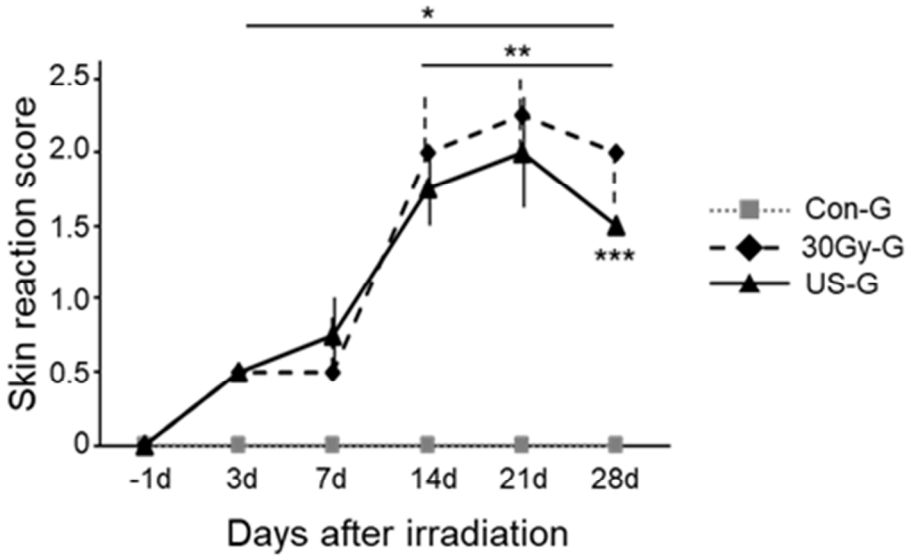

Figure 2. Inert- and intra-group comparison of radiation-induced skin damage in three groups.
*Asterisks indicate significant differences: * and ** $p < 0.05$ for intragroup comparisons in 30 Gy-G and US-G with the day before irradiation, and with 3 and 7 days after irradiation, respectively; *** $p < 0.05$ for intergroup comparison between 30 Gy-G (2.0; IQR 1.6–2.0) and US-G (1.5; IQR 1.5–1.5).*

The ankle joint ROM in the 30 Gy-G significantly decreased from 14 days after x-ray irradiation, compared with the other groups, whereas there was no significant difference in ROM between the Con-G and US-G throughout the experiment period **(Figure 3).**

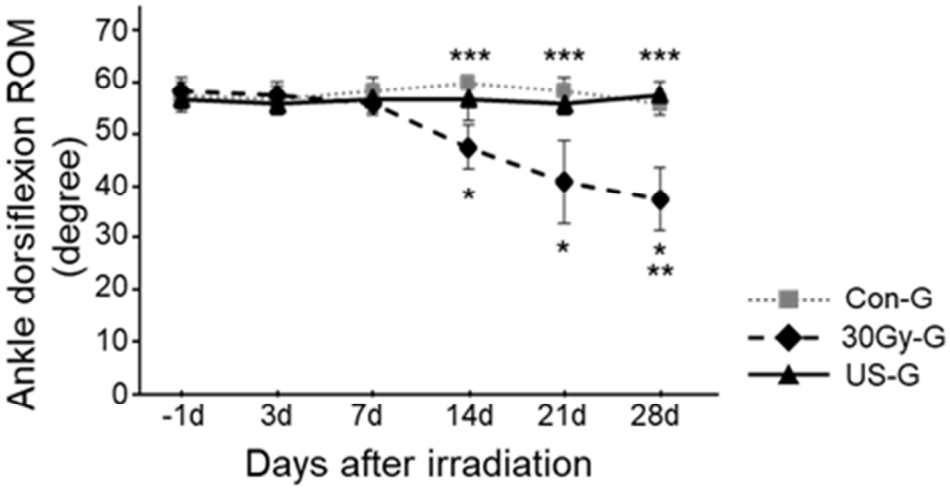

Figure 3. Inert- and intra-group comparison of radiation-induced joint immobility in three groups.
(See next page)

*Asterisks indicate significant differences: * and ** $p < 0.01$ for intragroup comparisons in 30 Gy-G with the day before irradiation, 3 and 7 days after irradiation, and between 14 days and 28 days after irradiation, respectively ($p < 0.05$ only for comparison between 7 days and 14 days); *** $p < 0.01$ for intergroup comparison in 14, 21, 28 days after irradiation. ROM in 30 Gy-G (47.5 ± 4.2° in 14 days; 40.8 ± 8.0° in 21 days; 37.5 ± 6.1° in 28 days) is significantly lower than that in Con-G (60.0 ± 0.0° in 14 days; 58.3 ± 2.6° in 21 days; 55.8 ± 2.0° in 28 days) and US-G (56.7 ± 4.1° in 14 days; 55.8 ± 2.0° in 21 days; 57.5 ± 2.7° in 28 days).*

Twenty eight days after irradiation, many neutrophils were histologically found to infiltrate both epidermis and dermis in the 30 Gy-G, showing inflammation, whereas the cell number was lower in the US-G **(Figure 4).**

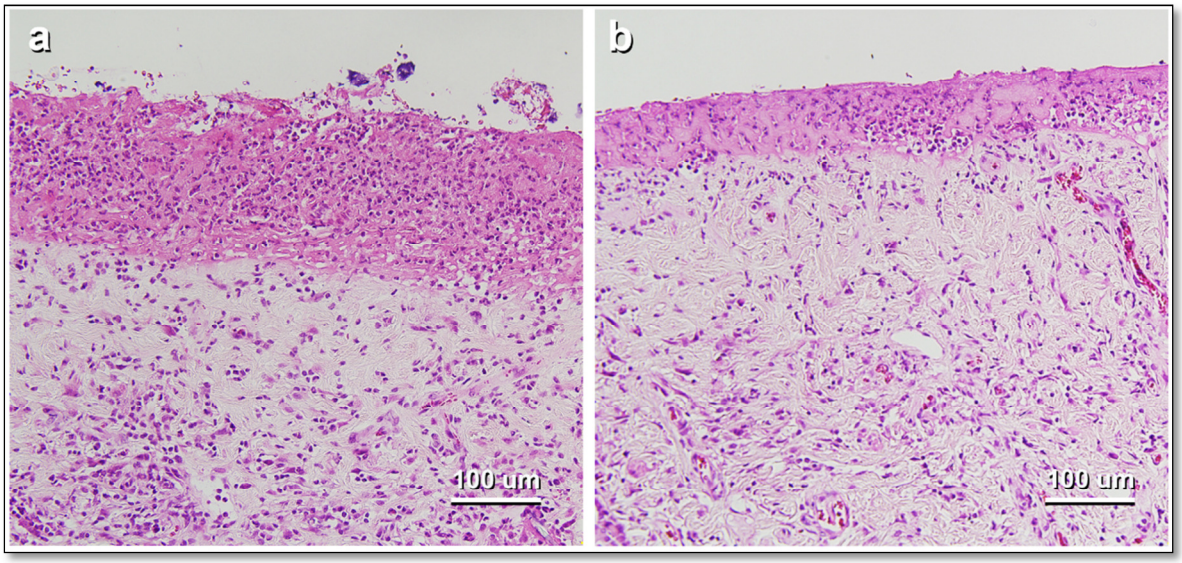

Figure 4. Representative images of H.E.-stained skin sections of 30 Gy-G (a) and US-G (b) 28 days after x-ray irradiation.
(a) *Severe infiltration of inflammatory cells is observed within the epidermis and dermis of 30 Gy-G rat.* ***(b)*** *Infiltration of inflammatory cells is relatively mild within US-G rat skin.*

DISCUSSION

In this study, we demonstrated the mitigative effect of TUS on radiation-induced skin damages in rats. Twenty eight days after irradiation, our results showed a significant improvement in skin reaction score and lower number of neutrophils infiltrating both epidermis and dermis in the US-G, compared with the 30 Gy-G, suggesting progression from the inflammatory phase to the scar formation.

Effects of TUS is known to depend on insonation frequency, intensity, treatment time and pulsed or continuous wave [17,18]. TUS in the present insonation condition have been reported to accelerate the wound healing in normal mammal skin, but its effect has not been found on ischemic and radiation-induced skin wound healing in rodents [19,20]. This inconsistency with our results may relate to difference in treatment time: only 3 or 5 minutes per day for 3 or 5 days per week in previous studies [19,20]. This idea is supported by Osumi's report that TUS for 10 minutes per day for 4 days per week with similar conditions to ours improved the healing delay of infected wound in mice [10]. Additionally, low intensity (30 mW/cm^2) pulsed TUS for 20 minutes daily has been reported to reverse impaired wound healing in diabetic and aging mice [11]. Therefore, pulsed TUS in long time (≥ 10 minutes), frequent (≥ 4 days per week) and/or low intensity condition may be suitable for the effective treatment of refractory wound.

Our results also showed the effect of TUS on early radiation-induced ROM restriction. In some previous reports, radiation-induced contracture of mice has been evaluated from more than 2 months after x-ray irradiation, because it is due to the skin fibrosis following prolonged inflammation [4,21]. Meanwhile, the present study demonstrated the significant reduction of ankle dorsiflexion ROM in the 30 Gy-G from only 2 weeks after irradiation and reversing effect of TUS on that, though the mechanism leading the indications remains unclear so far. Clinically, since long term immobility lead to severe contracture, TUS may be applicable to maintain joint mobility from early stage after x-ray irradiation.

To our knowledge, this is the first study showing the usefulness of TUS for radiation skin damage by use of animal model, but we evaluated the radiation-induced skin reaction and ROM only for a limited period. Further examinations for morphological changes and molecular

mechanisms mediated by TUS are required for the valid treatment of radiation-induced skin damage.

ACKNOWLEDGEMENT

This work was supported by JSPS KAKENHI Grant Number 15K16346.

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