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Original paper

Effects of gamma-low dose irradiation on skin flap survival in rats

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ABSTRACT

Purpose: Skin flap necrosis due to inadequate blood supply has remained a common postoperative problem in constructive surgery. As low-dose irradiation (LDI) has been shown to promote the wound-healing process, this study aims to investigate whether LDI could increase neovascularization and skin flap survival in rats.

Methods: McFarlane flaps were created in 21 male rats, which were divided into one control and two treatment groups (Ta and Tb). The treatment groups received a whole body single dose of 100 cGy gamma ray irradiation before (Tb) and after (Ta) flap surgery. The flap survival area was evaluated after seven days. The skin samples were collected for histological analysis and determining the vascular endothelial growth factor (VEGF) using the immunohistochemical method. Serum malondialdehyde (MDA) was examined with the kit.

Results: The mean areas of flap survival were 56.7 ± 3.24, 61.7 ± 2.6, and 66.5 ± 3.82 in the control, Tb, and Ta groups, respectively. There were significant differences between the Tb and Ta groups in comparison with the control group ($P < 0.05$ and $P < 0.01$, respectively). Compared with the control group (8.0 ± 0.73), the mean numbers of the blood vessels in the Ta group (22 ± 1.24) and the Tb group (14 ± 1.29) were significantly higher ($P < 0.001$ and $P < 0.01$). Moreover, the mean numbers of the VEGF-positive cells in the Ta group (4.5 ± 1.04) were significantly higher ($P < 0.05$) than the control group (2.5 ± 0.83). However, no significant differences in the MDA levels were observed among the groups.

Conclusion: The findings of this study suggest that LDI has the potential to promote neovascularization to improve flap survival.

1. Introduction

Skin flap is an important and common surgical technique that is widely used in plastic and reconstructive surgery to heal major tissue defects resulting from congenital problems and trauma [1,2]. Postoperative flap damages due to ischemia-reperfusion injury and inadequate blood perfusion are clinical problems that lead to partial or complete skin flap necrosis [2,3].

This complication may require reoperation, delay hospital discharge, and the outpatient visits' extension [4]. As documented by extensive previous research using skin flaps, the rat has been used as an animal model to evaluate the effect of treatments on flap viability [3,5,6].

To enhance skin flap survival in rats, several pharmacological agents such as antioxidants [7] and anti-inflammatory drugs [8], or even growth factors [9] and other devices, including low-level laser therapy [10] and ultrasound [11] have been used. However, the results obtained in most studies are controversial and require further investigation.

The evidence indicates that the whole-body irradiation to low levels of ionizing radiation stimulates many physiological functions, such as increased immune competence, lower morbidity and mortality, longer lifespan, and enhanced reproduction [12].

The biological effects of ionizing radiation VII (BEIR VII) committee has defined 'low-dose' as dose levels of less than 100 cGy (100 mSv) of low-linear energy transfer (LET) radiation [13].

Previous studies using animal models have revealed that low-dose irradiation (LDI) accelerated the wound healing process through the up-regulation of VEGF, stimulation of cell proliferation in wound tissue and bone marrow stem cells, and mobilized them into blood circulation [14,15]. Moreover, LDI can protect cells and tissues against oxidant damages mediated by their antioxidant property [16]. An increase of tissue glutathione by LDI has been shown. LDI is considered to have an important role in inducing protective effects in tissues and cells [17–20].

Overall, several studies have demonstrated that low-dose X-irradiation (100 cGy) promotes fracture healing [21,22]. In

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addition, it has been indicated that a low dose of irradiation promotes tissue revascularization through VEGF release from mast cells and matrix metalloproteinase-9 (MMP-9) mediated progenitor cell mobilization [23]. Thus, we were encouraged to evaluate the effects of low-dose ionizing radiation on skin flap survival using a single gamma-ray dose (100 cGy) irradiation. Therefore, this study explored the therapeutic effect of LDI on the survival of random skin flaps in a rat model.

2. Methods and materials

This study was performed in accordance with the guidance for care and use of laboratory animals. It was approved by the Animal Ethics Committee of the Urmia University of Medical Sciences. Twenty one adult male Wistar rats, weighing 220–240 g, were obtained from the animal house of the Urmia University of Medical Sciences and randomly divided into three groups. They were maintained on a 12 h light-dark cycle at a room temperature of 22°–24 °C with free access to food and water.

After performing random-pattern skin flap in the animals, they were randomly divided into three groups, based on the low-dose radiation exposure time.

Group 1: Control (non-irradiated) group (n = 7) placed in the treatment room without irradiation similar to the treatment groups
Group 2: Treatment group I (n = 7) undergone whole body irradiation before flap surgery (Tb)
Group 3: Treatment group II (n = 7) exposed to whole body radiation after flap surgery (Ta)

2.1. Random pattern skin flap model

After anesthesia with an intraperitoneal injection of 60 mg/kg ketamine and 10 mg/kg xylazine, the rats were immobilized in a prone position, their dorsal hair was shaved, and then the skin was disinfected with a povidone iodine solution. A caudally-based 7 × 2 cm random skin flap was created on the dorsum of each rat. The flaps were designed according to the procedures described by McFarlane [24].

The flaps were raised and cut superficial fascia, panniculus carnosus, subcutaneous tissue, and skin and then, sutured back to their original position with a 4-0 silk suture.

2.2. Flap survival assessment

At seven days after flap elevation, the rats were re-anesthetized and subsequently, the survival surface area was demarcated and then cut and weighed using a precision electronic scale. The survival area was calculated using the following formula:

$$Percentage\ of\ skin\ flap\ survival = \frac{Weight\ of\ survival\ area}{Total\ weight\ of\ flap\ template} \times 100$$

The living tissue was easily identified by gross observation and characterized by being warm and soft to the touch and hair-bearing skin, while hairless, stiff, dark, and colder skin was considered necrotic tissue [1,25,26].

2.3. Exposure to low-dose radiation

The two irradiated groups (Ta and Tb) received the whole-body single dose of 100 cGy of gamma-ray using a Cobalt-60 source (Theratron Phoenix, Theratronics, Inc., Ottawa, Canada) with the approximate dose rate of 39.24 cGy/min at the source-to-skin distance (SSD) of 78.5 cm. The Tb and Ta groups were irradiated to gamma ray before and after the flap surgical procedure respectively. The control group remained untreated and was kept in the restrainers with the same conditions as those in the irradiation groups.

2.4. Histology

At seven days' post-operation, the rats were re-anaesthetized and blood was taken from their portal veins for biochemistrical analysis. Then they were all sacrificed, and the tissue specimens were collected from the same position of the surviving portion of the flap. The specimens were fixed in 10% paraformaldehyde, embedded in paraffin, sectioned to 6 μm slices, and prepared for hematoxylin and eosin (H&E) staining, and anti-VEGF. For assessment of angiogenesis, the vessels were counted in five fields on H&E stained slides (at 40×) [1].

To determine the immunohistochemical localization of the VEGF, tissue sections from the paraffin-embedded blocks were deparaffinized and rehydrated in decreasing concentrations of alcohol, and then washed in a phosphate buffered saline (PBS) pH 7.4. Antigen retrieval was performed by incubating the sections in 10 mM sodium citrate at 36 °C for 30 min. The sections were treated with hydrogen peroxidase (3%) to block endogenous peroxidase and then washed with the PBS. The sections were incubated overnight at 4 °C with an anti-VEGF antibody (Abcam). The slides were washed in the PBS and then incubated with a secondary antibody for 45 min at 37 °C. After washing, the slides were treated with diaminobenzidine for 10 min at room temperature. Finally, they were counterstained with hematoxylin. The sections were observed by a light microscope, and the VEGF-positive cells with brown color were counted in two fields by a person blinded to treatment [27].

2.5. Measurement of MDA concentration

MDA was used as the marker of oxidative stress and analysed based on the kit manufacturer's instructions (SHANGHAI CRISTAL DAY BIOTHEH, Co, China) using the samples of the blood serum collected on day seven after the flap surgery.

2.6. Statistical analysis

The mean and standard deviation values from the different groups were expressed and compared using analysis of variance (ANOVA). One-way ANOVA was followed by the Tukey's multiple comparison post hoc test for comparing different treatment groups. The statistical significance was set at $p < 0.05$.

3. Results

3.1. General observation

On day seven after the flap surgery, the surviving area of the flap was soft with warm skin and fine hair, while the necrotic area was hard and dark with cold hairless skin, and there was no bleeding when cut with a scalpel.

3.2. Survival assessment

Digital photographs show the regions of survival and necrosis of the flaps from the different groups on the seventh postoperative day (Fig. 1). Seven days after the surgery, the mean ± standard deviation (SD) of the skin flap survival percentage in the control, Tb, and Ta groups were 56.7 ± 3.24, 61.7 ± 2.6, and 66.5 ± 2.82,

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