The Preventive Effect of Recombinant Human Growth Factor (rhEGF) on the Recurrence of Radiodermatitis

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Recombinant human epidermal growth factor (rhEGF)/Irradiation/Radiodermatitis/Recurrence.

The effects of topical application of recombinant human epidermal growth factor (rhEGF) on wound healing and the recurrence of radiodermatitis were assessed in the irradiated skin of BALB/c Nu/Nu mice. Mice irradiated with 45 Gy of radiation were divided into 5 groups and treated with 10, 50, and 100 μg/g rhEGF ointment, vehicle alone, or no treatment (control) for 6 months. Wounds were observed initially in all groups and complete healing time (HT100) for initial wound repair did not differ significantly among groups. However, the rate of recurrence over 6 months was significantly lower in the EGF-treated groups than in the control group (p < 0.05). Histological examination showed that treatment with the optimum dose of EGF (50 μg/g) accelerated normal wound healing when compared with the higher dose of EGF (100 μg/g), vehicle alone, or no treatment, with the latter group showing irregular epidermal thickness, poor definition of epidermis and dermis, and unstable dermal structure. Collagen distribution was also significantly increased in mice treated with 50 μg/g rhEGF (p < 0.05) compared with the control or vehicle-treated group. Taken together, these results indicate that treatment with exogenous EGF (50 μg/g dose) can enhance radiation-induced wound repair while preserving structural tissue stability and preventing the recurrence of radiodermatitis.

INTRODUCTION

Radiation is often used to treat malignant tumors, either alone or in combination with surgery and/or chemotherapy. Despite the advantage of radiotherapy and the improvements in techniques, many patients experience moderate to severe side effects, including xerostomia, diarrhea, mucositis, dermatitis, ulceration, and fibrosis.1) Rapidly dividing cells such as those in the skin, bone marrow and gastrointestinal tract are easily damaged by radiation.2) Ionizing radiation generates highly reactive free radicals, which damage cells, even in adjacent normal tissue, especially in the skin covering the entire body.3) Delayed wound healing of irradiated skin can cause other severe complications, such as skin necrosis and ulceration, and non-cancerous skin damage is considered a dose-limiting factor of radiotherapy that can impair patient quality of life.4) Various interventions have been tested for their ability to prevent radiation-induced skin toxicity and increase wound healing; these include topical steroid creams, washing practices, aqueous creams, aloe vera gel, chamomile, almond cream, sucralfate cream, and amifostine.2,5–7) but none has proven optimal for treating radiation-induced skin damage. Recently, various growth factors have been applied in attempts to manipulate the environment of wounds and to enhance wound healing.2) Key elements in wound healing, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), keratinocyte growth factor (KGF), and epidermal growth factor (EGF), can trigger tissue regeneration and the remodeling process.8–11) Despite their potential efficacy in the wound healing process, there have been few studies on the wound healing capacity of growth factors, especially in radiodermatitis. Recombinant human PDGF-BB may be used to treat selected individuals with refractory, poorly healing, dermal wounds caused by irradiation,12) and the combination of a topical recombinant PDGF gel and hydrophilic copolymer membranes was found to ameliorate chronic ulcerating radiodermatitis.13) Topical and local application of granulocyte-macrophage colony-stimulating factor (GM-CSF) can facilitate rapid healing of radiation-induced ulcers or dermatitis.14,15) and EGF has been reported to accelerate recovery from oral or intestinal mucosal damage induced by irradiation in animal models.16,17) Our group

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doi:10.1269/jrr.100010
has reported that EGF stimulated epithelialization of a chronic radiation ulcer in a patient who had a mastectomy and radiation therapy 23 years previously. These studies, however, have been conducted in few patients, with very limited conditions. Moreover skin wounds resulting from irradiation often recur after long periods of time due to abnormal pathological changes during the early stages of healing. We hypothesized that EGF treatment of the radiation-induced skin wounds would enhance the normal healing process and lower the recurrence rate without abnormal pathological changes. We therefore tested the effects of 6 months of rhEGF treatment on radiation-induced dermatitis and recurrence in BALB/c Nu/Nu mice.

**MATERIALS AND METHODS**

**Experimental animals and radiation**

Male BALB/c Nu-Nu mice (6 weeks old, average body weight 20 ± 2 g) were obtained from OrientBio Inc. (Republic of Korea) and adapted for 1 week before experiments. Mice were immobilized during irradiation using a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) injected intramuscularly. The buttock regions of these anesthetized mice were irradiated with a total of 45 Gy over 3 consecutive days (15 Gy per day) with a 4 MV photo beam generated by a linear accelerator (CLINAC 600C, Varian, Palo Alto, CA, USA) at dose rate of 2 Gy/min. During irradiation, the bodies of the mice were covered with a 1.5-cm thick bolus to provide an adequate radiation dose build-up on the skin surface. Irradiated mice were subsequently divided randomly into 5 groups of 6 mice per group and treated with 10, 50, and 100 μg/g rhEGF ointment, vehicle alone, or no treatment (control) for 6 months. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Asan Medical Center, University of Ulsan.

**EGF treatment**

Human recombinant EGF (Nepidermin®) ointment was provided by Daewoong® Pharmaceutical Company (Seoul, Republic of Korea). Briefly, EGF was mixed with ointment base at concentrations of 10, 50, and 100 μg/g, and 0.1 g of each ointment was topically applied to the skin twice daily, at 10 AM and 4 PM, after irradiation. The quantities of EGF applied on each application to each wound area were 1, 5, and 10 μg, respectively.

**Measurement of 100% healing time (HT100) and frequency of recurrence**

Digital images of the irradiated skin areas of each mouse were taken every day, and evaluated for epithelialization with an image analyzer (Image measurement Standard v4.01, Bersoft, Puerto Plata, Dominican Republic) to assess healing time. HT100 was defined as the time of full epithelialization of a wound, as determined by the single-blinded opinion of two trained researchers. Recurrence was assessed in all groups for up to 6 months. EGF or vehicle ointment was applied until the termination of the experiment.

**Evaluation of morphological changes**

At sacrifice, the dorsal buttock skins of the mice were fixed in 10% neutral buffered formalin and embedded in paraffin. The tissue sections were stained with hematoxylin and eosin (H&E) and Masson’s trichrome reagent (Dako, CA, USA) to evaluate morphological changes. The percentage of trichrome stained area was calculated with an image analyzer at 100x magnification.

All values were expressed as mean ± standard deviation, and the Student’s t-test was used for comparisons between vehicle and treatment groups.

**RESULTS**

100% healing time (HT100)

Acute radiodermatitis was effectively induced in BALB/c Nu-Nu mice within 1 week after 45 Gy of fractionated irradiation. Complete wound healing time, expressed as mean HT100 was 12.3 ± 1.5 days in the control group, 12.33 ± 2.1 days in the vehicle-treated group, and 12–15 days in the three rhEGF-treated groups (Fig. 1).

Recurrence of radiodermatitis

Measurements of the frequency of recurrence of radiodermatitis for 6 months after irradiation showed that, in the control and vehicle-treated groups, radiodermatitis recurred 3.33 ± 0.58 and 3.00 ± 1.00 times per mouse, respectively.

![Fig. 1.](image-url) Comparison of 100% healing time (HT100) of radiation-induced skin wounds treated with various concentration of rhEGF. Following irradiation, 0.1 g of ointment containing various concentrations of EGF or vehicle alone was topically applied to the buttock regions twice daily for 6 months. Optical images were used to evaluate epithelialization and to calculate HT100. Each bar shows the mean ± standard deviation for that group.
to assess collagen distribution in the dermis. Both the control and vehicle-treated groups showed irradiation-induced loss of collagen fibers in the dermal area (Fig. 4). Topical treatment with 50 μg/g EGF ointment increased collagen deposition, with specimens from this group showing much more dense dermal structure than any of the other groups. Treatment with low dose (10 μg/g) EGF ointment resulted in smoother collagen distribution than in either the control or vehicle-treated groups, whereas treatment with high-dose (100 μg/g) EGF ointment resulted in a pattern similar to that of the control group. When we measured the percentages of trichrome stained areas in the samples, relative to total area, we found positive staining in 26.58 ± 7.89% and 23.79 ± 4.62% of the control and vehicle-treated tissue specimens, respectively. Treatment with 10 μg/g EGF slightly increased the trichome stained area, to 33.26 ± 10.69%, but the difference was not statistically significant, and treatment with high-dose (100 μg/g) EGF resulted in positive staining similar to that observed in control- or vehicle-treated skin. In contrast, treatment with 50 μg/g EGF markedly and significantly increased collagen deposition, to 41.36 ± 10.47% (p < 0.05).

**DISCUSSION**

We have shown here that topical application of rhEGF for 6 months after irradiation with 45 Gy decreased the recurrence of radiodermatitis and normalized epidermal structure in mouse skin. This was confirmed by histopathological analysis, in that mice treated with 50 μg/g EGF did not show the characteristics of radiation injured skin, such as loose connections between the epidermal and subcutaneous layers, thin epidermal layers, and irregular dermal structures. These findings also indicate that topical treatment with EGF accelerates normal wound healing by activating cell proliferation and epidermal regeneration in radiation-induced skin wounds.

Radiation-induced skin damage often occurs in non-cancerous tissues as a direct result of ionizing radiation. Early adverse effects of irradiation may include erythema, pigmentation, epilation, dry and moist desquamation, and erosions, whereas the late adverse effects of irradiation may include aggravated wound reactions such as ulceration, dermal necrosis, atrophy, telangiectasia, and fibrosis. Impaired wound healing is often seen in late stages after irradiation and may remain chronic, with delayed or non-healing of ulcerations and infections. Because wound healing is a complex process involving multiple cells, inflammatory mediators, cytokines, growth factors, and various other factors, manipulating this process remains a challenge. Although the healing process that occurs following radiation-induced damage is not the same as that following incisional wounds, the two processes share several steps such as epidermal regeneration, fibroblast proliferation, and col-

**Fig. 2.** Effect of rhEGF on the rate of radiodermatitis recurrence over 6 months. Topical treatment with rhEGF ointment significantly inhibited the recurrence of skin wounds after initial wound healing, compared with the control and vehicle-treated groups. There was no difference between the latter two groups. Each bar shows the mean ± standard deviation for that group, with p values shown over each bar.
Fig. 3. Histopathological evaluation of mouse skin samples. Skin samples were stained with H&E staining and photographed at ×200 magnification. A: Irradiated control group, B: vehicle-treated group, C: 10 μg/g EGF-treated group, D: 50 μg/g EGF-treated group, E: 100 μg/g EGF-treated group. Bold-headed arrows indicate irregular epithelium and thin arrows indicate the thickness of the epidermis and dermis.

Fig. 4. Representative images of collagen deposition, as assessed by Masson’s trichrome staining. Images captured under ×100 magnification. A: Irradiated control group, B: vehicle-treated group, C: 10 μg/g EGF-treated group, D: 50 μg/g EGF-treated group, and E: 100 μg/g EGF-treated group. Collagen fibers were stained blue.
lagent deposition. Thus materials or agents that increase cell proliferation may be promising candidates for wound healing. Growth factors may be effective in wound repair, due to their ability to stimulate cell proliferation and their ability to chemo-attract immune system cells. The EGF treatment regimen described here has been used in wound care, both in vitro and in vivo. In clinical trials, EGF has been found to accelerate the wound healing time of partial thickness skin, and to have positive effects on diabetic ulcerations and chronic skin wounds. However, the effects of EGF on radiation-induced skin wounds have not yet been tested clinically.

We found that the initial irradiation-induced wounds developed equally in all five groups, with no differences in the time to achieve complete healing (HT100). Wound recurrence rates, however, were significantly decreased in all three rhEGF-treated groups compared with the control and vehicle-treated groups. Because recurrence or sudden breakdown of the skin may appear clinically long after irradiation, we used a 6-month model to assess the effects of long-term treatment. The average number of recurrences per mouse in the control and vehicle-treated groups were 3.33 ± 0.58 and 3.00 ± 1.00, showing the strength of this radiation wound model. Undesirable changes in the skin during radiotherapy may alter the concentrations or balance of immune-related cytokines. For example, the concentrations of PDGF and EGF were decreased in chronic wounds, suggesting that these deficiencies may be related, at least in part, to impaired wound healing. In addition, radiation-induced fibroblast dysfunction may be responsible for poor wound healing. Thus, better wound healing can reduce the incidence of late complications resulting from early wound repair steps. Our findings indicate that the ability of 50 μg/g of EGF ointment to completely inhibit the recurrence of radio-dermatitis was due to an efficient and proper healing process during the initial stages of wound repair.

These findings were confirmed by histopathological examination of skin biopsy samples from these mice. Six months after irradiation, tissue samples from control and vehicle-treated mice showed severe alterations in skin structure, including an impaired epidermal layer, irregular dermal structure, and unstable subcutaneous layer, although the skin surfaces appeared relatively normal. In contrast, specimens from mice treated with 50 μg/g EGF ointment showed normal skin structure, characterized by thick epithelium, a definite delineation of the epidermis from the dermis, and a densely composed dermis. Moreover, decreased collagen deposition, one of the long-term effects of radiotherapy, was observed in control and vehicle-treated mice, but not in EGF-treated mice. The dense extracellular matrix of the reticular dermis is accomplished by fibroblasts within the dermis. Collagen, the main extracellular matrix component, is responsible for the strength and elasticity of the skin. Because collagen is synthesized by fibroblasts, the impairment of fibroblasts in radiation-induced wounds will have a negative impact on collagen synthesis, resulting in delayed wound healing. These results indicate that EGF treatment stimulates the proliferation of fibroblasts that actively synthesize collagen during the wound healing process and further strengthens dermal structure. Although accumulated collagen may be considered a histological feature of fibrosis, collagen synthesis is also the key step in wound repair and possibly a major factor in achieving long-term stability of skin structure.

Another goal of this study was to determine the dose range of EGF inducing the most efficient response. Different concentrations of EGF have been used in other animal studies. For example, 0.1–10 μg/ml of EGF induced a therapeutic response in a partial thickness burn pig model although treatment with 10 μg/ml of rhEGF has been reported to delay wound healing in an excisional wound model compared with treatment with 0.01 to 1 μg/ml EGF. We previously reported that the most efficient dose facilitating the re-epithelialization of partial thickness wounds was 1–5 μg/g rhEGF. These divergent results may have been due to differences in types of wounds and species. Human recombinant EGF may have a reduced affinity to mouse than to human or pig EGF receptors; thus, a higher dose would be required in mice to induce the same therapeutic activity. However, doses greater than the optimum range worsen the wound healing process. For example, high concentrations of rhEGF did not show any positive effects in radiation-induced oral or intestinal mucositis and we have shown here that mice treated with the highest dose of EGF (100 μg/g) showed similar morphological patterns to those in irradiated, untreated control mice. Concentrations of growth factors
greater than the therapeutic dose window may adversely decrease inflammatory responses in the wound site due to negative feedback. Alternatively, higher concentrations of EGF may over-activate the EGFR signaling pathway through MAPK activation, which is essential for proper epithelial wound healing in vivo. In cell culture systems, high concentrations of EGF activate MAPK through the transient phosphorylation of MAPK, whereas lower concentrations of EGF induce the persistence of a phosphorylated MAPK, such that EGFR signaling can stimulate cell proliferation and survival. We have also observed optimum ranges of EGF for the proliferation of irradiated fibroblasts and keratinocytes, with higher concentrations of EGF having no effect on proliferation. Although our results indicate that the optimum concentration of EGF for the stimulation of signaling pathways in tissues is about 50 μg/g, further studies are necessary to establish the dose relationship to the healing process.

Although improvements in radiation technology have markedly reduced patient complications and adverse effects, radiotherapy of tumors close to the skin may still induce unwanted skin damage. Treatment of skin wounds caused by radiation is still difficult, despite supportive care and treatments. The findings presented here indicate that treatment with EGF ointment may be effective in healing skin wounds induced by radiation. However the complexity of the healing process and the balance among the numerous growth factors and cytokines involved in the sequential steps of wound repair should be considered. Despite the limitation of our experimental conditions, our findings indicate that treatment with exogenous EGF can enhance wound repair, accompanied by the preservation of tissue structure, and can prevent the recurrence of radiodermatitis.

ACKNOWLEDGEMENT

This research was supported by a grant (A084025) from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea.

REFERENCES

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