Aloe vera oral administration accelerates acute radiation-delayed wound healing by stimulating transforming growth factor-β and fibroblast growth factor production

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Abstract

BACKGROUND: Delayed wound healing is a significant clinical problem in patients who have had previous irradiation. This study investigated the effectiveness of Aloe vera (Av) on acute radiation-delayed wound healing.

METHODS: The effect of Av was studied in radiation-exposed rats compared with radiation-only and control rats. Skin wounds were excised on the back of rats after 3 days of local radiation. Wound size was measured on days 0, 3, 6, 9, and 12 after wounding. Wound tissues were examined histologically and the expressions of transforming growth factor β-1 (TGF-β-1) and basic fibroblast growth factor (bFGF) were examined by immunohistochemistry and reverse-transcription polymerase chain reaction.

RESULTS: Wound contraction was accelerated significantly by Av on days 6 and 12 after wounding. Furthermore, the inflammatory cell infiltration, fibroblast proliferation, collagen deposition, angiogenesis, and the expression levels of TGF-β-1 and bFGF were significantly higher in the radiation plus Av group compared with the radiation-only group.

CONCLUSIONS: These data showed the potential application of Av to improve the acute radiation-delayed wound healing by increasing TGF-β-1 and bFGF production.

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KEYWORDS: Aloe vera; Radiation wound; TGF-β; FGF

Wound healing is a highly complex but orchestrated cascade of cellular and molecular events. Successful wound healing is the result of a sequence of several basic processes, including inflammation, cell proliferation, angiogenesis, wound contraction, epithelialization, and matrix remodeling. These processes, which depend on the appropriate and integrated functions of neutrophils, macrophages, fibroblasts, and endothelial cells, are thought to be organized by interactions among cells, extracellular matrix proteins, and growth factors. Any alteration of these healing processes will result in abnormal wound healing and adversely affect the surgical outcome.1

Combined radiotherapy and surgery have resulted in improved functional and cosmetic outcomes in cancer pa-
Patients. However, preoperative radiotherapy carries a good response rate; it is associated with significant morbidity including wound infection and delayed healing. Radiotherapy has been shown to affect nearly every cell type involved in the process of wound healing. It declines neovascularization, fibroblast proliferation, collagen deposition, and varying levels of regulatory growth factors of wound healing. Various growth factors are found to be down-regulated after acute radiation exposure. Among the many growth factors, transforming growth factor β-1 (TGF-β-1) and basic fibroblast growth factor (bFGF) are strongly down-regulated after acute radiation exposure. Conversely, TGF-β-1 has been found to be increased in the chronic phase of irradiated wound healing. Fibroblast proliferation, transformation to myofibroblasts, and stimulation of extracellular matrix (ECM) production are all controlled by growth factors, primarily TGF-β-1. Another important growth factor in the wound-healing process is bFGF. bFGF can regulate the migration and replication of endothelial and epithelial cells as well as fibroblasts, which are responsible for neovascularization, epithelialization, and collagen production, respectively.

Several trials have been performed to identify possible therapeutic approaches to increase healing after radiation. In recent years, the use of natural/herbal products has been increased dramatically in wound healing because they contain a wealth of interesting and possibly beneficial pharmacologically active compounds. Moreover, they have wide acceptability and better tolerance, are inexpensive, and can be manipulated safely for human use. The Aloe vera (Av) plant has been known as “the healing plant.” Aloe has been used to treat wounds and burns for centuries. The oral and topical application of Av gel has been reported to be effective in wound repair in normal and/or diabetic conditions. Recent studies have shown that treatment with either Av crude gel or its extracts, such as acemannan, β-sitosterol, and others, resulted in faster healing of wounds by stimulating fibroblast proliferation, collagen deposition, angiogenesis, and production of growth factors. Clinical trials also have examined the effectiveness of Av gel in the treatment of wound healing and burn wound healing.

Fulton documented stimulation of wound healing of full-thickness dermabrasion in 18 patients treated with a polyethylene oxide gel dressing saturated with stabilized Av compared with a polyethylene oxide gel dressing. Khorasani et al clinically reported that healing of partial-thickness burns in patients was significantly faster in sites treated with aloe than in sites treated with silver sulfadiazine. Based on the meta-analysis of 2 studies using duration of wound healing as an outcome measure, the Av group was quicker to heal burn wounds: 8.79 days shorter than those in the control group (CG). Despite reported radioprotective effects of Av, its influence on the early phase of acute radiated-delayed wound healing has not been studied.

The purposes of the current study were to investigate the influence of Av oral administration on cutaneous wound healing in an irradiated rat model and to correlate the outcome of healing with the expression levels of TGF-β-1 and bFGF in the wound.

Methods

Animals

Thirty-six male Wistar rats, 6 weeks old, weighing 200 to 250 g (Nihon CLEA, Inc, Tokyo, Japan) were assigned randomly to 3 groups (n = 12 each) as follows: CG (sham-radiation), radiation-only group (RG), and radiation plus Av treatment group (RAG). Animals were housed individually for 1 week before the experiment for accommodation. Rats were given free access to commercial rat food and water ad libitum and were subjected to a 12-hour light–dark cycle. This study was approved by the ethics committee of the National Institute of Radiological Sciences in accordance with the guidelines of the National Institutes of Health.

Radiation

RG and RAG rats were anesthetized with pentobarbital sodium (Nembutal; Dainippon Sumitomo Pharma, Co, Ltd, Osaka, Japan), at a dose rate of 40 mg/kg of body weight, administered intraperitoneally (IP). The dorsal hair was clipped and the corners of a 3 × 3-cm area were tattooed on the back of each animal. A sheet of 3-mm lead with 3 × 3-cm cut out to accommodate the skin was used to shield the rats’ bodies. A single dose of 9 Gy soft x-rays, generated by a M-70-WE machine (SOFTEX Cooperation, Kanagawa, Japan), was administered to the selected area on each rat. The radiation conditions were as follows: 30 kV, 30 mA, .1 mm Al Filter, and a 46-cm focus-surface distance. The dose rate in this condition was about 1.1 Gy/min, and exposure time was 8 minutes and 55 seconds.

Wound creation and assessment of wound size

Three days postradiation, all rats were anesthetized with pentobarbital (40 mg/kg) IP. The back hair was shaved and disinfected with 70% ethanol. A full-thickness skin wound excision measuring 1.5 × 1.5 cm was made on the back of each animal in the center of the irradiated skin area. The wounds were photographed on days 0, 3, 6, 9, and 12 after wounding by a digital camera. All wound images were transferred to the computer, changed to a tagged information file format extension using Adobe Photoshop Elements 4.0 (Adobe Systems, Tokyo, Japan). The wound size area was measured with special size analysis software, (National Institutes of Health ImageJ software, downloaded from http://www.rsb.info.nih.gov/ij). The change in wound size was expressed as a percentage of the original wound size.
Aloe vera administration

RAG rats were administered lyophilized Av powder (Coral Vegetable, Miyakojima, Japan) at a dose of 30 mg/head,14 dissolved in 1.5 mL purified water. Av was given orally by an oral tube on days 0, 3, 6, 9, and 12 after wounding.

Five rats from each group were euthanized by an overdose of pentobarbital anesthesia (500 mg/kg, IP) on days 6 and 15 after wounding. The entire wound including a margin of approximately 5 mm of unwounded skin was excised in-depth to include underlying connective tissues above the external fascia of the dorsal muscles. The skin wound samples of day 6 postwounding were divided into 2 halves; 1 was fixed for 48 hours in a 10% buffered formalin solution (pH 7.4) and embedded in paraffin, the other half was frozen at −80°C.

Histopathologic examination

Skin wound tissues were cut 4-μm thick perpendicular to the wound and stained with hematoxylin-eosin and Masson’s trichrome stain (for scoring of collagen). The following parameters were assessed individually: the epithelialization, inflammatory cells infiltration, fibroblast proliferation, neovascularization, and collagen deposition. Epithelialization and collagen deposition were graded histologically using a modified Ehrlich and Hunt numeric scale from 0 to 4.25 The inflammatory cell infiltration and fibroblast proliferation were evaluated by counting the inflammatory cells (neutrophils, macrophages, and lymphocytes) and fibroblasts. The counting of inflammatory cells and fibroblasts were assessed by capture 8 (day 6) and 4 (day 15) randomly selected high power-fields (magnification, 400×) images and counting using a manual cell counter in ImageJ software. To evaluate the neovascularization, the number of blood vessels was counted in 4 randomly selected fields (magnification, 100×) 3 times and the mean number of blood vessels in each group was compared. All the wound tissue samples were graded histologically in a blind fashion on 2 slides per animal.

Immunohistochemical staining

Immunohistochemistry was used to detect TGF-β-1 and bFGF protein in rat skin wounds. Two slides obtained from each specimen were used for immunohistochemistry. The sections were deparaffinized in xylene and rehydrated. The slides were immersed in a covered plastic container with Target Retrieval Solution pH 9 (DAKO Corporation, Kyoto, Japan) and placed in an autoclave at 121°C for 10 minutes. Endogenous peroxidase activity was quenched with peroxidase blocking solution (DAKO) for 10 minutes at room temperature. After a thorough washing in phosphate-buffered saline (PBS), the protein block serum-free (DAKO) was applied to block nonspecific binding for 10 minutes at room temperature. The primary antibody (TGF-β-1 [V], Sc-146 [dilution 1:500], and bFGF [147] Sc-79 [dilution 1:500]; Santa Cruz Biotechnologies, Santa Cruz, CA) were applied to slides and incubated in a moist chamber at 4°C overnight. After washing in PBS, the sections were incubated with a biotinylated secondary antibody (DAKO) for 30 minutes. After a second washing in PBS, the sections were incubated with avidin-biotin complex with horseradish peroxidase solution (Vectastain ABC kit, catalog # SP-2001; Vector Laboratories, Burlingham, CA) for 30 minutes. After final PBS washing was performed. Diaminobenzidine tetrahydrochloride (DAKO) was treated as a chromogen. All slides were counterstained with hematoxylin, rinsed in distilled water again, dehydrated, and mounted. The sections were examined qualitatively under a bright field microscope at 100 to 400× magnification for changes in the expression of TGF-β-1 and bFGF in wound tissue among the groups. For quantitative analysis of expression labeling indexes were determined as the ratio of positively stained cells to the total number of cells per visual field. For this study, only cell-associated staining was considered. The criteria for a positively expressing cell were the existence of a clear cell structure with nucleolus and clear specific staining of the cytoplasm. The overall number of evaluated cells per section was 900 ± 100 cells (at least 3 fields per section were examined with 400× magnification).

Total RNA extraction and reverse-transcription polymerase chain reaction

A Mixer Mill MM 301 (Retsch, Inc, Pittsburgh, PA) was used to homogenize granulation tissue specimens. Total RNA was extracted from the homogenized granulation tissue using the Maxwell 16 Total RNA Purification Kit (Promega Corporation, Madison, WI) according to the manufacturer’s protocol. The A260/A280 ratio was used to determine the quantity of total RNA. The ratio of A260/A280 was from 1.8 to 2.0. First-strand complementary DNA was synthesized by reverse transcription of total RNA (1 μg/μL) using ReverTra Ace kit (ReverTra Ace-α; Toyobo, Osaka, Japan) according to the manufacturer’s instructions. The polymerase chain reaction (PCR) reaction was performed with a diluted complementary DNA sample in a 50-μL reaction volume. The final reaction concentrations were 20 μmol/L primers, 10 mmol/L deoxynucleoside triphosphates (dNTPs) mixture, 10 × PCR buffer II, and .5 U/μL Takara Ex Taq HS polymerase (Takara Bio, Inc, Shiga, Japan) per reaction. Rat-specific primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), TGF-β-1, and bFGF were designed and tested elsewhere (Table 1).26,27 The cycling conditions for PCR amplification were as follows: 1 cycle of 95°C for 3 minutes followed by 30 cycles of 94°C (45 s), 60°C (45 s), and 72°C (75 s) for
GAPDH; 30 cycles of 94°C (30 s), 53°C (30 s), and 72°C (45 s) for TGF-β-1; and 35 cycles of 94°C (30 s), 50°C (30 s), and 72°C (45 s) for bFGF in MyCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). The amplified products were resolved by electrophoresis in 2% agarose gel and stained with ethidium bromide (Sigma Chemical Co, St. Louis, MO). The images were captured by the ChemiDoc XRS system (Bio-Rad Laboratories) and processed with Quality One analysis software (Bio-Rad Laboratories). The density of each PCR product was evaluated by a semiquantitative method in relation to internal GAPDH control.

### Statistical analysis

All the data were evaluated and expressed as mean ± standard deviation. Significant differences among the groups were determined by 1-way analysis of variance with the Newman–Keuls post test, followed by the Wilcoxon rank-sum test (U test) using Graph Pad Prism software version 5.00 for Windows (GraphPad Software, San Diego, CA). A P value less than .05 was considered statistically significant.

### Results

The average weight of rats during this experiment did not differ significantly. Two rats each in CG and RG, and 1 rat in RAG died of anesthesia complications. Gross inspection revealed that none of the wounds showed evidence of infection.

#### Wound contraction

The change in the wound healing size was expressed as a percentage of the wound area at the time of measurement compared with the wound area on day 0 (100%) in the different groups (Fig. 1). The wound contraction of CG was significantly higher than RG at all time points. Av treatment resulted in a significant enhancement of wound contraction on days 6 and 12 postwounding compared with RG.

#### Histologic assessment

The epithelium was observed to creep over the granulation tissue more in CG than RAG, but still was not seen in RG on day 6 postwounding (Fig. 2). The epithelialization score was significantly different between CG and RG on day 15 postwounding. The epithelialization was enhanced by Av treatment; however, it was not significantly higher than RG.

The overall number of cellular components in RG was decreased. The mean number of inflammatory cells per field was significantly higher in RAG than RG on day 6 postwounding. Macrophage was the predominant cell type in RAG. However, the mean number of inflammatory cells per field was significantly lower in RAG than RG on day 15 postwounding. The average number of fibroblast cells per field was significantly greater in CG than RAG and RG on days 6 and 15 postwounding. It also was significantly greater in RAG than RG on days 6 and 15 postwounding (Table 2) (Fig. 3).

**Table 1** Primer sequences for PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>PCR primer sequences</th>
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<tr>
<td>GAPDH-fw</td>
<td>5′-CAGCAATGCATCTGACAG-3′</td>
</tr>
<tr>
<td>GAPDH-rv</td>
<td>5′-GAGTTGCTTTGAGTCACAGG-3′</td>
</tr>
<tr>
<td>TGF-β-1-fw</td>
<td>5′-CCAGATCTCCTCCCAACTAA-3′</td>
</tr>
<tr>
<td>TGF-β-1-rv</td>
<td>5′-TTTTGTCTAGATTGTGGT-3′</td>
</tr>
<tr>
<td>bFGF-fw</td>
<td>5′-GTCAAATACAGCTCCAAAGC-3′</td>
</tr>
<tr>
<td>bFGF-rv</td>
<td>5′-TTTATACTGCCAGTTCGTT-3′</td>
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fw = forward; rv = reverse.
The newly formed blood vessels were more distributed in RAG than RG. The number of newly formed blood vessels in the granulation tissue was significantly higher in RAG than RG on day 6 postwounding (Table 2). On day 15 postwounding, the granulation tissue was more organized and less blood vessels in RAG than RG (blood vessels not counted).

By using Masson’s trichrome stain for assessing the collagen deposition, the dense network of thin collagen fibers indicating new deposition of collagen was observed on day 6 postwounding. The collagen deposition score was significantly higher in CG than both RAG and RG on day 6 postwounding. It also was significantly higher in RAG than RG (Figs. 3 and 4). On day 15 postwounding, collagen fibers were higher in content and more tightly packed and mature in appearance in CG than both RAG and RG. There was a significantly higher collagen deposition score between CG and RG and between RAG and RG (Figs. 3 and 4). Av treatment in irradiated rats resulted in an increase and restoration of collagen bundles produced by fibroblasts.

**Immunohistochemical assessment**

The qualitative assessment of immunohistochemical staining on day 6 postwounding in the granulation tissue revealed that the predominant cell types that were positive to TGF-β-1 and bFGF expression were fibroblasts and macrophages. There was a higher number of TGF-β-1– and bFGF-positive cells throughout the wound tissue in RAG compared with RG. The expression profiles of TGF-β-1 and bFGF are shown in (Fig. 5). A significant increase of both TGF-β-1– and bFGF-positive cells were observed in RAG

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**Table 2** The mean number of inflammatory cells, fibroblasts, and blood vessels per field

<table>
<thead>
<tr>
<th></th>
<th>Inflammatory cells/field</th>
<th>Fibroblast cells/field</th>
<th>Blood vessels/field</th>
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<tr>
<td></td>
<td>Day 6</td>
<td>Day 15</td>
<td>Day 6</td>
</tr>
<tr>
<td>CG</td>
<td>157.50 ± 55.31* †</td>
<td>30.67 ± 16.33*</td>
<td>123 ± 42.54* †</td>
</tr>
<tr>
<td>RAG</td>
<td>108.35 ± 51.31 †</td>
<td>39 ± 16.42‡</td>
<td>47 ± 25.01‡</td>
</tr>
<tr>
<td>RG</td>
<td>74.50 ± 47.85</td>
<td>59.6 ± 14.87</td>
<td>12.55 ± 13.60</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of 5 rats in each group.
ND = no data.
*P < .05 significant difference between CG and RG.
†P < .05 significant difference between CG and RAG.
‡P < .05 significant difference between RAG and RG.
than RG (Table 3) (Fig. 6). These results show that expression levels of TGF-β-1 and bFGF were significantly changed by Av treatment in radiation skin wounds.

**PCR results**

The expression of TGF-β-1 messenger RNA (mRNA) was enhanced in RAG when compared with RG (Fig. 6). The expression of bFGF mRNA was not detected in RG, in contrast it was expressed in RAG (Fig. 6). The relative density of TGF-β-1 mRNA was significantly higher in RAG (34.30% ± 3.04%) than those of RG (13.94% ± 12.74%).

The relative density of bFGF in RAG and CG was (63.92% ± 35.81% and 71.37% ± 5.27%; respectively; Fig. 6).

**Comments**

The main finding of our study was that oral Av administration accelerates wound healing in an acute radiation-delayed wound healing model by stimulating TGF-β-1 and bFGF production.

The effect of radiation on the wound process depends both on the sequences of radiation and surgery (ie, which is administered first) and on the time elapsed between radiation and surgery. Preoperative irradiation usually is assumed to have a therapeutic ratio (tumor control relative to the risk of long-term radiation-induced complication) superior to that of postoperative irradiation. The risk of early wound complication is, in contrast, substantially less with postoperative radiation therapy. Postoperative wound complication rates after radiation exposure have been reported to occur in up to 67% of cases. Moreover, the timing of surgery relative to preoperative radiation therapy affects the wound healing process. Previous studies have shown that wounding of the skin a few days after single or multiple irradiation is associated with a significant delay in wound healing. Surgery and radiation exposure may occur in close temporal proximity of each other. Most frequently, this occurs in the setting of adjuvant radiation therapy, and surgeons do not frequently create excisional wounds acutely after radiation but the development of surgical emergencies also may necessitate surgery before, during, or after a course of radiation therapy. Therefore, our model of acute radiation effects on wound healing might have clinical relevance.

The biological effect of soft x-rays on delayed wound healing was reported previously. Soft x-rays with their long wavelength enable the delivery of a maximal dose to skin and subcutaneous tissue. There is a high dose of skin entry and a rapid decrease in the energy of radiation at depth, minimizing the systemic radiation effects compared with other sources. Therefore, it is convenient to observe the molecular and cellular effects of radiation in skin wound healing.

Radiation has been reported to disrupt all the wound healing processes; it inhibits inflammatory reactions, fibroblast proliferation, ECM production, angiogenesis, and growth factor synthesis. Thus, there is a need for a multifunction drug that adjusts all the criteria of good wound healing. Av has been proven to promote wound healing in various wound models. Furthermore, it has been found to be anti-inflammatory, antibacterial, and a good immunostimulant and radioprotective agent. Therefore, the present study was performed to explore the usefulness of Av treatment in irradiated wounds.

Previous studies have shown that Av is effective in accelerating wound contraction by both oral and topical
application routes of administration. Wound contraction is an important feature of the healing process that results from the movement of the wound edges toward the center. The progression of wound healing can be judged by periodic assessment of the contraction of the excision wound. In the present study, RG showed significantly less wound contraction at all time points compared with CG. A similar delay in wound contraction after exposure to soft dermis; GT = granulation tissue. Yellow lines indicate a border between normal dermis and wound gap.

Figure 4  Histologic finding of the collagen deposition. CG shows a much greater amount of collagen than that of RAG and RG, and RAG shows greater collagen than RG. (A and D) CG on days 6 and 15 postwounding, respectively; (B and E) RAG on days 6 and 15 postwounding, respectively; and (C and F) RG on days 6 and 15 postwounding, respectively (Masson’s trichrome stain). D = dermis; GT = granulation tissue.

Figure 5  Immunohistochemical staining for TGF-β-1 and bFGF on day 6 postwounding. RAG shows more expression of TGF-β-1 and bFGF than RG as revealed by increasing the number of immunopositive cells in RAG than RG. (A, B, and C) TGF-β-1 expression in CG, RAG, and RG, respectively. (D, E, and F) bFGF expression in CG, RAG, and RG, respectively.
x-ray radiation was observed earlier. Wound contraction is mediated by generation of cellular forces in the contractile elements of fibroblasts and myofibroblasts. Radiation may impair healing primarily because of toxicity to fibroblasts. Our histologic results revealed that the number of fibroblasts significantly decreased in RG compared with CG. By contrast, Av significantly increased fibroblast proliferation compared with RG. This finding is consistent with previous studies that showed Av gel crude extract or its components have the ability to stimulate proliferation of normal and diabetic skin fibroblasts in both in vivo and in vitro studies.

Collagen is the major protein of the ECM and the predominant constituent of the final scar. The main function of collagen is to provide strength and integrity to the wound. Acute irradiation predominantly affects the fibroblast’s capability to produce collagen, and the collagen that is produced does not mature quickly enough to meet the demands of the early phase of wound healing. Our results revealed that collagen deposition was decreased significantly in RG compared with CG. Av was observed to enhance the collagen deposition significantly more than RG on both days 6 and 15 postwounding. Therefore, the promotion of fibroblast proliferation by Av also partly could be ascribed to its enhancement activity to collagen synthesis of fibroblasts.

Angiogenesis during wound repair serves the dual function of providing the nutrients required by the healing tissues and contributing to structural repair through the formation of granulation tissue. Radiation damage to the microvascular endothelium is a critical event in the pathogenesis of poor healing. Radiation can induce DNA damage and apoptosis in microvascular endothelial cells. In the present study, a decreased angiogenesis process after radiation exposure was reflected by the significant decrease in the number of newly formed blood vessels compared with control wounds. By contrast, Av was found to enhance angiogenesis as evidenced by the increase in the number of newly formed blood vessels in the granulation tissue. Previous studies have shown that the β-sitosterol compound from Av has been shown to enhance angiogenesis in the chick embryo chorioallantoic membrane assay.

<table>
<thead>
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<th>Table 3</th>
<th>The percentage of positive cells of TGF-β-1 and bFGF per total cells on day 6 postwounding</th>
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<tbody>
<tr>
<td>% of positive cells/total cells</td>
<td></td>
</tr>
<tr>
<td>TGF-β-1</td>
<td>bFGF</td>
</tr>
<tr>
<td>CG</td>
<td>7.12 ± 1.19*†</td>
</tr>
<tr>
<td>RAG</td>
<td>3.79 ± .84‡</td>
</tr>
<tr>
<td>RG</td>
<td>1.41 ± .36</td>
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Values are mean ± standard deviation of 5 rats in each group.
*P < .05 significant difference between CG and RG.
†P < .05 significant difference between CG and RAG.
‡P < .05 significant difference between RAG and RG.
pound also showed positive results in the human umbilical vein endothelial cell motility assay. In another experiment, β-sitosterol was shown to enhance the expression of several proteins related to angiogenesis, namely Von Willebrand factors, vascular endothelial growth factor (VEGF), VEGF-receptor fetal liver kinase-1, and blood vessel matrix laminin in the brain of Mongolian gerbils. Growth factors are critical regulators of the wound-healing process. Among many growth factors, TGF-β-1 and bFGF are strongly down-regulated after acute radiation exposure. Although nearly all cell types produce TGF-β-1, its main sources of production in wound healing are platelets, monocytes/macrophages, and fibroblasts. Our data revealed that all of those cells are decreased and TGF-β-1 expression was decreased significantly in RG compared with CG. Similarly, Johnson et al reported that acute radiation combined with surgery leads to marked depression in local collagen deposition and TGF-β-1 expression, whereas late effects of radiotherapy tend to be associated with increased collagen deposition and TGF-β-1 expression levels. In our model, only the early phase after radiation was studied.

Another important growth factor in the wound-healing process is bFGF. bFGF is one of the most potent angiogenic growth factors involved in soft-tissue healing. Our data revealed that bFGF expression was decreased significantly in RG compared with CG. Tattini et al reported that the combination of TGF-β-1 and bFGF improved wound healing in an acute postirradiation impaired wound-healing model. Av treatment was shown to have similar effects by increasing the expression of both TGF-β-1 and bFGF compared with RG. We suggested that Av with its components stimulated the production of TGF-β-1 and bFGF, which enhanced the fibroblast proliferation, collagen deposition, and angiogenesis. Previous reports have stated that acemannan and other polysaccharides extracted from Av exert an immunostimulative effect by activating macrophages. Those stimulated macrophages activate phagocytosis, nitrous oxide, cytokines, and growth factor production.

In the present study, Av accelerated infiltration of macrophages in the early phases of wound healing even under irradiated conditions. The macrophage is the prime source of several growth factors in wound healing. Moreover, acemannan was found to stimulate fibroblasts in vitro to produce keratinocyte growth factor-1 and VEGF. β-sitosterol also was shown to enhance the expression of VEGF.

The exact mechanism whereby Av stimulates cell proliferation and growth factor production in the macrophage and/or fibroblast still is unclear. Previous reports speculated that Av active components such as mannose-6-phosphate and/or acemannan bind with special ligands to the mannose receptor on the cell surface of macrophages and fibroblasts. After this binding the cells will be stimulated to produce growth factors and proliferate. Further study still is required to confirm this speculation.

The cellular and molecular bases of wound healing are different between acute and chronic radiation exposure. The present study investigated the effect of Av on wound healing in the early phase after single-dose acute radiation exposure. Further studies are needed to determine the effect of Av on wound healing after chronic radiation exposure and multiple doses of radiation exposure.

In conclusion, Av oral administration showed wound healing enhancement in the early phase after single-dose acute radiation exposure. The improved wound activity might be attributed to its stimulating effect on increasing inflammatory cell infiltration, fibroblast proliferation, angiogenesis, and growth factor production.

References