

## Review Article

# Effects of Ginsenoside Rb<sub>1</sub> on Skin Changes

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Ginseng roots (*Panax ginseng* CA Meyer) have been used traditionally for the treatment, especially prevention, of various diseases in China, Korea, and Japan. Both experimental and clinical studies suggest ginseng roots to have pharmacological effects in patients with life-style-related diseases such as non-insulin-dependent diabetic mellitus, atherosclerosis, hyperlipidemia, and hypertension. The topical use of ginseng roots to treat skin complaints including atopic suppurative dermatitis, wounds, and inflammation is also described in ancient Chinese texts; however, there have been relatively few studies in this area. In the present paper, we describe introduce the biological and pharmacological effects of ginsenoside Rb<sub>1</sub> isolated from Red ginseng roots on skin damage caused by burn-wounds using male Balb/c mice (*in vivo*) and by ultraviolet B irradiation using male C57BL/6J and albino hairless (HR-1) mice (*in vivo*). Furthermore, to clarify the mechanisms behind these pharmacological actions, human primary keratinocytes and the human keratinocyte cell line HaCaT were used in experiments *in vitro*.

## 1. Introduction

The oral administration of red ginseng root (*P. ginseng*) extracts has long been used to treat various diseases, including liver and kidney dysfunction, hypertension, non-insulin-dependent diabetes mellitus, and postmenopausal disorders, in China, Korea, and Japan. Topical applications have also been used for atopic suppurative dermatitis, wounds, and skin inflammation. The materials for Korean red ginseng products are selected from among ginseng roots (*Panax ginseng* CA Meyer) carefully cultivated in well-fertilized field for 6 years and then steamed and dried in the sun six times. The red ginseng extract produced by Korea Ginseng Corporation (Taejon, Korea) is dried and powdered by freezing prior to use. In this paper, we introduce the biological and pharmacological effects of ginsenoside Rb<sub>1</sub> isolated from red ginseng roots on skin damage in mice.

## 2. Effects of Ginsenoside Rb<sub>1</sub> on Burn Wound Healing in Mice

Burns and wounds initially induce coagulative necrosis and cause the formation of a scar. Macrophages migrate to an injured area to kill invading organisms and produce cytokines that recruit other inflammatory cells responsible for the diverse effects of inflammation [1, 2]. Angiogenesis in the injured area is closely associated with the process of wound healing [3]. Moreover, growth factors and cytokines are central to the wound-healing process [4–6]. Thus, the burn wound-healing process is complex, involving inflammatory factors, including monocyte migration and cytokine production, and growth factors and angiogenesis during reepithelialization. Vascular endothelial growth factor (VEGF) plays an important role in skin tissue repair through angiogenesis during the healing of burn wounds

TABLE 1: Effects of various ginseng saponins on angiogenesis.

Effects of ginsenoside Rb <sub>2</sub> (100 µg/mouse, <i>iv</i> ) on tumor-induced angiogenesis in B16-BL6 melanoma-inoculated mice ( <i>in vivo</i> ) Ginsenoside Rb <sub>2</sub> showed antiangiogenesis [25].
Effects of ginsenoside Rb <sub>2</sub> (100 µg/mouse, <i>iv</i> and 300 µg/mouse, <i>po</i> ), 20(R)Rg <sub>3</sub> (100 µg/mouse, <i>iv</i> and 300 µg/mouse, <i>po</i> ), and 20(S)Rg <sub>3</sub> (100 µg/mouse, <i>iv</i> and 300 µg/mouse, <i>po</i> ) on tumor-induced angiogenesis in B16-BL6 melanoma-inoculated mice ( <i>in vivo</i> ). Ginsenoside Rb <sub>2</sub> , 20(R)Rg <sub>3</sub> and 20(S)Rg <sub>3</sub> , showed antiangiogenesis [40].
Effects of the total saponin fraction (10–100 µg/mL) on tube formation by HUVECs ( <i>in vitro</i> ). The total saponin fraction enhanced angiogenesis [22].
Effects of ginsenoside Rg <sub>1</sub> (10 µM) and Rb <sub>1</sub> (10 µM) on angiogenesis in scaffold implants in mice ( <i>in vivo</i> ). Effects of Rg <sub>1</sub> (125 nM) and Rb <sub>1</sub> (125 nM) on chemoinvasion in HUVEC ( <i>in vitro</i> ). Ginsenoside Rg <sub>1</sub> enhanced angiogenesis, and ginsenoside Rb <sub>1</sub> showed antiangiogenesis in the earliest stage [23].
Effects of ginsenoside Re (10–100 µg/mL) on angiogenesis in HUVECs ( <i>in vitro</i> ). Effects of Re (70 µg/extracellular matrix) on angiogenesis in extracellular matrix-implanted rats ( <i>in vivo</i> ). Ginsenoside Re showed angiogenesis [41].
Effects of ginsenoside Rg <sub>1</sub> (150–600 nM) on angiogenesis in HUVECs ( <i>in vitro</i> ). Effects of Rg <sub>1</sub> (600 nM/Matrigel) on angiogenesis in Matrigel-implanted mice ( <i>in vivo</i> ). Ginsenoside Rg <sub>1</sub> promoted angiogenesis [42].
Effects of 20(R)-ginsenoside Rg <sub>3</sub> (1-1000 nM) on angiogenesis in HUVECs ( <i>in vivo</i> ). 20(R)-ginsenoside Rg <sub>3</sub> showed antiangiogenesis [43].
Effects of Ginsenoside Rg <sub>1</sub> (150 nM) on angiogenesis in HUVECs ( <i>in vitro</i> ). Ginsenoside Rg <sub>1</sub> promoted angiogenesis [44].
Effects of ginsenoside Rg <sub>1</sub> (30 µg/mL) and Re (30 µg/mL) on angiogenesis in HUVECs ( <i>in vitro</i> ). Effects of Rg <sub>1</sub> (50 µg/mL) and Re (50 µg/mL) on angiogenesis in Matrigel-implanted mice ( <i>in vivo</i> ). Ginsenoside Rg <sub>1</sub> and Re promoted angiogenesis [45].
Effects of ginsenoside Rg <sub>3</sub> (3 mg/kg, <i>ip</i> ) on growth and angiogenesis of ovarian cancer ( <i>in vivo</i> ). Ginsenoside Rg <sub>3</sub> showed antiangiogenesis [46].
Effects of ginsenoside Rb <sub>1</sub> (250 nM) on angiogenesis in HUVECs ( <i>in vitro</i> ). Ginsenoside Rb <sub>1</sub> showed antiangiogenesis [47].
Effects of ginsenoside Rg <sub>1</sub> (500 µg/Matrigel) on angiogenesis in Matrigel-implanted mice ( <i>in vivo</i> ) Ginsenoside Rg <sub>1</sub> promoted angiogenesis [48].
Effects of saponins (0.1–100 µg/mL) isolated from <i>Panax notoginseng</i> and Rg <sub>1</sub> (10 µg/mL) on angiogenesis in HUVECs ( <i>in vitro</i> ). Total saponin and ginsenoside Rg <sub>1</sub> promoted angiogenesis [49].
Effects of ginsenoside Rg <sub>3</sub> (20 mg/kg, <i>po</i> ) on angiogenesis and growth in lung carcinoma-implanted mice. Ginsenoside Rg <sub>3</sub> showed antiangiogenesis [50].

[4, 7, 8]. Furthermore, it has been demonstrated that chemokines including macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ) and monocyte chemoattractant protein-1 (MCP-1) are expressed at high levels in murine full-thickness dermal wounds at times preceding and coinciding with maximal macrophage infiltration [9–12]. Interleukin 1- $\beta$  (IL-1 $\beta$ ) is also known to be released from monocyte-derived macrophages during inflammation and stimulates VEGF expression in endothelial cells, keratinocytes, synovial fibroblasts, and colorectal carcinoma cells [13–16]. IL-1 $\beta$  gene expression was reported to be upregulated in MCP-1-treated human monocytes [17]. Trautmann et al. [18] found that the expression of MCP-1 of macrophage and keratinocyte origin correlated with the accumulation of mast cells during wound healing. Weller et al. [19] reported that mast cell activation and histamine release were required for wound healing. Numata et al. [20] showed that the accelerated wound-repair activity of histamine was mediated by the activity of basic fibroblast growth factor (bFGF), which leads to angiogenesis, and macrophage recruitment in the wound-healing process. Thus, the process of wound repair is thought to be closely associated with the network systems among

various cells such as keratinocytes, fibroblasts, macrophages and mast cells, and might be modulated by interactions among chemokines, cytokines, growth factors, and related biofactors secreted from these cells.

The genus *Panax* derives its name from the Greek words *pan* (all) and *akos* (healing). In 1988, Kanzaki et al. [21] reported that an orally administered red ginseng root extract stimulated the repair of intractable skin ulcers in patients with diabetes mellitus and Werner's syndrome in clinical trials. Morisaki et al. [22] showed that the local administration of ginseng saponins markedly improved wound healing in diabetic and aging rats. Sengupta et al. [23] reported that ginsenoside Rg<sub>1</sub> promoted functional angiogenesis into a polymer scaffold (*in vivo*) and the proliferation and chemoinvasion of tube-like capillary formation by human umbilical vein endothelial cells (HUVECs) through enhanced expression of nitric oxide synthetase, phosphatidylinositol-3 kinase, and the Akt pathway (*in vitro*). Conversely ginsenoside Rb<sub>1</sub> inhibited the earliest step in angiogenesis, the chemoinvasion of HUVECs [23]. Furthermore, Choi [24] reported that ginsenoside Rb<sub>2</sub> improved wound healing through its facilitating effects on

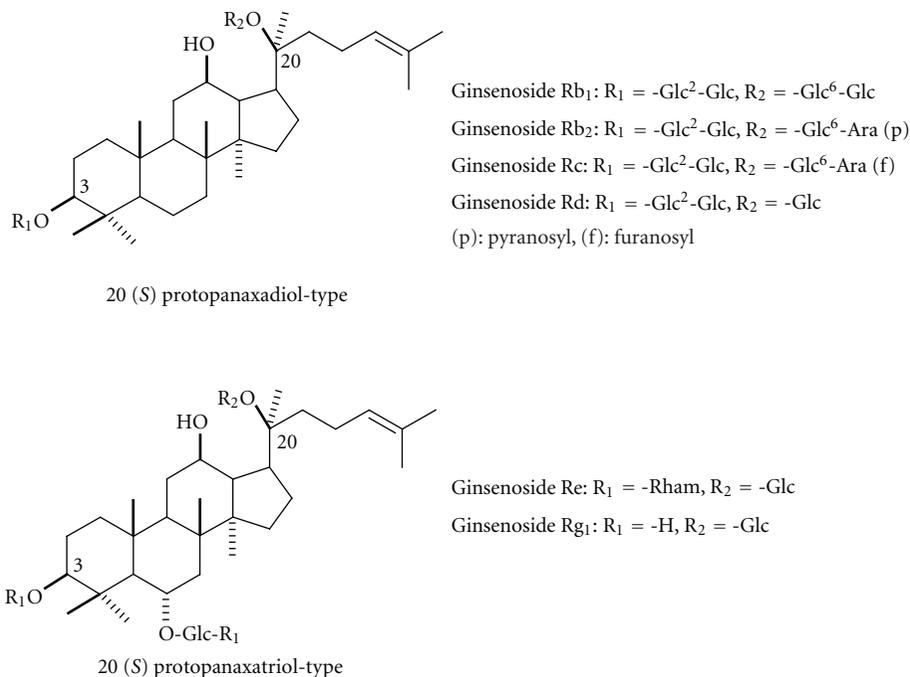


FIGURE 1: The structure of various ginsenosides.

TABLE 2: Effects of ginsenoside Rb<sub>1</sub> on angiogenesis from the area surrounding burn wounds in mice [26].

Treatment	Blood vessel length (mm/field)	Blood vessel area (mm <sup>2</sup> /field)
Untreated burn wounds (control)	75.6 ± 24.9	10.5 ± 3.8
+Ginsenoside Rb <sub>1</sub>		
(100 fg/wound)	228.8 ± 38.6*	46.6 ± 15.0*
(10 pg/wound)	203.0 ± 17.0*	37.6 ± 5.5*
(1 ng/wound)	274.1 ± 37.4*	49.9 ± 4.7*
+bFGF	241.5 ± 28.3*	35.8 ± 5.9*

The burn wounds were created on the backs of male Balb/c mice (6 weeks old) under anesthesia with pentobarbital. A polyethylene filter pellet (about 8 mm in diameter, 3 mm thick) containing the indicated amount of basic fibroblast growth factor (bFGF) or ginsenoside Rb<sub>1</sub> was applied to the burn wound surface. On day 9, any angiogenesis in the site surrounding the burn wound was photographed using a stereoscopic microscope, and the area and length of blood vessels were measured using a Coordinating Area and Curvimeter Machine (X-PLAN 360 dII, Ushitaka, Tokyo, Japan).

Values are the mean ± SE for six mice. \*Significantly different from untreated burn wounds (control),  $P < 0.05$ .

epidermal cell proliferation, by upregulating the expression of proliferation-related factors. However, Sato et al. [25] found that the intravenous administration of ginsenoside Rb<sub>2</sub> inhibited metastasis to the lung by inhibiting tumor-induced angiogenesis in B16-BL6 melanoma-bearing mice. Thus, there are perplexing contradictions in the reported effects of various ginseng saponins on angiogenic activity as shown in Table 1.

To clarify these differing effects, we first attempted to examine the effects of various ginseng saponins on wound

healing. Among six ginseng saponins (ginsenoside Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, and Rg<sub>1</sub>) (Figure 1), we found that ginsenoside Rb<sub>1</sub> enhanced burn-wound healing most strongly.

In summary, we reported the promotion of burn-wound healing by the topical application of ginsenoside Rb<sub>1</sub> at low doses (100 fg, 10 pg, and 1 ng per wound) to be due to the promotion of angiogenesis during skin wound repair through stimulation of VEGF production and an increase in hypoxia-inducible factor (HIF-) 1 $\alpha$  expression in keratinocytes and the elevation of interleukin (IL-) 1 $\beta$  from macrophage accumulation in the burn wound area [26]. Furthermore, we found the facilitating effects of ginsenoside Rb<sub>1</sub> at low doses (100 fg, 10 pg, and 1 ng per wound) to be due to the promotion of angiogenesis via the activation of basic fibroblast growth factor (bFGF) through an increase in histamine released from mast cells recruited by the stimulation of monocyte chemoattractant protein-1 (MCP-1) as another mechanism [27]. We will explain our experiments regarding the facilitating effects of ginsenoside Rb<sub>1</sub> on burn-wound healing in detail. The burn area in mice treated with a topical application of ginsenoside Rb<sub>1</sub> in the range of 10<sup>-8</sup>% to 10<sup>-12</sup>% was significantly reduced on days 8–20 compared to that in vehicle-treated burn-wound control mice (Figure 2).

To clarify the mechanism behind the facilitating effect of ginsenoside Rb<sub>1</sub> on wound healing, we examined levels of IL-1 $\beta$  and VEGF in exudates of the burn. The levels increased with time over 9 days. At 1 ng of ginsenoside Rb<sub>1</sub> per wound, the level of IL-1 $\beta$  was increased on days 1, 3, and 5 but significantly decreased on day 9 compared to that in vehicle-treated control mice (Figure 3). The topical application of bFGF (2.5  $\mu$ g per wound) also increased IL-1 $\beta$  production on day 3. The VEGF level in the exudates from the wound

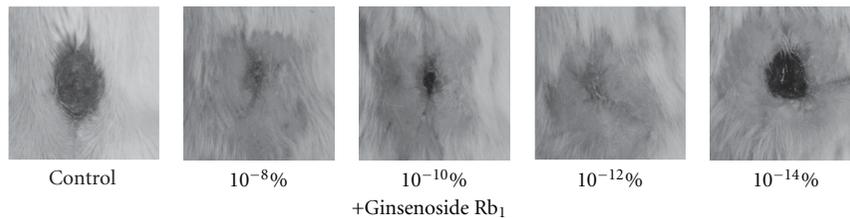
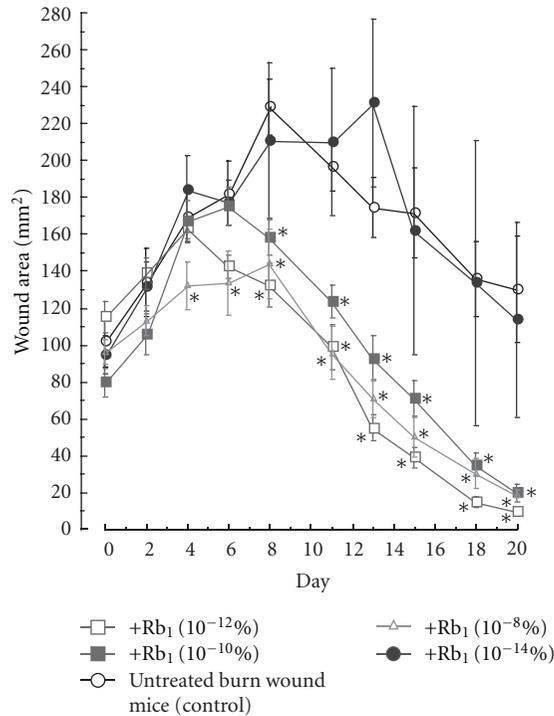


FIGURE 2: Effects of ginsenoside Rb<sub>1</sub> on wound healing in mice [26]. After the burn wound was made by applying a customized soldering iron to the skin on the backs of male Balb/c mice (6 weeks old) for 10 s at 250° C, a sterile biopsy punch (8 mm diameter) was used to excise the burnt skin, leaving the underlying fasciae intact. All surgical treatments were performed under anesthesia with pentobarbital. Indicated amounts of ginsenoside Rb<sub>1</sub> were applied to the burn wounds surface and then covered with a film dressing for 19 consecutive days. The burn wound site was photographed every other day and burn wound area was measured using a Coordinate Area and Curvimeter Machine (X-PLAN 360 dII). Values are the mean  $\pm$  SE for 6–12 mice. \* Significantly different from vehicle-treated mice,  $P < 0.05$ .

TABLE 3: Effects of *Panax ginseng* extract on skin aging.

Effects of the total ginseng saponin fraction (100 to 500 $\mu\text{g}/\text{mL}$ ) on luciferase reporter gene assays in human dermal fibroblast ( <i>in vitro</i> ).
Effects of the total ginseng saponin fraction (100 to 500 $\mu\text{g}/\text{mL}$ ) on type I collagen in human dermal fibroblast ( <i>in vitro</i> ).
The total saponin fraction (100 to 500 $\mu\text{g}/\text{mL}$ ) increased type I procollagen synthesis [51].
Effects of red ginseng extract (20 and 60 mg/kg, <i>po</i> ) on acute UVB-induced skin aging in mice
The extract inhibited the increases in epidermis and dermis thickness induced by UVB [52].
Effects of red ginseng extract (20 mg/kg, <i>ip</i> or topical application of 0.2% cream) on chronic UVB-irradiated skin damage in hairless mice.
The extract reduced wrinkling and tumor incidence [53].
Effects of ginsenoside Rb <sub>1</sub> (100 fg, 10 pg, or 1 ng/mouse, topical application) on chronic UVB-irradiated skin aging in hairless mice.
Ginsenoside Rb <sub>1</sub> inhibited the increase in skin thickness, wrinkling, and epidermis in UVB-irradiated hairless mice [31].
Effects of red ginseng extract (a diet containing 0.5 and 2.5% red ginseng extract) on UVB-irradiated skin aging in hairless mice.
The extract reduced wrinkling, the mRNA level of procollagen type I, and the MMP-1 level [54].
Healthy female volunteers over 40 years of age were randomized in a double-blind fashion to receive either red ginseng extract (3 g/day) or placebo for 24 weeks. ( <i>Clinical study</i> ).
Red ginseng extract caused an improvement in facial wrinkling and increase in type I procollagen synthesis [55].

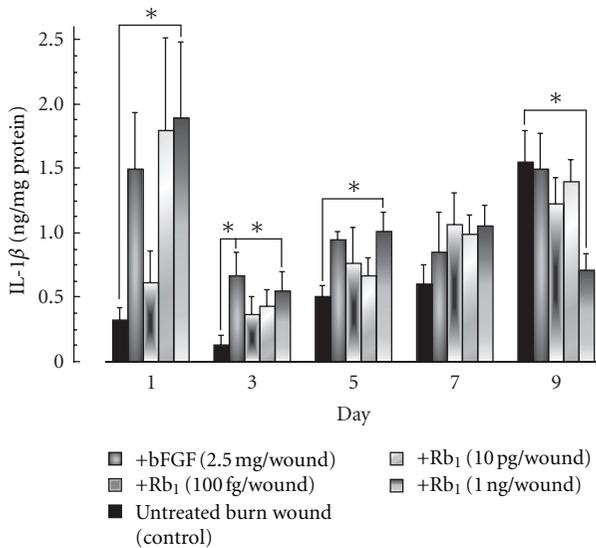


FIGURE 3: Effects of ginsenoside Rb<sub>1</sub> and bFGF on IL-1 $\beta$  production in the exudates of burns in male Balb/c mice [26]. The burn wounds were created on the backs of male Balb/c mice (6 weeks old) under anesthesia with pentobarbital. A polyethylene filter pellet (about 8 in mm diameter, 3 mm thick) containing the indicated amount of basic fibroblast growth factor (bFGF) or ginsenoside Rb<sub>1</sub> was applied to the burn wounds surface. On days 1, 3, 5, 7, and 9, the filter pellets were removed and replaced with fresh filter pellets. For control mice, filter pellets containing saline alone were applied according to the same schedule. Immediately after removal, phosphate-buffered saline (PBS, pH 7.0) (200  $\mu$ l) was added to each filter pellet and mixed for 10 min. The IL-1 $\beta$  levels in the filter pellets were measured using a mouse IL-1 $\beta$  ELISA kit. Values are the mean  $\pm$  SE for 6 mice. \*Significantly different from control,  $P < 0.05$ .

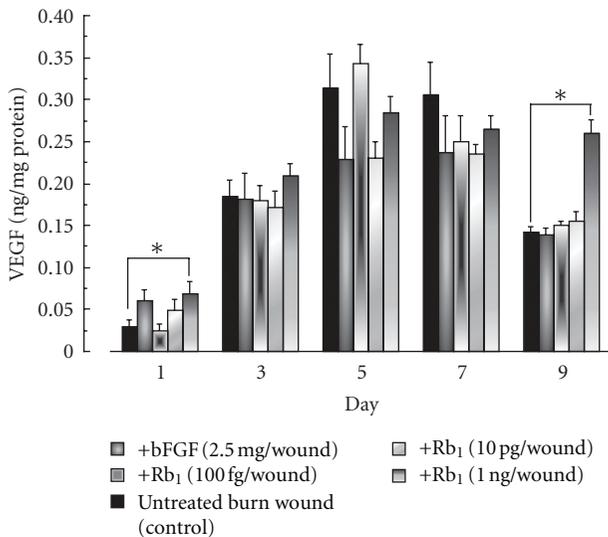


FIGURE 4: Effects of ginsenoside Rb<sub>1</sub> and bFGF on VEGF production in the exudates of burns in male Balb/c mice [26]. The experiments were performed as described in Figure 3, and the VEGF levels in the filter pellets were measured using a mouse IL-1 $\beta$  ELISA kit. Values are the mean  $\pm$  SE for 6 mice. \*Significantly different from control,  $P < 0.05$ .

TABLE 4: Effects of ginsenoside Rb<sub>1</sub> on the thickness of the epidermis and extracellular matrix (ECM) of the dermis at week 12 in UVB-irradiated hairless mice [31].

	Epidermis ( $\mu$ m)	ECM ( $\mu$ m) in dermis
Normal mice	14.74 $\pm$ 1.11*	332.51 $\pm$ 23.18*
Vehicle-treated UVB-irradiated mice (control)	142.59 $\pm$ 25.37	632.32 $\pm$ 31.96
+Ginsenoside Rb <sub>1</sub>		
(100 fg/mouse)	46.00 $\pm$ 6.26*	561.86 $\pm$ 45.22
(10 pg/mouse)	49.24 $\pm$ 4.73*	560.67 $\pm$ 44.81
(1 ng/mouse)	39.84 $\pm$ 6.26*	585.63 $\pm$ 31.35

The initial dose of UVB was set at 36 mJ/cm<sup>2</sup>, which was subsequently increased to 54 mJ/cm<sup>2</sup> at weeks 1–4, 72 mJ/cm<sup>2</sup> at weeks 4–7, 108 mJ/cm<sup>2</sup> at weeks 7–10, and finally to 122 mJ/cm<sup>2</sup> at weeks 10–12 in male albino hairless HOS: HR-1 mice. The frequency of UVB irradiation was set at three times per week. Ginsenoside Rb<sub>1</sub> (100 fg, 10 pg, and 1 ng/mouse) was applied topically to the dorsal region of each mouse every day for 12 weeks. The dorsal skin samples (about 3 cm<sup>2</sup>) removed at week 12 were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5  $\mu$ m thickness, deparaffinized, and stained with hematoxylin-eosin (HE) and Azan. Four different microscopic fields ( $\times$ 200 magnification) per plate were photographed. The thickness of the epidermis and dermis thickness were measured from the samples stained by HE and Azan, using a Digimatic Caliper.

Values are the mean  $\pm$  SE for 6 mice. \*Significantly different from UVB-irradiated hairless mice (control),  $P < 0.05$ .

TABLE 5: Effects of ginsenoside Rb<sub>1</sub> on the numbers of apoptotic and 8-OHdG-positive cells at week 12 in the skin of UVB-irradiated hairless mice [31].

	Apoptotic cells (number/field)	8-OHdG-positive cells (number/field)
Normal mice	0 $\pm$ 0*	106 $\pm$ 7*
Vehicle-treated UVB-irradiated mice (control)	102 $\pm$ 10	286 $\pm$ 32
+Ginsenoside Rb <sub>1</sub>		
(100 fg/mouse)	19 $\pm$ 11*	150 $\pm$ 24*
(10 pg/mouse)	11 $\pm$ 11*	183 $\pm$ 27*
(1 ng/mouse)	9 $\pm$ 9*	109 $\pm$ 26*

Ginsenoside Rb<sub>1</sub> (100 fg, 10 pg, and 1 ng/mouse) was applied topically to the dorsal region of each mouse every day for 12 weeks. The expression levels of apoptotic cells and 8-hydroxy-2'-deoxyguanosine (8-OHdG) (marker of oxidative DNA damage) in the dorsal skin of UVB-irradiated hairless mice were examined by the TUNEL method using an apoptosis in situ detection kit and an immunoperoxidase technique using anti-8-OHdG antibody.

Values are the mean  $\pm$  SE for 6 mice. \*Significantly different from UVB-irradiated mice (control),  $P < 0.05$ .

increased until day 5 and then decreased. The application of ginsenoside Rb<sub>1</sub> increased VEGF levels on days 1 and 9 (Figure 4). However, the application of bFGF did not affect VEGF production.

The application of bFGF (2.5  $\mu$ g per wound) or ginsenoside Rb<sub>1</sub> (100 fg, 10 pg, and 1 ng per wound) for 9 days increased the length of blood vessels by 3- to 3.5-fold and

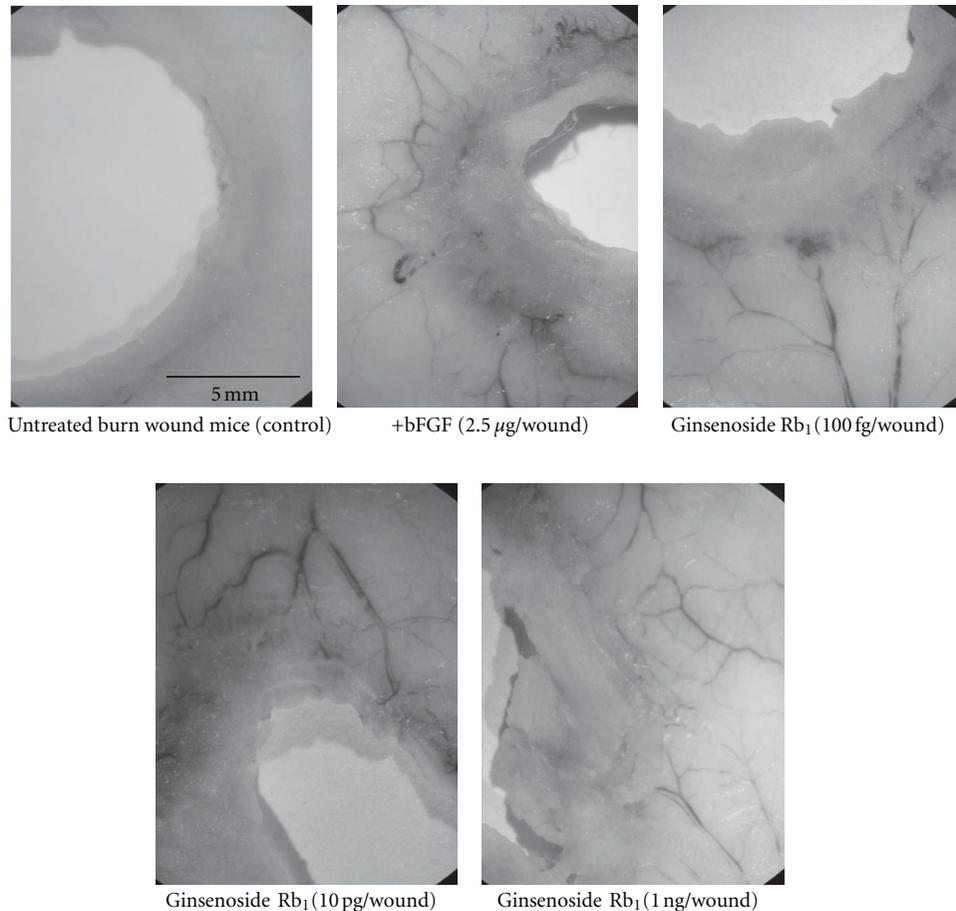


FIGURE 5: Photographs showing neovascularization from the tissue surrounding the burn and the effects of the topical application of ginsenoside Rb<sub>1</sub> [26]. The experiments were performed as described in Figure 3. On day 9, any angiogenesis in the site surrounding the burn wound was photographed using a stereoscopic microscope.

the corresponding area by 3.5- to 5.0-fold, compared to the control (Figure 5 and Table 2).

Ginsenoside Rb<sub>1</sub> at concentrations from 100 fg/mL to 1 ng/mL enhanced the VEGF production and HIF-1 $\alpha$  expression induced by IL-1 $\beta$  in the human keratinocyte cell line HaCaT (Figure 6).

These findings suggest the enhancement of wound healing by ginsenoside Rb<sub>1</sub> to be due to the promotion of angiogenesis during the repair process as a result of the stimulation of VEGF production caused by the increase in HIF-1 $\alpha$  expression in keratinocytes. Furthermore, the MCP-1 level in the exudates of vehicle-treated (control) mice reached a maximum 1 day after the burn treatment and declined rapidly from day 3. Ginsenoside Rb<sub>1</sub> (1 ng per wound) and bFGF (2.5  $\mu$ g per wound) significantly increased the level of MCP-1 on day 1 compared to that in control mice (Figure 7). Histamine levels in the exudates of the burn wound area increased until day 7. Ginsenoside Rb<sub>1</sub> (1 ng per wound) significantly increased the histamine level on day 5 compared to that in control mice (Figure 7). Furthermore, ginsenoside Rb<sub>1</sub> (100 fg, 10 pg, and 1 ng per wound) and bFGF (2.5  $\mu$ g per wound) significantly increased histamine production on day 7 (Figure 7). The facilitating

effects of ginsenoside Rb<sub>1</sub> may be due to the promotion of angiogenesis *via* the activation of bFGF through the increase in histamine released from mast cells recruited by the stimulation of MCP-1 production.

Based on these experimental results, the enhancing effects of ginsenoside Rb<sub>1</sub> on burn wound healing are summarized in Figure 8.

It has been reported that ginsenoside Rb<sub>2</sub> as well as ginsenoside Rb<sub>1</sub> promotes wound healing [24].

### 3. Effects of Ginsenoside Rb<sub>1</sub> on Ultraviolet B (UVB-) Irradiated Skin Damage in Mice

The symptoms of cutaneous aging, such as wrinkles and pigmentation, for example, develop earlier in sun-exposed skin than in unexposed skin, a phenomenon referred to as photoaging. Ultraviolet B (UVB) radiation is one of the most important environmental factors because of its hazardous effects, which include the generation of skin cancer [28], suppression of the immune system [29], and premature skin aging [30].

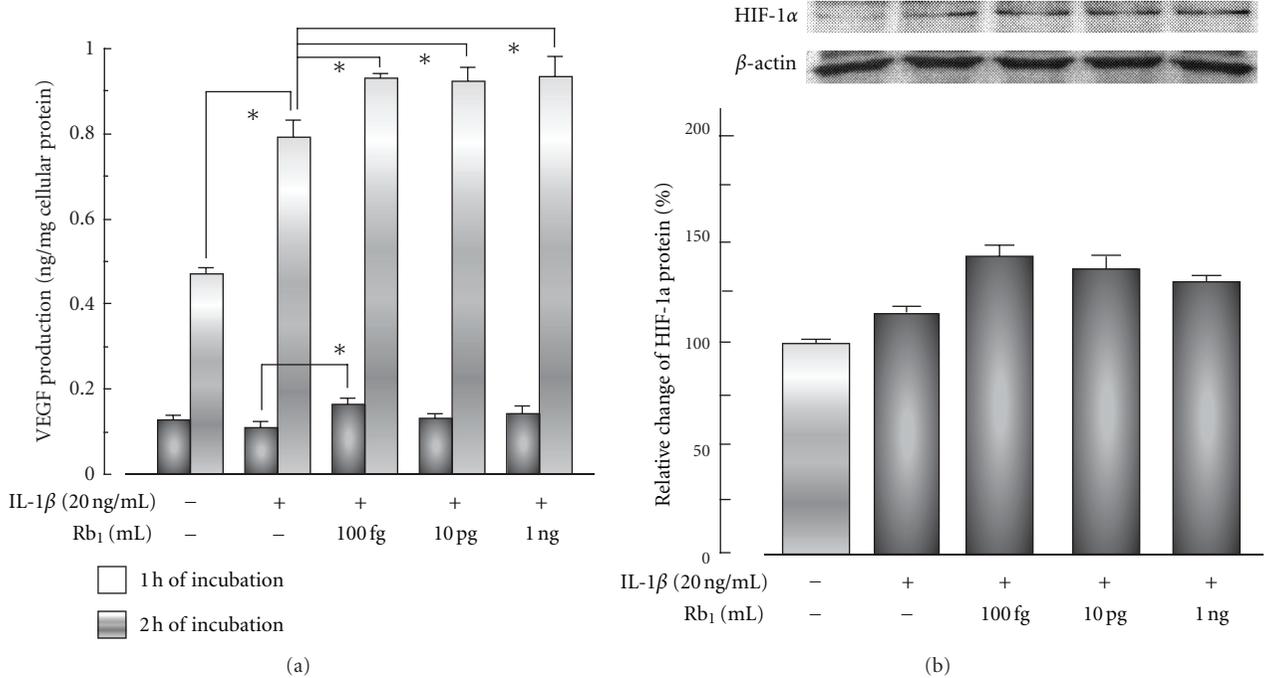


FIGURE 6: Effects of ginsenoside Rb<sub>1</sub> on VEGF production (a) and HIF-1α expression (b) with or without IL-1β in HaCaT cells [26]. The human keratinocyte cell line HaCaT was treated with the indicated amounts of ginsenoside Rb<sub>1</sub> in the presence or absence of IL-1β (20 ng/mL) for 1 or 2 h. VEGF levels in the medium were measured using a human VEGF kit. The expression of hypoxia-inducible factor (HIF-) 1α in the nuclear fraction of HaCaT cells was measured by western blot analysis with mouse anti-HIF-1α and anti-β-actin antibodies. Values are the mean ± SE for six experiments. \*Significantly different from control, *P* < 0.05.

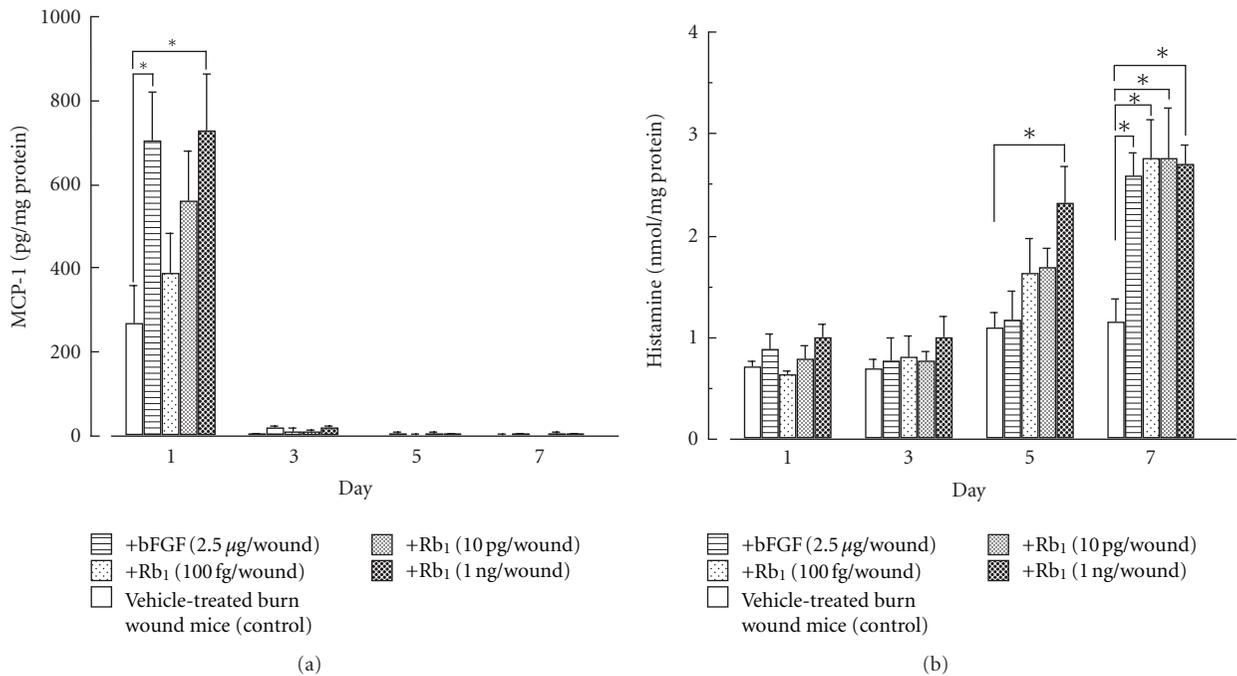


FIGURE 7: Effects of ginsenoside Rb<sub>1</sub> and bFGF on MCP-1 (a) and histamine (b) production in the exudates of burns in male Balb/c mice [27]. The experiments were performed as described in Figure 3, and then MCP-1 and histamine levels in the filter pellets were measured using mouse MCP-1 and histamine ELISA kits, respectively. Values are the mean ± SE for 6 mice. \*Significantly different from control, *P* < 0.05.

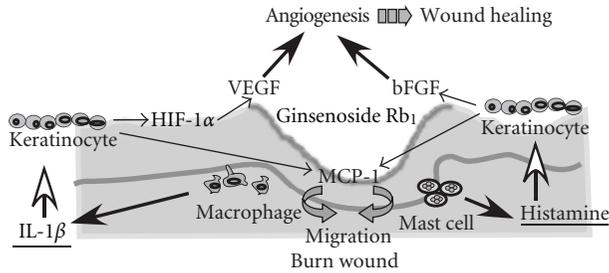


FIGURE 8: The proposed mechanisms of the enhancing effects of ginsenoside Rb<sub>1</sub> on burn wound healing.

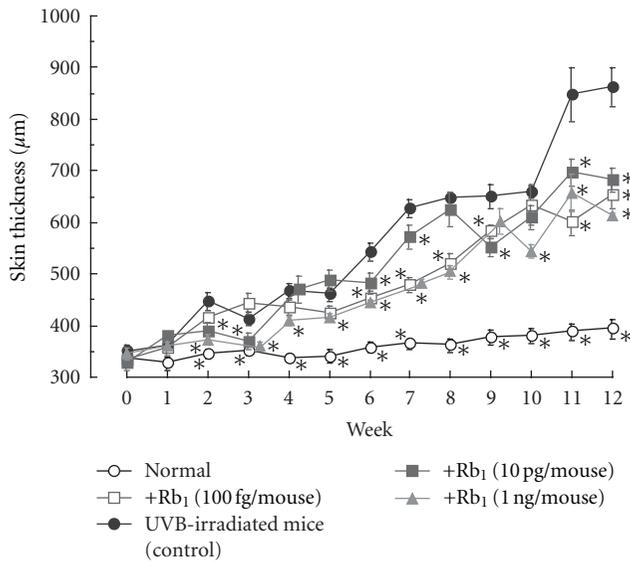


FIGURE 9: Effects of ginsenoside Rb<sub>1</sub> on skin thickness in chronic UVB-irradiated male hairless (HRM-1) mice [31]. The initial dose of UVB was set at 36 mJ/cm<sup>2</sup>, which was subsequently increased to 54 mJ/cm<sup>2</sup> at weeks 1–4, 72 mJ/cm<sup>2</sup> at weeks 4–7, 108 mJ/cm<sup>2</sup> at weeks 7–10, and finally 122 mJ/cm<sup>2</sup> at weeks 10–12 in male albino hairless HOS: HR-1 mice. The frequency of UVB irradiation was set at three times per week. Ginsenoside Rb<sub>1</sub> (100 fg, 10 pg, and 1 ng/mouse) was applied topically to the dorsal region of each mouse every day for 12 weeks. The dorsal skin of the hairless mice was lifted up by pinching gently under anesthetization with pentobarbital, and skin-fold thickness was measured using a Quick Mini caliper. Skin thickness after UVB irradiation was measured every week. Values are the mean ± SE for 6 mice. \*Significantly different from vehicle-treated mice,  $P < 0.05$ .

As shown in Table 3, it has been reported that red ginseng extract prevents skin aging induced by UVB irradiation. However, the active substance(s) has yet to be identified. We found that ginsenoside Rb<sub>1</sub> isolated from red ginseng roots inhibited the increases in skin thickness, epidermis, and wrinkle formation induced by chronic UVB irradiation [31]. In this paper, we will introduce the effects of ginsenoside Rb<sub>1</sub> on chronic UVB irradiation-induced cutaneous aging in hairless mice.

The topical application of ginsenoside Rb<sub>1</sub> at lower doses, 100 fg, 10 pg, and 1 ng/mouse, significantly inhibited the

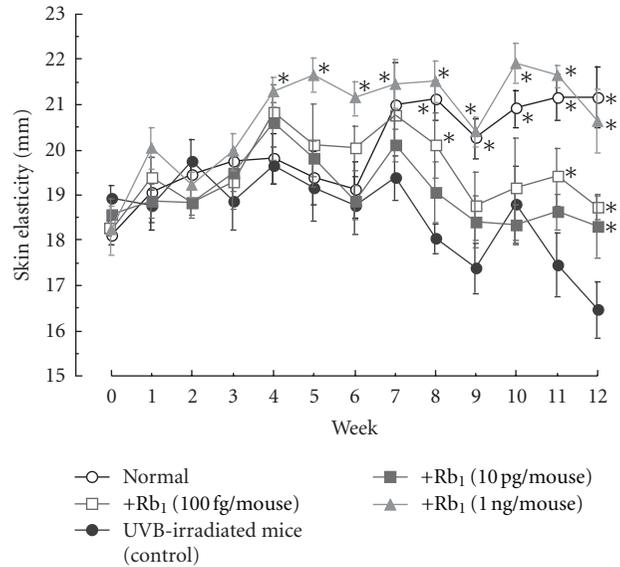


FIGURE 10: Effects of ginsenoside Rb<sub>1</sub> on skin elasticity in chronic UVB-irradiated male hairless (HRM-1) mice [31]. The experiments were performed as described in Figure 9. The dorsal skin was lifted up and skin stretch was measured using a digimatic caliper. Skin elasticity after UVB irradiation was measured every week. Values are the mean ± SE for 6 mice. \*Significantly different from vehicle-treated mice,  $P < 0.05$ .

increase in skin thickness induced by UVB irradiation during weeks 2 to 12 compared to the skin thickness of vehicle-treated UVB-irradiated mice (control) (Figure 9).

The reduction in skin elasticity induced by UVB irradiation was significantly inhibited by the topical application of ginsenoside Rb<sub>1</sub> (100 fg, 10 pg, and 1 ng/mouse) during weeks 6 to 12 compared to that of control mice (Figure 10).

Wrinkling induced by UVB irradiation at week 9 was inhibited by the topical application of ginsenoside Rb<sub>1</sub> (100 fg, 10 pg, and 1 ng/mouse) (Figure 11).

The topical application of ginsenoside Rb<sub>1</sub> (100 fg, 10 pg, and 1 ng/mouse) inhibited the increase in epidermal thickness induced by UVB irradiation but had no effect on the increase in the extracellular matrix of the dermis (Table 4). The occurrence of apoptotic cells was localized to the stratum granulosum of the epidermis and was increased by UVB irradiation. The increase in apoptotic cell levels induced UVB irradiation was significantly inhibited by ginsenoside Rb<sub>1</sub> (100 fg, 10 pg, and 1 ng/mouse). Furthermore, 8-hydroxy-2'-deoxyguanosine (8-OHdG, a marker of oxidative DNA damage) [32] was also localized to the stratum basale and dermis, and its level was increased by UVB irradiation. The increase in 8-OHdG-positive cells induced by UVB irradiation was inhibited by ginsenoside Rb<sub>1</sub> (Table 5). UVB (20 mJ/cm<sup>2</sup>) irradiation reduced the level of Bcl-2 expression in human primary keratinocytes. Conversely, UVB irradiation had no effect on Bak or Bax expression. Ginsenoside Rb<sub>1</sub> increased the Bcl-2 levels in UVB-treated human primary keratinocytes at the lower concentrations of 100 fg, 10 pg, and 1 ng/mL (Figure 12).

UVB irradiation at week 9

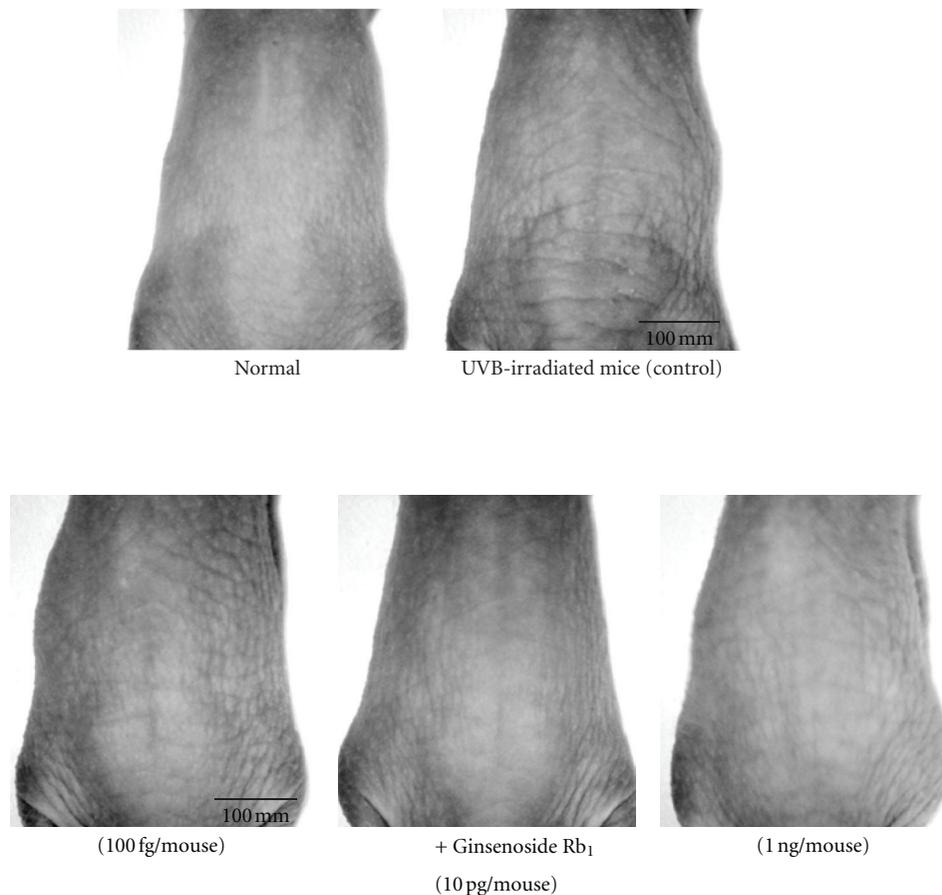


FIGURE 11: Photograph showing skin wrinkling induced by chronic UVB irradiation and the effects of topically applied ginsenoside Rb<sub>1</sub> [31]. The experiments were performed as described in Figure 9. To evaluate the formation of wrinkles after the UVB irradiation, the UVB-irradiated dorsal area (site of wrinkles) of each hairless mouse was photographed at 9 weeks.

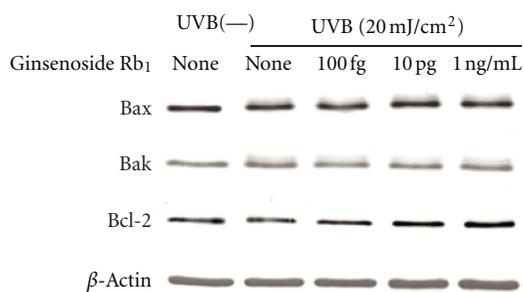


FIGURE 12: Effects of ginsenoside Rb<sub>1</sub> on Bax, Bak, and Bcl-2 expression levels in UVB-irradiated human primary keratinocytes [31]. Human keratinocytes ( $3 \times 10^5$  cells) were seeded in a 100-mm culture dish and cultured in KG-2 medium for 48 h. The cells were irradiated with UVB (20 mJ/cm<sup>2</sup>) and treated with the indicated amounts of ginsenoside Rb<sub>1</sub> for 24 h in KB-2 medium. After being washed with phosphate-buffered saline (PBS, pH 7.0), the cells were treated with lysed buffer. The supernatant obtained by centrifugation was subjected to a western blot analysis with anti-Bcl-2, anti-Bax, anti-Bak, and anti-β-actin antibodies.

UVB exposure of skin cells results in several types of DNA damage such as the formation of the cyclobutane pyrimidine dimer, pyrimidine pyrimidone photodimers and 8-OHdG [33–35], and consequently DNA damage induced by long-term UV exposure leads to skin carcinogenesis. Furthermore, there are many reports that apoptotic stimuli such as UV radiation and tumor necrosis factor- $\alpha$  induce cell death by activating caspases [36]. Bcl-2 is a member of the large Bcl-2 family and protects cells from apoptosis. On the other hand, it has been reported that Bax and Bak appear to permeabilize the outer mitochondrial membrane, allowing the efflux of apoptogenic proteins [37–39]. The protective effect of ginsenoside Rb<sub>1</sub> on UVB-mediated apoptosis may be partly due to the upregulation of Bcl-2 expression in human keratinocytes. Thus, the protective effect of ginsenoside Rb<sub>1</sub> on skin photoaging induced by chronic UVB exposure may be due to the increase in collagen synthesis and/or the inhibition of metalloproteinases expression in dermal fibroblast and the inhibition of epidermal hyperplasia. Further research is needed to clarify the mechanism of the protective effect of

ginsenoside Rb<sub>1</sub> on photoaging induced by chronic UVB irradiation of the skin.

#### 4. Conclusion

The topical application of ginsenoside Rb<sub>1</sub> isolated from red ginseng roots enhances burn wound healing, and ginsenoside Rb<sub>1</sub> prevents chronic UVB-induced skin photoaging, at very low doses. Further studies will be needed to clarify the clinical significance of these findings for skin damage induced by burn wounds or UV irradiation.

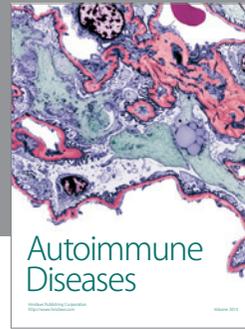
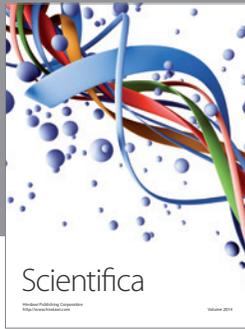
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