Portable ultraviolet light A1 light source to treat hypertrophic scar

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ABSTRACT

Background/Objectives: Recently, fibrotic diseases such as hypertrophic scar, keloid, and scleroderma have been treated with UV-A1 radiation with encouraging results. However, conventional UV light sources are bulky and expensive. In this study, we aimed to verify the effectiveness of a portable UV-A1 radiation device in treating hypertrophic scars.

Materials and methods: A light-emitting diode array that emitted 365 ± 5 nm (UV-A1) was used to irradiate fibroblasts and hypertrophic scar in a rabbit model.

Results: In cell cultures, UV-A1 light exposure inhibited post-wound cell migration and reduced the total amount of soluble collagen production in fibroblasts. Type I collagenase production and its activity increased after treatment. On the rabbit ear, UV-A1 light irradiation reduced the thickness of hypertrophic scars, confirming the antifibrotic effect in vivo.

Conclusion: These results support the potential of a portable UV-A1 light device in treating hypertrophic scar.

Conflicts of interest: The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in this article.

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Introduction

Fibrotic diseases in humans, including lung fibrosis,1 liver cirrhosis,2 scleroderma,3 and keloid,4–6 occur as a result of deposition of collagen in tissues or organs due to an imbalance of fibrogenesis and fibrolysis. No effective cure is available to treat these fibrotic diseases. Immunosuppressive agents are usually recommended but they may have significant adverse effects.7 For skin fibroblastic diseases, a safer and effective treatment is UV irradiation. UV-A light is an electromagnetic radiation with a wavelength of 320–400 nm. Phototherapy with UV-A to treat different skin diseases is usually accompanied by a systemic or topical photosensitizer to enhance the efficacy of the irradiation.8 The development of a lamp emitting radiation predominantly in the long-wavelength UV-A1 (340–400 nm), was described in 1981.8

High-dose-UV-A1 irradiation without photosensitizer emerged a decade later to treat atopic dermatitis.9,10 Hypertrophic scars and keloid result from excessive extracellular matrix deposition in the dermis after wound healing.6 Hypertrophic scars are limited to the wound area and usually resolve spontaneously with time. Keloid is defined as scar tissues growing beyond original wounds and is challenging to treat. Large scars may cause disfigurement and loss of function due to scar contracture. Both scars may be complicated with pruritus and pain.11 Traditional scar treatments include pressure garments, silicone gel sheeting, silicone cream and gel, local steroid injection, and excision and repair with/without skin graft and/or radiation.5,12 Pressure garments are inconvenient and uncomfortable especially in tropical and subtropical countries due to humid and hot weather. Surgical removal of keloid, although temporarily rewarding, is almost invariably followed by even more aggressive regrowth of scar tissue.12 The combination of radiation and surgery provides a higher success rate to cure keloid. However, long-term safety has not been established and invasive tumor may develop after this regimen.13 Intralesional steroid injections are probably the most common therapy clinically. Many patients are reluctant to undergo this painful therapy, especially for children and patients with large keloids.4,14 Recently, UV-A1 has been used in treating localized15 and generalized scleroderma16 with encouraging results. UV-A1


irradiation has been shown to stimulate collagenase production by human fibroblasts in vitro.\(^1\) Asawanonda et al\(^1\) demonstrated that UV-A1 with a cumulative dose of 2860 J/cm\(^2\) was helpful in treating keloid in a 37-year-old man. The results were not supported in a subsequent study in which three keloid patients showed no response after receiving UV-A1 irradiation with cumulative doses of 1500–1800 J/cm\(^2\).\(^2\) Moreover, the application of UV-A1 therapy is limited to a few centers because of the large size and high cost of the machines.\(^3\)

To develop the application of UV therapy for larger groups of patients, smaller and more economical UV devices are in demand. One potential candidate is the light-emitting diode (LED) light source which has the advantages of portability, high luminance, a relatively narrow spectrum, long lifespan, and low cost. In fact, LEDs in the visible spectrum have been applied to phototherapy in several other skin diseases such as wound healing,\(^4\) acne,\(^5\) and photodynamic therapy.\(^6\) In this study, we tested the efficacy of UV-A1 LEDs in treating hypertrophic scar on rabbit ear.

Materials and methods

**UV-A1-LED light source**

A commercial UV-A1-LED light source (Figure 1B) composed of 18 LEDs (LH365BG02) in a 5-cm circle was bought from Clearstone Technologies Inc. (Hopkins, MN, USA). This device emits wavelengths of 365 ± 5 nm with a total optical power of 2500 mW. The uniformity and intensity of light was confirmed by an UV-A meter (UV-Meter HighEnd, Hoene UV Technology Inc., Grafelfing, Germany). The light intensity was set at 30 mW/cm\(^2\) for *in vitro* and 100 mW/cm\(^2\) for *in vivo* experiments by adjusting the distance from the light source to the treated surface. An electric fan was used to disperse heat during irradiation.

**Cells**

Human fetal skin fibroblasts (WS1)\(^2\) were obtained from the Bioresource Collection and Research Center (BRCB, Taipei, Taiwan) and were maintained in Eagle’s Minimum Essential Medium supplemented with 10% fetal bovine serum, 2.5 mM HEPES, and 100 U/mL penicillin/streptomycin at 37°C in 5% CO\(_2\).

**UV-A1 phototoxicity in fibroblasts**

The purpose of the study was to determine the optimal UV-A1 dose to inhibit scar formation without damaging cells. The phototoxicity of UV-A1 in fibroblasts was determined by exposing cells to different UV doses after seeding 1 × 10\(^4\) cells in 100 μL medium per well of a 96-well plate for 16 hours. Cell survival was determined with WST-1 assay\(^2\) 24 hours after treatment. The irradiation dose...
that was not phototoxic to cells was selected for further experiments.

**Total collagen, collagenase I production, and collagenase activity**

Supernatant was collected 24 hours after treating cells with UV-A1. Total soluble collagen content in the supernatant was measured with Sircol assay (Biocolor Ltd., Carrickfergus, Antrim, UK). Since type I collagen is overproduced in keloid and hypertrophic scar, we measured type I collagenase (MMP-I) and its activity in the supernatant with ELISA kits (Biotrak-ELISA System, Amersham, GE Inc., Piscataway, NJ, USA) following the manufacturer’s instructions.

**Cell migration**

Cell migration was assessed by creating a 0.5-mm wound with a 200-μL pipette tip on the center of a confluent cell sheet. Cells were treated with mitomycin C (10 μg/mL) to inhibit cell proliferation for 2 hours at 37°C and 5% CO2 prior to scratching the cell sheet. The damaged area was recorded over time with a digital camera coupled to a microscope and analyzed with Image-J software (National Institutes of Health, Bethesda, MD, USA).

**UV-A1 effect on hypertrophic scar in rabbit ear**

Five full-thickness dermal wounds were created on the ventral site of each ear of three New Zealand white rabbits (3–6 months of age, 2–2.5 kg) with a 6-mm punch. All study protocols were in compliance with and approved by the Animal Center Review Board of the institution. Wounds were left open and hypertrophic scars formed in 28 days. Scars on one ear were irradiated every 2 days with 150 J/cm² at 100 mW/cm² UV-A1 for 30 days (total: 2250 J/cm²) while the scars on the other ear served as a control. The scar thickness was measured with a caliper (Mitutoyo Inc., Kawasaki, Japan) and digital images were recorded and assessed by observers blinded to the study.

**Statistical analysis**

One-way analysis of variance (ANOVA) was used for multiple group comparisons. Unpaired Student’s t test was used to compare between two groups. Dose dependence was analyzed by linear regression. At least two separate independent experiments in triplicate were done for the in vitro studies. A p value <0.05 was considered statistically significant.

**Results**

**UV-A1 inhibits collagen production and enhances collagenase I secretion**

As expected, the phototoxicity of UV-A1 on human fibroblasts was dose dependent (Figure 2A), with a lethal dose beginning at 9 J/cm². We therefore adopted UV light doses <9 J/cm² in the rest of the experiments. The main product of fibrosis, the total collagen content, was reduced by UV-A1 irradiation (40% at 3 J/cm², p < 0.05; 70% at 6 J/cm², p < 0.001, one-way ANOVA; Figure 2B). UV-A1 irradiation enhanced collagenase I production in a dose-dependent manner (Figure 2C) and improved the enzyme activity significantly (Figure 2D). The collagenase I in the supernatant increased 33% at 3 J/cm² and 44% at 6 J/cm² compared to the control (p < 0.05, one-way ANOVA; Figure 2C). The enzyme activity increased 25% at 3 J/cm² (p < 0.01) and 21% at 6 J/cm² (p < 0.05), respectively (Figure 2D). These results demonstrated the anti-fibrotic effects of UV-A1.

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**Figure 2** Cell survival, total collagen production, type I collagenase (MMP-I) titer, and type I collagenase activity after UV-A1 exposure. Significant cell death occurred after being exposed to light dose higher than 9 J/cm² (A). The total collagen production decreased 40–70% compared to the control (B) after irradiation with nonphototoxic doses. The collagenase in supernatant increased around 40% (C) and its activity increased around 20% (D) compared to the control. *p < 0.05; **p < 0.01; ***p < 0.001. MMP-1 = matrix metalloproteinase-1.
UV-A inhibits migration of fibroblasts

Fibroblast migration may also play a critical role in fibrotic diseases such as active keloid. In the clinic, keloid spreads beyond the margins of the original wound. The fibroblasts proliferate, migrate, and lay down collagen on their path and generate tongue-like fibrotic tissue advancing edges under the microscope. Fibroblast migration induced by an artificial wound in culture was similarly inhibited in a dose-dependent manner by UV-A1 irradiation (Figure 3).

Rabbit ear scarring is inhibited by UV-A1 irradiation

On the rabbit ear, the minimal dose of UV radiation producing erythema was 150–200 J/cm² (data not shown). We therefore chose 150 J/cm² to test the effect on the scar (n = 15). After multiple treatments (irradiation every 2 days, cumulative dose 2250 J/cm²), scar tissue became thinner on Day 20 after the onset of irradiation and the change of thickness became statistically significant on Day 84 (Figure 4). The treated scars continued to resolve even after UV therapy was discontinued. The time course of the responses observed in this study (35–84 days after onset of treatment) was consistent with the slow response of fibrotic tissues to other forms of treatment.

Discussion

Our study demonstrates the beneficial effects of LED UV-A1 irradiation on hypertrophic scar. The efficacy of this small portable, inexpensive LED light source on treating fibrosis tissue is comparable to a large UV-A1 machine. Various UV-A1 sources are available, such as fluorescent lamp cubicles which allow only low (10–30 J/cm²) to medium (40–70 J/cm²) individual treatment doses to be administered. High-output metal halide sources allow high doses (up to 130 J/cm²) for a single treatment session. Low and medium UV-A1 dose shows minimal to moderate effects in treating atopic dermatitis, and high UV-A1 dose provides better control of the disease. High UV-A1 dose is particularly promising for localized scleroderma including widespread, pansclerotic, and linear morphea because there is no reliable treatment available. Setge et al showed that a high dose (130 J/cm² for 30 times, cumulative dose 3900 J/cm², n = 10) was more effective than a low dose (20 J/cm², cumulative dose 600 J/cm², n = 7) of UV-A1 in treating 17 patients with localized scleroderma. Four of 10 patients in the high-dose group showed complete clearance. A higher UV-A1 dose is also required for treating keloid. The higher dose required for treating fibrosing diseases suggests the existence of one effective threshold dose of UV-A1 irradiation, probably higher than 2250 J/cm². The thicker collagenous tissue may need a higher light dose to achieve therapeutic results. It is also likely that an optimal dose may exist along with a temporal course of treatment in relation to the onset time of scar tissue. In the present study, hypertrophic scar was treated after it was established. Whether early UV-A1 intervention prevents hypertrophic scar formation or enhances wound healing remains to be explored.

This rabbit scar model parallels hypertrophic scar in humans and has been used to study potential therapeutic modalities. However, the model cannot study the safety of chronic UV-A1...
therapy. Patient data on safety such as carcinogenic effects need to be determined before extensive use of UV-A1 on humans. The major acute adverse effects of UV-A exposure are erythema and pigmentation. The erythema response can be avoided by giving a minimal erythema dose test before treatment and adjusting doses according to the response. Pigmentation of the skin is reversible. The major potential chronic adverse effects are photocaging and skin cancer. Although it is unclear whether or not UV-A1 increases the risk of melanoma, a case of cutaneous melanoma diagnosed after 18 months intensive UV-A1 and psoralen UV-A (PUVA) for urticaria pigmentosa has been reported. In this report, the total dose of UV-A1 was 910 J/cm², while the total dose of PUVA was 2144 J/cm². A role for UV-A in causing malignant melanoma cannot be excluded. However, UV-A irradiation causing skin cancer is not detected in persons who are pigmented, tan easily, or are of Asian or African ancestry (Fitzpatrick Skin Type IV or higher). The effective cumulative dose of UV-A1 required for treating keloid in our study was ~2000 J/cm² without psoralen. This treatment may be relatively safe in our population. Similarly, the risk of UV-A1 inducing non-melanoma skin cancers, particularly squamous cell carcinoma, is not known, although UV-A induces squamous cell carcinoma-like tumors in mice. Another limitation of our study was the lack of fibroblasts from human hypertrophic scar for mechanistic studies. Nevertheless, WS1 fibroblasts are a commercial cell line derived from 12-week gestation fetal skin. These cells are highly proliferative and senescent around 70 passages. It has been widely used as a model in wound healing studies. Hypertrophic scar, unlike keloid, usually spontaneously resolves with time. In case of limited resources of human tissue, using WS1 fibroblasts may be an alternative model.

In summary, we showed in in vitro and in vivo systems that UV-A1 irradiation using an LED light source was effective in treating hypertrophic scars. However, the long-term side effects need to be explored in future experiments.

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