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ORIGINAL ARTICLE EXPERIMENTAL/SPECIAL TOPICS

Whitening Effects of Adipose-Derived Stem Cells: A Preliminary In Vivo Study

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Abstract Many studies have reported various growth factors secreted from adipose-derived stem cells (ADSCs). In particular, regenerative effects in skin have received much attention in the clinical fields. The in vitro whitening effects of ADSCs have been reported. A previous study demonstrated that ADSCs secrete growth factors that inhibit both melanin synthesis and tyrosinase activity. This study aimed to investigate the in vivo whitening effect of ADSCs using mouse models. In the study, ADSCs were isolated from the adipose tissue of C57BL/6 mice and cultured. The ADSCs (1×10^6 cells in 30 μl of Hanks' balanced salt solution [HBSS]) then were injected intradermally in the dorsal area of the right ear, and 30 μl of HBSS was injected on the left ear as a control. After 7 days, both ears were irradiated with ultraviolet B (UVB) (150 mJ/cm^2) three times at 2-day intervals. The sections of each ear were stained with hematoxylin-eosin, Fontana–Masson, and HMB-45 (a melanocytic cell-specific monoclonal antibody). The histologic parameters evaluated included inflammation (+/−), erosion (+/−), and melanin formation (graded on a scale of 1 to 3). No significant differences in inflammation or erosion were observed by hematoxylin and eosin staining (inflammation: $p = 0.388$; erosion: $p = 0.355$). However, significantly more melanin formation was observed in the control group than in the ADSC injection group by Fontana–Masson and HMB-45 staining (Fontana–Masson: $p = 0.025$; HMB-45: $p = 0.015$). The study findings suggest that ADSCs inhibit melanin formation induced by UV exposure. Potentially, ADSCs may be used as anti-aging agents, including skin whitening. Before human clinical studies can be conducted, further study is required to determine the mechanisms underlying the whitening effects of ADSCs and the safety of ADSC use.

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Keywords Adipose-derived stem cells · In vivo study · Whitening effect

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Recently, secretary proteins from adipose-derived stem cells (ADSCs) have been demonstrated, and these proteins have received much attention in the clinical fields. Especially, many studies concerning the skin regeneration effects, including wound healing and anti-aging effects, have been reported [3–9].

Skin aging is induced by harmful environmental factors such as ultraviolet (UV) irradiation, as well as an intrinsic aging process including atrophy, loss of elasticity, wrinkle, and dryness [2]. Pigmentation, a clinical feature of skin aging, is induced by UV irradiation [1].

Modulation of melanin synthesis is very important to whitening of the skin pigmentation. Tyrosinase is the key enzyme in melanin synthesis [10]. Kim et al. [8] demonstrated that secretary proteins of ADSCs, which are mainly

mediated by transforming growth factor-ß1 (TGF-ß1), inhibit melanin synthesis by downregulating the expression of tyrosinase and tyrosinase-related protein 1 (TRP1) in B16 melanoma cells. In C57BL/6 mice, epidermal melanocytes are found in less hairy areas such as the sole, tail, and dorsal portion of the ears. An increase in the epidermal melanocyte population density was demonstrated after UV irradiation in C57BL/6 mice [13]. This study aimed to evaluate the whitening effects of ADSCs in vivo using a C57BL/6 mouse model.

Materials and Methods

Isolation and Culture of ADSCs from the C57BL/6 Mouse

All the animal experiments were approved by the Institutional Animal Care and Use Committee of Seoul National University College of Medicine. Epididymal fat tissue was harvested from two 6-week-old male C57BL/6 mice. The washed and chopped fat tissue was digested with 0.5 % type 1 collagenase (Worthington, Lakewood, NJ, USA) for 1 h at 37 °C. The tissue then was filtered, washed, and centrifuged. Floating mature adipocytes were discarded. From the precipitated pellets, ADSCs were obtained, then seeded in dishes and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS) (Invitrogen-Gibco, Grand Island, NY, USA) and antibiotics/antimycotics (A/A) (Welgene Inc. Daegu, Korea) under normoxic conditions at 37 °C.

Injection of ADSCs and UV Irradiation

For the study, 10 male 6-week-old C57BL/6 mice were used. ADSCs (1×10^6) were suspended in 30 µl of Hanks' balanced salt solution (HBSS) and injected intradermally into the dorsal area of the right ear. As a control, 30 µl of HBSS was injected into the left ear. After 7 days, both ears were irradiated with 150 mJ/cm^2 of ultraviolet B (UVB) by TL20 W/12RS (Philips, Amsterdam, The Netherlands) three times at 2-day intervals.

Histologic Evaluations

All the mice were killed 2 days after the last irradiation. Both ears were cut 5 mm from the distal margin. The harvested ears were sectioned at the midline with a longitudinal axis. The sections were subjected to hematoxylin and eosin, Fontana–Masson, and HMB-45 (a melanocytic cell-specific monoclonal antibody) staining. The histologic parameters evaluated included inflammation (positive/negative), erosion (positive/negative), and melanin formation graded on a scale of 1 to 3 (Table 1). All histology was evaluated by an experienced pathologist in a blinded study setting.

Statistical Analysis

Statistical significance was assessed by a paired t test. A p value lower than 0.05 was considered significant.

Results

Gross inspection showed no significant changes in either ear. In the histologic analysis, no significant differences in inflammation ($p = 0.388$) or erosion ($p = 0.355$) were observed by hematoxylin and eosin staining (Figs. 1, 2). However, significantly more melanin formation was observed in the control group than in the ADSCs injection group by Fontana–Masson ($p = 0.025$) and HMB-45 ($p = 0.015$) staining (Figs. 1, 3).

Discussion

The mechanism of regeneration or repair by ADSCs is not entirely clear. It has been deduced that stem cells differentiate and repopulate into the phenotype of injured tissue and repair it. In addition, stem cells may exert therapeutic effects (i.e. paracrine actions) through the cell-free conditioned medium from the stem cells [6, 11, 14].

Recently, secretary proteins from ADSCs have been demonstrated, and various beneficial effects of these cytokines have been reported [3–9]. The skin regeneration effect of ADSCs has received much attention in the clinical fields. The wound-healing effect of ADSCs has been verified by both in vitro and in vivo findings [7]. Various growth factors secreted into the ADSC conditioned medium (CM) such as basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF), TGF-ß1, hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF) can mediate the wound-healing effect [6, 7].

Kim et al. [7] suggested that ADSC-CM enhanced type 1 collagen secretion from human dermal fibroblasts and

Table 1 Histologic scale of melanin formation

Scale	Description
1	Discontinuous melanin distribution with conglomerated form <5
2	Discontinuous melanin distribution with conglomerated form >5
3	Melanin linear distribution with conglomerated form >5

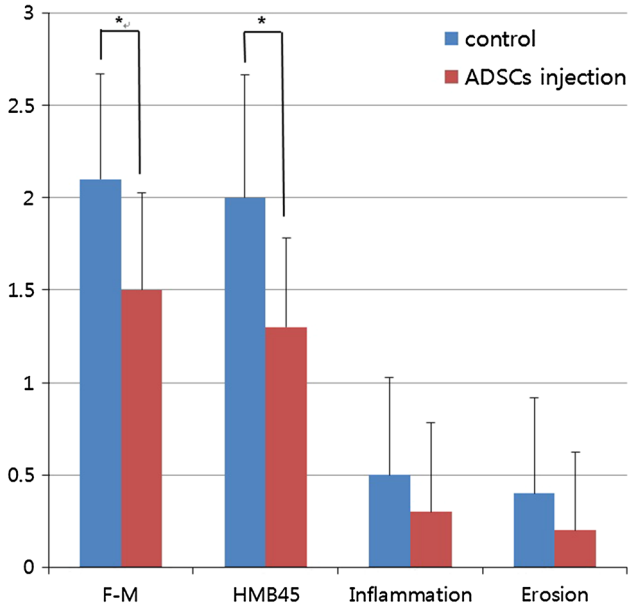

Fig. 1 Histologic scale for melanin formation evaluation using Fontana–Masson (F–M) and HMB45 stain. *$p < 0.05$

fibroblast migration in an in vitro wound-healing model. Also, in their animal study, they suggested that topical application of ADSCs reduced wound size significantly and enhanced epithelialization at the wound edge.

Kim et al. [5] reported that wrinkles, induced by UVB irradiation, were significantly improved by subcutaneous injection of ADSCs into hairless mice. In addition, dermal thickness and collagen contents were increased in the ADSC injection groups. When human dermal fibroblasts were incubated in ADSC-CM, reduction of dermal fibroblast proliferation by UVB irradiation was reversed in a dose-dependent manner. In cell-cycle analysis, ADSC-CM decreased UVB-induced apoptotic cell death. The authors suggested that the antiwrinkle effect of ADSCs and their soluble factors demonstrated the mediation by activating dermal fibroblasts, which inhibit UV-induced apoptosis and stimulate collagen synthesis [5].

In a similar study, Kim et al. [3] suggested that subcutaneous injection of ADSCs increased collagen synthesis, dermal thickness, collagen density, and fibroblast number in hairless mice. Also, increased angiogenesis in ADSC-treated skin was confirmed by CD31 and NG2 immunofluorescence stains.

Kim et al. [8] demonstrated the whitening effect of ADSCs in vitro using B16 melanoma cells. These authors suggested that the whitening effect is exerted via a paracrine mechanism with secretory factors of ADSCs, mainly by TGF-ß1 inhibition of melanin synthesis via downregulation tyrosinase and TRP1 expression.

To date, no in vivo study has investigated the whitening effect of ADSCs. Our study was conducted to evaluate the whitening effect in vivo using C57BL/6 mice. The results showed a significant inhibitory effect on UV-induced melanin formation through an intradermal injection of ADSCs. Specific cytokines from ADSCs may have contributed to the whitening effect. High levels of TGF-ß1, which inhibits melanin synthesis, was detected in ADSC-CM [4]. Neutralizing TGF-ß1 in ADSC-CM sufficiently reversed the inhibitory effect in melanin synthesis [8].

Other cytokines, interleukins, and tumor necrosis factor-α can inhibit pigmentation by acting on tyrosinase. However, the concentrations of these cytokines in ADSC-CM are much lower than the half-maximal inhibitory concentration (IC_{50}) value for the inhibitory effect [4, 8]. Proteomic analysis has detected 100 μmol of ascorbic acid and various antioxidant proteins in ADSC-CM, thereby showing that ADSC-CM has antioxidant effects [4]. Antioxidants inhibit melanin formation and melanosome transfer and change the type of melanin formed [8, 12]. Consequently, ADSCs may exert whitening effects as antioxidants.

Adipose-derived stem cells and their secretory factors may be used as anti-aging agents. For clinical use of ADSCs and their secretory factors, an adequate application method and optimal concentration should be determined through understanding the mechanism underlying the whitening effects of ADSCs. Although much research concerning ADSCs has been performed, the safety of ADSCs has not been determined. The safety of ADSCs should be evaluated before they are applied clinically.

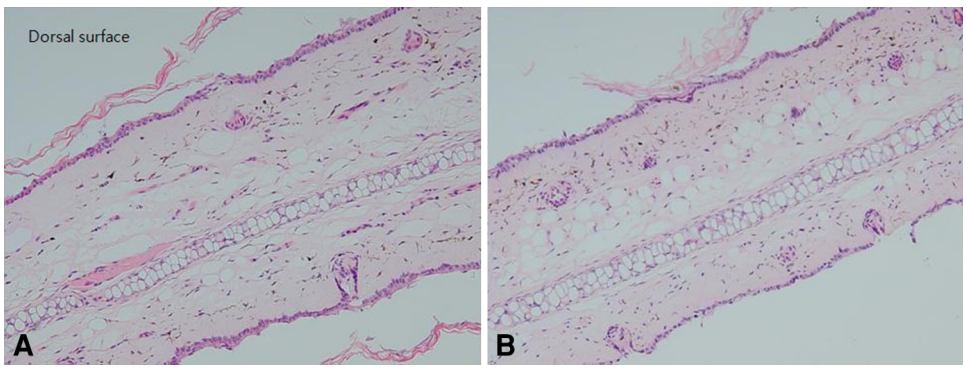

Fig. 2 Hematoxylin and eosin staining. **a** Adipose-derived stem cell (ADSC) injection group; **b** Control group. Original magnification, ×200

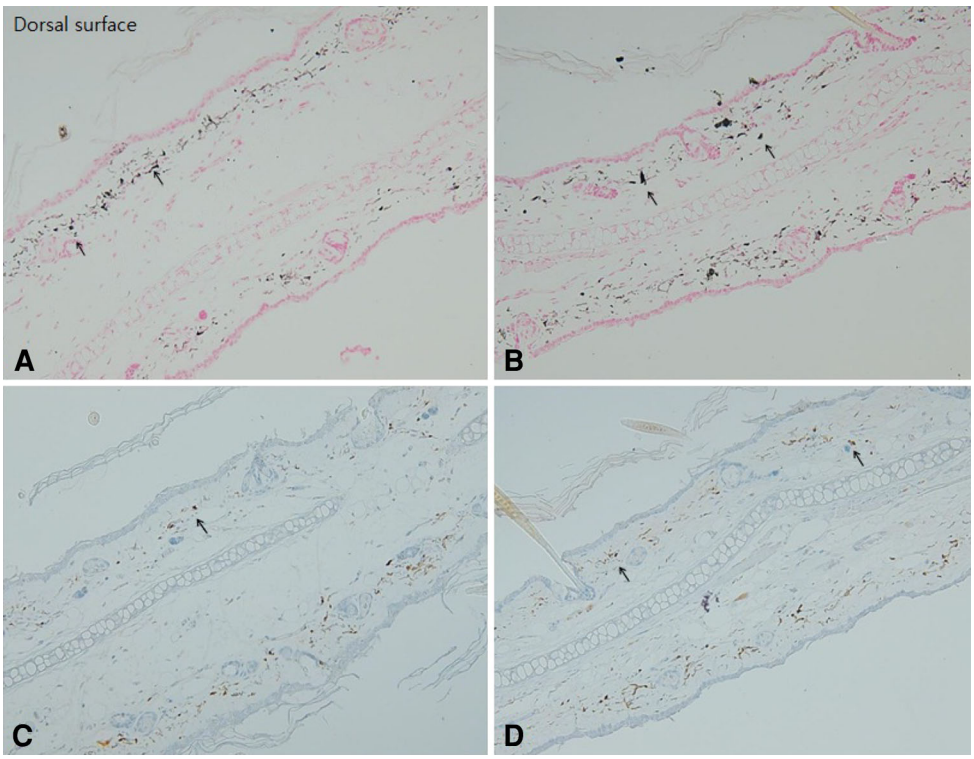

Fig. 3 **a** Adipose-derived stem cell (ADSC) injection group, grade 2 (Fontana–Masson stain). **b** Control group, grade 3 (Fontana–Masson stain). **c** ADSC injection group, grade 1 (HMB-45 stain). **d** Control group, grade 2 (HMB-45 stain). Original magnification, ×200. The *black arrows* show the conglomerated form of melanin

Conclusions

Our study suggests that ADSCs inhibit melanin formation induced by UV exposure. Thus, ADSCs may potentially be used as anti-aging agents, including skin whitening. However, before human clinical studies can be conducted, further study is required to determine the mechanisms underlying the whitening effects of ADSCs and the safety of ADSC use.

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