The Effect of Adipose-Derived Stem Cell-Cultured Media on Oxazolone Treated Atopic Dermatitis-Like Murine Model

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Background: A stem cell is an undifferentiated cell that has the potential for self-renewal and differentiation. Adipose-derived stem cells (ADSCs) have advantages in accessibility and abundance compared to other kinds of stem cells and produce many growth factors and hormones. Objective: We investigated whether ADSC cultured media could be used as a therapy for atopic dermatitis. Methods: ADSC cultured media was topically applied twice daily for 5 days to oxazolone-treated atopic dermatitis-like hairless mice. Results: Topical application of ADSC cultured media improved the epidermal permeability barrier and keratinocyte differentiation, and restored the predominant Th2 phenotype when compared to vehicle. ADSC cultured media-treated epidermis also showed an increase in the expression of antimicrobial peptides cathelin-related antimicrobial peptide, mouse beta-defensein 3. Conclusion: Topical ADSC cultured media could be useful in the treatment of atopic dermatitis. (Ann Dermatol 24(2) 181 ~ 188, 2012)

-Keywords-
Adipose-derived stem cell, Atopic dermatitis, Oxazolone

INTRODUCTION

Atopic dermatitis (AD) is a common, chronic inflammatory skin disorder characterized by pruritic skin lesions, immunodysregulation, disrupted epidermal barrier function, and immunoglobulin E (IgE)-mediated sensitization to food and environmental allergens. The striking increase in the incidence of AD observed in recent decades has been attributed to the resettlement of populations from rural to urban areas, where a lack of early exposure to a variety of microbes purportedly results in reduced immune tolerance. Long-term systemic therapy or combinations of multiple treatments are often used in the management of AD. These conventional approaches for the treatment of AD include systemic and topical anti-inflammatory, anti-pruritic, and immunosuppressive agents, as well as phototherapy1. However, no single agent is always effective for the treatment of AD. Therefore, many studies have sought to develop novel agents or methods for the management of AD.

The stem cell is an undifferentiated cell that has the potential for self-renewal and differentiation. Recently, adult stem cells have received attention because they are associated with fewer ethical difficulties than embryonic stem cells and less potential risk of carcinogenesis. Among adult stem cells, adipose-derived stem cells (ADSCs), mesenchymal stem cells that are extracted from human adipose tissue, have essentially the same properties as stem cells derived from bone marrow2. Moreover, ADSC have relative advantages in accessibility and abundance compared to other types of adult stem cells and produce many growth factors and hormones. Recently, several studies of the mechanism of action of stem cells such as anti-inflammatory and immunomodulatory actions have
been reported3-5. Therefore, we investigated whether ADSC cultured media could be used as a novel therapeutic modality for AD.

As a model of AD, an epidermal hyperproliferative model (ideally also accompanied by dermal inflammation) would be preferred to assess the therapeutic efficacy and to study the action mechanisms responsible for the efficacy of ADSC cultured media. Therefore, this study employed an oxazolone-induced AD-like mouse model6 to assess the therapeutic efficacy of topical ADSC cultured media.

MATERIALS AND METHODS

Animals and materials

Twenty five female hairless mice (8 weeks old) were purchased from the animal laboratory of Yonsei University. Mice were kept under controlled humidity (40%) and temperature (22±2°C). 4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone) and acetone were purchased from Sigma-Aldrich (St. Louis, MO, USA). All laboratory measurements were performed under blinded conditions or by blinded researchers. Study sample sizes were determined based on available reference data for epidermal permeability barriers7.

Development and treatment of hapten-induced dermatitis with features of AD in mice

All animal procedures were approved by the Yonsei University Wonju Campus Institutional Animal Care and Use Committee. Development of a hapten (oxazolone)-induced, murine model with multiple features of AD (oxazolone-AD) was described in previous studies6. Each group (n=6 for each) of mice was sensitized by one topical treatment with 60 μl of 5% oxazolone, while the ethanol-treated vehicle group (n=6) served as the control (Fig. 1). After 13 days of multiple oxazolone challenges, all mice were evaluated the basal transepidermal water loss (TEWL), stratum corneum hydration and severity of erythema, and there is no statistical differences between each groups except the ethanol-treated vehicle group (data not shown).

Preparation of ADSC from adipose tissue and human fibroblasts

Subcutaneous adipose tissue was obtained from healthy female human donors and washed three times with phosphate buffered saline (PBS) to remove debris and red blood cells. Washed aspirates were digested with 0.075% collagenase (Type 1; Sigma-Aldrich) for 45 min at 37°C with constant shaking. Mature adipocytes and connective tissues were separated from the pellets by centrifugation at 1,200 rpm for 10 min. Pellets were resuspended in PBS, passed through a 100 μm mesh filter, and washed twice with PBS. The cell pellets containing ADSC were resuspended in low glucose Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco) and 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco), and plated at a density of 2×10^4 cells/cm^2 in T75 flasks. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂. After 7~10 days, the cells were detached and remnant extract (media without cells) was used as ADSC cultured media. Human dermal fibroblastic MRC-5 cells were obtained from American Type Culture Collection (No. CCL-171; Manassas, VA, USA) and used as controls, and cultured in the aforementioned supplemented DMEM.

Evaluation of the effects of ADSC cultured media

After sensitization by oxazolone, 30 μl of ADSC cultured media were topically applied twice a daily with a micropipette on the dorsal skin ADSC cultured media applied (AM) group of each mouse for 10 days. For the control groups, minimal media applied (MM) group, fibroblast cultured media applied (FM) group and glucocorticosteroid (0.1% methylprednisolone aceponate cream, S) were topically applied (0.2 g twice daily). Twenty four hours after the last treatment, biopsy specimens were taken to evaluate changes in morphology and protein expression.

Both at baseline and at the end of the treatment period, basal TEWL was measured with a TewameterTM210 electrolytic water analyzer (Courage and Khazaka, Cologne, Germany) and was stratum corneum (SC) hydration assessed as capacitance with a Corneometer CM820 (Courage and Khazaka) was measured immediately before each application of oxazolone and at 24 hours after the final application of oxazolone as described previously8.

Tissue preparation, immunohistochemistry, and immunofluorescence

Terminal differentiation marker proteins of keratinocytes (filaggrin, involucrin, and loricrin; Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used for immune identifi-