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ORIGINAL CONTRIBUTION

Exosomes derived from human adipose tissue-derived mesenchymal stem cells for the treatment of dupilumab-related facial redness in patients with atopic dermatitis: A report of two cases

Kui Young Park MD, PhD[1] | Hye Sung Han MD[1] | Jae Wan Park MD[1] | Hyuck Hoon Kwon MD, PhD[2] | Gyeong-Hun Park MD, PhD[3] | Seong Jun Seo MD, PhD[1]

[1]Department of Dermatology, Chung-Ang University College of Medicine, Seoul, Korea

[2]Oaro Dermatology Institute, Seoul, Republic of Korea

[3]Department of Dermatology, Dongtan Sacred Heart Hospital, Hallym University College of Medicine, Hwaseong, Republic of Korea

Correspondence
Seong Jun Seo, Department of Dermatology, Chung-Ang University Hospital, (06973) 102, Heukseok-ro, Dongjak-gu, Seoul, Korea.
Email: drseo@hanafos.com

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Abstract

Background: Atopic dermatitis is a chronic, pruritic, and inflammatory dermatosis that affects approximately 20% of children and 10% of adults worldwide. Dupilumab facial redness is gaining attention as additional cases are coming to light in the medical literature.

Objectives and methods: Exosomes are nano-sized vesicles that are constantly released by almost all cells. They can travel between cells and transport their cargo (lipids, proteins, and nucleic acids), making them a possible cell-free therapeutic option for various diseases. Herein, we investigated whether topical application of human adipose tissue-derived mesenchymal stem cell-derived exosomes could reduce dupilumab facial redness in patients with severe atopic dermatitis.

Results: Two patients with atopic dermatitis and refractory dupilumab facial redness were successfully treated with electroporation-assisted topical application of human adipose tissue-derived mesenchymal stem cell-derived exosomes. Six repeated sessions of treatment, with an interval of 1 week between each session, led to marked improvement in erythematous facial lesions.

Conclusions: We suggest that human adipose tissue-derived mesenchymal stem cell-derived exosomes may serve as an effective agent in the management of dupilumab facial redness. However, further controlled studies with a larger number of patients are necessary to confirm the efficacy and safety of this agent, as well as the optimal treatment protocol.

KEYWORDS
atopic dermatitis, dupilumab facial redness, exosome

1 | INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease that places a heavy burden on patients due to its chronic nature and frequent relapses.[1] Dupilumab is a human monoclonal antibody that inhibits IL-4 and IL-13 signaling, and it has been recently approved for the management of AD. It is highly effective and well tolerated in the treatment of AD, and successful treatment

cases are gradually increasing.[2] Dupilumab facial redness (DFR) (ie, development of an eczematous facial rash after initiation of dupilumab)[3] is an adverse event that was not described in the clinical trials of this monoclonal antibody.[4] However, in daily clinical practice, DFR occurs in approximately 10% of patients treated with dupilumab. Since the face and neck are visible areas, DFR negatively affects the patients' quality of life, and some patients opt to discontinue treatment due to this adverse event. Furthermore, the etiology and pathogenesis of DFR are currently poorly understood; therefore, there is limited information regarding the treatment of the potentially serious aforementioned adverse event. Based on a recent systematic review, among 101 patients from 16 studies on DFR, most of them were empirically treated with topical formulations (corticosteroids, calcineurin inhibitors, and antifungal agents); however, these regimens only showed efficacy in approximately 25–30% of patients, while 11% of the patients discontinued dupilumab due to DFR.[5] Herein, we investigated whether topical application of human adipose tissue-derived mesenchymal stem cell (MSC)-derived exosomes (ASCEs) could reduce DFR in patients with severe AD.

2 | MATERIALS AND METHODS

2.1 | Exosomes used in the cases

Exosomes were acquired from human adipose-derived stem cell (ASC)-conditioned medium (CM) using ExoSCRT™ technology (ExoCoBio Inc., Seoul, Republic of Korea) as previously described.[6,7] Briefly, CM was collected from ASCs cultured in serum-free Dulbecco's Modified Eagle's Medium (Thermo Fischer Scientific, Waltham, MA, USA). Subsequently, using a 0.2-μm filter, the CM was filtered and non-exosomal particles were removed. Furthermore, concentration determination, purification using tangential-flow filtration, and quantification by nanoparticle tracking analysis were performed as described in our previous study.[8] The characterization of ASCEs is shown in Figure 1. For application purposes, ASCEs were prepared at 2.0×10^9 particles/mL in a gel solution containing 30% ASCE, 2% 1,2-hexanediol (Cosbon Co., Ltd, Hwaseong, Republic of Korea), 1% glycerin (Procter and Gamble Chemicals, Cincinnati, OH, USA), 0.6% ammonium acryloyldimethyltaurate/VP copolymer (Clariant International Ltd, Muttenz, Switzerland), 0.0045% L-arginine (Daesang, Seoul, Republic of Korea), and 66.3955% water (Dai Han Pharm. Co. Ltd, Seoul, Republic of Korea).

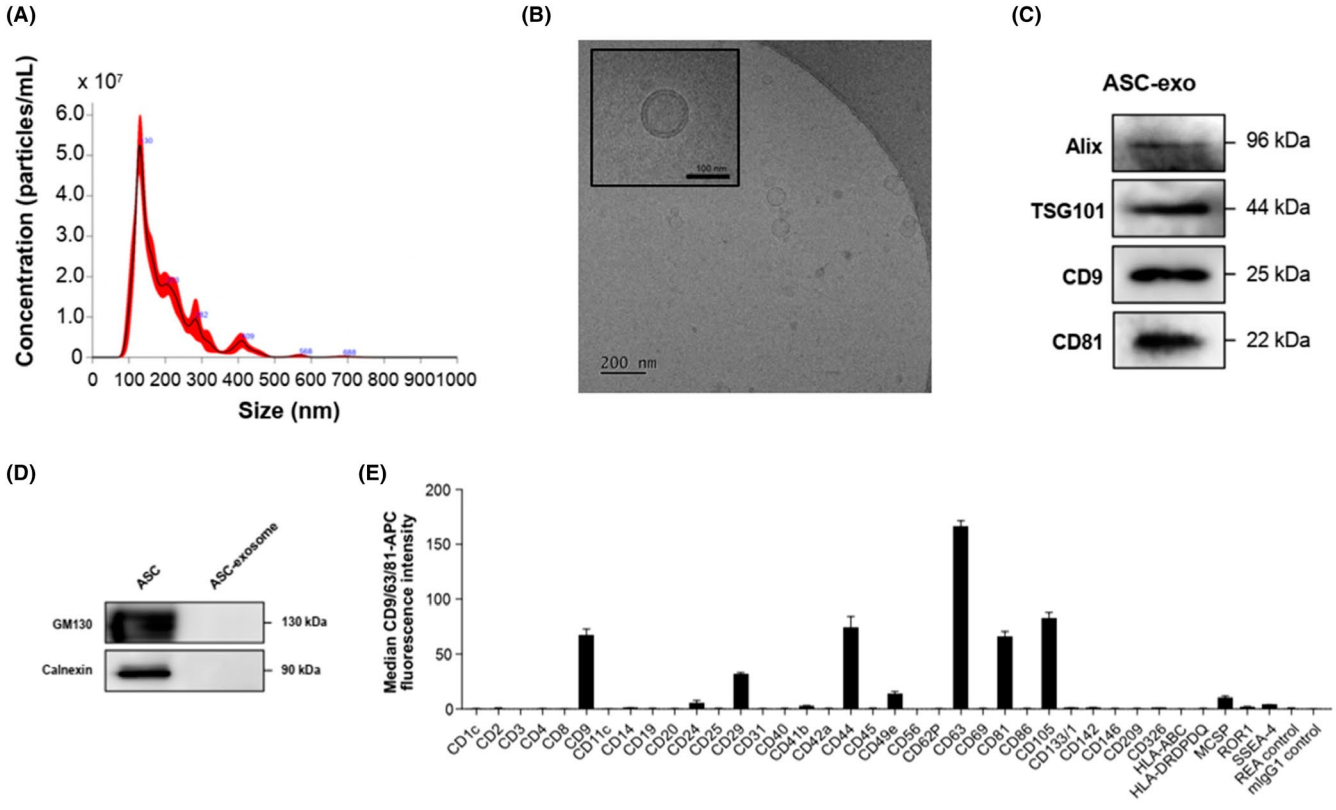

FIGURE 1 Characterization of human adipose tissue-derived mesenchymal stem cell-derived exosomes (ASCEs). (A) Representative histogram of particle concentration and size distribution of ASCEs measured via nanoparticle tracking analysis (NTA). (B) Representative cryo-TEM image of ASCEs. Scale bar: 100 nm. (C) ASCEs were analyzed, using western blotting, to determine the presence of exosomal markers such as Alix, TSG101, CD9, and CD81. (D) ASCEs were analyzed via western blotting to determine the presence of negative markers of exosomes, such as GM130 and calnexin. (E) Surface signature of ASCEs quantified using the MACSPlex Exosome Kit (human) in conjunction with flow cytometry

Currently, there is no recommended dose of exosomes for humans or animals.[9] However, in previous reports that used exosomes for the treatment of acne scars[8] or skin brightening,[10] exosomes with concentrations of 2.0×10^{10} to 9.78×10^{10} particles/mL were used. Since the skin barrier is impaired in patients with AD, we decided to use a lower concentration of 2.0×10^{9} particles/mL in our cases. Nevertheless, further controlled studies to determine the optimal concentration of exosomes are recommended for future research.

2.2 | Methods

Two patients with AD and refractory DFR were treated with electroporation-assisted topical application of ASCEs. One milliliter of ASCEs was applied to the entire face using a transdermal electroporation delivery system (Porederm™, Woojin System Co., Ltd, Gyeonggi-do, Korea) for 10 min. Six repeated sessions of treatment were performed, with an interval of 1 week between each session. Written informed consent was obtained from each patient for the publication of this case study and associated digital images.

3 | RESULTS

3.1 | Case 1

A 33-year-old man with severe AD since childhood was being treated with 300 mg of dupilumab subcutaneously every 2 weeks at our outpatient clinic. Initially, a significant improvement in AD was observed. However, after 9 weeks of treatment, the patient developed worsening redness and scaling of the face (Figure 2A). These symptoms did not respond to four months of treatment with topical corticosteroids (1 month), calcineurin inhibitors (2 months), or antifungal agents (1 month). A phototest and patch test were conducted to exclude allergic contact dermatitis (ACD). We decided to treat him with ASCEs after obtaining informed consent. After receiving six

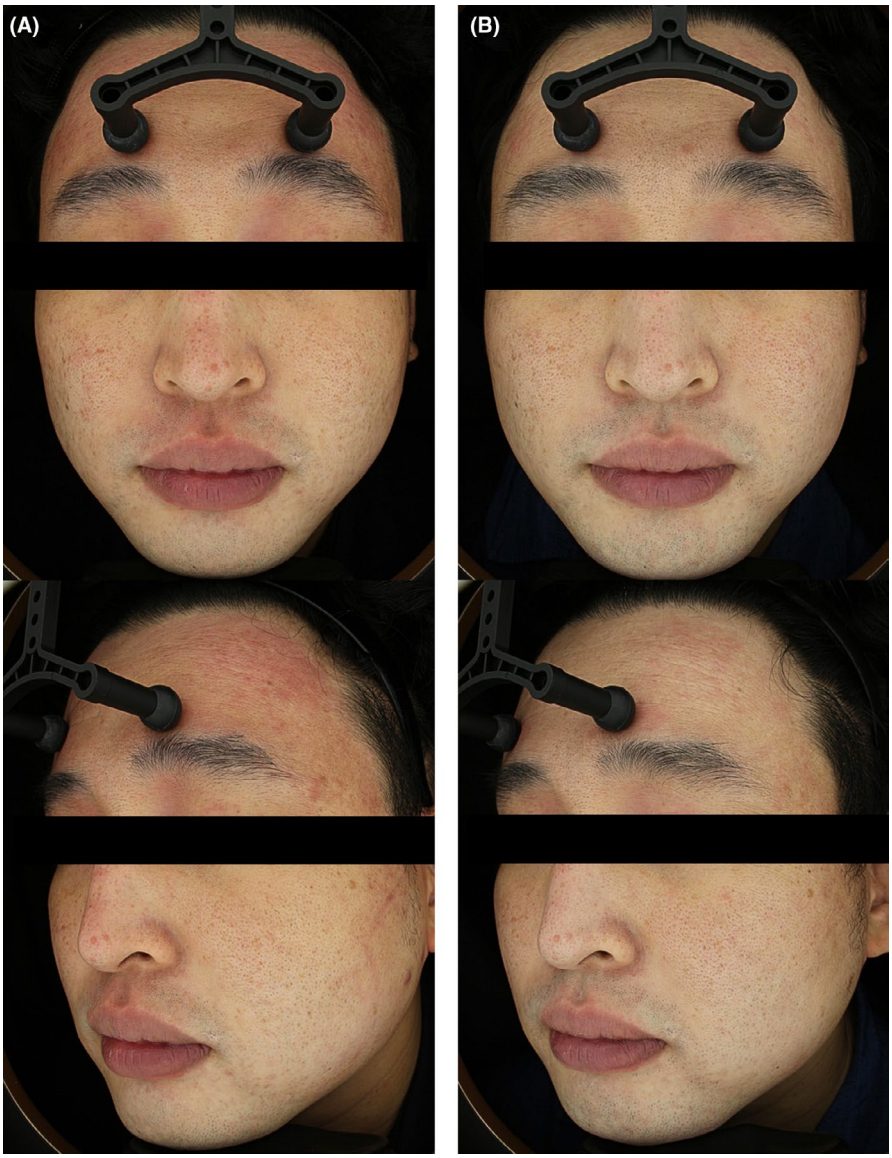

FIGURE 2 (A) Image obtained prior to topical application of exosomes. Exosomes were applied to the entire face of the patient using a transdermal electroporation delivery system. (B) Image obtained 6 weeks after topical application of exosomes, showing lesser facial redness. The patient received six repeated sessions of the same treatment with an interval of 1 week between each session

repeated sessions of ASCE treatment as described above, with an interval of 1 week between each session, marked improvement of the erythematous facial lesions was observed (Figure 2B). The patient was very satisfied with the treatment outcomes, and the treatment was well tolerated, with no adverse effects.

3.2 | Case 2

A 28-year-old man presented with erythematous maculopatches on his face (Figure 3A), which had been visible to him for a month. In addition, the patient had previously started receiving subcutaneous dupilumab for severe AD, 4 months prior to the consultation. Prior to starting dupilumab, his Eczema Area Severity Index (EASI) score was 27.2, despite cyclosporine treatment. By week 4 of treatment, his EASI score had reduced to 21.8, and by week 16, it was 8.6. With the exception of facial redness, eczema on other parts of the body had greatly improved. A phototest and patch test were conducted to exclude ACD. He had undergone topical treatment with corticosteroids (1 month), calcineurin inhibitors (1 month), and antifungal agents (1 month), with little improvement to his condition. He was diagnosed with DFR and treated with ASCEs after obtaining informed consent. He showed excellent improvement after receiving six repeated sessions of ASCE treatment twice a week, as described above (Figure 3B). Since then, he has received ASCE treatment every 2 weeks for 3 months, and the improvement is well maintained. Dupilumab treatment is still ongoing, and his AD remains well under control.

4 | DISCUSSION

Dupilumab is an emerging therapeutic agent for AD, and the number of treatment cases is increasing in Korea. Although dupilumab has shown high clinical efficacy and safety, some patients with AD develop facial redness that is resistant to dupilumab, despite clearance

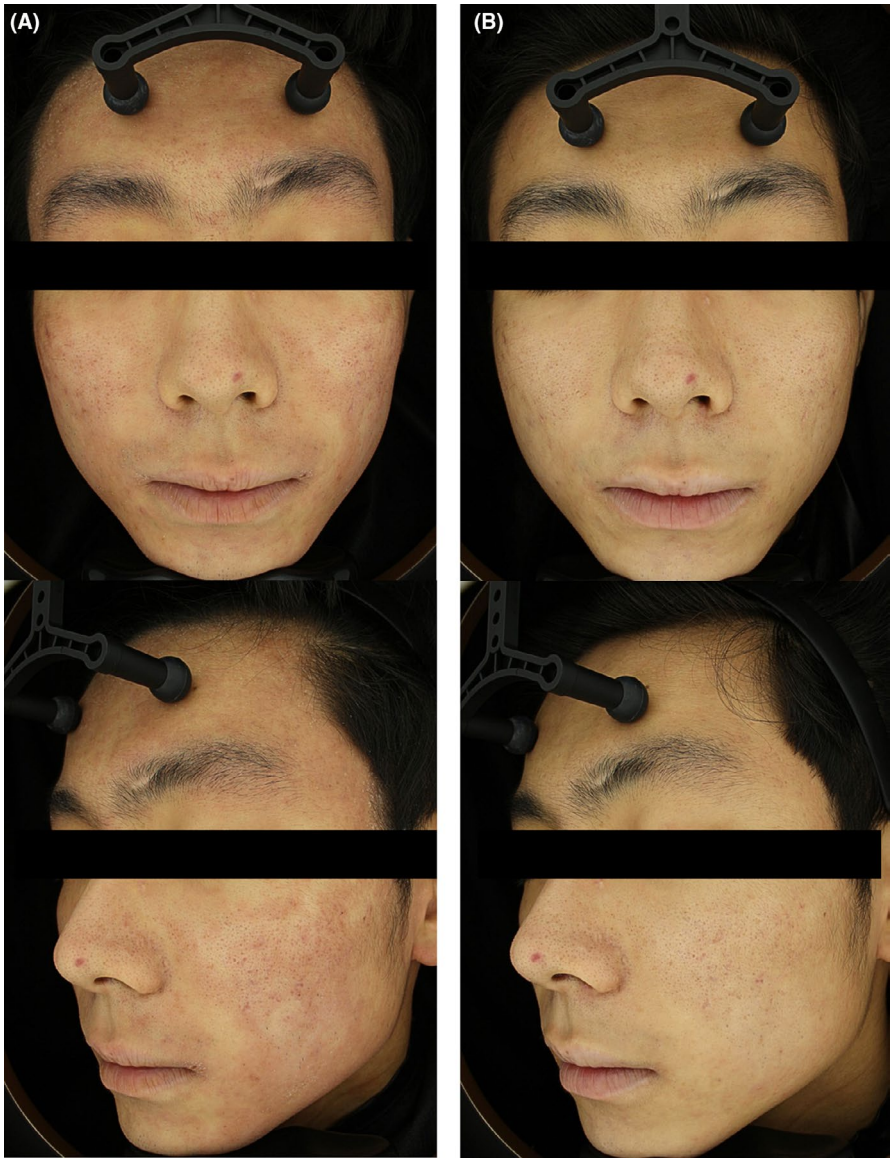

FIGURE 3 (A) Image obtained prior to topical application of exosomes. Exosomes were applied to the entire face of the patient using a transdermal electroporation delivery system. (B) Image obtained 3 weeks after six applications of exosomes (twice a week). The treatment improved previous erythematous facial lesions in the patient

of lesions on other parts of the body. Moreover, erythema on visible areas, such as the face and neck, generate a negative first impression, and thus, negatively impact the overall quality of life and treatment satisfaction of these patients.

Although the etiology and pathogenesis of DFR are still poorly understood, various etiologies have been proposed: (a) a hypersensitivity reaction to dupilumab, (b) site-specific treatment failure, (c) a seborrheic dermatitis-like reaction to facial *Malassezia* species, and (d) paradoxical flaring of allergic contact dermatitis, or a combination of these.[11,12] In one study, histological examination involving a small number of patients with DFR (n = 7) revealed that the hallmarks of AD, such as spongiosis, mononuclear cells, mast cells, and eosinophilic infiltrates, were absent and rather represented an atypical manifestation of chronic AD.[12] In our cases, the two patients with DFR had negative phototest and patch test results and did not respond to antifungal treatment, and both patients continued on dupilumab without progressing to a generalized hypersensitivity reaction. Based on these findings, we presumed that DFR in our patients likely represented site-specific treatment failure rather than allergic contact dermatitis, seborrheic dermatitis-like reaction related to *Malassezia* species, or a drug-related hypersensitivity reaction.

Several studies have demonstrated that allergic progression in AD can be suppressed by MSCs.[13] However, MSCs have several drawbacks because it is difficult to generate a consistent source of cells with a stable phenotype and immunogenicity, and they have the possibility of forming tumors. Recently, it has been shown that numerous extracellular vesicles, including microvesicles (200–1,000 nm in diameter) and exosomes (30–200 nm in diameter), are released by MSCs.[14] Exosomes are nano-sized vesicles that are continuously released by almost all eukaryotic cells. Exosomes can travel between cells and transport their cargo (lipids, proteins, and nucleic acids), making them an appealing cell-free therapeutic option for various diseases.[15] A recent study using a murine model showed that ASCE treatment can relieve AD-like symptoms by reducing levels of multiple inflammatory cytokines (IL-4, IL-31, IL-23, and TNF-α), and the results were comparable to those observed with prednisolone treatment.[6] In a subsequent study, ASCE treatment led to a significant improvement in epidermal barrier functions in an oxazolone-induced dermatitis mouse model by de-repressing the synthesis of ceramides and dihydroceramides and facilitating the formation of lamellar bodies.[7] Overall, ASCE treatment normalized the altered gene expression observed in oxazolone-induced AD-like lesioned skins, with respect to skin barrier function, lipid metabolism, cell cycle, and immune responses. From these results, we can presume that the inhibitory immune inflammatory response of ASCE treatment and its beneficial effects on the skin barrier led to improvement of DFR in our patients.

A limitation of this study is the limited number of cases, and thus, the findings cannot be generalized. Therefore, based on these preliminary findings, further large, well-controlled, prospective, randomized studies should be performed in the future to fully elucidate the therapeutic ability of ASCEs in treating DFR.

5 | CONCLUSIONS

Based on the reported case reports, we propose ASCEs as a promising agent for the management of DFR. As the use of dupilumab for patients with AD gradually expands, the incidence of DFR will increase. Successful management of DFR is important to increase patient satisfaction from dupilumab treatment and improve their quality of life. However, further controlled studies with a larger sample size are needed to confirm the efficacy and safety of this agent, as well as the optimal treatment protocol for the treatment of DFR.

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CONFLICTS OF INTEREST

No conflict of interest.

AUTHOR CONTRIBUTIONS

Kui Young Park, Hye Sung Han, Jae Wan Park, and Seong Jun Seo contributed to conception and design. Kui Young Park, Hyuck Hoon Kwon, and Gyeong-Hun Park contributed to acquisition of data. Kui Young Park, Hye Sung Han, Jae Wan Park, and Seong Jun Seo contributed to interpretation of data. All the authors were involved in the drafting of the manuscript and in the critical revision of the manuscript for important intellectual content and have given final approval of the version to be published.

ETHICAL APPROVAL

Written informed consent was obtained from each patient for publication of this case study and associated digital images.

ORCID

Kui Young Park https://orcid.org/0000-0001-5965-1754
Hye Sung Han https://orcid.org/0000-0002-3556-0740
Jae Wan Park https://orcid.org/0000-0003-2690-6495
Hyuck Hoon Kwon https://orcid.org/0000-0001-8194-1790
Gyeong-Hun Park https://orcid.org/0000-0001-8890-8678
Seong Jun Seo https://orcid.org/0000-0003-2915-839X

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