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Successful transdermal allergen delivery and allergen-specific immunotherapy using biodegradable microneedle patches

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ABSTRACT

Allergen-specific immunotherapy (SIT) is an effective treatment modality for allergic diseases such as atopic dermatitis (AD). However, frequent visits over a 3-year period as well as looming adverse events tend to discourage patient compliance. Therefore, a more convenient, effective, and safe method of SIT is needed.

For several decades, use of microneedles has been promoted as an efficient and precise transdermal drug delivery method. In this study, we developed *Dermatophagoides farinae* (*D. farinae*) extract (*DfE*)-loaded microneedle patches, and evaluated their safety and efficacy as a novel SIT method. After 4 weeks of patch application, efficient allergen delivery and successful induction of immune response to *DfE* were demonstrated in mice, with no apparent adverse events. AD-induced NC/Nga mice received microneedle immunotherapy (MNIT) (10 μg), subcutaneous immunotherapy (SCIT) (10 μg), SCIT (100 μg), or placebo. Both MNIT (10 μg) and SCIT (100 μg) treatments improved clinical and histologic manifestations of AD skin lesions, altered immunoglobulin production, dampened Th2 cellular response, and boosted Treg infiltrates, without significant side effects; whereas SCIT (10 μg) or placebo subsets failed to show any effects. Based on the favorable safety and efficacy profiles demonstrated in mice by MNIT in the current study, we believe that MNIT may serve as a new SIT modality.

1. Introduction

Despite numerous treatment modalities that are available for allergic diseases, allergen-specific immunotherapy (SIT) is advantageous in that it is a disease-modifying, causal treatment that induces long-lasting tolerance to allergens [1].

Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin disease that affects 20% of children and 1–3% of adults [2,3]. Recently, SIT has received support in treating AD, backed by randomized controlled trial [4] as well as meta-analyses [5], and especially by its success in patients sensitized with house dust-mite allergen, the most commonly sensitized allergen in atopic dermatitis patients [6–8]. There are several routes for SIT. Subcutaneous immunotherapy (SCIT) is the most commonly used and generally effective route for allergen injection. However, the need for frequent visits over a long period has undermined patient adherence. To overcome this limitation, sublingual immunotherapy (SLIT), which delivers allergen via mucosal layer, has been developed. However, effectiveness of SLIT is less certain than that of SCIT [9,10]; therefore, a more effective, safe, and convenient method for allergen delivery is needed.

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Microneedle patch is a transdermal drug delivery method consisting of micrometer-sized needles in arrays, on a backing material [11,12]. It allows patients to safely self-administer treatment without pain, while presenting minimal risk of bleeding, infection, or injury, compared with conventional hypodermic needles. Accordingly, microneedle patch has been actively investigated as a potent drug delivery strategy, especially for vaccination [13–15]. Recently, however, epicutaneous [16] and allergen-coated microneedle [17,18] methods of allergen delivery have emerged as alternative means of inducing immune reactions, and as novel vehicles for SIT.

In this study, we developed *Dermatophagoides farinae* (*D. farinae*) extract (*DfE*)-loaded sodium hyaluronate-based microneedle patches, and assessed their ability to deliver allergen into the skin of mice. We then evaluated safety and efficacy of this strategy to determine its potential use in SIT.

2. Methods

2.1. Preparation of microneedle patches

Droplet-born air blowing (DAB) method was used to prepare *DfE*-loaded microneedle patches [19]. First, *DfE* was passed through an Acrodisc Syringe Filter with 0.45 μm Supor membrane (Pall Corp, Port Washington, NY, USA), and admixed with HA powder in phosphate-buffered saline (PBS) (pH 7.4) to load microneedle patches (4 °C) at various concentrations (0, 0.2, 2, and 10 μg/patch). The specified concentrations of *DfE* and sodium hyaluronate (intrinsic viscosity: 0.14 m^3/kg, HA-EP1; Bloomage Freda Biopharm Co Ltd, Jinan, China) were placed in solution (HA concentration: 16.8% w/w), we fabricated corresponding *DfE*-loaded microneedle patches through precision control of solution droplets, with each droplet delivered separately and air-blown to form a microneedle. Each patch was designed as 76-microneedle array (needle pitch, 1.4 mm; needle length, 0.25 mm). *DfE* was provided by the Institute of Allergy of Yonsei University (Seoul, Korea) [20].

2.2. Measurement of DfE allergenicity

Allergenicity of *DfE* was determined by inhibition ELISA [21]. A 96-well plate was coated with 100 μl of *DfE* (10 μg/ml) and incubated overnight (4 °C). Then, sera from four subjects who were highly sensitized to *D. farinae* were pooled, and diluted 1:3 with 1% bovine serum albumin in PBS containing 0.05% Tween20 (PBST). The 1:3 diluted sera and serially six-fold diluted test solutions were pre-incubated with *DfE* (4 °C), then incubated in a coated plate (2 h, 37 °C). Finally, goat biotinylated anti-human IgE (Vector Laboratories Inc., Burlingame, CA, USA) was added (1 h, 37 °C), followed by horseradish peroxidase-streptavidin (HRP; Vector Laboratories) incubation (1 h, room temperature [RT]). Color was developed by adding 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution (Kirkegaard & Perry Laboratories [KPL] Inc., Gaithersburg, MD, USA) to each well, and then interrupted using TMB stop solution (KPL). Absorbance at 450 nm was measured via microplate reader, with percent inhibition calculated as follows: $(1\text{-}A_t/A_0) \times 100$, where A_t stands for absorbance of test solution at 450 nm, and A_0 for absorbance of non-test solution. Allergenicity was determined by calculating the concentration of allergen required to produce 50% inhibition of a standard extract.

2.3. Evaluation of DfE-loaded microneedle patches

2.3.1. Safety test

Before applying microneedle patches, mice were anesthetized with 3–5% isoflurane using isoflurane vaporizer (VetEquip Inc., Livermore, CA, USA). Hair on the back of mice (n = 3) was shaved, and microneedle patches were applied. Self-adherent bandages (3 M Coban, St Paul, MN, USA) were used to wrap around the torsos, covering the patches to prevent detachment. After 2 h, bandages and patches were gently removed. Patch applications were performed twice per week for 4 weeks. Transepidermal water loss (TEWL) was measured (Tewameter TM210; Courage and Khazaka Electronic, Cologne, Germany) before and immediately after patch application, and again after 2 h. For this purpose, mice were anesthetized in an atmosphere-controlled room (24 ± 2 °C, 55 ± 15% relative humidity).

2.3.2. Allergen delivery capacity

To evaluate dendritic cell migration, 6-week-old female BALB/c mice were treated with 10 μg of *DfE*-loaded microneedle patch or subcutaneously injected with 10 μg or 100 μg of *DfE* twice a week for 4 weeks. Next, cells were isolated from skin draining lymph nodes and stained with anti-CD11c Alexa 488 (N418) and anti-Der f1 (MA-6A8) antibodies. Secondary PerCP (sc-358939; Santa Cruz Biotechnology, Dallas, TX, USA) for anti-Der f1 was stained and read sample using FACSVerse flow cytometer (BD Biosciences).

2.4. Allergen-specific immunotherapy in AD mice

Induction of AD in a mouse model was achieved using previously described methods [6]. In brief, 100 mg of *DfE* ointment (Biostir-AD; Biostir Inc., Kobe, Japan) was applied to shaved dorsal skin and each ear surface of pathogen-free, female NC/Nga mice twice per week, for 8 weeks. After 3 weeks of induction, mice were divided into four groups as follows: 1) AD controls; 2) microneedle immunotherapy (MNIT), 10 μg; 3) SCIT, 100 μg; and 4) SCIT, 10 μg. Immunotherapy was performed twice per week for 5 weeks, along with AD induction. In MNIT (10 μg) group, *DfE*-loaded microneedle patches (10 μg) were applied, while again wrapping the patched areas in self-adherent bandages (3 M) to prevent detachment. After 2 h, bandages and patches were gently removed. Mice in SCIT groups were subcutaneously injected with either 10 μg or 100 μg of *DfE*, respectively, in 100 μl of PBS. Control animals received the same volume of PBS, without *DfE* (Supplementary Fig. 1). All experiments were performed under protocols approved by the Animal Research Ethics Board of Yonsei University (Seoul, Korea).

2.5. Scoring of dermatitis severity

Scoring AD (SCORAD) index for mice, grading erythema/hemorrhage, scarring/dryness, edema, and excoriation/erosion were used to gauge severity of dermatitis. Each symptom was rated as 0 (none), 1 (mild), 2 (moderate), or 3 (severe), combining individual marks for a total score [22,23]. Each animal was scored once each week, by two independent researchers. Photographs were also obtained every week, under the same camera settings and lighting conditions.

2.6. Histopathological assessment of dermatitis

Dorsal skin tissues, taken from same sites in each group, were fixed in 4% paraformaldehyde, processed routinely, and serially sectioned (4-μm thick) for hematoxylin-eosin (H&E) staining. Epidermal thickness was determined by image analysis software (MetaMorph; Molecular Devices Corp, Sunnyvale, CA, USA), and inflammatory infiltrates (eosinophils, other leukocytes) were assessed microscopically (BX53; Olympus Corp, Tokyo, Japan). Three different images were taken of each slide. Results were expressed as mean ± standard error of mean (SEM), based on eight different digital images.