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Allergology International

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Letter to the Editor

STAT6 decoy oligodeoxynucleotide (ODN)-containing ointment more potently inhibits mouse skin inflammation when formulated with ionic liquid technology than as a traditional Vaseline ointment

Dear Editor,

Atopic dermatitis (AD) is a chronic, eczematous disorder with multiple etiological factors, such as genetics, barrier dysfunction, and immune responses to environmental elements.^{1,2} The immune characteristics of AD are Th2-dominant inflammation via the up-regulation of Th2 cytokines (IL-4, IL-5, and IL-13) and chemokines [TARC/CCL17 and macrophage-derived chemokine (MDC)/CCL22], eosinophilia, and high levels of IgE. IL-4 and IL-13 both decrease the expression of the epidermal barrier protein filaggrin, which triggers allergen sensitization and bacterial infection, resulting in the exacerbation of skin inflammation.^{1,2}

Signal transducers and activators of transcription (STAT) 6 is a signalling molecule and transcription factor that regulates IL-4/IL-13 receptors and induces Th2 gene transcription.³ Previously, we showed that the subcutaneous administration of STAT6 decoy oligodeoxynucleotides (ODNs) clearly inhibited allergic inflammation, such as contact hypersensitivity (CHS) and IgE-mediated late-phase reactions, in mice.^{4,5} Additionally, our clinical trial for AD patients demonstrated that steroid-resistant facial erythema was successfully improved by the topical application of STAT6 decoy ODNs in Vaseline.⁶ Here, we established a new STAT6 decoy ODN ointment using a novel drug delivery system—the ionic liquid transdermal system (ILTS[®], MEDRx, Kagawa, Japan)—and verified its therapeutic effects in a mouse model of skin inflammation. In ILTS, ionic liquid is prepared from organic acids and amines.⁷ The molecular assembly involves the equilibrium reaction of ionic liquid/acid/amines and hydrogen bond interactions to enhance transdermal absorption of drugs at the hydrophobic field of skin tissue.⁷ The animal experiments were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University.

We compared the therapeutic effects of STAT6 decoy ODN Vaseline with those of the ILTS ointment on 2,4,6-trinitrochlorobenzene (TNCB)-induced CHS, which was clearly inhibited by the subcutaneous injection of STAT6 decoy ODNs.⁴ Vaseline and ILTS ointments containing scrambled ODNs were used as negative controls. The sequences of the STAT6 decoy ODNs were previously described: 5'-GATCAAGACCTTTCCCAA-GAAATCTAT-3' and 3'-CTAGTTCTGGAA AAGGGTCTTTAGATA-5'.^{6,8} While the application of traditional Vaseline containing STAT6 decoy ODNs (ODN concentration between 2 and 0.02%) slightly inhibited TNCB-induced CHS in a dose-dependent manner

compared to scrambled decoy ODNs, as assessed by ear swelling, the effects were not statistically significant (2%, $P = 0.12$; 0.2%, $P = 0.07$; 0.1%, $P = 0.39$; 0.02%, $P = 0.77$; Fig. 1A). However, ILTS ointment containing STAT6 decoy ODNs (ODN concentration between 0.2 and 0.02%) significantly suppressed CHS responses in dose-dependent manner compared to scrambled decoy ODNs (0.2%, $P < 0.01$; 0.1%, $P < 0.01$; 0.02%, $P = 0.06$; Fig. 1A). Moreover, we found the gene expression levels of IL-4 and IL-13 in the ears were significantly inhibited in ILTS ointment-treated compared with Vaseline-treated ears (Fig. 1B). Histologically, the cellular infiltration was also inhibited in ILTS ointment-treated compared with Vaseline-treated skin (Fig. 1C). These results indicate that the ILTS ointment elicits improved therapeutic effects on skin inflammation compared to Vaseline.

To assess whether these therapeutic effects observed for ILTS ointment were a result of the high absorption efficiency of decoy ODNs, we examined the number of decoy ODNs in normal and inflamed (TNCB-induced CHS) skin after the application of each ointment using real-time PCR analysis. In normal skin, the quantity of STAT6 decoy ODNs was four times greater in ILTS ointment-treated skin than in Vaseline-treated skin ($P < 0.05$; Fig. 2). In CHS lesions, the amount of STAT6 decoy ODNs was 10 times greater in ILTS ointment-treated skin than in Vaseline-treated skin ($P < 0.01$; Fig. 2). These findings indicate that the ILTS allows a higher absorption of decoy ODNs than does Vaseline. However, we could not identify the distribution of decoys in the skin. Further studies are necessary to reveal this matter.

In this study, we showed that ILTS ointment containing STAT6 decoy ODNs has better therapeutic effects on mouse skin inflammation than traditional Vaseline. In general, nucleic acid-based medications suffer from poor absorption of high-molecular-weight ODNs.⁹ To overcome this issue, we applied ILTS, a novel drug delivery system, using an ionic liquid as the ointment base and obtained improved absorption of decoy ODNs, suppressing mouse skin inflammation.

A wide variety of molecular targets will become available as drug agents for the treatment of AD in the near future.^{1,2} One of these drugs, dupilumab, is a monoclonal antibody against the IL-4/IL-13 receptor α -chain, a molecule related to STAT6. In the phase III trial for AD patients, decreased eczema area and severity index scores and alleviation of pruritus were observed.¹⁰ Dupilumab is currently approved as an AD therapy in Japan. Our previous clinical trial for AD patients showed that Vaseline containing STAT6 decoy ODNs improved refractory facial erythema and decreased pruritus,

Peer review under responsibility of Japanese Society of Allergology.

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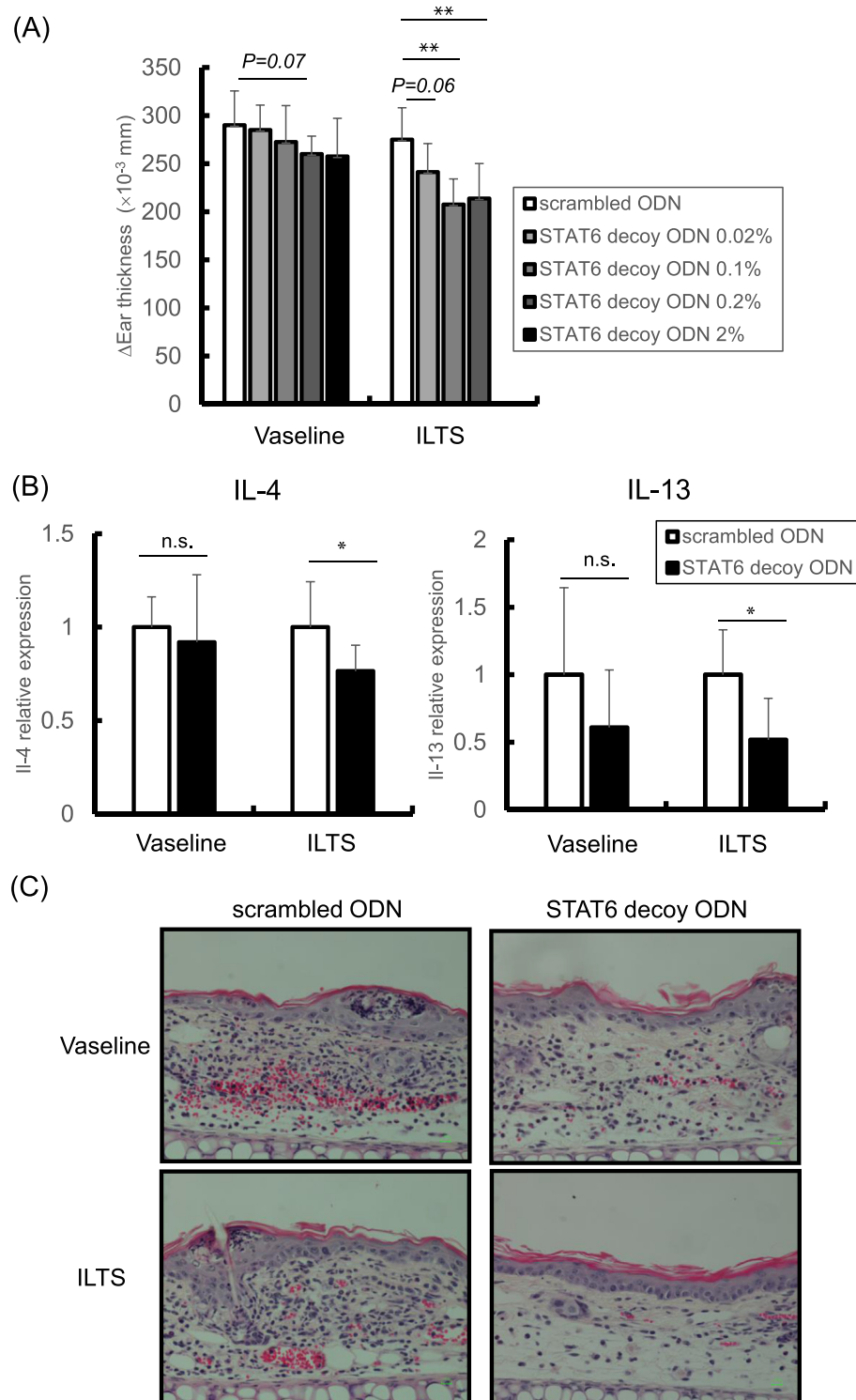


Fig. 1. ILTS ointment containing STAT6 decoy ODNs diminishes TNCB-induced CHS compared with Vaseline containing STAT6 decoy ODNs. **(A)** BALB/c mice were sensitized with 5% TNCB on the shaved abdominal skin. Four days after sensitization, the indicated concentrations of STAT6 decoy ODNs contained in either ILTS ointment or Vaseline were applied to both earlobes. On the following day, mice were challenged with 0.5% TNCB on both earlobes. Twenty hours after the challenge, the thicknesses of the earlobes were measured using a dial thickness gauge. The changes in ear thicknesses are shown. The data represent the means $\pm$ SD; ** $P < 0.01$ by Student's *t*-tests compared with the scrambled ODN-treated earlobe. **(B)** BALB/c mice were sensitized as described above. Four days after sensitization, 0.2% STAT6 decoy ODNs in either ILTS or Vaseline ointment were applied to both earlobes. Scrambled ODNs (0.2%) were used as negative controls. On the following day, mice were challenged with 0.5% TNCB on both earlobes. Eighteen hours after the challenge, mice were sacrificed for analysis. Transcript levels of *IL-4* (left panel) and *IL-13* (right panel) were measured by real-time PCR and normalized to those of *Gapdh*. Data represent the means $\pm$ SD; * $P < 0.05$ by Student's *t*-tests compared with the scrambled ODN-treated earlobe. **(C)** Mice were treated as described in **(A)**. Tissue sections were prepared from ear lobes treated with 0.2% STAT6 or scrambled decoy ODNs in either ILTS or Vaseline ointment and stained with haematoxylin and eosin. Scale bar, 10 μ m.

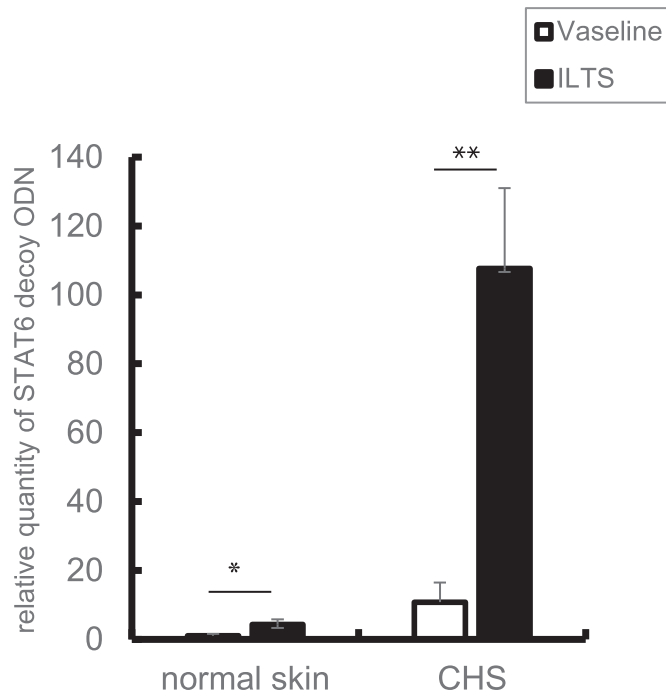


Fig. 2. More STAT6 decoy ODNs were detected in ILTS ointment-treated skin than in Vaseline-treated skin. For normal skin, 0.2% STAT6 decoy ODNs in either ILTS ointment or Vaseline were applied to both earlobes of BALB/c mice. Twenty-four hours later, mice were sacrificed for analysis. For CHS lesions, BALB/c mice were sensitized with 5% TNCB on the shaved abdominal skin. Five days after sensitization, mice were challenged with 0.5% TNCB on both earlobes. Twenty-four hours later, 0.2% STAT6 decoy ODNs in either ILTS ointment or Vaseline were applied to both earlobes. Twenty-four hours later, mice were sacrificed for analysis. Scrambled ODNs (0.2%) were used as negative controls for both ointments. To remove the decoy ODNs from the surface of the skin, ear lobes were washed with neutral detergents. The number of decoy ODNs on the earlobes was measured using SYBR Green dye and a real-time PCR system, and normalized to levels of *Gapdh*. The data show the mean $\pm$ SD. * $P < 0.05$; ** $P < 0.01$, Student's *t*-test. Datasets are representative of three experiments.

as assessed by visual analogue scale, similar to the observations in the phase III studies of dupilumab.⁶ Our findings in this study show that ILTS ointment containing STAT6 decoy ODNs may elicit better therapeutic effects for skin symptoms and pruritus in AD patients, suggesting its use as a novel topical agent for AD, with high safety and efficacy.

Acknowledgments

This work was supported by AMED under Grant Number 17ek0410017h0003 (H.Y.).

Conflict of interest

The authors have no conflict of interest to declare.

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Received 26 September 2018

Received in revised form 14 November 2018

Accepted 28 December 2018

Available online xxx

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