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Topical application of dehydroxymethylepoxyquinomicin improves allergic inflammation via NF-κB inhibition

To the Editor:

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease with significant morbidity and an adverse impact on patient well-being. AD has become increasingly prevalent in industrialized countries, where it now occurs in 10% to 20% of children and 1% to 3% adults.[1] Corticosteroids are generally prescribed to control the symptoms, yet repeated use can cause severe skin atrophy and susceptibility to infection.[2] Tacrolimus, a calcineurin inhibitor, has recently gained widespread use as an AD treatment that avoids the typical side effects associated with topical corticosteroids.[3] However, refractory AD can remain even in patients treated with both corticosteroid and tacrolimus.

Dehydroxymethylepoxyquinomicin (DHMEQ), a newly developed low-molecular-weight nuclear factor- κB (NF-κB) inhibitor, is a 5-dehydroxymethyl derivative of the antibiotic epoxyquinomicin C.[4] DHMEQ has been found to inhibit TNF-α–induced NF-κB activation by suppressing nuclear translocation but not IκB phosphorylation or degradation.[5] Recently the antitumor effects of DHMEQ on breast,[6] thyroid,[7] and prostate[8] cancers as well as its anti-inflammatory and immunosuppressive effects have been reported in mice models.[9,10] In this study, we used atopic dermatitis model mice to examine the antiallergic inflammation efficacy of DHMEQ.

We confirmed that DHMEQ effectively inhibited the NF-κB activity in macrophage-like cell line, RAW264.7 with LPS stimulation (Fig 1, *A*).

First we examined whether DHMEQ suppressed contact hypersensitivity response. Balb/c mice were sensitized with 2,4,6-trinitrochlorobenzene (TNCB) to the dorsal skin and challenged 5 days later on the dorsal surface of the right ear. Immediately after the challenge, DHMEQ (1 mg/mL in acetone) or tacrolimus ointment (1%) was applied to the same ear. The DHMEQ-treated ears showed significantly less ear swelling than the tacrolimus-treated ears (Fig 1, *B*). The suppressive effect of DHMEQ was dose-dependent (Fig 1, *C*). Furthermore, expression of inflammatory cytokine mRNA (IL-1β, IL-6, TNF-α) in lesional skin was suppressed by DHMEQ ointment as well as by tacrolimus ointment (Fig 1, *D*). These data show that DHMEQ suppresses inflammation via suppression of inflammatory cytokine expression regulated by NF-κB.

We next examined whether DHMEQ would inhibit hapten-induced Langerhans cell (LC) migration. Treatment with TNCB caused a significant decline in epidermal LC density 4 hours after application in the untreated mice (30.5%). In contrast, TNCB treatment failed to provoke a significant epidermal LC migration response in the DHMEQ-treated mice (13.1%; Fig 1, *E*). We further evaluated LC morphology in the epidermal sheet preparations derived from the untreated and DHMEQ-treated mice. As illustrated in Fig 1, *F (left)*, at 4 hours after exposure to TNCB, the LCs in the control mice appeared to be activated and to have extended dendritic processes, whereas no such morphologic changes were evident in the LCs examined in the DHMEQ-treated mice (Fig 1, *F, right*). These data support that the DHMEQ suppressed the contact hypersensitivity response at least in part by inhibition of LC migration.

To determine whether DHMEQ has any therapeutic effect in AD, we applied DHMEQ to AD-like lesions of NC/Nga mice and evaluated the progression of skin changes. NC/Nga mice were the spontaneous mouse models of AD. Another spontaneous mouse model, the DS-Nh mouse, has a mutation of transient receptor potential vanilloid 3,[11] whereas the genetic defect of NC/Nga is not known. We used conventional NC/Nga mice that presented severe skin lesions very similar to those of human AD.[12] Conventional NC/Nga mice with moderate to severe AD were topically applied with 1% DHEMQ in plastibase (5% polyethylene and 95% mineral oil), 0.1% tacrolimus ointment, or 0.12% betamethasone ointment daily for 2 weeks. The clinical severity of skin lesion was scored daily according to the 5 main clinical symptoms: scratch behavior, erythema/hemorrhaging, edema, excoriation/erosion, and scaling/dryness.[12] Topical DHMEQ application significantly improved the severity of skin lesions compared with the ointment base as well compared with topical treatment with the 0.1% tacrolimus or 0.12% betamethasone ointment (Fig 2, *A and B*). Improvement of clinical skin condition by DHMEQ was also confirmed by histologic observation, which showed amelioration of hyperkeratosis, acanthosis, dermal edema, and infiltration of the inflammatory cells compared with the ointment base treatment (Fig 2, *C*). At the affected skin sites, the numbers of eosinophils and mast cells were significantly lower in the DHMEQ-treated mice than those in the control mice (Fig 2, *C*).

Potential side effects of topical application of NF-κB inhibition might include susceptibility to infection via local immune suppression. However NF-κB inhibitor presumably avoids the typical side effects associated with topical corticosteroids as well as tacrolimus.

Several reagents targeting NF-κB have been reported. For example, NF-κB decoy oligodeoxynucleotides were reported to be effective in resolving atopic skin lesions in NC/Nga mice.[13] IMD-0354, a selective IKK inhibitor, also improved AD manifestation in model mice.[14] In contrast with these, DHMEQ inhibits NF-κB activation by suppressing nuclear translocation but not IκB phosphorylation or degradation.[5] Because DHMEQ has a unique mechanism to inhibit NF-κB activation, DHMEQ might be effective for AD that does not respond to tacrolimus or corticosteroid, and it might have additive effects with other reagents.

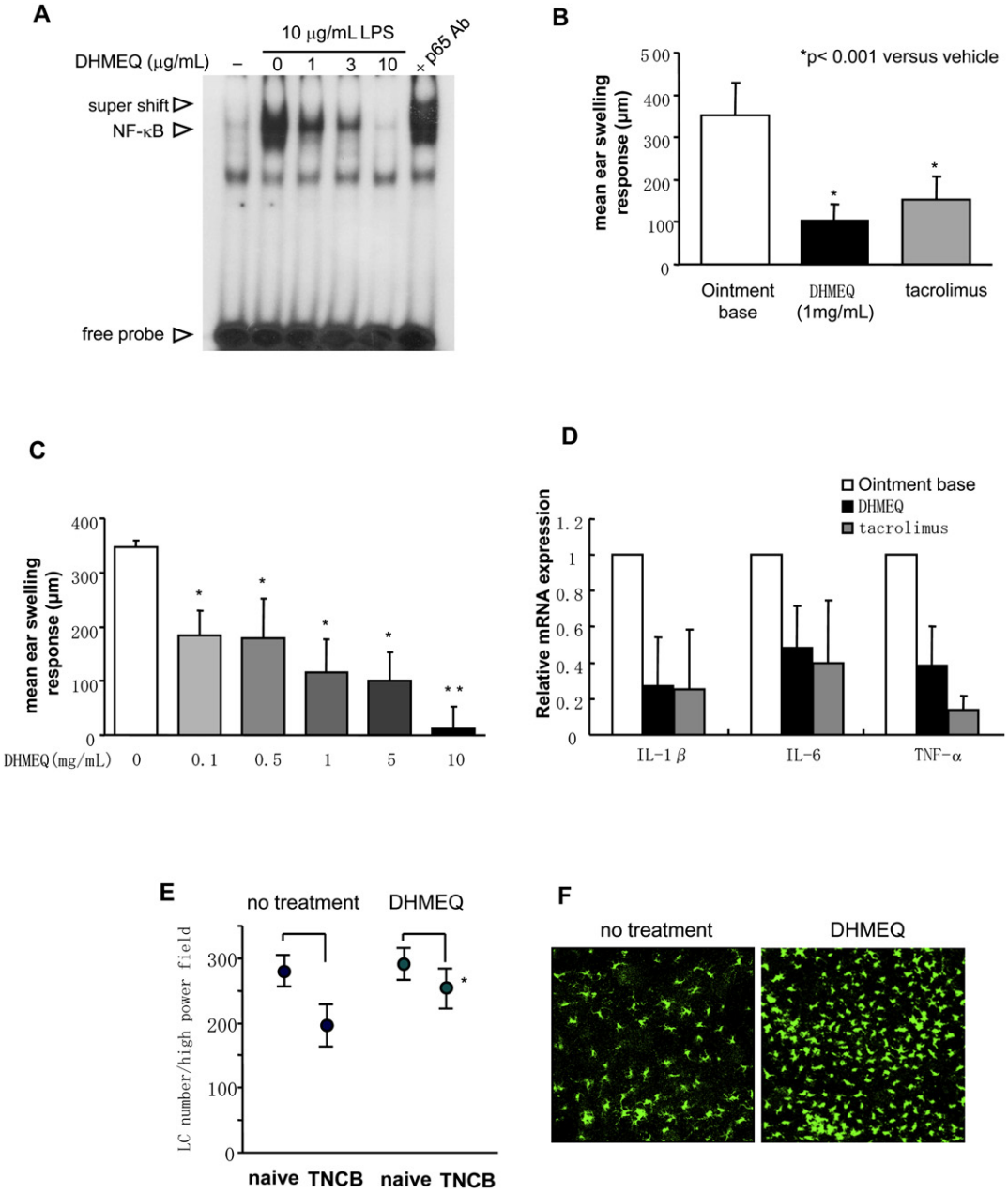

FIG 1. Effect of DHMEQ on contact hypersensitivity response. **A,** DHMEQ effectively inhibited the NF-κB activity in macrophagelike cell line RAW264.7 with LPS stimulation (10 μg/mL). **B,** DHMEQ, tacrolimus, or ointment base was applied topically. All ear-swelling values are shown as means ± SEs (n = 5). $*P < .01$; $**P < .005$. **C,** DHMEQ was applied in various concentrations. **D,** The density of mRNA expression of skin was analyzed by RT-PCR. **E,** The number of LCs was counted. $*P < .05$ (n = 4 for each group). **F,** LC morphology in epidermal sheet preparations derived from untreated *(left)* and DHMEQ-treated *(right)* mice.

In conclusion, we clearly demonstrated that DHMEQ inhibits the contact hypersensitivity response via suppression of inflammatory cytokines and decrease in LC migration. Furthermore, DHMEQ was found to improve AD manifestation of model mice with an efficacy equivalent to that of tacrolimus or betamethasone. DHMEQ may offer a novel therapeutic approach for the treatment of AD.

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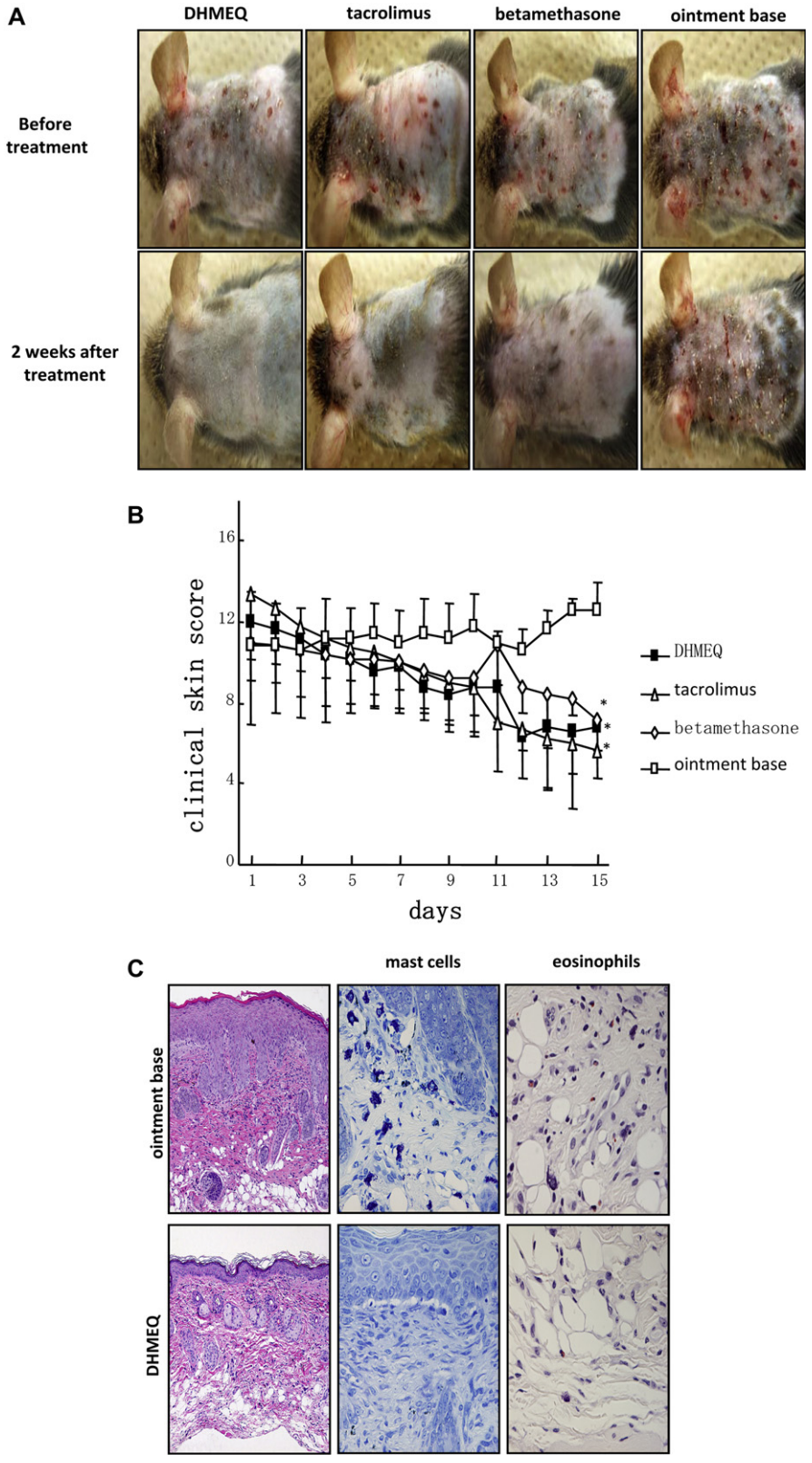

FIG 2. Improvement of atopic dermatitis model mice by DHMEQ. NC/Nga mice were applied topically with DHMEQ, tacrolimus, or ointment base. **A,** Clinical features of each mouse. **B,** The clinical skin score of each group is given as mean ± SE. $*P < .005$. **C,** Specimens were collected from the dorsal skin and were stained with hematoxylin and eosin, direct fast scarlet for eosinophils, or toluidine blue for mast cells.

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A novel non–IgE-mediated pathway of mite-induced inflammation

To the Editor:

In a recent article published in the *Journal of Immunology*, Barrett et al[1] demonstrated that mite and *Aspergillus fumigatus* extracts stimulate the production of cysteinyl leukotrienes from bone marrow–derived dendritic and pulmonary $CD11c^+$ cells through a glycan C–type lectin receptor (Dectin-2) interaction involving FcRγ and Syk signaling that activates arachidonic acid metabolism.

Previously, a number of clinical and experimental observations had called our attention to the existence of important connections between IgE-mediated diseases and leukotriene-mediated inflammation. We had observed that a large proportion of patients with hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs), a condition that is accompanied by increased production of leukotrienes, were atopic and had IgE-mediated sensitivity to mite allergens.[2] This observation has been recently confirmed in another study in which we found significantly increased total and mite-specific IgE levels in patients with NSAID hypersensitivity compared with those seen in healthy control subjects.[3] In tropical countries in which high relative humidity and temperature are optimal for mite proliferation, mite allergens are the main source of sensitization and allergic respiratory disease.

A second clinical observation, also reported by Spanish investigators and others, was that most patients with anaphylaxis after the ingestion of mite-contaminated foods frequently exhibit hypersensitivity to NSAIDs.[4] We designated the association of allergic rhinitis, aspirin/NSAID hypersensitivity, and severe reactions to mite-contaminated foodstuffs as a "new aspirin triad." To understand this association, we performed a study in collaboration with Canadian investigators in which it was demonstrated that mite allergenic extracts inhibited COX-1 *in vitro*. We postulated that mite-induced human allergic diseases could be accompanied, at least in a subset of the patient population, by a dysregulation of leukotriene biosynthesis, metabolism, or both similar to the disturbances described in patients with NSAID hypersensitivity.

In concordance with that hypothesis, various genetic polymorphisms that involve leukotriene C_4 (LTC_4) synthase and cysteinyl leukotriene receptors have been observed in patients with hypersensitivity to NSAIDs; in that context it is important to mention that so-called atopic genes are located in the 5q22-q35 region of human chromosome 5, close to the *LTC4S* gene.

Diverse lines of evidence also support a dysregulation of leukotriene pathways in subjects with mite allergy. Acevedo et al[5] described an association of the A-444C allele of the *LTC4S* gene and IgE response to mite allergens, and we have observed that NSAID-sensitive patients show stronger skin test responses and increased specific IgE antibodies to *Blomia tropicalis* than atopic non–NSAID-sensitive subjects.[3]

Cysteinyl leukotrienes modulate the allergic response, as evidenced in various studies in which it has been shown that leukotrienes enhance IgE and IgG production by human B cells, whereas *LTC4S* knockout mice have a markedly reduced antigen-induced T_H2 pulmonary inflammation. Additionally, it has been demonstrated that IL-4 and IL-13 modulate the number of cysteinyl leukotriene type 1 and 2 receptors on T, B, and antigen-presenting cells.

Furthermore, various groups of investigators have observed that aspirin enhances food-dependent exercise-induced anaphylaxis[6] and facilitates anaphylaxis induced by food allergens.[7-10] These effects could be due to an increased gut permeability, resulting in enhanced opportunity for sensitization at the immunocompetent cell–rich gastrointestinal submucosa.

Bachert et al[11] have proposed a role for staphylococcal enterotoxins, which, through the Vβ receptor on T lymphocytes, allow polyclonal IgE production, including IgE to house dust mite, in nasal polyps, the lungs, and possibly the skin. It would be interesting to investigate in the future whether aspirin-hypersensitive patients with urticaria and angioedema and those with oral anaphylaxis to mites have superantigen-induced immune stimulation