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Dieckol, a phlorotannin of *Ecklonia cava*, suppresses IgE-mediated mast cell activation and passive cutaneous anaphylactic reaction

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Background

Mast cell activation mediated by immunoglobulin E (IgE) plays a crucial role in allergic diseases related to type I hypersensitivity including allergic rhinitis, asthma, and atopic dermatitis (1, S1, S2). Binding of IgE to FcεRI receptors, followed by the crosslinkage of the IgE with the antigen, leads to the mast cell activation and secretion of preformed chemical mediators such as lipid mediators (leukotrienes and prostaglandins), granular contents (β-hexosaminidase and histamine), and inflammatory cytokines (interleukin [IL]-4, IL-6, IL-13, and tumor necrosis factor [TNF]-α), which initiates and exacerbates allergic inflammation (2,3,S3-6). Thus stabilization of mast cells or blocking the released mediators' action is a major strategy for the treatment of patients with type I hypersensitivity. While several agents such as nonsteroidal anti-inflammatory drugs, steroids, and immunosuppressants have been used, they more or less bring about adverse effects (4,S7). Therefore, safe candidates with both anti-allergic property and less toxicity have been expected.

Recent studies have suggested that phlorotannins, which were extracted from the brown seaweed *Ecklonia cava*, has an inhibitory effect on FcεRI expression on basophils and IgE mediated granule release (5,S8). Another experiment determined a dieckol, one of the active ingredients from the extract, exerted potent anti-inflammatory effects by abrogating cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (iNOS) activation (6).

Questions addressed

Because there were no adverse effects when the dieckol was administered to mice (S9) and a chemical that possess a similar structure to dieckol suppressed basophil activation (5), we hypothesized that dieckol may also suppress mast cell activation in type I allergic responses. Therefore, we attempted to evaluate effects of dieckol on IgE-mediated activation of mast cells *in vitro* and experimental type I allergy *in vivo*.

Experimental design

Detailed methods are described in Data S1. In *in vitro* experiments, bone marrow-derived cultured mast cells (BMCMCs) from male WBB6F₁-+/+ mice and LAD2, a human mast cell line were used to determine the release of β -hexosaminidase and histamine, or cytokine expressions. Flow cytometry analysis was also conducted to detect the expression of Fc ϵ RI and IgE binding to the surface of BMCMCs and LAD2 cells. The *in vivo* effects of dieckol were analyzed by using a passive cutaneous anaphylactic (PCA) reaction model of mice induced by both anti-2,4-dinitrophenol (DNP)-IgE and DNP-BSA.

Results

Because mast cells play a critical role in type I allergic responses (2,S2,S4), we examined the effect of dieckol on degranulation of mast cells by measuring β -hexosaminidase and histamine release. As shown in Figs. 1a and S1a, dieckol significantly and dose-dependently decreased the release of β -hexosaminidase and histamine in antigen-stimulated BMCMCs. Interestingly, chemical induced degranulation of BMCMCs, which were triggered by an ionophore (A23187) or a histamine liberator (compound 48/80), were not suppressed by dieckol (Figs. S1c). The inhibitory effects of dieckol on IgE-mediated degranulation were observed in LAD2 cells as well (Figs. S1d and S1e). Moreover, dieckol did not affect the viability of both BMCMCs and LAD2 cells (Figs. S1b and S1f). We also examined the inhibitory effects of dieckol on cytokine expressions of mast cells, and found that dieckol suppressed the expression of various kinds of cytokines including IL-4, IL-6, IL-13 and TNF- α in not only activated BMCMCs but also LAD2 cells, respectively (Figs. 1b and S2). To determine these inhibitory mechanisms of dieckol, we next analyzed the effects of dieckol on IgE-binding to Fc ϵ RI. Pretreatment of BMCMCs with dieckol before anti-DNP-IgE sensitization strongly reduced the binding of IgE to Fc ϵ RI compared to control cells (Fig. 1c).

We attributed this to the downregulation of FcεRI, thus flow cytometry analysis was carried out. As expected, dieckol treatment significantly reduced the expression of FcεRI (Fig. 1d).

To examine the anti-allergic and mast cell-stabilizing effects of dieckol *in vivo*, we examined cutaneous extravasation of Evans blue dye in IgE-sensitized mice challenged with specific antigen with or without dieckol treatment. Compared to control mice, oral administration of dieckol 2 h prior to IgE sensitization markedly abrogated the PCA reaction in the mouse ear and reduced the leakage of Evans blue dye (Figs. 2a and 2b). Abrogation of mast cell degranulation and edematous changes was observed by histological analysis (Fig. 2c). Interestingly, suppression of PCA reaction was also observed when dieckol was administered 2 h before antigen stimulation (Figs. S3a and S3b).

Discussion

We demonstrated the potency of dieckol to suppress mast cell activation and mast cell-mediated type I allergic responses caused by IgE-specific antigen, which was mainly due to the significant downregulation of FcεRI. Interestingly, dieckol treatment even after the IgE sensitization inhibited the PCA reaction, which may be explained by anti-inflammatory effects as reported previously (6). As far as we know, there has been no report that showed suppression of COX2 and iNOS resulted in the FcεRI downregulation. Therefore, another target molecule for dieckol may exist. Because relatively high-dose was required to suppress the type I hypersensitivity reactions in our model, it will require some chemical structural modifications for using dieckol as a therapeutic agent. Identifying the precise mechanism and target molecules of dieckol will bring about a novel therapeutic approach for the prevention or treatment of mast cell-dependent allergic diseases.

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Authorship contribution

All authors contributed this study. Details are described in the supplemental data.

Conflict of interests

The authors have declared no conflicting interests.

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Figure legends

Figure 1. (a) β -hexosaminidase and (b) IL-6 release of BMCMCs. Cells were incubated with dieckol, sensitized with IgE and stimulated with DNP-BSA. Three independent experiments were performed in triplicate and each data point represents the mean $\pm$ SE. ***, $p < 0.005$. (c, d) Flow cytometry analysis of BMCMCs. IgE binding (c) and Fc ϵ RI expression (d) was detected respectively. Three independent experiments were performed in triplicate and each data point represents the mean $\pm$ SE. ***, $p < 0.005$.

Figure 2. Effect of dieckol on IgE-mediated PCA *in vivo*. (a) Representative photographs of each mice. Dieckol was administered 2 h before IgE sensitization. (b) Quantification of Evans blue leakage. Three independent experiments were performed in triplicate and each data point represents the mean $\pm$ SE. ***, $p < 0.005$. (c) Representative data of histological analysis in each group mice. Tissues were stained with toluidine blue. Arrows indicate mast cells. Most of mast cells identified in vehicle treated mice were degranulated, though mast cells were stabilized in mice treated with dieckol. Original magnification, $\times 200$. Bar; 50 μ m.

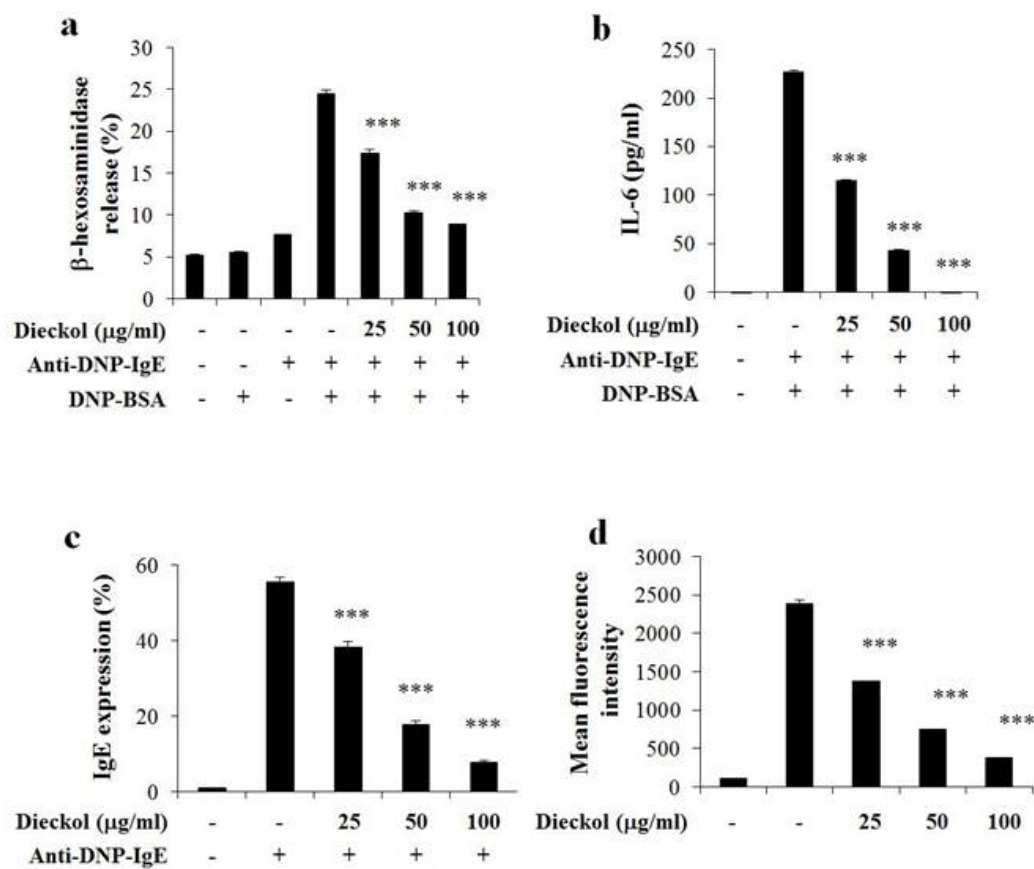


Fig. 1. Ahn et al.

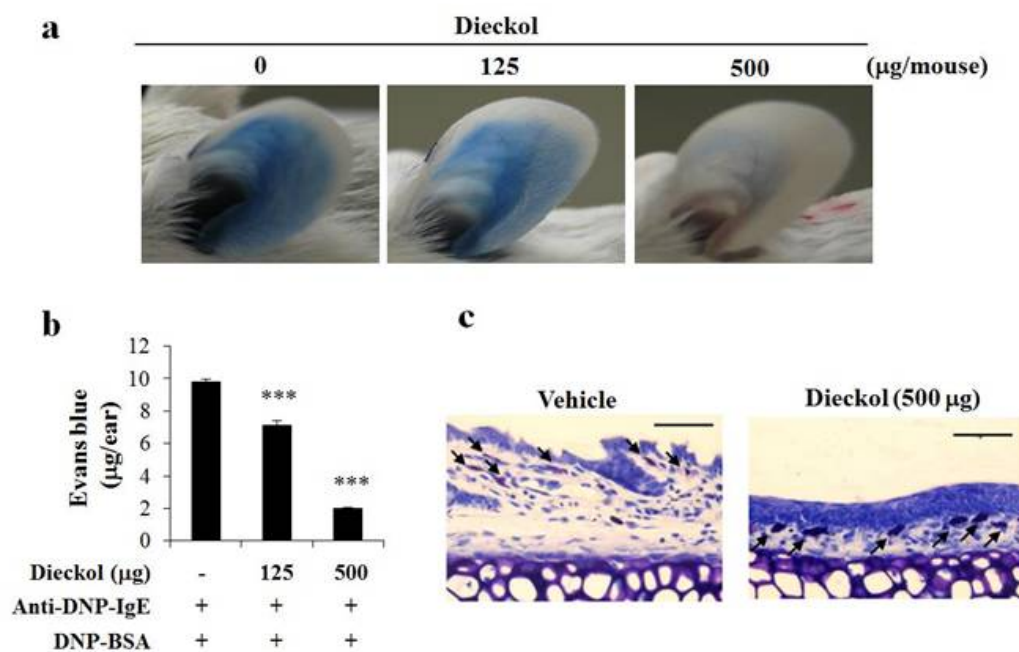


Fig. 2. Ahn et al.