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Thymol attenuates the worsening of atopic dermatitis induced by *Staphylococcus aureus* membrane vesicles

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ABSTRACT

Staphylococcus aureus membrane vesicles (MVs) aggravate atopic dermatitis (AD) through the delivery of bacterial effector molecules to host cells and the stimulation of inflammatory responses. This study investigated the inhibitory effect of thymol, a phenolic monoterpene found in essential oils derived from plants, on the worsening of AD induced by *S. aureus* MVs both *in vitro* and *in vivo*. The sub-minimal inhibitory concentrations of thymol disrupted *S. aureus* MVs. Intact *S. aureus* MVs induced the expression of pro-inflammatory cytokine (interleukin (IL)-1β, IL-6, and tumor necrosis factor-α) and chemokine (IL-8 and monocyte chemoattractant protein-1) genes in cultured keratinocytes, whereas thymol-treated *S. aureus* MVs did not stimulate the expression of these genes. Topical application of thymol-treated *S. aureus* MVs or treatment with thymol after intact *S. aureus* MVs to AD-like skin lesions diminished the pathology of AD. This included decreases in epidermal/dermal thickness and infiltration of eosinophils/mast cells, and inhibited expression of pro-inflammatory cytokine and chemokine genes in mouse AD model. Moreover, thymol significantly suppressed the Th1, Th2, and Th17-mediated inflammatory responses in AD-like skin lesions induced by *S. aureus* MVs, and reduced the serum levels of immunoglobulin (Ig) G2a, mite-specific IgE, and total IgE. In summary, thymol disrupts *S. aureus* MVs and suppresses inflammatory responses in AD-like skin lesions aggravated by *S. aureus* MVs. Our results suggest that thymol is a possible candidate for the management of AD aggravation induced by *S. aureus* colonization or infection in the lesions.

1. Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disorder characterized by dryness, scaling, eczematous pruritic lesions, epidermal hyperplasia, and lichenification [1,2]. The pathogenesis of AD is multifactorial and involves a combination of genetic, immunologic, and environmental factors that are associated with an increased susceptibility to colonization or infection by *Staphylococcus aureus* [3–5]. Up to 90% of AD patients are colonized by *S. aureus* and the disease severity is proportional to the degree of *S. aureus* colonization in the AD lesions [6,7]. Thus, *S. aureus* is regarded as a pivotal aggravating factor of AD [8,9]. Many *S. aureus*-derived molecules, including superantigens [10,11], α-hemolysin [12], staphylococcal enterotoxin B (SEB) [13], lipoteichoic acid [14], peptidoglycan [15,16], staphylococcal protein A (SPA) [17,18], and phenol-soluble modulin α [19], are able to stimulate inflammatory responses, which play a crucial role in worsening AD [20–23]. However, single bacterial component such as superantigens and SPA does not have any significant effect on the worsening of AD [24,25]. We recently demonstrated that *S. aureus*-derived membrane vesicles (MVs), a molecular nanocomplex, aggravated inflammatory responses in AD-like skin lesions [26,27]. *S. aureus* MVs effectively deliver bacterial effector molecules to host cells, which stimulate inflammation. Moreover, *S. aureus* MVs could induce AD-like skin inflammation in a mouse model [28]. These results suggest that *S. aureus* MVs are considered a therapeutic target for the management of AD aggravation.

Thymol, 2-isopropyl-5-methylphenol, is a phenolic monoterpene primarily found in essential oils from thyme, oregano, and tangerine peel [29]. This bioactive compound is known to possess a variety of pharmacological properties such as antioxidant, anti-cancer, anti-inflammatory, anti-fungal, and anti-parasitic activities [30–34]. Additionally, thymol has antimicrobial activity against many pathogenic

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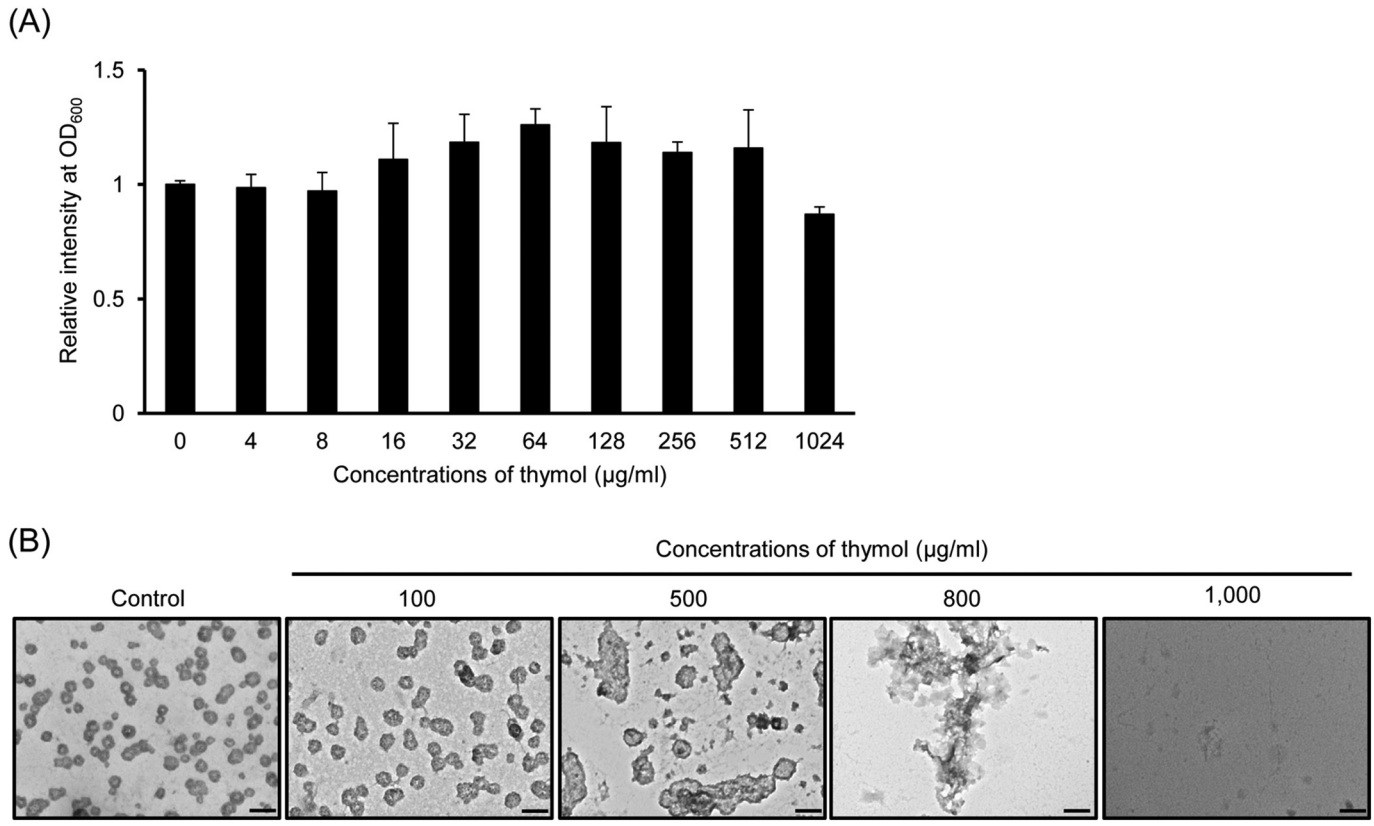

Fig. 1. Disruption of membrane vesicles (MVs) purified from *S. aureus* 03ST17 by thymol. A. Bacteria (5×10^5 CFUs/ml) were cultured in Luria-Bertani broth with various concentrations of thymol (4–1024 μg/ml) for 24 h and then the optical density at 600 nm (OD_{600}) was determined. The data presented are the mean ± SD of three independent experiments. B. Transmission electron microscopic analysis of *S. aureus* MVs treated with thymol. *S. aureus* 03ST17 MVs in the amounts corresponding to protein concentrations of 2 mg were incubated with various concentrations of thymol (0.1–1 mg/ml) for 2 h at 37 °C. Bar, 1 μm.

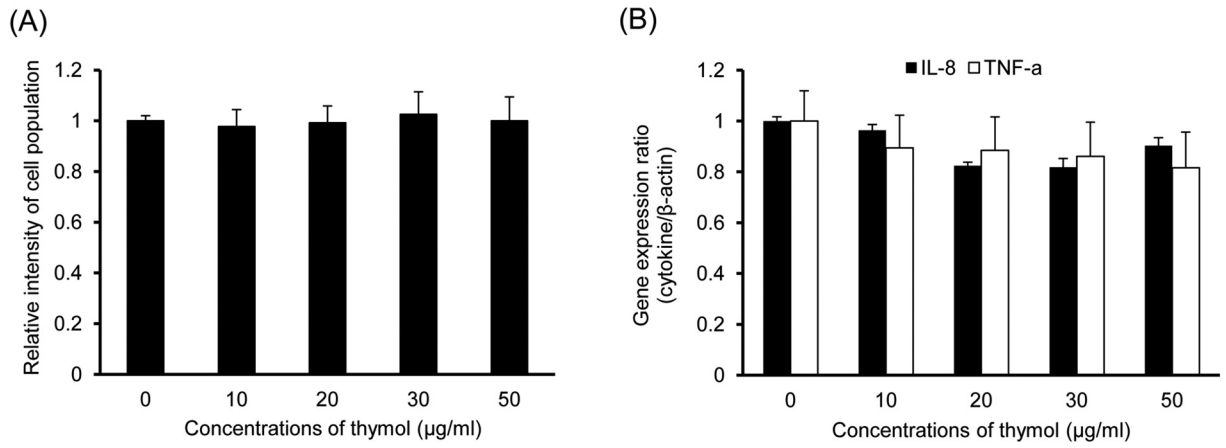

Fig. 2. Effect of thymol on the cytotoxicity and inflammatory responses in HaCaT cells. A. Cells were treated with various concentrations of thymol (10–50 μg/ml) for 24 h, and then, cytotoxicity was measured using the lactate dehydrogenase assay. The data are presented as the mean ± SD of three independent experiments. B. Cells were treated with thymol (10–50 μg/ml) for 6 h, and then, the expression of IL-8 and TNF-α genes was analyzed by qPCR. The data are the mean ± SD of three independent experiments.

bacteria, including *S. aureus*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* Typhimurium [35–37]. Its antimicrobial activity is associated with the disruption of the lipid bilayer of the cytoplasmic membrane and an induction of DNA morphological changes through its interaction with the minor groove of bacterial genomic DNA [38–40]. Moreover, sub-inhibitory concentrations of thymol suppress the secretion of α-hemolysin, SEA, SEB, and toxic shock syndrome toxin-1 in *S. aureus* [41,42]. However, to our knowledge, the effect of thymol on *S. aureus*-derived MVs and its biological effect on AD remain uncharacterized.

The aim of this study was to investigate the inhibitory effect of thymol on inflammatory responses against *S. aureus* MVs in cultured keratinocytes. Moreover, we determined the inhibitory activity of thymol in the *S. aureus* MV-induced worsening of AD in a mouse model.