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Orally Applied Novel Organogermanium Improves Skin Inflammation in NC/Nga Mice with Atopic Dermatitis

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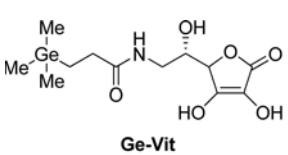
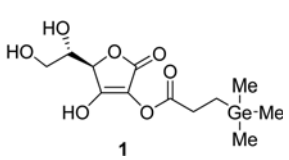
It has been reported that compounds containing germanium (Ge) have a broad range of biological activities.[1] In general, inorganic forms of germanium are severely toxic to certain organs and tissues, whereas organic forms of germanium compounds appear to be less toxic for mammals.[2] Therefore, many organogermanium compounds have been synthesized and investigated for their pharmacological activities.[3] However, in spite of its beneficial effects, long-term ingestion or a high dose of organic Ge-132 is known to cause toxic effects.[4] Ge-132 is a water-insoluble polymer which is chemically synthesized from GeO_2 and an organic acid. During purification, Ge-132 can easily be contaminated by significant amounts of toxic inorganogermanium or other hazardous impurities.

Organogermaium compounds display immune responses which may be due to their antioxidant effects.[5] In a previous study, vitamin C (L-ascorbic acid), which is a water-soluble antioxidant, was coupled to organogermanium because the antioxidant activity of this conjugated compound (Ge-Vit) would be improved by the combined effects of ascorbic acid and organogermaium as along with improved water solubility.[6] As expected, this compound attenuated the disruption of skin barrier function in a murine model of chronic contact dermatitis.[7] However, instability of Ge-Vit, such as susceptibility of ascorbic acid to thermal and oxidative degradation, made it difficult to maintain its physiological value over long periods of time. Since a number of stable ascorbic acid derivatives have been developed by modifying the 2-hydroxyl group of ascorbic acid,[8] an ascorbic acid-conjugated organogermanium **1** at the C-2 position was also prepared and investigated for its *anti*-oxidant effects.[9] In the present study, the *anti*-inflammatory effects of this compound on atopic dermatitis (AD) were investigated because atopic dermatitis is a type of chronic inflammatory disease of the skin.

It has been reported that the *Dermatophagoides farinae* (Df) extract ointment-induced dermatitis model in NC/Nga mice is useful for elucidating the pathogenesis of atopic dermatitis and for evaluating therapeutic agents.[10] Therefore,



Ge-Vit



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in this study, induction of AD using Df body ointment was performed as described previously. Briefly, on day 0, mice were treated with 150 μL of 4% sodium dodecyl sulfate after shaving the hair on the backs of pathogen-free male NC/Nga mice (aged 7-8 weeks, 20-25 g). Df body ointment (150 mg per mouse) was then applied to the dorsal skin and both surfaces of each ear 6 times over the course of 3 weeks to induce AD-like skin lesions. Ascorbyl 2-palmitate, which is one of lipophilic and stable 2-*O*-substituted ascorbate derivatives, showed vitamin C activity after enzymatic hydrolysis to free vitamin C by a certain esterase *in vivo*.[11] Therefore, the compound dissolved in PBS was administered orally once a day for 3 weeks. Prednisolone (10 mL/kg) and PBS alone were used as positive control and vehicle control, respectively, over the same treatment period.

Clinical symptoms of AD were assessed according to four symptoms: erythema/hemorrhage, scarring/dryness, edema and excoriation/erosion.[12] In order to evaluate the effects of the compound on skin barrier function, the basal TEWL (Transepidermal Water Loss) values of the back and both ears of the mice were measured first at the end of the treatment period. The TEWL values of the mice treated with compound **1** and prednisolone decreased (51% at compound **1** 10pmk, and 48% at prednisolone 3pmk) compared to the vehicle-treated group, indicating that treatment of compound **1** affected the basal TEWL. In addition, in the clinical skin score which was determined macroscopically from the sum of individual scores according to four AD symptoms, treatment of compound **1** was shown to significantly improve the dermatitis score in the compound-treated NC/Nga mice compared with the ointment base-treated group, as shown in Figure 1(b). Histological examination further supported the *anti*-AD activities of this compound, and both dermal inflammation and parakeratosis were substantially alleviated (Figure 1(d)) along with attenuation of inflammatory cell infiltration.

Allergen-specific $CD4^+$ T cells can be isolated from the skin lesions of patients with AD.[13] On the basis of their cytokine production profiles, $CD4^+$ helper T lymphocytes were subdivided into type 1 T-helper (Th1) and type 2 T-helper (Th2) cells, and the predominance of Th2 and increased serum IgE levels were reported in patients with atopic dermatitis.[14] However, in the present study, the level of IgE in the compound-treated group still remained high, while predominant expression of IgE was observed in the Df extract ointment-

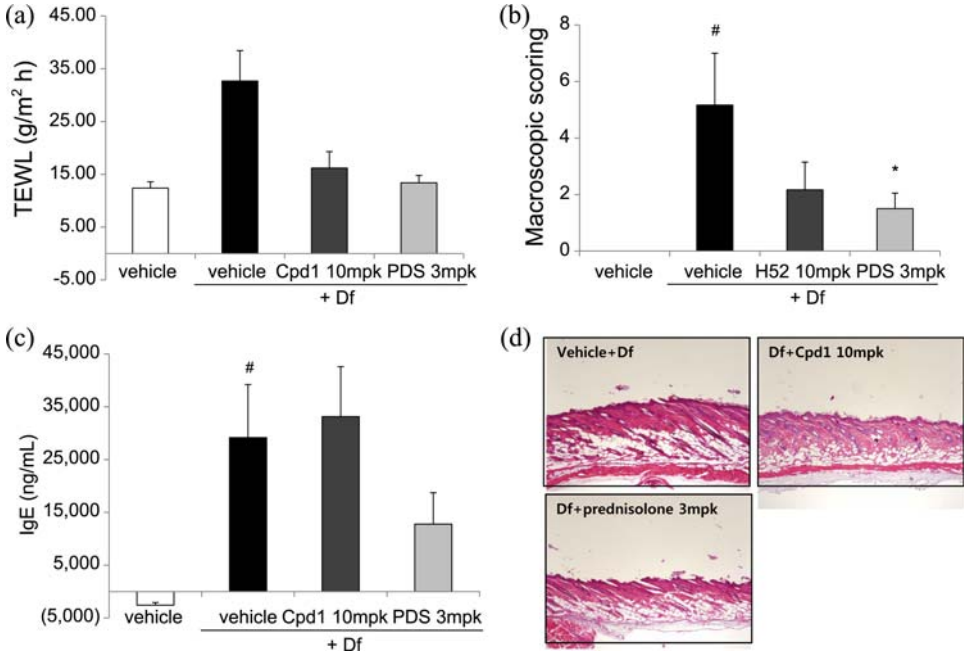

Figure 1. Effects of compound **1** on the recovery from the Df-induced murine AD model. (a) The basal TEWL was measured using a vapor meter, SWL4102 (Delfin Technologies Ltd., Finland). The values represent the mean ± S.E. (N = 5). (b) The extent of (1) erythema/hemorrhage, (2) scarring/dryness, (3) edema and (4) excoriation/erosion was scored as 0 (none), 1 (mild), 2 (moderate), and 3 (severe). The total skin score was defined as the sum of the individual scores.[12] (c) The serum IgE levels were measured using mouse IgE ELISA kits (BD Biosciences, San Jose, CA). The values represent the mean ± S.E. (N = 5). (d) Histological examination of the treated skin (H&E staining).

induced dermatitis model and a lower IgE production level was exhibited in the prednisolone-treated mice than the control group (Figure 1(c)). Previously, we reported that a novel water-soluble organogermanium compound (Ge-OH) conjugated with glucose enhance INF-γ, resulting in the *anti*-inflammatory effect.[15] Meanwhile, oral administration of high dosages of vitamin C to the mice increased the secretion ratio of Th1/Th2 (INF-γ/IL-5) cytokines in the bronchoalveolar lavage fluid (BALF), suggesting that a high dose of the vitamin C supplement might attenuate allergic inflammation *in vivo* by modulating the Th1/Th2 balance toward the Th1 pole.[16] Although down-reglulation of IgE was not detectable within 3 week, the prolonged treatment of compound might result in the dereased level of IgE through INF-γ production. These suggest that the expression of other Th1 and Th2 cytokines needs to be clarified in detail in order to determine the molecular mechanisms of the immune-modulating effects between IgE/INF-γ.

Although steroids have widely been prescribed for patients with AD, skin abnormalities, such as skin atrophy and epidermal barrier disturbance, are frequently observed after prolonged steroid treatment. Regarding the side effects, our study results suggest that compound **1** can be a useful drug candidate for the treatment of atopic dermatitis.

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