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A novel organogermanium protected atopic dermatitis induced by oxazolone

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ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory disease of the skin that is often associated with other atopic diseases, such as asthma and allergic rhinitis. Although topical steroids have widely been prescribed for patients with AD, skin abnormalities are frequently observed after prolonged steroid treatment. In this study, a novel water-soluble organogermanium compound (Ge-Vit) was prepared because organogermanium is a known INF-γ inducer. The Ge-Vit treatment decreased the basal TEWL and IgE production and attenuated the disruption of the skin barrier function in a murine model of chronic contact dermatitis. The histological examination further supported the anti-AD activities. These results suggested that Ge-Vit can be a useful drug candidate for treating atopic dermatitis.

Atopic dermatitis (AD) is a chronic inflammatory disease of the skin that is often associated with other atopic diseases, such as asthma and allergic rhinitis.[1] Clinical symptoms of AD include a chronic, relapsing form of skin inflammation, epidermal thickening, and hypertrophy.[2] Although topical 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.[3] Therefore, many attempts have been made to search for the development of novel therapies.[4–11]

Compounds containing germanium (Ge) are known to have a broad range of biological activities, including antimicrobial, antiviral, antineoplastic, analgesic, and immuno-modulating effects.[12] In general, inorganic forms of germanium (such as GeO_2 and $GeCl_4$) are severely toxic to some important organs and tissues, whereas organic germanium compounds appear to be less toxic for mammals.[13] For this reason, many organogermanium compounds have been synthesized and investigated for their pharmacological activities. Among these organogermaniums, Ge-132 enhances INF-γ and has been tested in clinical trial, with promising preclinical results as an anti-inflammation agent.[5] However, in spite of its beneficial effects, it has been reported that the long-term ingestion or high doses of organic Ge-132 can cause toxic effects similar to GeO_2.[2] Ge-132 is a water-insoluble polymer that is chemically synthesized from GeO_2 and organic acid. After the synthesis and purification, Ge-132 can easily be contaminated by significant amounts of inorganic germanium or other hazardous impurities. Therefore, in this study, water-soluble organogermanium compound (Ge-Vit), organic germanium that was conjugated with vitamin C, was synthesized, and the anti-inflammatory and barrier protecting effects of this compound were investigated for chronic oxazolone-induced murine atopic dermatitis.

The efficient preparation of organogermanium **4** conjugated with ascorbic acid proved to be straightforward. First, compound **1** was treated with *N*-hydroxysuccinimide (NHS) in the presence of dicyclohexylcarbodiimide (DCC) to produce the corresponding active ester **2**. The vitamin C derivative **3**, which was prepared from ascorbic acid according to the literature procedure,[14] was treated with a solution of triphenyl phosphine in acetonitrile, and then reacted with germylhydroxysuccinimide derivative **2** without purification to give the condensation product in 88% yield. After deprotection by hydrogenation in the presence of $Pd(OH)_2$, the resulting crude product was recrystallized in a hexane/ethyl acetate solution to produce the final product (Ge-Vit, **4**)[15] in good yield (Scheme 1).

Although the relationship of the chronic oxazolone (Ox)-induced murine AD model to human AD is not entirely identical, it has been reported that a contact hypersensitivity model by the repeated application of oxazolone mimics the histological phenotype of AD in human,[16] and murine oxazolone model in relation to inflammation and barrier damages has been used to assess the anti-AD effect of compounds.[17,18] Therefore, in order to determine the anti-inflammatory effects of the Ge-Vit compound, a group of five hairless mice (aged 7 weeks old, 21.19 ± 1.74 g) were sensitized using a topical treatment with 50 μl of 10% oxazolone (Ox) in ethanol and challenged once every other day with 100 μl of a 0.3% ethanolic Ox solution on both flanks for an additional 5 days.

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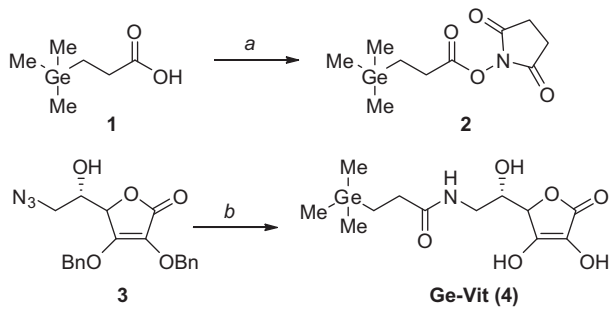

Scheme 1. Synthesis of the water-soluble organogermanium compound. Reagents and conditions: (a) DCC, NHS, CH_2Cl_2, rt, 16 h, 86%; (b) i—Ph_3P, MeCN, 0 °C, 1 h, to 50 °C, 2 h; ii—**2**, MeCN, rt, 3 h, 88%; iii—$Pd(OH)_2$, H_2, MeOH, rt, 4 h, 86%.

Then the Ox-treated group was topically treated twice a day with Ge-Vit in an ethanol/PEG 400 vehicle (7:3) for 10 days. During the Ge-Vit treatment, the mice were treated once a day with 100 μl of 0.3% Ox. Betamethasone (0.01%) and ethanol/PEG 400 alone were used as the positive control and the vehicle control, respectively, over the same treatment period. The basal TEWL (Transepidermal Water Loss) value and the skin thickness of the back of the mice were measured at the end of the treatment period in order to evaluate the effects of the Ge-Vit compound on the skin barrier function.[16] While the mean body weights of all of the groups similarly increased throughout the study (data not shown), the TEWL values of the back of the mice that were treated with Ge-Vit and betamethasone decreased (6% at 0.3% Ge-Vit, 39% at 1.8% Ge-Vit, and 57% at 0.01% betamethasone) compared to the vehicle-treated group, indicating that the Ge-Vit treatment affected basal the TEWL. Additionally, Ge-Vit also attenuated the disruption of the skin barrier function, as determined by the skin thickness (Fig. 1b) compared to the vehicle group. The histological examination further supported the anti-AD activities of this compound, and both the dermal inflammation and parakeratosis were substantially alleviated (Fig. 1d), along with the attenuation of the inflammatory cell infiltration.

Allergen-specific $CD4^+$ T cells can be isolated from the skin lesions of patients with atopic dermatitis.[19] On the basis of their cytokine production profiles, the $CD4^+$ helper T lymphocytes were subdivided into type 1 T-helper (Th1) and type 2 T-helper (Th2) cells. The Th1 cells, which produce IL-2 and interferon-γ (IFN-γ), play a critical role in the cellular immunity, whereas the Th2 cells, which produce interleukin (IL)-4, IL-5, and IL-13, are essential for the regulation of the humoral immunity. The balance between the Th1- and Th2-dominant immunity (Th1/Th2 balance) was thought to be important for the development and maintenance of various diseases. For example, the predominance of Th2 and increased serum IgE levels were reported in patients with atopic

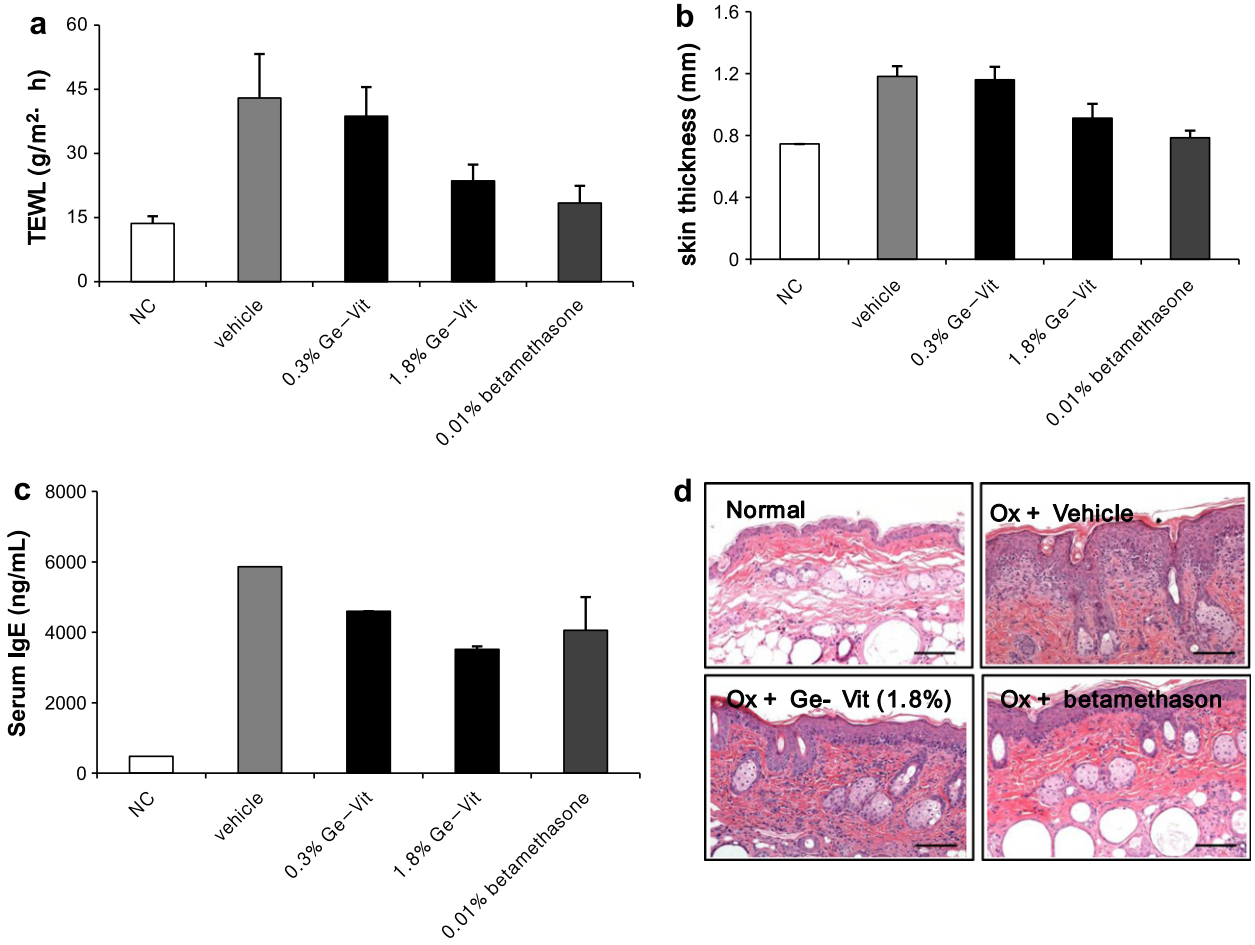

Figure 1. Effects of Ge-Vit on the recovery from the Ox-induced murine AD model. (a) The basal TEWL was measured using a vapor meter, SWL4102 (Delfin Technologies Ltd, Finland). The values represent means ± SE ($N = 7–8$). The normal group was treated with ethanol instead of Ox. (b) The skin thickness was measured as the skin-fold thickness (2×) using a micrometer (Mitutoyo®, Japan). (c) The serum IgE levels were measured using mouse IgE ELISA kits (BD Biosciences, San Jose, CA). The values represent the mean ± SE ($N = 7–8$). (d) Histological examination of the treated skin (H&E staining). The infiltration of the lymphocytes into the dermis and the development of the parakeratotic scales were remarkably alleviated in the 1.8% Ge-Vit treated group.

dermatitis.[20] Thus, while the Th2 cell cytokines (IL-4 and IL-13) were implicated in the isotype switching of the B-cells to produce IgE, the Th1 cells promoted the cell-mediated immunity in order to inhibit the IgE production. In this study, compound treated mice exhibited a lower IgE production level than the control group (Fig. 1c), suggesting that the Ge-Vit treatment might have attenuated AD by modulating the Th1/Th2 balance into a Th1 dominance.

In this study, a water-soluble organogermanium compound that was conjugated with vitamin C was synthesized without any possible contamination of toxic inorganic germanium, and the topical treatment of this compound provided anti-(AD) effects for chronic Ox-induced AD in mice, as shown by the recovery of the skin barrier disruption, the decrease in the serum IgE, and histopathological improvement. Meanwhile, in a previous study, 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.[21] However, in this study, the topical treatment with vitamin C did not have significant effects on the recovery of the skin barrier function after the barrier disruption in mice, indicating that the anti-AD effects were caused by the germanium core. Detailed studies are currently underway in order to determine the molecular mechanisms of the immune-modulating effects that were caused by the use of the novel organic germanium.

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