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Original Article

Inhibitory effects of dietary kale on 2,4,6-trinitrochlorobenzene-induced dermatitis in mice

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ABSTRACT

Kale (*Brassica oleracea L.var.acephala DC*) has been shown to alleviate the effects of pollinosis. However, very little is known about its effect on other allergic diseases. In this study, we examined the effects of dietary kale on single or chronic contact hypersensitivity (CHS) elicited by 2,4,6-trinitrochlorobenzene (TNCB) in mice. The application of kale reduced ear swelling and cell invasion in single CHS and chronic CHS. This ability was enhanced by a longer period of intake or higher dose of kale. In addition, kale reduced mast cell invasion in chronic CHS, but the total IgE levels in blood plasma were not changed by kale treatment. Therefore, the inhibitory effects of kale on ear swelling and cell invasion were related to mechanisms other than the production of IgE. Taken together, dietary intake of kale probably improved or relieved allergic dermatitis.

KEYWORDS: kale, ear swelling, chronic contact hypersensitivity, 2,4,6-trinitrochlorobenzene, cell invasion.

INTRODUCTION

Allergic contact dermatitis (ACD) caused by hard-to-avoid common allergens called haptens, such as nickel and rubber products, occurs at the site of contact with the hapten in sensitized individuals. It is characterized by itching, edema, erythema, dry skin, etc. Its socioeconomic impact as an acquired, job-related disease is enormous [1]. ACD affects approximately 7% of the population and the incidence of ACD is steadily rising [2, 3].

The development of ACD is composed of two phases: the sensitization and elicitation phase. In the sensitization phase, the hapten binds to proteins in the skin, and the hapten-protein complexes are then processed by epidermal Langerhans cells (LCs) and dermal dendritic cells as haptenated peptides in the groove of major histocompatibility complex (MHC) classes I and II molecules at the cell surface [4-6]. The sensitization step lasts for 10-15 days in humans and 5-7 days in mice. This first step has no clinical consequence in most cases [7]. In the elicitation phase, after exposure to the same hapten in sensitized mice, the haptenated peptide is presented by antigen-presenting cells, which express MHC classes I and/or II/haptenated, such as epidermal LCs, and hapten-specific T cells are selectively recruited to the extravascular space at the challenged sites [8]. This phase of ACD lasts for 72 h in humans and 24-48 h in mice. The inflammatory reaction persists for only a few days and rapidly decreases following down-regulatory mechanisms [9]. In a general way, the contact hypersensitivity (CHS) murine model induced by synthesized potent hapten is used to study ACD in humans.

The inflammatory responses of repeated application of hapten are different from those elicited by a single application of hapten. Exposure of hapten to the ear of BALB/c mice leads to a shift in the time course of CHS from a delayed-type hypersensitive response of the acute phase to an immediate-type

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response followed by a late-type reaction of the chronic phase [10]. The chronic phase in a site-restricted pathology develops elevated serum levels of antigen-specific IgE [11, 12], mast cell accumulation and continuous degranulation in the lesion [13, 14], a persistent increase in scratching behavior and resulting shifts in the local cytokine pattern from a T-helper (Th) 1-type (interferon-γ) and interleukin-2 (IL-2) to a Th2-type (IL-4 and IL-10) profile [15, 16].

The chronic ACD model in mice appears to mimic many events of the lesional skin in patients, and is considered to be a better model for proper assessment of compounds against various allergic cutaneous diseases, particularly used as a model for atopic dermatitis [17-19]. In addition, a previous study reported that an allergen applied through the skin induced systemic allergic responses, and mimicked courses of other diseases including asthma, allergic rhinitis, and allergic conjunctivitis [20].

Present topical therapies for ACD and chronic ACD include glucocorticoids, non-steroid antiinflammatory drugs, and immunomodulators [21-22]. However, these agents often cause severe side effects [23-24]. Therefore, there is a great need for the development of new and effective therapies for these diseases.

Kale (*Brassica oleracea L.var.acephala DC*), known as the basic ingredient of green juice, has been reported to mitigate the effects of pollinosis in a clinical study, and inhibit effects of mast cell degranulation *in vitro* [25]. Thus, kale is expected to decrease symptoms of allergic diseases. In addition, oral intake is a simple way to respond to exposure to an antigen at any site of the body. Therefore, in this study, we investigated whether consuming kale can alleviate or prevent dermatitis. The study was done to clarify the following 2 points: 1) whether feeding diets containing 10% kale for 1, 2, and 3 weeks can prevent the effects of a single CHS reaction, and 2) whether feeding 5 or 10% kale powder for two weeks can ameliorate the effects of chronic CHS.

MATERIALS AND METHODS

Reagents and chemicals

2,4,6-trinitrochlorobenzene (TNCB) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Acetone and phosphoric acid were purchased from Sigma Aldrich Japan Co. (Tokyo, Japan). Isoflurane was purchased from Mylan Seiyaku Ltd. (Tokyo, Japan). Olive oil, 0.05% toluidine blue solution (pH 7.0), Mayar's hematoxylin solution and 0.5% Eosin Y ethanol solution were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Paraformaldehyde powder was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Mouse IgE ELISA kit was purchased from Morinaga Institute of Biological Science, Inc. (Yokohama, Japan). The powder of kale (K) was a gift from Fancl Co. Ltd. (Kanagawa, Japan).

Animals

Male mice (6 weeks old, BALB/c Cr Slc strain, purchased from Japan SLC, Inc., Shizuoka Japan) were kept in a room at 25 ± 1 °C on a 12 h dark/ light cycle (8:00-20:00 light), and had access to feed and drinking water *ad libitum*. The experimental procedures followed the Guidelines for Animal Experiments of the Faculty of Agriculture and of the Graduate Course of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Japanese Government.

Experimental diet for single CHS elicitation

After a 5 day acclimation (day 0) period, the animals were divided into 5 groups of 8 mice each according to their body weights, i.e., control (CNT), negative control (NC), K1, K2, and K3 groups. The CNT and the NC groups were given a commercial diet (MF, Oriental Yeast Co. Ltd., Japan). The K1 group was given the commercial diet containing 10% kale powder during the period of day 14 to day 21. The K2 group was given the commercial diet containing 10% kale powder during the period of day 7 to day 21. The K3 group was given the commercial diet containing 10% kale powder during the period of day 0 to day 21.

Experimental diet for chronic CHS elicitation

After the 5 day acclimation (day 0), the animals were divided into 4 groups of 8 mice each according to their body weights, i.e., CNT, NC, K5%, and K10% groups. The CNT and the NC groups were given a commercial diet (MF, Oriental Yeast Co. Ltd., Japan). The K5% and K10% groups were given the commercial diet containing 5% and 10% kale powder, respectively.

Induction of single CHS

Induction of CHS was performed according to previous studies [26, 27]. After being anesthetized with isoflurane, mice were sensitized by application of 100 µl of 7% TNCB (w/w) dissolved in acetone/olive oil (4:1) on the shaved abdominal skin on day 15. For elicitation, after being anesthetized with isoflurane, 20 µl of 1% TNCB was applied to the outer surfaces of both ears on day 20. In the CNT group, mice were treated with vehicle at these time periods.

Induction of chronic CHS

Induction of chronic dermatitis was performed according to previous studies [10, 26, 27]. After being anesthetized with isoflurane, mice were sensitized by application of 100 µl of 7% TNCB (w/w) dissolved in acetone/olive oil (4:1) on the shaved abdominal skin on day 8. For elicitation, after being anesthetized with isoflurane, 20 µl of 1% TNCB was applied to the outer surfaces of both ears on days 13, 16, 19, 22, 25, 27, 31, 34, 37, and 40. In the CNT group, mice were treated with the vehicle.

Measurement of ear thickness and swelling

The thickness of the right ear of each mouse was measured using a dial thickness gauge (G-7C, Ozaki MFG. Co. Ltd., Tokyo, Japan). In single CHS-elicited mice, the ear thickness was measured 24 h after the elicitation. In the chronic CHS-elicited mice, the ear thickness was measured 72 h after each elicitation and 24 h after the last elicitation. The ear swelling was calculated by taking the difference between measurements of thickness before the elicitation and after the elicitation.

Histological analysis

After sacrifice by cervical dislocation, the left ear of the mice was excised and fixed in 4% (w/v) phosphate-buffered paraformaldehyde (pH 7.4) embedded in paraffin, and sectioned at 5 µm. Tissue sections were stained with hematoxylin and eosin for microscopic examination, and the number of cells per square millimeter in the dermis at 4 randomly chosen sites was counted as previously described [28]. In chronic CHS-elicited mice, sections were also stained with toluidine blue, and the density of mast cells was measured by the same method as in the case of hematoxylin and eosin stains.

Determination of total IgE level in blood plasma

In chronic CHS-elicited mice, after sacrifice by cervical dislocation, blood was collected using heparin. Blood plasma samples were obtained by centrifugation and they were stored at -30 °C until analysis. Total IgE levels in blood plasma were measured using an enzyme-linked immune sorbent assay kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan).

Statistical methods

Data were statistically analyzed using a student's t-test between the CNT and NC groups. Significant difference was set at $P < 0.05$. The values among the NC group and the kale-treated groups were analyzed by a one-way analysis of variance. When significant ($P < 0.05$) effects were detected, the Dunnett's test was used to evaluate the differences. These analyses were performed with StatView (version 5, SAS Institute Cary, United States, SAS 1998).

RESULTS

Table 1 shows the body weight and the food intake of single CHS-elicited mice. There were no significant differences between the CNT and the NC groups. Dietary kale has no significant effect on body weight. Fig. 1 shows the ear swelling 24 h after the single CHS elicitation in mice. The NC group showed a significant ($P < 0.0001$) increase in ear swelling compared with the CNT group. There were significant [$F(3, 25) = 12.179$] decreases in the ear swelling of the K2 ($P < 0.01$) and K3 ($P < 0.05$) groups compared with the NC group.

Fig. 2 shows the sections of ear, stained with hematoxylin and eosin, of single CHS-elicited mice. Apparent detachment of corneum, swelling of the dermis, and cell invasion were increased in the TNCB-treated groups compared with the CNT group.

Fig. 3 shows the density of cells in the dermis of the ear surface of single CHS-elicited mice. There were significant ($P < 0.0001$) increases in the density of cells in the dermis of the NC group compared with the CNT group. There were significant [$F(3, 16) = 6.784$] decreases within the K1 ($P < 0.05$), K2 ($P < 0.01$) and K3 ($P < 0.01$) groups compared with the NC group.

Table 2 shows the body weight and food intake of chronic CHS-elicited mice. There were no significant

Table 1. Effect of kale on the body weight and food intake in single CHS-elicited mice.

	CNT	NC	K1	K2	K3
Body weight (g)	23.3 ± 0.49	22.5 ± 0.23	23.0 ± 0.19	22.7 ± 0.25	23.1 ± 0.20
Food intake (g/day)	2.66 ± 0.11	2.69 ± 0.08	2.73 ± 0.07	2.73 ± 0.07	2.88 ± 0.09

The control (CNT) and negative control (NC) groups were given a commercial diet. The K1 group was given the commercial diet containing 10% kale powder for 1 week. The K2 group was given the commercial diet containing 10% kale powder for 2 weeks. The K3 group was given the commercial diet containing 10% kale powder for 3 weeks. 20 μl of vehicle for the CNT group and 1% 2,4,6-trinitrochlorobenzene (TNCB) for other groups were applied to the outer surfaces of both ears for elicitation of contact hypersensitivity (CHS). Values are means ± S.E.M. (n = 7-8).

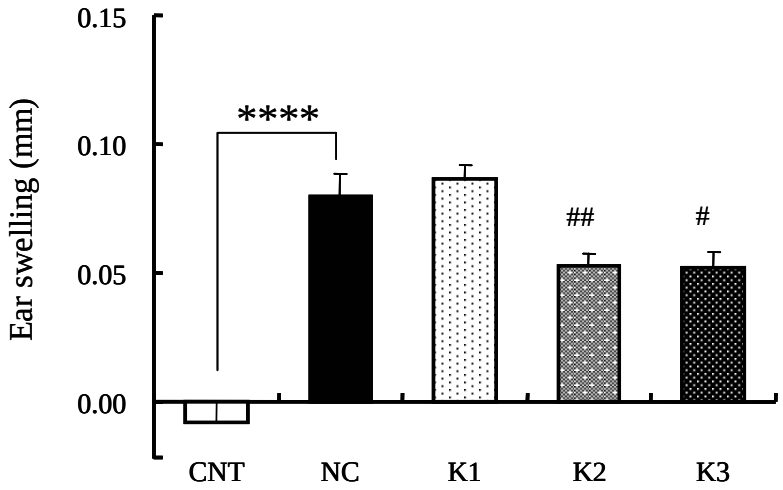

Fig. 1. Effects of kale on the contact hypersensitivity (CHS)-induced ear swelling in mice. The control (CNT) and negative control (NC) groups were given a commercial diet. The K1 group was given a commercial diet containing 10% kale powder for 1 week. The K2 group was given a commercial diet containing 10% kale powder for 2 weeks. The K3 group was given a commercial diet containing 10% kale powder for 3 weeks. The thickness of right ear was measured 24 h after the application of 20 μl of vehicle (CNT group) or 1% 2,4,6-trinitrochlorobenzene (TNCB) to the outer surface of both ears of mice. Values are mean ±S.E.M. (n = 7-8). ****$P < 0.0001$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ - significantly different from the NC group.

differences between the CNT and the NC group. No significant effects of dietary kale were detected in body weight. Table 3 shows the relative tissue weight of chronic CHS-elicited mice. In the liver, a significant ($P < 0.001$) increase was observed in the NC group compared to the CNT group. However, no significant differences were found between the kale-treated groups and the NC group. In the spleen, a significant ($P < 0.05$) increase was also observed in the NC group compared to the CNT group. However, no significant differences were found between the kale-treated groups and the NC group. The epididymal fat was not significantly different between the CNT and NC groups. There was no significant difference between the kale-treated groups and the NC group in epididymal fat weight.

Fig. 4 shows the ear swelling 24 h after the last elicitation of chronic CHS in mice. The NC group showed a significant ($P < 0.0001$) increase in ear swelling compared with the CNT group. There were significant [$F(2, 18) = 6.298$] decreases in

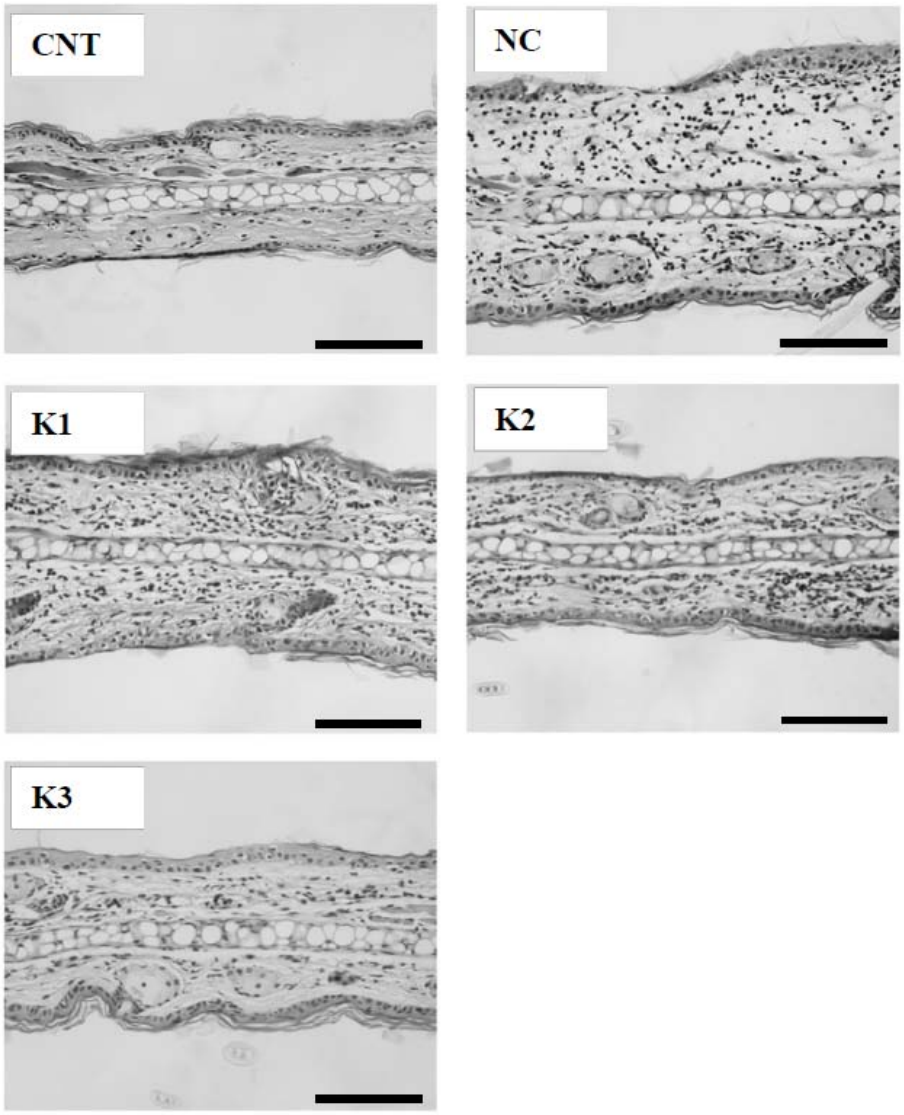

Fig. 2. Effects of kale on the ear tissue of contact hypersensitivity (CHS)-elicited mice. The control (CNT) and negative control (NC) groups were given a commercial diet. The K1 group was given a commercial diet containing 10% kale powder for 1 week. The K2 group was given a commercial diet containing 10% kale powder for 2 weeks. The K3 group was given a commercial diet containing 10% kale powder for 3 weeks. 24 h after the application of 20 μl of vehicle (CNT group) or 1% 2,4,6-trinitrochlorobenzene (TNCB) to the outer surfaces of both ears of mice, the left ear was excised and stained with hematoxylin and eosin for microscopic examination. Scale bars mean 100 μm.

the ear swelling of the K5% group ($P < 0.05$) and the K10% group ($P < 0.01$) compared with the NC group.

Fig. 5 shows the time course of ear swelling 72 h after each elicitation of chronic CHS in mice. The NC group showed a significant ($P < 0.0001$) increase in ear swelling compared with the CNT group at all times after the application. There were significant decreases in the ear swelling of the K10% group compared with the NC group after the 5^{th} ($P < 0.05$), 6^{th} ($P < 0.05$), and 7^{th} application ($P < 0.01$). In addition, a tendency ($P < 0.1$) towards a decrease in the ear swelling was detected after the 6^{th} application in the K5% group, 7^{th} application in the K5% group, 8^{th} application in the K10% group, and

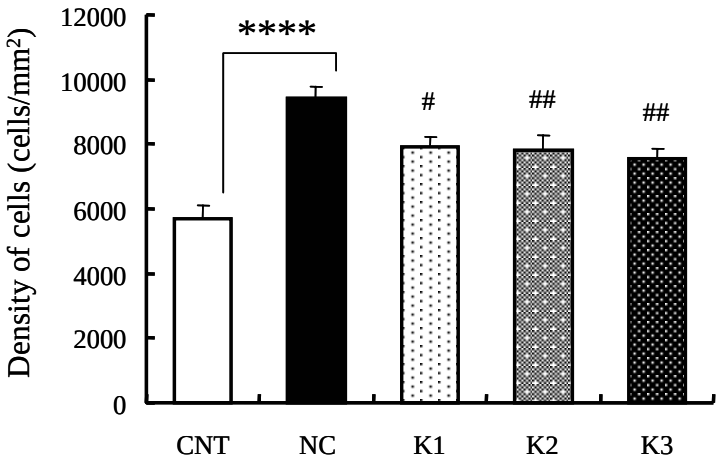

Fig. 3. Effects of kale on the density of cells in dermis of contact hypersensitivity (CHS)-elicited ear surface in mice. The control (CNT) and negative control (NC) groups were given a commercial diet. The K1 group was given a commercial diet containing 10% kale powder for 1 week. The K2 group was given a commercial diet containing 10% kale powder for 2 weeks. The K3 group was given a commercial diet containing 10% kale powder for 3 weeks. 24 h after the application of 20 μl of vehicle (CNT group) or 1% 2,4,6-trinitrochlorobenzene (TNCB) to the outer surfaces of both ears of mice, the left ear was excised and stained with hematoxylin and eosin, and the density of cells in dermis of outer surface was measured. Values are mean ± S.E.M. (n = 5). ****$P < 0.0001$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ - significantly different from the NC group.

Table 2. Effect of kale on the body weight and food intake in chronic CHS-elicited mice.

	CNT	**NC**	**K5%**	**K10%**
Body weight (g)	22.8 ± 0.3	22.4 ± 0.44	22.4 ± 0.35	22.6 ± 0.24
Food intake(g/day)	2.92 ± 0.07	3.05 ± 0.05	2.92 ± 0.02	3.05 ± 0.05

The control (CNT) and the negative control (NC) groups were given a commercial diet. The K5% group was given the commercial diet containing 5% kale powder. The K10% group was given the commercial diet containing 10% kale powder. 20 μl of vehicle for the CNT group and 1% 2,4,6-trinitrochlorobenzene (TNCB) for other groups were applied to the outer surfaces of both ears repeatedly for elicitation of chronic dermatitis. Values are means ± S.E.M. (n = 6-8).

Table 3. Relative tissue weight (% of body weight) in chronic CHS-elicited mice.

	CNT	**NC**	**K5%**	**K10%**
Liver	5.83 ± 0. 08**	5.89 ± 0. 10	5.89 ± 0. 10	5.89 ± 0. 04
Spleen	0. 347 ± 0. 013*	0. 350 ± 0. 006	0. 354 ± 0. 012	0. 361 ± 0. 007
Epididymal fat	1.36 ± 0. 07	1.34 ± 0. 09	1.34 ± 0. 11	1.25 ± 0. 08

The control (CNT) and negative control (NC) groups were given a commercial diet. The K5% group was given a commercial diet containing 5% kale powder. The K10% group was given a commercial diet containing 10% kale powder. 24 h after the last application of 20 μl of vehicle (the CNT group) or 2,4,6-trinitrochlorobenzene (TNCB) to the outer surfaces of both ears, mice were sacrificed and then tissues were excised and weighed. Values are mean ± S.E.M. (n = 6~8). *P<0.05, **P<0.01 - significantly

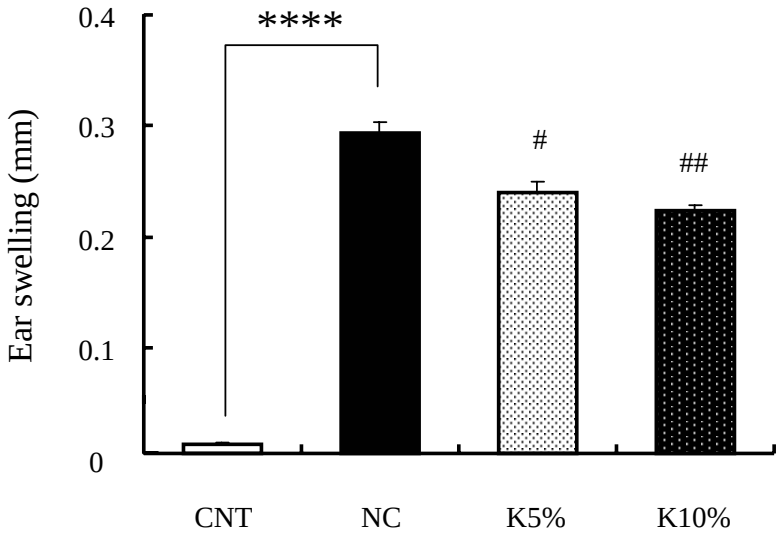

Fig. 4. Effects of kale on ear swelling after repeated elicitation of contact hypersensitivity (CHS) in mice. The control (CNT) and negative control (NC) groups were given a commercial diet. The K5% group was given a commercial diet containing 5% kale powder. The K10% group was given a commercial diet containing 10% kale powder. The thickness of right ear was measured 24 h after the last application of 20 µl of vehicle (CNT group) or 1% 2,4,6-trinitrochlorobenzene (TNCB) to the outer surfaces of both ears of mice. Values are mean ± S.E.M. (n = 6-8). ****$P < 0.0001$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ - significantly different from the NC group.

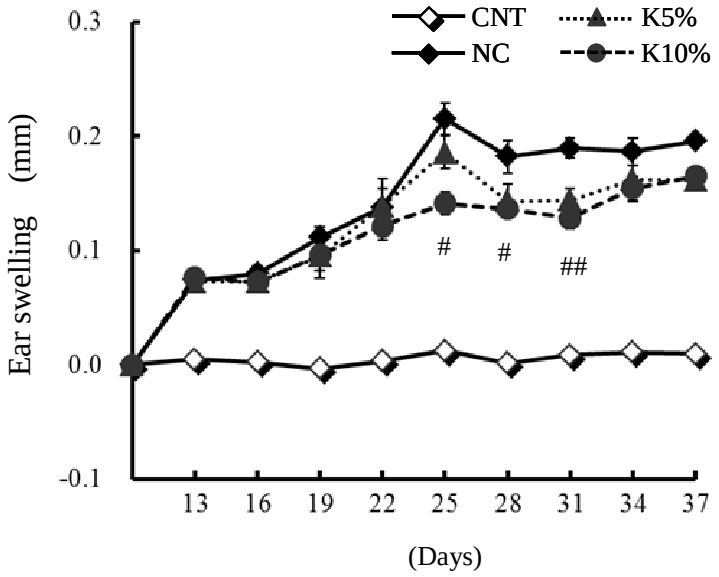

Fig. 5. Effects of kale on ear swelling after repeated elicitation of contact hypersensitivity (CHS) in mice. The control (CNT) and negative control (NC) groups were given a commercial diet. The K5% group was given a commercial diet containing 5% kale powder. The K10% group was given a commercial diet containing 10% kale powder. The thickness of the right ear was measured 72 h after each applications of 20 µl of vehicle (CNT group) or 1% 2,4,6-trinitrochlorobenzene (TNCB) to the outer surfaces of both ears of mice. Values are mean ±S.E.M. (n = 6-8). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ - significantly different from the NC group.

9th application in the K10% group, compared with the NC group.

Fig. 6 shows the sections of ear, stained with hematoxylin and eosin, of chronic CHS-elicited mice. An increase in apparent detachment of the corneum or hyperkeratosis, epidermal hyperplasia, swelling of dermis, cell invasion, and vasodilation, were observed in the TNCB-treated groups compared with the CNT group.

Fig. 7 shows the density of cells in the dermis of the ear surface of chronic CHS-elicited mice. There were significant ($P < 0.0001$) increases in the density of cells in the dermis of the NC group compared with the CNT group. There were significant [$F(3, 16) = 25.888$] decreases in the density of cells among the K5% ($P < 0.01$) and the K10% ($P < 0.01$) group compared with the NC group.

Fig. 8 shows the sections of ear, stained with toluidine blue, of chronic CHS-elicited mice. Mast cell invasion was increased in the TNCB-treated groups compared with the CNT group.

Fig. 9 shows the density of mast cells in the dermis of the ear surface of chronic CHS-elicited mice. There were significant ($P < 0.0001$) increases in the density of cells in the dermis of the NC group compared with the CNT group. There was a significant [$F(3, 16) = 22.592$] decrease in the density of mast cells in the K5% ($P < 0.01$) and K10% ($P < 0.01$) groups compared with the NC group.

Fig. 10 shows the total IgE level in blood plasma 24 h after the last elicitation of chronic CHS in mice. There were significant ($P < 0.0001$) increases in total IgE levels in blood plasma of the NC group compared with the CNT group. There were no

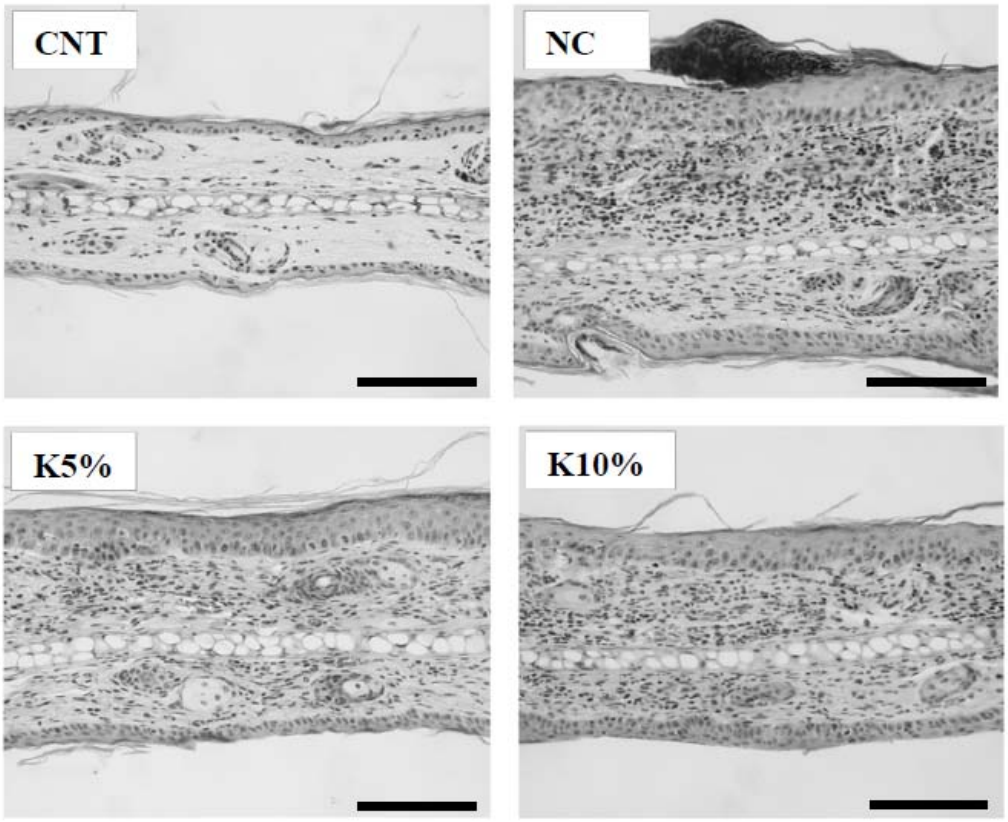

Fig. 6. Effects of kale on the ear tissue after repeated elicitation of contact hypersensitivity (CHS) in mice. The control (CNT) and negative control (NC) groups were given a commercial diet. The K5% group was given a commercial diet containing 5% kale powder. The K10% group was given a commercial diet containing 10% kale powder. 24 h after the last application of 20 μl of vehicle (CNT group) or 2,4,6-trinitrochlorobenzene (TNCB) to the outer surfaces of both ears of mice, left ear was excised and stained with hematoxylin and eosin for microscopic examination. Scale bars in each photograph indicate 100 μm.

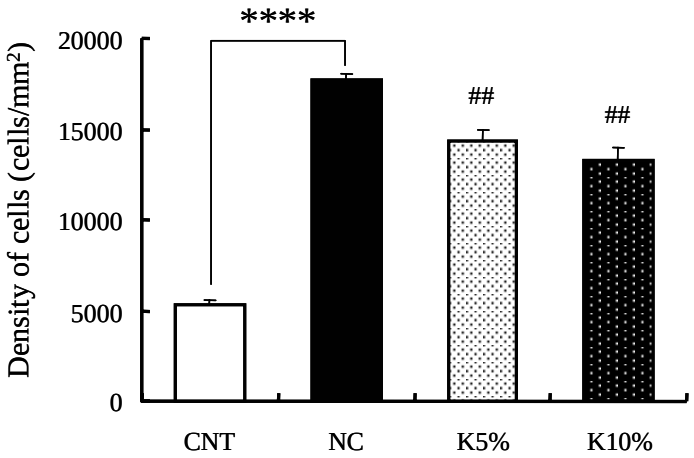

Fig. 7. Effects of kale on the density of cells in dermis of the ear surface after repeated elicitation of contact hypersensitivity (CHS) is mice. The control (CNT) and negative control (NC) groups were given a commercial diet. The K5% group was given a commercial diet containing 5% kale powder. The K10% group was given a commercial diet containing 10% kale powder. 24 h after the last application of 20 µl of vehicle (CNT group) or 2,4,6-trinitrochlorobenzene (TNCB) to the outer surfaces of both ears of mice, the left ear was excised and stained with hematoxylin and eosin, and the density of cells in dermis of outer surface were measured. Values are mean ± S.E.M. (n = 5). ****$P < 0.0001$, $^{\#\#}P < 0.01$ - significantly different from the NC group.

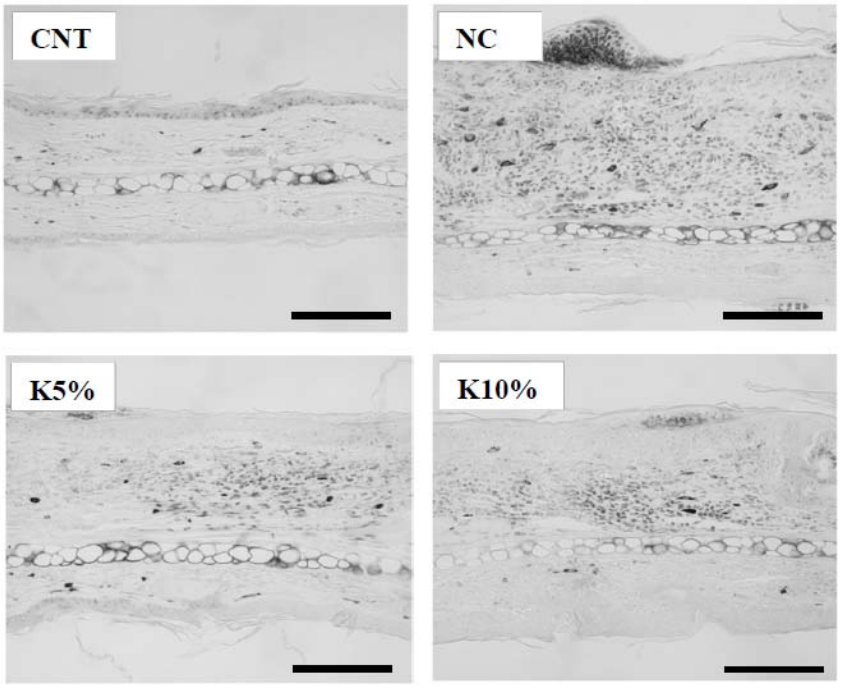

Fig. 8. Effects of kale on the mast cell of ear after repeated elicitation of contact hypersensitivity (CHS) in mice. The control (CNT) and negative control (NC) groups were given a commercial diet. The K5% group was given a commercial diet containing 5% kale powder. The K10% group was given a commercial diet containing 10% kale powder. 24 h after the last application of 20 µl of vehicle (CNT group) or 2,4,6-trinitrochlorobenzene (TNCB) to the outer surfaces of both ears of mice, the left ear was excised and stained with toluidine blue for microscopic examination. Scale bars in each photograph indicate 100 µm.

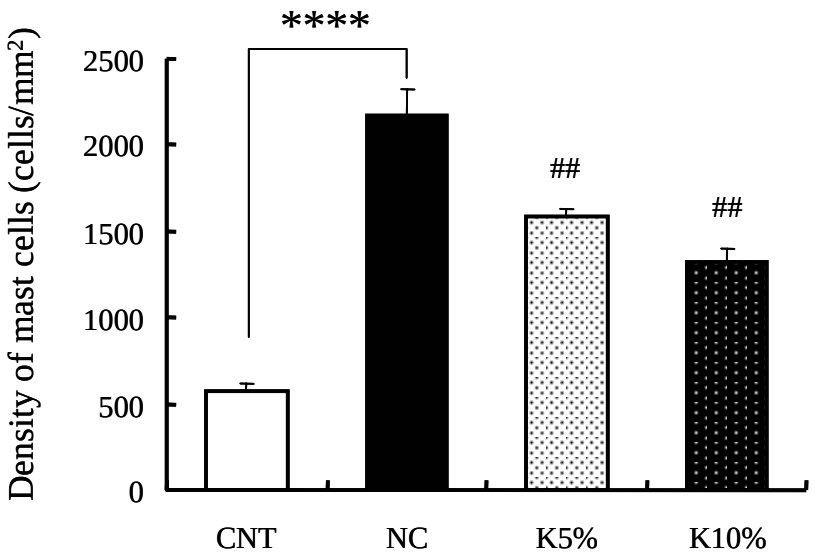

Fig. 9. Effects of kale on the density of mast cells in dermis of the ear surface after repeated elicitation of contact hypersensitivity (CHS) in mice. The control (CNT) and negative control (NC) groups were given a commercial diet. The K5% group was given a commercial diet containing 5% kale powder. The K10% group was given a commercial diet containing 10% kale powder. 24 h after the last application of 20 μl of vehicle (CNT group) or 2,4,6-trinitrochlorobenzene (TNCB) to the outer surfaces of both ears of mice, the left ear was excised and stained with toluidine blue, and the density of mast cells in dermis of outer surface was measured. Values are mean ± S.E.M. (n = 5). ****$P < 0.0001$, $^{\#\#}P < 0.01$ - significantly different from the NC group.

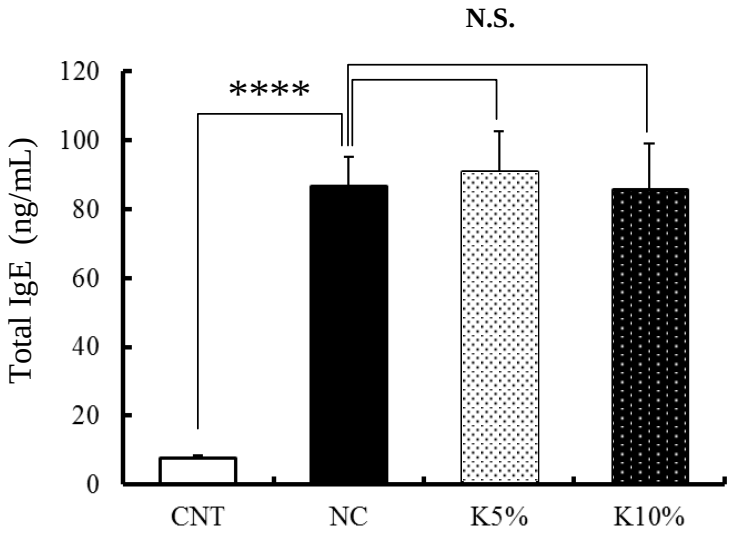

Fig. 10. Effects of kale on the total IgE level of blood plasma after repeated elicitation of contact hypersensitivity (CHS) in mice. The control (CNT) and negative control (NC) groups were given a commercial diet. The K5% group was given a commercial diet containing 5% kale powder. The K10% group was given a commercial diet containing 10% kale powder. 24 h after the last application of 20 μl of vehicle (CNT group) or 2,4,6-trinitrochlorobenzene (TNCB) to the outer surfaces of both ears, mice were sacrificed and then blood was collected, and blood plasma samples were measured using the mouse IgE ELISA kit. Values are mean ± S.E.M. (n = 5-8). ****$P < 0.0001$ - significantly different from the NC group.

significant differences between the kale-treated groups and the NC group.

DISCUSSION

In this study, we showed that the dietary intake of kale suppressed the ear swelling in single CHS and chronic CHS induced by application of TNCB in mice. In both experiments, food intake and body weight (Tables 1 and 2) were not influenced by dietary intake of kale. Therefore, administration of kale did not effect the growth of mice, at least during the experimental period applied here. On the other hand, in the relative tissue weights of chronic CHS-elicited mice, the liver and spleen were significantly increased in the NC group compared to the CNT group, but no significant differences in the weight were found between the kale treated groups and the NC group. Therefore, this increase was probably due to the stress by chronic dermatitis, but dietary intake of kale may not attenuate this effect. The epididymal fat was not different between groups; therefore, the dietary intake of kale did not result in a large-scale change in obesity index.

After the single CHS elicitation (Fig. 1), there were significant decreases in ear swelling in the K2 and K3 groups compared with the NC group. The longer period of kale intake might result in the more potent inhibition of ear swelling in response to CHS elicitation. Kale might activate mast cells to produce tumor necrosis factor-α (TNF-α) and serotonin, or more specifically release inflammatory mediators (IL-18, IL-1β, TNF-α, ATP, prostaglandin E_2 (PGE_2), histamine, and chemokine (C-C motif) ligand 2 (CCL2)) *via* resident-skin cells that induce the influx of a second wave of leucocytes comprising neutrophils, after CHS elicitation [9].

After the last elicitation of CHS in mice (Fig. 4), the NC group showed a significant increase in ear swelling compared with the CNT group. There were significant decreases in the ear swelling of both kale-treated groups compared with the NC group, and the K10% group was more effective than the K5% group. It is suggested that the larger dose of kale intake might result in the more potent inhibition of tissue swelling. In addition, 72 h after each elicitation (Fig. 5), there was a significant decrease in ear swelling in the kale-treated groups compared with the NC group after the 5^{th} application. These results were enhanced in the K10% group compared with K5% group. In chronic CHS, as a result of repeated TNCB application there is the highest production of cytokines such as IL-4, IL-1β and IL-6, and these cytokines cause the accumulation of large numbers of $CD4^+$ T cells and mast cells that are closely related to the initiation of skin lesions [10, 29]. Accordingly, dietary intake of kale might influence these effectors.

Cell invasion was also decreased significantly by the kale treatment. In single CHS-elicited mice (Fig. 3), the higher doses of kale were more effective than the lower dose. In the CHS response, the influx of leucocytes comprising neutrophils, NK cells and Treg cells occurs at 24-48 h after CHS elicitation [9]. Accordingly, dietary intake of kale for 1 week before the CHS may decrease cell invasion and thus decrease vasodilatation. However, the inhibitory effect of kale on cell invasion is intensified by longer periods of kale intake.

In chronic CHS-elicited mice, the density of total cells (Fig. 7) and mast cells (Fig. 9) in the ear 24 h after the last elicitation were significantly decreased in the K5% and the K10% group compared with the NC group. In chronic CHS, infiltration of neutrophils, eosinophils and monocytes, and epidermal hypertrophy occur in affected tissue [12]. However, the total IgE level in blood plasma (Fig. 10) was not altered by the kale treatment. Therefore, inhibitory effect of kale on cell invasion was related to mechanisms other than production of IgE.

CONCLUSION

Kale suppressed ear swelling and cell invasion in both single and chronic CHS. The regulatory effect of kale in ear swelling might be enhanced by long-term feeding and increases in dose.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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