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Original article

Oral administration of freeze-dried powders of honey bee larvae inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice

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ABSTRACT

Purpose: This study was designed to evaluate the suppressive effect of the oral administration of the freeze-dried honey bee larvae powder on atopic dermatitis (AD)-like skin lesions in NC/Nga mice.
Methods: The effects of freeze-dried honey bee larvae powder on AD-like skin lesions in mice were studied by evaluating the condition of the skin macroscopically and histopathologically, ear swelling degree, serum levels of total IgE, interleukin-4 (IL-4) and interferon-γ (IFN-γ), and level of IL-18 and IL-12 in skin lesions.
Results: NC/Nga mice fed the freeze-dried honey bee larvae powder-supplemented diet showed a decrease in dermatitis scores of the dorsal skin, ear thickness, skin hypertrophy, inflammatory cell infiltration in the skin, serum total IgE, IL-4, and IFN-γ levels, and IL-18 and IL-12 levels in skin lesions. These results suggest that the freeze-dried powder of honey bee larvae inhibits the development of AD-like skin lesions in NC/Nga mice by suppressing both T-helper (Th) 1 and Th 2 cell responses.
Conclusions: Our results indicate that oral administration of the freeze-dried honey bee larvae powder could provide an adjunctive therapy for the management of AD.

1. Introduction

Atopic dermatitis (AD) is a relatively common chronic, relapsing, and inflammatory dermatitis, caused by a complex interrelationship among genetic, psychological, immunological, and skin barrier dysfunction factors [7,8]. The main symptoms of AD are dry skin, pruritus, erythematous papules and eczema [7,8]. Previous studies revealed that serum IgE levels were associated with the severity of AD [5,7], and patients with AD demonstrated high levels of serum IgE [5,10]. The development of AD is associated with the deregulation of both T-helper type 1 (Th1) and T-helper type 2 (Th2) cells from the immune system [2], and the production of interleukin (IL)-4, IL-5, and IL-13 by the Th2 cells increased in the skin of patients with AD [10]. In patients with AD, IL-4, IL-5, and IL-13 are responsible for elevated serum IgE levels, blood eosinophilia, and eosinophil infiltration in the skin [2,8,10]. The Th1-type cytokine interferon-γ (IFN-γ) also plays an important role in the pathogenesis of AD [2]. In a clinical study, it was observed that IFN-γ mRNA expression increased in AD skin lesions compared with that in normal skin [3]. The IFN-γ mRNA expression was significantly downregulated after successful therapy of AD [3].

NC/Nga mice are the most extensively studied animal models of AD [9] because they develop AD-like skin lesions when kept in an air-uncontrolled conventional housing, rather than under specific pathogen-free (SPF) conditions [9]. The clinical symptoms observed in NC/Nga mice kept under conventional conditions began with scratching behavior, erythema, and hemorrhage, followed by edema, superficial erosion, deep excoriation, scaling, and dryness of the skin [9]. Histological examinations revealed hyperplasia as well as infiltration of mast cells, eosinophils, and mononuclear cells in skin lesions [9]. Along with the appearance of the AD-like skin

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lesions, elevated plasma levels of total IgE is observed in NC/Nga mice [9]. Reproducible AD-like skin lesions in NC/Nga mice have been successfully produced by the repeated application of chemical haptens, such as 2,4,6-trinitrochlorobenzene (TNCB), 2,4-dinitrochlorobenzene (DNCB), and 2,4-dinitrofluorobenzene (DNFB) [4,13,16,19]. It is known that the pathogenesis of hapten-induced contact hypersensitivity is predominantly the result of T-cell-mediated immune responses [21]. As the pathophysiological findings of AD-like skin lesions in NC/Nga mice resemble those in humans, this mouse strain has been considered a useful animal model of human AD [17].

Honey bee-derived products are used as traditional complementary medicine worldwide, especially in oriental countries [12]. The main active ingredients of honey bee products have been considered to be composed of phytosterols, phytoestrogens (lignans and flavonoids), amino acids, oligopeptides, and enzymes [1,12]. In addition, honey bees also produce the insect hormone, ecdysteroids, which regulate molting, metamorphosis, and diapause induction [1,12]. A previous study demonstrated that a honey bee-derived product, royal jelly (RJ), inhibited the development of AD-like skin lesions in picryl chloride (PiCl)-treated NC/Nga mice [18], suggesting that the mechanism of inhibition of AD development by RJ was associated with the downregulation of IFN-γ production and upregulation of iNOS expression [18]. Another study revealed that lyophilized powder of honey bee larvae alleviated depression associated with tinnitus by regulating the activity of hypothalamic-pituitary-adrenal axis [1]. To the best of our knowledge, to date, there is no research on the suppressive effect of honey bee larvae on the development of AD. In the present study, for the first time, we examined the effect of freeze-dried powders of honey bee larvae on the development of AD-like dermatitis induced by the repeated application of TNCB in sensitized NC/Nga mice to confirm their usefulness against AD.

Table 1
Nutritional components and amino acids of freeze-dried powders of honeybee larvae.

Component (g)	/100 g
Moisture	2.1
Protein	50.1
Fat	13.5
Ash	4
Carbohydrate	30.3
Total Energy (kcal)[a]	443
Arginine	2.32
Lysine	3.29
Histidine	1.26
Phenylalanine	1.87
Tyrosine	2.38
Leucine	3.55
Isoleucine	2.21
Methionine[b]	1.05
Valine	2.61
Alanine	2.35
Glycine	2.25
Proline	2.93
Glutamic acid	6.74
Serine	2.08
Threonine	1.87
Aspartic acid	4.73
Tryptophan	0.6
Cystine[b]	0.5

[a] Protein: 1 g = 4 calories; Fat: 1 g = 9 calories; Carbohydrates: 1 g = 4 calories.
[b] HCl acid hydrolysis, performic acid oxidation for methionine and cystine analysis.

2. Materials and methods

2.1. Materials

Freeze-dried powders of honey bee larvae (approximately 21 days old) were used in all experiments performed in this study. The nutritional components and amino acids of the powder are shown in Table 1.

2.2. Animals

5-week-old male NC/Nga mice were obtained from Japan SLC, Inc. (Shizuoka, Japan). The animals were maintained under a 12-h light/dark cycle, at a temperature of 22 ± 2 °C. The use of these animals and all animal procedures were approved by the Animal Research Committee of Tottori University.

2.3. Methods

Fifty animals were randomly divided into five groups of 10 mice each as follows: (1) negative control group, (2) positive control group, (3) 0.1% freeze-dried powders of honey bee larvae-supplemented diet group, (4) 0.5% freeze-dried powders of honey bee larvae-supplemented diet group, and (5) 1.0% freeze-dried powders of honey bee larvae-supplemented diet group. The mice in the 0.1, 0.5, or 1.0% honey bee larvae-supplemented diet group were given a diet mixed with freeze-dried powders of honey bee larvae at the dosage of 0.1, 0.5, or 1.0%, respectively, from days 7–53. The mice in the negative and positive control groups were given the standard mouse diet (CE-2; Nihon Clea, Tokyo, Japan). The nutritional components of the standard mouse diet (CE-2) were compared to those of the freeze dried honey bee larvae powder-supplemented diet (Table 2). No differences in food intake were observed among the negative and positive control groups and the freeze dried honey bee larvae powder-treated groups.

TNCB was purchased from Tokyo Kasei Chemical Co. Ltd. (Tokyo, Japan) and used after recrystallization with ethanol. The hair of the thoracic, abdominal, and dorsal regions of the mice was shaved under halothane anesthesia with an electric clipper. On days 0 and 4, all animal groups except the negative control group were sensitized by the application of 150 μL (thoracic area, 50 μL;

Table 2
Nutritional components of standard mouse diet (CE-2) compare to that of honeybee larvae -added diet.

Component (g)	Standard mouse diet (CE-2)/100 g	0.1% Honeybee larva/100 g	0.5% Honeybee larva/100 g	1% Honeybee larva/100 g
Moisture	8.89	8.88	8.86	8.82
Protein	24.88	24.91	25.01	25.13
Fat	5.03	5.04	5.07	5.11
Ash	6.79	6.79	6.78	6.76
Carbohydrate[a]	54.41	54.39	54.29	54.17
Total Energy (kcal)[b]	343.9	344	344.4	344.9

[a] Total crude fiber (4.63 g/100 g) and nitrogen free extract (NFE) (49.78 g/100 g).
[b] Protein: 1 g = 4 calories; Fat: 1 g = 9 calories; Carbohydrates: 1 g = 4 calories.

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