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Water-soluble low-molecular-weight β-(1, 3–1, 6) D-Glucan inhibit cedar pollinosis

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ABSTRACT:

Background: The incidence of allergic diseases such as allergic rhinitis, atopic dermatitis, asthma, and food allergies has increased in several countries. Mast cells have critical roles in various biologic processes related to allergic diseases. Mast cells express the high-affinity receptor for immunoglobulin (Ig) E on their surface. The interaction of multivalent antigens with surface-bound IgE causes the secretion of granule-stored mediators, as well as the *de novo* synthesis of cytokines. Those mediators and cytokines advance allergic diseases. We investigated the effects of water-soluble, low-molecular-weight β-(1, 3–1, 6) D-glucan isolated from *Aureobasidium pullulans* 1A1 strain black yeast (LMW-β-glucan) on mast cell-mediated anaphylactic reactions. We reported that LMW-β-glucan dose-dependently inhibited the degranulation of mast cells. Furthermore, we discovered that orally administered LMW-β-glucan inhibited the IgE-mediated passive cutaneous anaphylaxis (PCA) reaction in mice. We then examined if LMW-β-glucan had effects on Japanese cedar pollinosis.

Findings: In a clinical study, a randomized, single-blind, placebo-controlled, parallel group study in 65 subjects (aged 22–62) was performed. This study was conducted 3 weeks before and until the end of the cedar pollen season. During the study, all subjects consumed one bottle of placebo or LMW-β-glucan daily. Each subject was required to record allergic symptoms in a diary. The LMW-β-glucan group had a significantly lower prevalence of sneezing, nose-blowing, tears, and hindrance to the activities of daily living than the placebo group.

Conclusions: These results suggested that LMW-β-glucan could be an effective treatment for allergic diseases.

Key Words: Mast cell, Anti-allergy, β-glucan, Cedar pollinosis

INTRODUCTION

The incidence of allergic diseases such as allergic rhinitis, atopic dermatitis, asthma, and food allergies has increased in several countries [1]. Mast cells and basophils have critical roles in various biologic processes related to allergic diseases [2, 3]. These cells express the high-affinity receptor for immunoglobulin (Ig) E on their surface. The interaction of multivalent antigens with surface-bound IgE causes the secretion of granule-stored mediators, as well as the *de novo* synthesis of cytokines [4]. Those mediators and cytokines activate the migration of neutrophils and macrophages. The reactions brought about by these cells causes tissue inflammation [5] and an allergic reaction.

β-glucan consists of many glucose molecules joined together with β-1, 3-and/or β-1, 6-linkages. β-glucan is derived from mushroom and fungi, formerly a substrate known to stimulate immune reactions [6–8]. However, β-1, 3 or β-1, 6-glucan isolated from mushrooms have high viscosity and high molecular weight (>2,000 kDa) and are insoluble [9]. Therefore, purification of these compounds is extremely difficult, and crude β-glucan fractions have been used in many experiments [9, 10].

Recently, a water-soluble, low-molecular-weight (≈100 kDa as an average molecular weight) β-(1, 3–1, 6) D-glucan (LMW-β-glucan) was prepared from the *A. pullulans* 1A1 strain of black yeast [11]. It has been reported that the LMW-β-glucan can exert anti-tumor, anti-metastatic [12] and anti-stress actions [13]. However, the effects of LMW-β-glucan on anaphylactic reactions have not been reported fully. We found that LMW-β-glucan dose-dependently inhibited the degranulation of rat basophilic leukemia and murine cultured

mast cells activated by IgE [14]. Furthermore, orally administered LMW-β-glucan inhibited the IgE-induced passive cutaneous anaphylaxis (PCA) reaction in mice [14].

In the present study, we investigated the effects of orally administrated LMW-β-glucan on pollinosis during the most prevalent season for allergic rhinitis, in order to evaluate its effects in humans.

METHODS:

Preparation of LMW-β-glucan: The *A. pullulans* 1A1 strain was cultured in Czapek's medium supplemented with 0.3% sodium ascorbate and 3% sucrose [11,15]. The resulting culture was treated under alkaline conditions by the addition of NaOH (pH 13) to lower its viscosity. The isolation and purification of LMW-β-glucan was carried out in the same manner [12]. The structure of the LMW-β-glucan consisted of a main chain of β-1, 3-linked β-D-glucose with β-1, 6-linked side-chains by NMR. Additionally, the integral ratio of the branches of β-1, 6 to β-1, 3 was estimated to be approximately 80%. The molecular weight of LMW-β-glucan was determined to be approximately 100 kDa as an average molecular weight by the alkaline gel chromatography [12]. The LMW-β-glucan purity was determined as follows; N content is estimated to be 0.24% by the Kjeldahl method. From contents of ash (6.3%) and moisture (3.7%), possible purity of the LMW-β-glucan used was estimated to be more than 80%. LMW-β-glucan was kindly supplied by Daiso Co. Ltd., (Hyogo, Japan).

Subject Selection: Sixty-five subjects (age: 22–62 years) were enrolled in this study. All subjects had a history of seasonal rhinitis. The present study was conducted in compliance with current standards of clinical practice. The Ethics Committees of the Fukuda Clinic of Internal Medicine (Fukuda, Japan) approved the study protocol and found it to be in accordance with the Declaration of Helsinki (inspection No: IRB20120218-1). Written informed consent for participation was obtained from all subjects. All subjects did not take any anti-allergic drug during the study period.

Test Samples: There were two types of test samples and thus two groups as follows: 50 mL of water containing 500 mg carbohydrate and 30 mg sodium citrate with 150 mg LMW-β-glucan (LMW-β-glucan group) and 50 mL of water containing 500 mg carbohydrate and 30 mg sodium citrate (placebo group). No differences in taste or appearance were observed between these two test sample types. Thirty-four subjects (26 males, 8 females; mean age, 42.8

years) were assigned randomly to the LMW-β-glucan group, and 31 subjects (24 males, 7 females; mean age, 39.1 years) were assigned randomly to the placebo group. There were no differences in sex, age or degree of seasonal rhinitis (p>0.2) between the LMW-β-glucan group and placebo group.

Study Design: This was a randomized, single-blind, placebo-controlled, parallel group study. Intake of the test drink was performed between 9 February and 4 April 2012 (3 weeks before and until the end of the cedar pollen season) (Fig. 1).

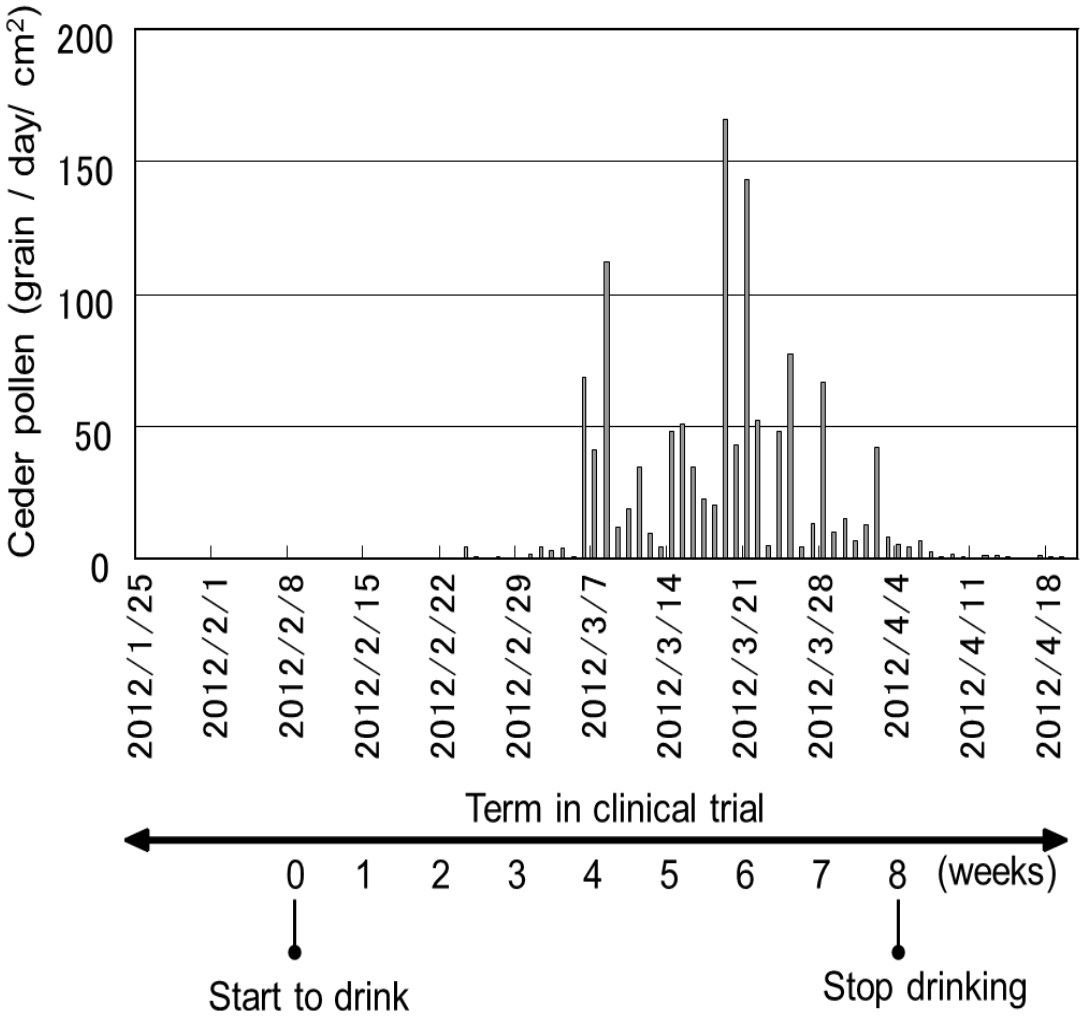

Figure 1. Number of Japanese cedar pollen scattered between 25 January and 24 April 2012. All subjects drank test samples with or without 150 mg of LMW-β-glucan between 9 February and 4 April.

The effects of intake of LMW-β-glucan, which began before pollen dispersion, on allergic

symptoms were examined by comparing the time-course changes in scores of nasal and eye symptoms in the LMW-β-glucan group with those of the placebo group. During the study period, all subjects consumed one bottle of placebo or LMW-β-glucan daily. We used the same lot of LMW-β-glucan in the clinical study.

During the test period, all subjects were required to record allergic symptoms in a diary. This involved recording the daily frequency of sneezing and nose-blowing, stuffy nose, itchy eyes, tears, and hindrance in the activities of daily living (ADL), in accordance with the method proposed by the Allergic Rhinitis Committee of the Japanese Society of Allergology. The symptoms were classified as follows: sneezing, total number of sneezing in one day (*continuous sneezing is counted as one number); nose-blowing, total number of nose-blowing in one day; stuffy nose, breathes freely = 0, mild nasal congestion without mouth breezing in one day = 1, moderate nasal congestion with several mouth breezing in one day = 2, severe nasal congestion with many mouth breezing in one day = 3, both nostrils blocked throughout the day = 4; itchy eyes, none = 0, mild itchy eyes without rub eyes = 1, mild itchy eyes with rub eyes sometimes = 2, moderate itchy eyes with rub eyes frequently = 3, severe itchy eyes with rub eyes many times = 4; tears, none = 0, mild symptoms without wipe tears = 1, mild symptoms with wipe tears sometimes = 2, moderate symptoms with wipe tears frequently = 3, severe symptoms with wipe tears many times; and ADL, none = 0, not interfere very much = 1, a little bit difficult = 2, much difficult = 3, impossible = 4. The diaries were collected at the end of the study. We assumed that the scores in the 0 week of the test period were zero. The differences of the scores between before and after the test period were compared.

Statistical Analysis: We compared the test groups using the subjective symptoms of allergic rhinitis in the Mann–Whitney *U*-test as the object of analysis of the score frequency. $p<0.05$ was considered significant. Data were analyzed with SPSS ver. 11.5 (IBM Corporation).

RESULTS:

The clinical study on subjects with Japanese cedar pollinosis was performed to evaluate the effect of LMW-β-glucan. Subjects in the LMW-β-glucan group were compared with those in the placebo group. Fig. 2 shows mean scores for sneezing, nose-blowing, stuffy nose, itchy eyes, tears, and ADL of the average of the changes in one week of subject symptoms. The LMW-β-glucan group had a significantly lower prevalence of sneezing at week 6 (Fig. 2a); nose-blowing at weeks 2 and 3 (Fig. 2b); tears at week 2 (Fig. 2e); and hindrance to ADL at

week 6 (Fig. 2f).

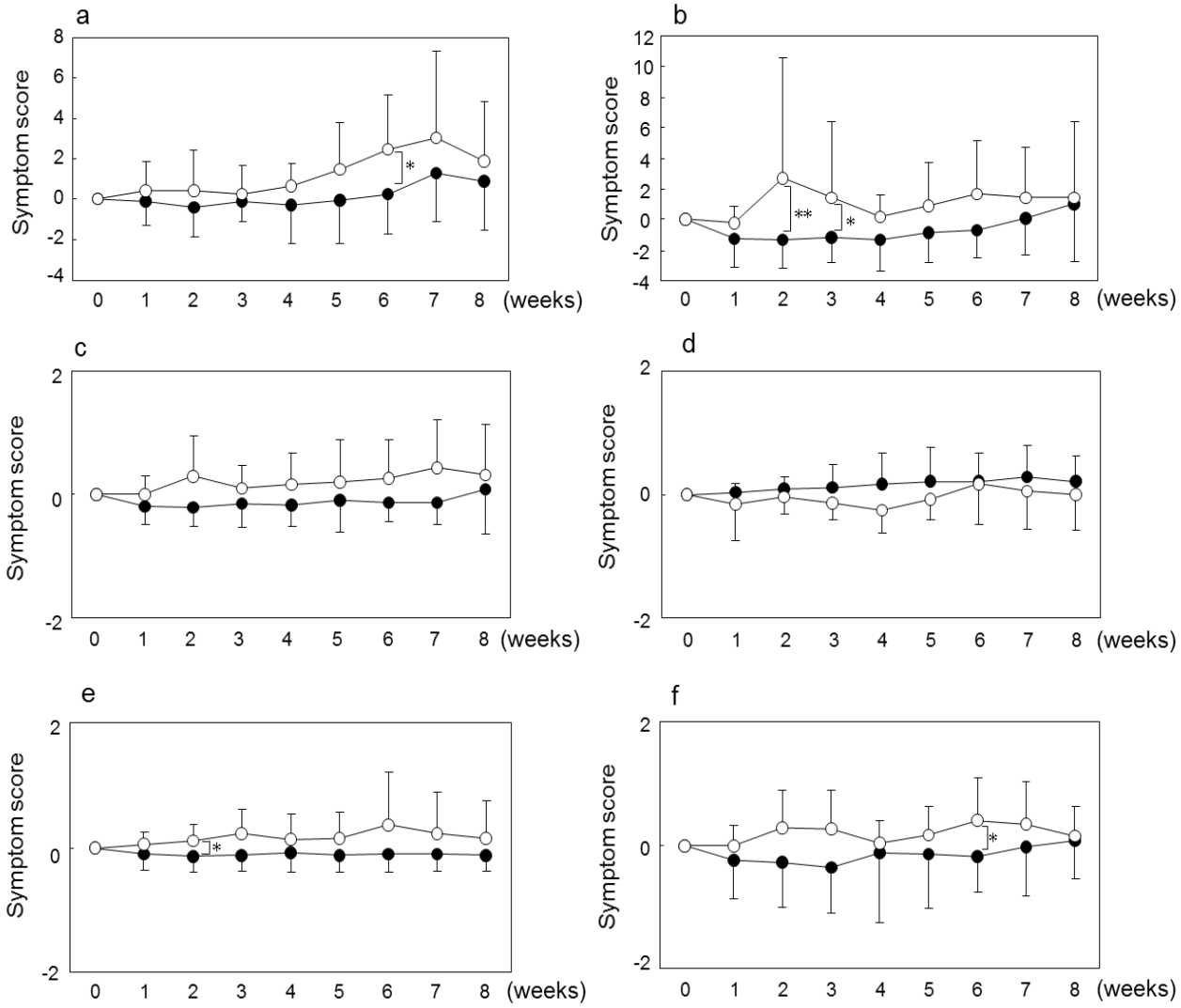

Figure 2. Mean scores for nasal and eye symptoms, and hindrance to the ADL of the changes in one-week symptoms of subjects given LMW-β-glucan. Solid circles represent the LMW-β-glucan group, open circles represent the placebo group. a: sneezing. b; nose-blowing, c; stuffy nose, d; itchy eyes, e; tears, f; hindrance to the activities of daily living. Each value represents the mean ± SD. A comparison between the LMW-β-glucan group and the placebo group was undertaken using the Mann–Whitney U test. ** $p<0.01$, * $p<0.05$ *vs*. another group.

DISCUSSION:

We investigated whether the intake of LMW-β-glucan could modify various symptoms during the grass pollen season in patients with seasonal allergic rhinitis. The clinical study revealed that the LMW-β-glucan group had a significantly lower prevalence of sneezing, nose-blowing, tears,

and hindrance to the ADL than the placebo group. Specially, the scores of sneezing and hindrance to the ADL were lower in the LMW-β-glucan group than in the placebo group at week 6. This result reveals that improvement regarding hindrance to the ADL might be related to the inhibitory effect on "sneezing" by LMW-β-glucan. Furthermore, the LMW-β-glucan group had a significantly lower prevalence of nose-blowing and tears at week 2. Although the amount of pollen at week 2 was significantly smaller than that at week 6, some people might have very high sensitivity to pollen; consequently, mast cell degranulation might be induced. Further investigation will be necessary to explain this phenomena.

We have shown that LMW-β-glucan inhibits mast cell degranulation, which means that LMW-β-glucan inhibits the release of chemical mediators from mast cells [14]. Furthermore, we have also revealed the inhibitory effect of LMW-β-glucan by an IgE-mediated PCA reaction [14]. The inhibitory effect of LMW-β-glucan on the PCA reaction may have been due to reduced degranulation.

Various allergic reactions occur via IgE-mediated hypersensitivity reactions [4, 5]. IgE production is promoted by T helper (Th) 2 cells and their cytokines, such as interleukin (IL)-4 and IL-5. Th1 cells and their cytokines, such as interferon (IFN)-γ and IL-12, can inhibit IgE production [16]. Orally administered LMW-β-glucan has been shown to significantly inhibit the ova-albumin (OVA) induced allergic reaction in mice, and this might be related to the inhibition of OVA-specific IgE elevation through the production of IFN-γ and IL-12 [17]. Moreover, treatment with β-glucan from zymosan has been demonstrated to be effective on the Th1/Th2 balance in patients with allergic rhinitis [18]. In addition to the inhibitory effects on mast cell degranulation, β-glucan has anti-allergic properties through its effects on the Th1/Th2 balance and IgE production. The measurement of the amounts in serum Th1/Th2 cytokines and IgE levels of subjects is important to clarify the effects of LMW-β-glucan.

In conclusion, drinking LMW-β-glucan markedly improved the symptoms of allergic rhinitis in our clinical study. These results suggest that LMW-β-glucan has an inhibitory effect on allergic reactions. We will proceed with further investigation in clinical trial to study the biochemical data. Furthermore, the *in vivo* and *in vitro* anti-allergic effects of LMW-β-glucan suggests there are possible therapeutic applications of this component regarding the treatment of inflammatory allergic diseases.

Competing Interests: The authors have no financial interests or conflicts of interests.

Author's contributions: All authors contributed to this study.

Abbreviations: LMW, low-molecular-weight; Ig, immunoglobulin; CMCs, cultured mast cells; PCA, passive cutaneous anaphylaxis; ADL, activities of daily living; Th, T helper; IL, interleukin; OVA, ova-albumin

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