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Original article

Effect of *Chongkukjang* on histamine-induced skin wheal response: A randomized, double-blind, placebo-controlled trial

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ABSTRACT

Background: Studies in animals have demonstrated the antiallergenic properties of *Chongkukjang* (CKJ), a traditional Korean food made by fermentation of soybean with *Bacillus subtilis*. CKJ might therefore be used as an ingredient in a functional food designed to suppress allergies. The purpose of this study was to investigate the effect of CKJ on histamine-induced skin wheal response in healthy participants.
Methods: A randomized, double-blind, placebo-controlled trial was conducted. Sixty participants (48 women and 12 men) were randomly assigned to one of two groups: One group received 35 g CKJ daily for 12 weeks, and the other received a placebo at the same dosing frequency. A skin prick test with histamine (10 mg/mL) was conducted on the ventral forearm 10 cm from the elbow, and assessed 15 minutes later. Outcomes included measurement of efficacy [skin wheal response, immunoglobulin E (IgE), histamine, interferon-gamma, interleukin-4, eosinophil, and eosinophil cationic protein (ECP)], and safety (adverse events, laboratory test results, electrocardiogram, anthropometric values, and vital signs).
Results: Fifty-five participants (28 in the CKJ group and 27 in the placebo group) completed the study. After 12 weeks of supplementation, participants in the CKJ group showed a significant reduction in histamine-induced skin wheal areas compared with placebo group ($p < 0.05$). At 12 weeks, the CKJ group showed a significant improvement in percentage change from baseline in histamine-induced wheal area, compared with the placebo group ($p < 0.05$). CKJ did not influence blood levels of IgE, histamine, interferon-gamma, interleukin-4, eosinophil, or ECP.
Conclusion: Oral administration of CKJ for 12 weeks resulted in a reduction of the skin wheal response to histamine, with no apparent adverse effects. Trial registration: ClinicalTrials.gov: NCT01402141.

1. Introduction

Allergic disease is a consequence of exposure to normally innocuous substances that elicit the activation of mast cells, which mediate tissue swelling, redness, pain, and respiratory symptoms [1–6]. A variety of pharmaceutical agents may be used to suppress the inflammation of an allergic response. Since the notion that a

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daily intake of certain foods having antiallergic activity may potentially reduce or eliminate the need for drugs is highly attractive, the anti-inflammatory and antiallergic activities of *Chongkukjang* (CKJ) have been investigated [7].

The traditional Korean food CKJ has the shortest fermentation period (2–4 days) and is fermented at a high temperature (40–43°C) with *Bacillus subtilis*. CKJ has its soybean protein degraded from the protein degradation enzyme, and free amino acid is produced along with related peptides afterwards. Because of this, CKJ has its own special characteristics and aroma. It also serves as a great source of nutrients that provide adequate amounts of amino acids in Korean peoples' diets, where rice constitutes a common and substantial part of most meals [8].

In its long history, CKJ has sometimes been used to treat superficial inflammatory skin disorders. The bioactive compounds in CKJ include the isoflavonoids genistein and daidzein [9]. During the fermentation period, the flavonoid aglycones accumulate through hydrolysis of the flavonoid glycosides [10]. CKJ also contains poly-gamma-glutamic acid (gamma-PGA), an anionic polymer composed of D- and L-glutamic acid units linked through the alpha-amino and gamma-carboxylic acid groups [11]. Gamma-PGA is produced by *Bacillus subtilis* during the fermentation of soybeans and is not present in humans [12]. Gamma-PGA is water soluble, biodegradable, edible, and nontoxic, and is therefore compatible with use in food and cosmetic products [13]. Recent reports also attribute antiallergic and immunomodulatory activities to gamma-PGA [7,14–17], although these activities are not confirmed in humans consuming CKJ.

Histamine is a mediator of the wheal and flare response, one of the highly irritating symptoms of allergy that impairs quality of life and prompts sufferers to seek relief in the form of medications. In the development and comparison of prospective antihistamine medications, the histamine-induced wheal and flare skin test has been used most frequently. The histamine-induced wheal and flare test is based on the planimetric assessment of local swelling caused by plasma extravasation (wheal) and reflex vasodilatation (flare) after a histamine challenge [18].

The present study, therefore, was carried out to investigate the antiallergic effects of CKJ, administered orally to human participants, and assessed using the histamine skin prick test. Findings from the present study shed light on the efficacy, safety, and underlying mechanisms of CKJ, which are relevant to the development of a dietary supplement to treat symptoms of allergy in the skin.

2. Materials and methods

2.1. Participants

The study participants were recruited from the Clinical Trial Center for Functional Foods (CTCF2) in Chonbuk National University Hospital (Jeonju, Republic of Korea) between January 2011 and September 2011. A total of 60 healthy volunteers (12 male, 48 female; 34.9 ± 12.3 years) participated in this study. The volunteers had not taken any drugs that might affect histamine response, such as antihistamines, mast cell stabilizers, or antidepressants for at least 7 days prior to enrolling in this study. Inclusion criteria were: (1) healthy volunteers aged 20–80 years and (2) positive response to the histamine skin prick test (a wheal size > 3 mm). Exclusion criteria were: (1) a severe generalized skin condition such as eczema, psoriasis, or atopic dermatitis; (2) a history of severe allergic reaction; (3) use of oral antihistamines or topical corticosteroids in the preceding 3 months; (4) any acute or chronic illness; (5) cardiovascular disease, liver, or kidney disease; (6) allergies to soy-containing foods; (7) use of any prescribed or investigative medication during the 2 months preceding enrollment; (8) excessive use of a drug or alcohol in the preceding 2 months; and (9) laboratory tests results as well as medical or psychological conditions that could interfere with successful participation in the study as judged by the investigators.

The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from each participant before the study began. The protocol was approved by the Functional Foods Institutional Review Board (FFIRB) of Chonbuk National University Hospital (FFIRB number: 2010-02-007). The protocol was registered with www.clinicaltrials.gov (NCT01402141).

2.2. Study design

This current study was conducted under a 12-week, randomized, double-blind, placebo-controlled trial to evaluate the safety and efficacy of CKJ in healthy individuals in Korea. Participants were recruited through advertisements in local newspapers and the hospital website and bulletin boards. Candidates were interviewed and evaluated to determine eligibility. After completing a screening test, eligible participants were randomized to either the CKJ group or the placebo group. The treatment period consisted of 1 week of baseline assessments, 12 weeks of treatment, and 3 weeks of follow-up, for a total study period of 16 weeks. All participants and investigators were blinded to the type of treatment received until completion of the study.

To avoid allocation bias, concealed allocation using a sealed envelope was employed in this study. A statistician randomized participants using a computer-generated random table in a 1:1 ratio with block size 2, and clinical research coordinators (CRC) used the random table to assign the CKJ and placebo treatments. CKJ or placebo pills were prescribed to the participants every 4 weeks. The CKJ group ($n = 30$) took 35 g (11.7 g pills per pack, 3 times per day) of freeze-dried CKJ daily for 12 weeks, which was equivalent to 70 g of fresh CKJ; the placebo group ($n = 30$) took the same amount. CKJ and the placebo were provided by the Institute of Sunchang Fermented Soybean Products (Sunchang, Republic of Korea). Briefly, white soybeans (Sunchang, Republic of Korea) were sorted, washed, and soaked in water for 12 hours at 15°C and boiled for 0.17 hours at 121°C. The cooked soybeans were cooled to 40°C and fermented with *Bacillus licheniformis* SRCM 100027 at 37°C for 24 hours. CKJ was freeze-dried using a freeze-dryer (model PVTFD 100R, Ilsinlab, Yangju, Republic of Korea), and then made into pills (Imshil Herbal Medicine Co., Imshil, Republic of Korea). The placebo had the same taste and appearance but did not have the principal ingredient that was present in CKJ. The placebo supplements were composed primarily of rice and wheat flours.

During a 12-week intervention period, participants were asked to continue their usual diets and activities and were asked not to take any other functional foods or dietary supplements. The skin prick test was performed, and immunoglobulin E (IgE), histamine, interferon-gamma, interleukin-4, eosinophil, and eosinophil cationic protein (ECP) were measured before and after the intervention period for both groups. Every 4th week the participants were asked to report for assessment of any adverse events or any changes in training, lifestyle, or eating patterns, and to assess pill compliance.

2.3. Assessments

The skin prick tests with histamine solution, CKJ, and saline were performed on the forearms using prick lancets. Histamine was dissolved in distilled water at 10 mg/mL. A droplet of 10 mg/mL histamine solution was introduced into the skin by piercing with a