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Safety evaluation of standardized allergen extract of Japanese cedar pollen for sublingual immunotherapy

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ABSTRACT

Japanese cedar (JC) pollinosis is caused by Japanese cedar pollen (JCP) and most common seasonal allergic disease in Japan. Subcutaneous immunotherapy (SCIT) with allergen extract of JCP (JCP-allergen extract) is well established for JC pollinosis treatment with improvement of symptoms. However, major drawbacks for SCIT are repeated painful injections, frequent hospital visits and anaphylactic risk. Currently, sublingual immunotherapy (SLIT) has received much attention as an advanced alternative application with lower incidence of systemic reactions because the liquid or tablet form of allergen is placed under the tongue. The aim of this study was safety evaluation of standardized JCP-allergen extract currently developed for SLIT in JC pollinosis. JCP-allergen extract showed no potential genotoxicity. No systemic effects were observed in rats administered JCP-allergen extract orally for 26 weeks followed by 4-week recovery period. Mild local reactions such as hyperplasia and increased globule leukocytes resulting from vehicle (glycerin)-induced irritation were observed in stomach. No-observed-adverse-effect level was greater than 10,000 JAU/kg/day for systemic toxicity, equivalent to 300-fold the human dose. No local irritation was found in rabbits oral mucosae by 7-day sublingual administration. These results demonstrate the safe profile of standardized JCP-allergen extract, suggesting it is suitable for SLIT in JC pollinosis.

1. Introduction

Seasonal allergic rhinitis, also named Japanese cedar (JC) pollinosis, is caused by Japanese cedar pollen (JCP). It is a type-I allergic disease and is the most common disease in Japan and thus is considered a national affliction (Kaneko et al., 2005; Okubo et al., 2011; Yamada et al., 2014). The main symptoms are sneezing, watery rhinorrhea, nasal blockage and itching of eyes. More than one third of the Japanese population has JC pollinosis, and this number has significantly increased in the last 2 decades (Okubo et al., 2011; Yamada et al., 2014). In addition, many JC pollinosis patients have also been sensitized to cypress pollen scattered after the JCP season by IgE cross-reactivity (Di Felice et al., 2001), which causes a long-term symptom onset.

Allergen-specific immunotherapy is the practice of administering gradually increasing quantities of allergen extracts to ameliorate the symptoms associated with the subsequent exposure to the causative allergen in order to induce a state of tolerance (Malling and Weeke, 1993; Bousquet et al., 1998; Cappella and Durham, 2012; Eifan et al., 2013; Canonica et al., 2014). It is considered to be a cure for allergic disease although symptomatic treatment such as antihistamines and nasal corticosteroids allows tentative relief from symptoms (Bousquet et al., 1998). Subcutaneous immunotherapy (SCIT) has been used to treat JC pollinosis but with limited usage because of inconvenience such as injection site pain and frequent hospital visits, and risk of severe side effect such as systemic allergic reaction including rarely occurred anaphylaxis (Bernstein et al., 2004; Cox et al., 2010) . Allergen-specific

Abbreviations: JAU, Japanese allergy units; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PCA, passive cutaneous anaphylaxis; %MNPCE, ratio of micronucleated polychromatic erythrocyte; %RET, ratio of reticulocytes; JC, Japanese cedar; JCP, Japanese cedar pollen; SLIT, sublingual immunotherapy; SCIT, Subcutaneous immunotherapy; NOAEL, no-observed-adverse-effect level; GLP, Good Laboratory Practice; 2AA, 2-aminoanthracene; AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; s.c., sub-cutaneous; p.o., per os; PBS, phosphate buffered saline; DMN, dimethylnitrosamine; MMC, mitomycin C; SPF, specific pathogen-free; HE, hematoxylin and eosin.

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sublingual immunotherapy (SLIT) is considered to be a safe and efficient treatment for respiratory allergy, and has been introduced as an alternative to SCIT (Didier et al., 2011; Nelson et al., 2011; Durham et al., 2012; Cox et al., 2012). SLIT reduces the burden on patients compared with SCIT because it can be administered at home and is associated with fewer severe side effects. In SLIT, the allergen extract is placed under the tongue for 1 or 2 min and then swallowed (SLIT-swallow) or spat out (SLIT-spit). Based on clinical results and pharmacokinetic considerations, only SLIT-swallow is currently performed (Canonica and Passalacqua, 2003).

A standardized allergen extract of JCP has been used for SCIT treatment of the JC pollinosis patients for 15 years. The potency of allergen extract of JCP (JCP-allergen extract) is expressed as Japanese allergy units (JAU) as defined by the Japanese Society of Allergology based on skin tests of allergy patients. A liquid containing 200 or 2000 JAU/mL is formulated by dilution of the standardized allergen of JCP original solution (10,000 JAU/mL; Torii Pharmaceutical Co., Ltd., Tokyo, Japan) containing the major allergen Cry j 1 (Yasueda et al., 1983) at 7.3–21.0 μg/mL (Yasueda et al., 1996) to provide the indicated potency.

Several clinical studies using small patient populations have shown that SLIT might be effective for the treatment of patients with JC pollinosis (Horiguchi et al., 2008; Okubo et al., 2008; Fujimura et al., 2011), in which SCIT products of standardized allergen extract of JCP were used for sublingual administration by SLIT. A modified liquid formulation of the SCIT product of standardized JCP-allergen extract has been developed (CEDARTOLEN®, Torii Pharmaceutical Co., Ltd.) for SLIT clinical applications.

In Japan, there is no specific regulatory guideline for allergen-specific immunotherapy drugs in Japanese new drug application process. Thereby, nonclinical data in allergen-specific immunotherapy drug should be applied to the regulatory requirements as well as a small molecule compound drug. Here, we investigated the safety of standardized JCP-allergen extract to evaluate its clinical application to SLIT. Genotoxicity studies, a repeated 26-week oral toxicity study and a repeated 7-day sublingual dose oral mucosal irritation study were conducted under Good Laboratory Practice (GLP) compliance.

2. Materials and methods

2.1. Standardized allergen extract of Japanese cedar pollen

Test liquids of 200 and 2000 JAU/mL (CEDARTOLEN®) were formulated by dilution of standardized JCP-allergen extract original solution (10,000 JAU/mL) containing 7.3–21.0 μg/mL Cry j 1 (Yasueda et al., 1996) to provide the indicated potency, respectively (Torii Pharmaceutical Co., Ltd.). In addition, 50% glycerin-containing sodium chloride was used for the vehicle control group. In the genotoxicity study (Ames bacterial reverse mutation test, *in vitro* chromosomal aberration test and *in vivo* micronucleus test), 10,000 JAU/mL of standardized JCP-allergen extract and its diluted solutions were used. Doses of 12,500, 25,000 and 50,000 JAU/kg were administered subcutaneously to rats in the bone marrow micronucleus test. In a repeated 26-week oral toxicity study, 200, 2000 and 10,000 JAU/mL of standardized JCP-allergen extract were used for oral administration. For the oral mucosal irritation study, 2000 JAU/mL of standardized JCP-allergen extract was administered sublingually, as the maximum dose used in the maintenance phase of SLIT in JC pollinosis.

The protein profile of JCP-allergen extract was assessed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) analysis. Two major allergens (Cry j 1 and Cry j 2) were identified (Fig. 1A).

The allergen profile of JCP-allergen extract was confirmed by measuring immune responses in animals. Levels of the JCP specific-antibody in serum was significantly increased following i.p. administration with alum compared with naïve serum (data not shown). In addition, allergic specific reactions were evaluated using PCA (titer = 128, Fig. 1B).

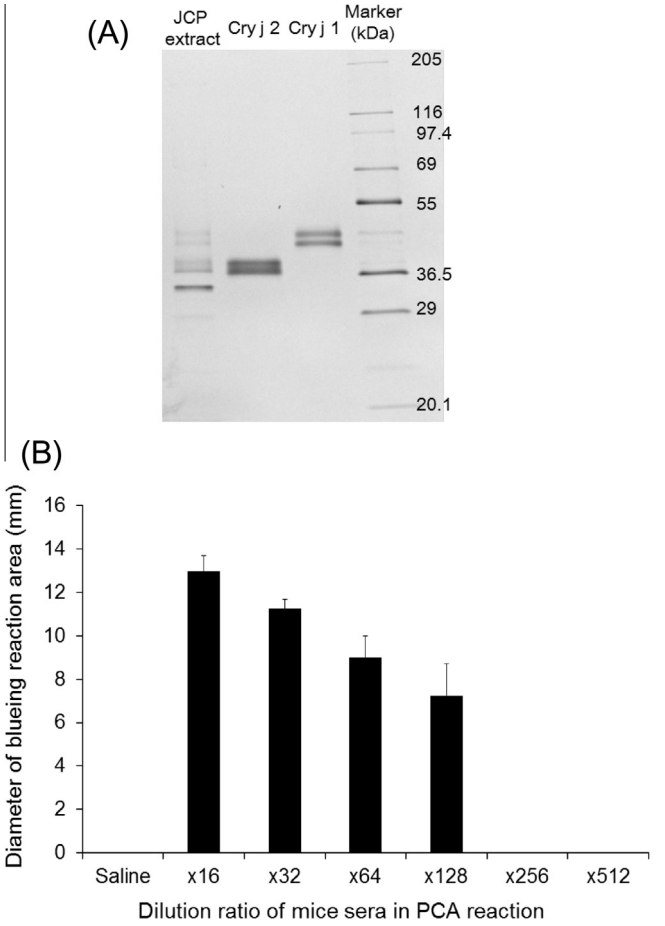

Fig. 1. SDS–PAGE and PCA reaction of JCP-allergen extract. JCP-allergen extract was assessed by SDS–PAGE analysis under non-reduction conditions followed by staining with silver (A). Rats were intradermally injected with serial dilutions of mouse anti-JCP-allergen extract serum. After 48 h, PCA reactions were elicited by intravenous injection of JCP-allergen extract. Values are expressed as mean ± SD of 4 sites in two animals (B).

2.2. Characterization of JCP-allergen extract

2.2.1. SDS–PAGE

JCP-allergen extract (2000 JAU/mL) was boiled in Tris–Glycine SDS sample buffer (TEFCO, Tokyo, Japan) and separated by PAGE on a Mini-Protean TGX Precast Gel (4–20% gradient; BIO-RAD, Hercules, CA). The gel was stained using a Silver Stain Kit (Wako Pure Chemical Industries, Osaka, Japan). Purified Cry j 1 (provided by Torii Pharmaceutical Co., Ltd.) and purified Cry j 2 (Hayashibara Co., Ltd., Okayama, Japan) were used as controls of the two major allergens in JCP.

2.2.2. Passive cutaneous anaphylaxis (PCA) reaction

All procedures used in the experiments were approved by the Animal Ethics Committee of Nihon Bioresearch Center, Gifu, Japan, in accordance with laboratory animal welfare guidelines. Two male Crl:CD (Sprague–Dawley) rats were used (9 weeks old, Charles River, Kanagawa, Japan). Serial dilutions of mouse anti-JCP-allergen extract sera were intradermally injected into shaved dorsal skin. The PCA reaction was measured after a 48-h sensitization period