



## Original article

## Polysaccharide extracted from Chinese white wax scale ameliorates 2,4-dinitrochlorobenzene-induced atopic dermatitis-like symptoms in BALB/c mice



Lin Lin<sup>a,1</sup>, Yiwei Zhou<sup>a,1</sup>, Huifang Li<sup>b</sup>, Dejian Pang<sup>b</sup>, Liyun Zhang<sup>b</sup>, Xiao Lu<sup>b</sup>, Zhengliang Chen<sup>b</sup>, Xiaoshan Zhao<sup>c</sup>, Daming Zuo<sup>b,d,\*</sup>, Ledong Sun<sup>a,\*</sup>

<sup>a</sup> Department of Dermatology, Zhujiang Hospital, Southern Medical University, Guangzhou 510250, China

<sup>b</sup> Department of Immunology, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, China

<sup>c</sup> School of Traditional Chinese Medicine, Southern Medical University, Guangzhou 510515, China

<sup>d</sup> Guangdong Province Key Laboratory of Proteomics, Southern Medical University, Guangzhou 510515, China

## ARTICLE INFO

## Article history:

Available online 28 April 2017

## Keywords:

Atopic dermatitis

Polysaccharide

White wax scale

Inflammatory skin lesion

Th cells

## ABSTRACT

Atopic dermatitis (AD) is a common inflammatory skin disease with high rates of morbidity and is associated with erythema, pruritus, scaling of affected areas of skin. It is extremely important to introduce a therapeutic agent which has significant anti-inflammatory effect with less side-effect for treatment of AD. This study evaluated the effect of a natural compound from herbal extracts, the crude polysaccharide extracted from the white wax scale (CWPS), on AD-like mice. Repeated applications of 2,4-dinitrochlorobenzene (DNCB) were performed on ear and dorsal skin of BALB/c mice to induce AD-like symptoms and skin lesions. Oral administration of CWPS decreased serum IgE level and limited the infiltration of mast cells and eosinophils to the dermal tissues in the DNCB-induced AD mice. In addition, CWPS reduced Th1 and Th17 responses, leading to an attenuated cutaneous inflammatory response. Furthermore, *in vitro* study also demonstrated that CWPS limited T cell activation and cytokines (i.e. IFN- $\gamma$  and IL-17) production induced by DNCB. We conclude that CWPS attenuates DNCB-induced AD-like skin lesion through modulating T cell-elicited immune responses and CD4<sup>+</sup> T cell polarization, and could be exploited as a new therapeutic approach for AD.

© 2017 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Atopic dermatitis (AD) is a chronic, pruritus and relapsing inflammatory skin disease occurring commonly in infants and children (Bieber, 2008; Halim and Phang, 2017). In most cases, AD is associated with elevated serum immunoglobulin E (IgE) and infiltration of innate immune cells (different types of dendritic cells (DCs), mast cells, eosinophils, basophils, innate lymphocytes, and myeloid-derived suppressor cells) as well as adaptive immune cells (B cells and T cells) (Leung and Soter, 2001). In AD, recruitment of

CD4<sup>+</sup> T helper (Th) cells into the dermis is believed to contribute to the cutaneous inflammation. Lesions of acute AD skin showed a predominantly Th2 response characterized by an increased skin expression of Th2 cytokines IL-4, IL-13, IL-5, and IL-31 (Leung and Soter, 2001). Subsequently, the chronic phase is characterized by a predominantly local Th1 response. The increased expression of interferon- $\gamma$  by Th1 cells follows a peak of interleukin-12 expression, which coincides with the appearance of inflammatory dendritic epidermal cells in skin (Trautmann et al., 2000). Th17 cells are important to the regulation of innate immunity and inflammation which involves in some allergic disorders (Oboki et al., 2008). The role of Th17 cells in the development of AD is suggested by a direct correlation between the presence of Th17 cells and severity of the disease (Koga et al., 2008). For many years, AD has been medically treated by the application of corticosteroids topically or systematically. However, these steroids often produce adverse effects such as skin atrophy, striae distensae, and perioral dermatitis in sensitive areas. Recently, it has been reported that

\* Corresponding authors at: Department of Immunology, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, China (D. Zuo).

<sup>1</sup> These authors contributed equally to this work.

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

treatment using natural compounds can decrease inflammation in skin disease (Kim et al., 2013, 2014; Yoon et al., 2015).

The white wax scale insect (*Ericerus pela*, Chavannes) is a famous insect owing to its role in the wax production and has been bred in China for over a thousand years (Yang et al., 2015, 2012). Historically, the white wax has been used in traditional medicine, candle production, printing, and its applications has since expanded to food, pharmaceutical, chemical, and cosmetic industries (Yang et al., 2012; Mustafa et al., 2017). Crude polysaccharide was extracted from the white wax scale with water and precipitated by alcohol. The crude polysaccharide from the white wax scale (CWPS) consists of glucose, mannose and galactose identified by the capillary zone electrophoresis (CZE) method (He et al., 2008). In recent years, it has been demonstrated that CWPS extracted from female wax scale has remarkable antioxidant, anti-inflammatory activity and anti-cancer activity (Feng et al., 2014; He et al., 2015; Nawaz et al., 2017; Rashid et al., 2017). To extend the applications of CWPS for treatment of skin inflammatory disease, we orally administrated CWPS to 2,4-dinitrochlorobenzene (DNCB)-induced AD model in BALB/c mice and investigated whether CWPS has therapeutic efficacy on AD-like symptoms, including ear swelling, AD-like skin lesions, the serum level of IgE and mast cells infiltration. We also analyzed the changes in the frequency of Th1, Th2 and Th17 cells in spleen and mRNA expression of IFN- $\gamma$ , IL-4 and IL-17 in dorsal skin and ear. Furthermore, we investigated the cell proliferation and cytokine production in DNCB-restimulated splenocytes obtained from both control and CWPS-treated AD mice.

## 2. Material and methods

### 2.1. Preparation of CWPS

CWPS was provided by Research Institute of Resources Insects, Chinese Academy of Forestry according to the previous protocol (He et al., 2008). To prepare CWPS solution, CWPS powder was dissolved in 0.9% saline (50 mg/ml) and stored at 4 °C before use.

### 2.2. Animals

BALB/c male mice with six-week-old were purchased from the Animal Center of Southern Medical University (Guangzhou, China). Mice were housed in individual ventilated cage under specific pathogen-free conditions at 22  $\pm$  2 °C with a 12-h light-dark cycle. After 1 week of acclimation, they were divided into two groups (n = 6 per group): (1) Vehicle: mice were sensitized with DNCB and treated with normal saline; (2) CWPS: mice were sensitized with DNCB and oral application with CWPS (1 mg/g body weight).

DNCB was applied to the dorsal skin and the back of both ears of BALB/c mice to induce AD-like symptoms. One day after complete dorsal hair removal (approximately 4 cm<sup>2</sup>), 150  $\mu$ l of 2% DNCB solution (dissolved in a 3:1 mixture of acetone and olive oil) was applied on the dorsal skin, and 10  $\mu$ l of 2% DNCB solution was applied on the back of both ears. After the first sensitization, mice were housed without any further treatment (for 4 days). In the secondary induction, 0.5% DNCB was dissolved in acetone: olive oil mixture (3:1 vol/vol) was applied on the dorsal skin (150  $\mu$ l) as well as the back of both ears (10  $\mu$ l each) once every 2 days. DNCB-treated BALB/c mice were orally administrated with CWPS (1 mg/g body weight) daily from the first day to the thirteenth day. AD mice in the vehicle group were given an equal volume of normal saline. All animal experiments in this study were approved by the Welfare and Ethical Committee for Experimental Animal Care of Southern Medical University (Guangzhou, China).

### 2.3. Histopathological analysis

To evaluate the epidermal thickening and mast cell infiltration, the ear and dorsal skin of each mouse were fixed with 4% paraformaldehyde and embedded in paraffin. Deparaffinized sections were stained with Hematoxylin and eosin (H&E) and toluidine blue (TB), severally. The number of mast cells per 0.95 mm<sup>2</sup> skin was counted at 200 $\times$  magnification. Tissue sections were examined using an Olympus IX71 light microscope.

### 2.4. Serum IgE measurement

Blood samples were collected at the end of the experiment. Serum IgE levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions. The absorbance was measured at 450 nm using a microplate reader.

### 2.5. Flow cytometric analysis of Th cells

At the end of the experiment, splenocytes were prepared in single cell suspension. For intercellular cytokine staining, the prepared splenocytes (5  $\times$  10<sup>6</sup>) were cultured in flat-bottomed 24-well plates in a volume of 500  $\mu$ l/well with cell stimulation cocktail and protein inhibitor (eBioscience) for 5 h according to the manufacturer's protocol. After surface staining with FITC labeled rat anti-mouse CD4 (Clone RM4-5, BD Pharmingen), permeabilized cells were stained with PE-labeled rat anti-mouse IFN- $\gamma$  mAb (Clone XMG1.2, BD Pharmingen), PE-Cy7-labeled rat anti-mouse IL-4 mAb (Clone 11B11, BD Biosciences) and APC-labeled rat anti-mouse IL-17 mAb (Clone Tc11-18H10, BD Pharmingen). Data were collected in a BD FACSCalibur™ Flow Cytometer and analyzed by FlowJo software.

### 2.6. Evaluation of cytokines and transcriptional factors mRNA levels in dorsal skin and ear by Real-time PCR

Total RNA is isolated from dorsal skin and ear using TRIzol (Takara, Dalian, China) according to manufacturer's instruction. For reverse transcription, 500 ng of total RNA was used and cDNA was generated using TranScript All-in-One First-Strand cDNA Synthesis SuperMix (Transgen Biotech, Beijing, China) in a total volume of 20  $\mu$ l. The mRNA level was determined using 1  $\mu$ l of cDNA by quantitative real-time PCR (qRT-PCR) with SYBR using a protocol provided by the manufacturer (Takara). The levels of target gene were normalized with respect to GAPDH gene expression. The primer sequences of cytokines and transcription factors are listed as follow (Table 1).

### 2.7. Determination of T cell activation stimulated by DNCB in vitro

Spleens from AD mice were removed aseptically at the last day of the experiment and gently mashed through a 70  $\mu$ m nylon mesh screen. After red blood cell lysis, the splenocytes were resuspended and cultured in round-bottomed 96-well microplates in a volume of 200  $\mu$ l/well with complete RPMI-1640 medium. The cultures were incubated in the presence of 0.5  $\mu$ g/ml of DNCB for 4 days. In the last 8 h of incubation, [<sup>3</sup>H] thymidine was added into each well. Then, the cells were harvested, using a 96-well plate harvester, onto fiber glass filters and radioactivity on the filter mat is counted in a liquid scintillation counter.

### 2.8. Statistical analysis

Statistical analysis was performed with SPSS Statistics Software (SPSS Inc., Chicago, USA). Data are presented as the mean  $\pm$  standard error of the mean (SEM) and statistical comparisons between

Download English Version:

<https://daneshyari.com/en/article/5551506>

Download Persian Version:

<https://daneshyari.com/article/5551506>

[Daneshyari.com](https://daneshyari.com)