International Immunopharmacology 23 (2014) 617–623

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Resveratrol attenuates HMGB1 signaling and inflammation in house dust mite-induced atopic dermatitis in mice

Vengadeshprabhu Karuppagounder [a], Somasundaram Arumugam [a], Rajarajan A. Thandavarayan [a,b], Vigneshwaran Pitchaimani [a], Remya Sreedhar [a], Rejina Afrin [a], Meilei Harima [a], Hiroshi Suzuki [a], Mayumi Nomoto [a], Shizuka Miyashita [a], Kenji Suzuki [c], Kenichi Watanabe [a,*]

[a] Department of Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Applied Life Sciences, Niigata 956-8603, Japan
[b] Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX 77030, USA
[c] Department of Gastroenterology, Niigata University Graduate School of Medical and Dental Sciences, Niigata City 951-8510, Japan

ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form 16 October 2014
Accepted 16 October 2014
Available online 23 October 2014

Keywords:
Resveratrol
Inflammation
High mobility group box protein 1
Nuclear factor kappa B
Cytokine
Receptor for advanced glycation end products

ABSTRACT

Resveratrol is a polyphenol abundantly found in red grape skin and is effective against antiaging and anti-inflammation associated with immune responses. In this study, we have investigated the effect of resveratrol on skin lesion, high mobility group box (HMGB)1 and inflammation pathway in an atopic dermatitis (AD) mouse model. AD-like lesion was induced by the application of house dust mite extract to the dorsal skin of NC/Nga mouse. After AD induction, resveratrol (20 mg/kg, p.o.) was administered daily for 2 weeks. We evaluated dermatitis severity, histopathological changes, serum levels of T helper (Th) cytokines (interferon (IFN)γ, interleukin (IL)-4) and changes in protein expression by Western blotting for HMGB1, receptor for advanced glycation end products (RAGE), toll like receptor (TLR)4, nuclear factor (NF)κB, phosphatidylinositide 3-kinase (PI3K), extracellular signal-regulated kinase (ERK)1/2, cyclooxygenase (COX)2, tumor necrosis factor (TNF)α, IL-1β, IL-2Rα and other inflammatory markers in the skin of AD mice. Treatment of resveratrol inhibited the development of the AD-like skin lesions. Histological analysis showed that resveratrol inhibited hypertrophy, intracellular edema, mast cells and infiltration of inflammatory cells. Furthermore, resveratrol treatment down-regulated HMGB1, RAGE, p-NFκB, p-PI3K, p-ERK1/2, COX2, TNFα, IL-1β, IL-2Rα, IFNγ and IL-4. Considering all these findings together, the HMGB1 pathway might be a potential therapeutic target in skin inflammation, and resveratrol treatment could have beneficial effects on AD by modulating the HMGB1 protein expression.

1. Introduction

Atopic dermatitis (AD) is a chronic relapsing skin disease that is manifested by type 2 helper T cells (Th2) dominant hyperactive immune disorder. Although the exact cause of AD is not completely elucidated, both genetic and environmental factors are known to play crucial roles in its pathogenesis [1]. In addition, various factors including immunological abnormalities contribute to the pathogenesis and development of AD. Itching is an important problem in AD patients because scratching worsens the condition [2]. The skin is an important interface between the host and its environment. Moreover, a leaky skin epithelial barrier combined with abnormal immune responsiveness likely contributes to the pathophysiology of AD [3]. When exposed to house dust mite allergens, atopic patients develop potent inflammatory diseases such as allergic asthma, perennial rhinitis, and AD [4]. *Dermatophagoides farinae* (DfE) is the major species of house dust mite found in Japan and its body and feces are well known as major environmental allergens [5].

High mobility group box (HMGB)1, a nonhistone chromatin-associated protein, is implicated as a mediator of both infectious and non-infectious inflammatory conditions. It is secreted actively by immune cells such as monocytes, macrophages, and dendritic cells [6], and has been proposed to have a crucial role in the pathogenesis of various chronic inflammatory and autoimmune diseases. Extracellular HMGB1 can bind to different cell surface receptors, including receptors for advanced glycation end products (RAGE) [7], toll-like receptor (TLR) 2 and TLR4 [8], with activation of downstream physiologic and pathologic responses [9]. Binding of HMGB1 to RAGE activates a signaling pathway through activation of extracellular signal-regulated kinase (ERK), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) [10]. NFκB-mediated inflammation in the skin appears to be a final common pathway for the translation of environmental insults into inflammation and is a crucial element for innate immunity. In addition, NFκB is the center of interest, since it promotes the transcription of Th2 cytokines and adhesion molecules [11].

* Corresponding author at: Department of Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Applied Life Sciences, 265-1 Higashijima, Akiha Ku, Niigata 956-8603, Japan. Tel.: +81 250 25 5267; fax: +81 250 25 5021.
E-mail address: watanabe@nupals.ac.jp (K. Watanabe).

http://dx.doi.org/10.1016/j.intimp.2014.10.014
1567-5769/

Resveratrol (trans-3,5,4-trihydroxystilbene), a well-known antioxidant ingredient of red wine, is a phytoalexin present in almost 70 plant species including grape, peanut, mulberry, etc. The compound possesses anti-inflammatory, immunomodulatory, antioxidant, cardioprotective and chemopreventive effects [12]. The anti-inflammatory activity is also related to a relaxing effect on vessels that cause an improvement in skin microcirculation, thus finding a valuable use in irritant dermatitis [13]. Kundu et al. [14] demonstrated that resveratrol pretreatment resulted in a decrease in the phosphorylation of ERK as well as the catalytic activity of ERK. Pretreatment of human epidermal keratinocytes with resveratrol inhibited ultraviolet (UV)B-mediated activation of the NFκB pathway [15]. In spite of these reports, resveratrol in atopic symptoms and the underlying mechanisms were not explored. Hence, the present study was aimed to investigate the ameliorative potential of resveratrol on HMGB1 signaling and skin inflammation in house dust mite induced AD mouse model.

2. Materials and methods

2.1. Materials

Biostir-AD, a cream containing the extract of the DfE, was purchased from Biostir, Inc. (Kobe, Japan). Phosphatase arrest-III was purchased from G-Biosciences, St. Louis, MO, USA. Trizma base, sodium chloride, sodium fluoride, sodium orthovanadate, 2-mercaptoethanol, bovine serum albumin (BSA) and tween 20 were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. Resveratrol was purchased from Tokyo Chemical Industry Co., Ltd, Tokyo, Japan. Unless otherwise stated, all other reagents were of analytical grade and were purchased from Sigma (Tokyo, Japan).

2.2. Experimental design

Specific pathogen free female 6 week-old NC/Nga mice were obtained from Charles River Japan (Yokohama, Japan). The animals were maintained in controlled room (temperature 23 ± 2 °C, 12 h lighting cycle). After 1 week, the mice were randomly divided into 3 groups, untreated group (Normal, n = 5); DfE cream treated mice (100 mg/mouse) were divided into two groups and each received either vehicle (AD, n = 5) or resveratrol (20 mg/kg/day, per oral by gavage) (AD + Resveratrol, n = 5) and they were allowed free access to water and chow throughout the period of study (4 weeks). The mice were weighed once a week. Food intake was estimated every second day, always at the same time of the day. The animal experiments were performed in accordance with national guidelines for the use of experimental animals and approved by the animal care committee of Niigata University of Pharmacy and Applied Life sciences.

2.3. Induction of AD in NC/Nga mice

AD-like skin lesions were induced in NC/Nga mice using DfE cream, as described previously [16]. Briefly, the hair on the upper back was shaved and 150 μL of 4% (w/v) sodium dodecyl sulfate was applied to the shaved dorsal skin and both surfaces of each ear for barrier disruption. After 3 h, 100 mg of DfE cream was applied topically. This procedure was carried out twice weekly for 2 weeks. Resveratrol (20 mg/kg/day, per oral by gavage) [17] treatment was started after the second week of AD induction and continued for 2 weeks (4 weeks total period).

2.4. Evaluation of dermatitis severity

The relative dermatitis severity was assessed macroscopically every week according to the eczema area and severity index scoring system using the following scoring procedure: 0, no symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms (model scoring symptoms are given in Fig. 1). The dermatitis score was defined as the sum of scores for erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness [18].

2.5. Histopathological studies

By the end of the study period (4 weeks), the mice were sacrificed and skin tissues were harvested for semi-quantitative immunoblotting studies. Half of the skin was immediately snap frozen in liquid nitrogen for subsequent protein extraction assays. The remaining excised skin was cut into about 2 mm-thick transverse slices and fixed in 10% formalin. Sections of 3–5 μm thickness were stained with hematoxylin and eosin (HE) or toluidine blue for detecting various inflammatory cells and mast cells, respectively. A histomorphological evaluation of all the skin sections was carried out in a blinded fashion.

2.6. Cytokine assay

By the end of the study period (4th week), blood specimens were obtained from the retro-orbital sinus of every animal using a capillary tube. Serum levels of mouse interleukin (IL)-4 and interferon (IFN)γ were measured by enzyme-linked immunosorbent assay (ELISA) development system quantitation kits provided by Thermo Scientific Inc. (Meridian Rd., Rockford, IL, USA) according to the manufacturer's protocol.

2.7. Protein analysis by Western blotting

The skin tissue samples obtained from the different groups were homogenized with lysis buffer. Protein concentrations in these homogenized samples were measured by the bicinchoninic acid method. For Western blots, protein samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes. Membranes were blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline with 0.1% Tween (TBST) and incubated using the following antibodies: Antibodies against HMGB1, RAGE, TLR4, phospho(p)- phosphatidylinositide 3-kinase (p-PI3K), p-ERK, p-NFκB, cyclooxygenase (COX)2, tumor necrosis factor (TNF)α, TNF receptor (TNFR)1, IL-1β, IL-2Rα, glucose-regulated protein (GRP)78, C/Ebp-homologous protein (CHOP) and cleaved caspase-7. All the antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) or Cell Signaling Technology, Inc. (Danvers, MA, USA) and used at a dilution of 1:1000. After washing three times with TBST, the membranes were incubated with appropriate horseradish-peroxidase (HRP) conjugated secondary antibodies for 1 h at room temperature. Further, the membranes were washed three times with TBST and then developed using a chemiluminescence detection system (Amersham Biosciences, Buckinghamshire, UK). The blots were scanned and the signals were quantified with densitometric analysis using Image Studio Digits ver. 4 (Superior Street, Lincoln, Nebraska, USA). Antibodies to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as control proteins.

2.8. Statistical analysis

Data are presented as mean ± standard error of mean (SEM) and were analyzed using one way analysis of variance (ANOVA) followed by Tukey multiple comparison test or two-tailed "t"-test when appropriate. A value of $p < 0.05$ was considered statistically significant. For statistical analysis, GraphPad Prism 5 software (San Diego, CA) was used.

3. Results

3.1. Effects of resveratrol on dermatitis score and body weight

The AD-like skin lesions induced by cutaneous application of DfE cream resulted in immediate itching, erythema, and hemorrhage on the ear and back that was followed by edema, superficial erosion,