Suppression of Transglutaminase-2 is Involved in Anti-Inflammatory Actions of Glucosamine in 12-O-Tetradecanoylphorbol-13-Acetate-Induced Skin Inflammation

Mi Kyung Park¹, Sun A Cho², Hye Ja Lee¹, Eun Ji Lee¹, June Hee Kang¹, You Lee Kim¹, Hyun Ji Kim¹, Seung Hyun Oh³, Changsun Choi³, Ho Lee³, Soo Youl Kim⁵ and Chang Hoon Lee¹,*

¹College of Pharmacy, Dongguk University, Goyang 410-820,
²R & D Center, AmorePacific Corporation, Yongin 449-729,
³College of Pharmacy, Gachon University of Medicine and Science, Incheon 406-840,
⁴Department of Food and Nutrition, College of Human Ecology, Chung-Ang University, Ansan 456-756,
⁵National Cancer Center, Goyang 410-769, Republic of Korea

Abstract

Glucosamine (GS) is well known for the treatment of inflammation. However, the mechanism and efficacy of GS for skin inflammation are unclear. The aim of this study was to evaluate the effects and mechanism of GS in the mouse 12-O-tetradecanoyl 13-acetate (TPA)-induced ear edema model. TPA-induced ear edema was evoked in ICR or transglutaminase 2 (Tgase-2) (-/-) mice. GS was administered orally (10-100 mg/kg) or topically (0.5-2.0 w/v %) prior to TPA treatment. Orally administered GS at 10 mg/kg showed a 76 or 57% reduction in ear weight or myeloperoxidase, respectively, and a decreased expression of cyclooxygenase-2 (COX-2), NF-κB and Tgase-2 in TPA-induced ear edema by western blot and immunohistochemistry. Role of Tgase-2 in TPA ear edema is examined using Tgase-2 (-/-) mice and TPA did not induce COX-2 expression in ear of Tgase-2 (-/-) mice. These observations suggested that Tgase-2 is involved in TPA-induced COX-2 expression in the inflamed ear of mice and anti-inflammatory effects of glucosamine is mediated through suppression of Tgase-2 in TPA ear edema.

Key Words: Glucosamine, TPA-induced ear edema, Transglutaminase-2, Cyclooxygenase-2, NF-κB, Tgase-2 (-/-) mice

INTRODUCTION

Glucosamine (GS), 2-amino-2-deoxy-D-glucose, is an amino monosaccharide that is one of the essential components of mucopolysaccharides and chitin. Glycosaminoglycans are components of connective tissue, skin, tendons, ligaments and cartilage. GS is readily synthesized in the body from glucose. Given the high concentration in joint tissues, the hypothesis that GS supplements would relieve the symptoms of osteoarthritis (OA) was developed more than 30 years ago (D’Ambrosio et al., 1981). These effects of GS were shown in carrageenan- and cotton pellet-induced acute and subacute inflammation in rats at 25 mg/kg dose (Kim et al., 2005). GS at 250 mg/kg showed a mild effect in carrageenan-induced edema and moderate inhibition of paw swelling against developing arthritis (Singh et al., 2007).

Recently, glucosamine showed positive effects in atopic dermatitis-like skin lesions in NC/Nga mice via inhibition of Th2 cell development (Kim et al., 2011a). Combination treatments of glucosamine with FK-506 also produces a beneficial effects in atopic dermatitis-like skin lesions in NC/Nga mice (Kim et al., 2012).

GS was also tested as a constituent of a new anti-inflammatory formulation (SAG) in a 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema model. SAG dose-depently inhibited the edematic responses of arachidonic acid (AA)- and TPA-induced ear edema in mice (Choi et al., 2005). But, in this case, efficacy of glucosamine by itself was not shown in TPA-induced ear edema and detailed histological studies on the effects of glucosamine were not done.

TPA promotes skin carcinogenesis via inflammatory responses and TPA-induced inflammatory responses are related with induction of pro-inflammatory cytokines, cyclooxygenase-2, reactive oxygen species and NF-κB (Chung et al., 2007; Song et al., 2008).

Recently, glucosamine was reported to act as a chemo-
sensitizer via inhibition of transglutaminase-2 (Tgase-2) in doxorubicin-resistant MCF7 cells (Kim et al., 2009; Jeong et al., 2010).

Tgase-2 is a multifunctional protein with both intracellular and extracellular functions. In addition to catalyzing Ca²⁺-dependent transamidation reactions (Lorand and Graham, 2003; Mehta, 2005; Lee and Kim, 2009), it can bind and hydrolyze GTP/GDP with a similar affinity and catalytic rate to the α subunit of large heterotrimeric G proteins and small Ras-type G proteins (Mhaouty-Kodja, 2004). Tgase-2 can activate NF-κB via polymerization of I-κB (Lee et al., 2004). But role of Tgase-2 is not known in TPA-induced skin inflammation.

Therefore, we were interested in the effect and related mechanism of glucosamine on Tgase-2 in TPA-induced ear edema. To our knowledge, the role of Tgase-2 in TPA ear edema has not been reported yet.

In this report, we evaluated the efficacy of glucosamine in a TPA-induced dermatitis model and found that Tgase-2 expression suppressed by glucosamine is involved in the anti-inflammatory action of glucosamine by TPA-induced inflammation.

**MATERIALS AND METHODS**

**Materials**

Primary antibodies purchased: (1) rabbit polyclonal murine COX-2 antibody (Cayman Chemical, Ann Arbor, MI, USA), (2) rabbit polyclonal anti-NF-κB p65 antibody (Novus biological, Littleton, CO, USA), (3) polyclonal anti-actin antibody (Santa Cruz, CA, USA).

**Animals**

Male ICR mice (Orientbio, Seoul, Korea), 7 weeks old were used in this experiment. They were acclimatized in the animal room at least 1 week prior to use. Throughout the experimental period, animals had free access to water and a commercial diet. The mice were randomly assigned into groups consisting of five animals per group, and were fasted overnight prior to experimentation. Tgase-2 knockout mice (C57BL/6) used in this experiment were established by Dr. Ho Lee (Kim et al., 2010). The experiments were conducted under the guidelines for the care and use of experimental animals of the Korea Association for Laboratory Animal Science.

**12-O-tetradecanoylphorbol 13-acetate (TPA)-induced mouse ear edema**

Edema was induced on the right ear by topical application of 20 μl of 12-O-tetradecanoylphorbol 13-acetate (TPA; Sigma, St. Louis, USA) in acetone (2.5 μg/ear) with a micropipette (De Young et al., 1989; Kim et al., 2011b). To evaluate the inflammatory effects of TPA, ear lobe samples were collected using a 6 mm biopsy punch, weighed and measured with a pipette (De Young et al., 1989; Kim et al., 2011b).

For histological analysis, ear tissue was fixed in 10% neutral buffered formaldehyde and embedded in paraffin wax according to standard methods. Sections were stained with hematoxylin and eosin.

**Immunohistochemistry**

Immunohistochemistry was performed by standard ABC technique. Tissue sections were deparaffinized in xylene and rehydrated in graded alcohols. Antigenic retrieval was performed by pressure cooking in 10 mM citric acid buffer (pH 6.0). Hydrogen peroxide (3%) was used to quench endogenous peroxidase activity for 10 min. For blocking buffer, 10% normal goat serum was used for 30 min. Sections were then incubated with primary antibody for 1 hour at room temperature. Biotinylated goat anti-rabbit IgG antibody and ABC solution (Vector Laboratories, Burlingame, CA, USA) were applied sequentially. Diaminobenzidine (DAB) was used to visualize a positive signal. Immunostained sections were lightly counterstained in hematoxylin according to the manufacturer’s instructions, dehydrated in graded ethanol, cleared in xylene and mounted with a coverslip using Canada balsam (Junsei Chemical, Tokyo, Japan).

**Statistics**

All data are presented as means and S.D. Statistical significance was analyzed using a student’s t-test; \( p<0.05 \) was