

Modulation of HMGB1 translocation and RAGE/NF κ B cascade by quercetin treatment mitigates atopic dermatitis in NC/Nga transgenic mice

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Abstract: Quercetin, glycosylated form of flavonoid compound, has potent antioxidant and anti-inflammatory properties. In this study, we have investigated the effects of quercetin on skin lesion, high-mobility group box (HMGB)1 cascade signalling and inflammation in 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 transgenic mouse. After AD induction, quercetin (50 mg/kg, p.o) was administered daily for 2 weeks. We evaluated dermatitis severity, histopathological changes 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) κ B, nuclear factor erythroid-2-related factor (Nrf2), kelch-like ECH-associated protein (Keap)1, extracellular signal-regulated kinase (ERK)1/2, cyclooxygenase (COX)2, tumor necrosis factor (TNF) α , interleukin (IL)-1 β , IL-2R α and other inflammatory markers in the skin of AD mice. In addition, serum levels of T helper (Th)

cytokines (interferon (IFN) γ , IL-4) were measured by enzyme-linked immunosorbent assay. Quercetin treatment attenuated the development of AD-like skin lesions. Histological analysis showed that quercetin inhibited hyperkeratosis, parakeratosis, acanthosis, mast cells and infiltration of inflammatory cells. Furthermore, quercetin treatment downregulated cytoplasmic HMGB1, RAGE, nuclear p-NF κ B, p-ERK1/2, COX2, TNF α , IL-1 β , IL-2R α , IFN γ and IL-4 and upregulated nuclear Nrf2. Our data demonstrated that the HMGB1/RAGE/NF κ B signalling might play an important role in skin inflammation, and quercetin treatment could be a promising agent for AD by modulating the HMGB1/RAGE/NF κ B signalling and induction of Nrf2 protein.

Key words: cytokine – high-mobility group box protein 1 – nuclear factor kappa B – quercetin – receptor for advanced glycation products

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Introduction

Atopic dermatitis (AD) is an inflammatory skin disease caused by environmental, psychologic, immunologic, pharmacologic and genetic factors (1). This is a major problem in developed and developing countries. Around 50% of all those with AD develop symptoms within their first year of life, and 95% of below 5-year-old child experience in AD symptoms. Severe AD may cause 50% risk of developing bronchial asthma and 75% risk of hay fever in children (2). AD skin lesions are characterized by the presence of infiltrated inflammatory cells such as monocytes, eosinophils, macrophages and mast cells. Around 50–70% of all children with early onset of AD are sensitized to allergens such as food and house dust mite allergens (3). Extensive experimental results have shown that exposure of the skin to house dust mite *Dermaphagoides farinae* (DfE) induces an immune dysregulation in NC/Nga mouse, encompassing a strong inflammatory reaction resembling severe AD, and is one of the most potent and frequently used AD model (4).

The high-mobility group (HMGB)1 protein was identified as a non-histone nuclear protein that can regulate transcription by

changing deoxyribonucleic acid (DNA) helical structure and was secreted by macrophages and released from damaged or necrotic tissues. Extracellular HMGB1 acts as an endogenous danger signal and binds to toll-like receptor (TLR)2, TLR4, receptor for advanced glycation end products (RAGE) and chemokine receptors (5). Receptor binding leads to the activation of mitogen-activated protein kinase (MAPK) and nuclear factor (NF) κ B signalling pathways, which mediate the production of a variety of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) α , interleukin (IL)-4, IL-1 β , IL-6, IL-8 and interferon (IFN) γ (6). Recent studies suggest that the elevated levels of extracellular HMGB1 have been implicated in several disease states, including sepsis, and autoimmune disease such as arthritis, ischaemia-reperfusion injury, cancer, psoriasis vulgaris and AD (7).

Flavonoid quercetin is found in most edible fruits and vegetables, specifically in apples, nuts, herbs, onions and red wine. Flavonoids have various biological activities, which are mainly related to their abilities to inhibit enzymes and their effects on immune responses (8). Pharmacological effects of quercetin are well known, such as antioxidant, anti-inflammatory, antiallergic, chemopreventive

activities, regulating IL-6, IL-8, TNF α , histamine and tryptase release in mast cells (9,10). Recent studies suggest that the polyphenolic compound quercetin can activate nuclear factor erythroid 2 related factor (Nrf2) protein and antioxidant responsive element-dependent gene expression (11). Moreover, the quercetin has a documented anti-inflammatory effect as well as balancing T helper cells (Th)1 and Th2 responses in inflammatory human dermal fibroblasts (12). On the basis of these studies, we hypothesized that quercetin ameliorates skin inflammation and attenuates the release and pro-inflammatory function of HMGB1 in AD model.

Materials and methods

Materials

Biostir AD, a cream containing DfE extract (DfE cream), was purchased from Biostir, Inc. (Kobe, Japan). All the reagents and chemicals were of analytical grade and purchased from either Sigma or Wako, Tokyo, Japan, until mentioned otherwise. Quercetin pure drug was purchased from Sigma-Aldrich (Tokyo, Japan).

Experimental design

Specific pathogen-free female 6-week-old NC/Nga mice were obtained from Charles River, Yokohama, Japan. The animals were maintained in controlled room (temperature $23 \pm 2^\circ\text{C}$, 12-h light cycle). After 1 week, the mice were randomly divided into three groups: untreated group – received vehicle (1% methyl cellulose) (Normal, $n = 5$); DfE cream-treated mice (100 mg/mouse) were divided into two groups and each received vehicle (1% methyl cellulose) (AD, $n = 5$) or quercetin (50 mg/kg/day, per oral) (AD+quercetin, $n = 5$) and were allowed free access to drinking water and standard laboratory diet. The mice were weighed once a week, and food intake was estimated every second day.

Induction of AD

Atopic dermatitis-like skin lesions were induced in NC/Nga mice using DfE cream, as we described previously (13). Briefly, 150 μl of 4% sodium dodecyl sulphate was applied to the shaved dorsal skin and ears. After 3 h, 100 mg of DfE cream was applied topically. This procedure was carried out twice weekly for 2 weeks. Quercetin (50 mg/kg/day, per oral) treatment (14) was started after AD induction (2 weeks from the start of the experiment) and continued for 2 weeks.

Evaluation of dermatitis severity

The dermatitis severity was measured every week according to the eczema area and severity index scoring using the following procedure: 0, no; 1, mild; 2, moderate; 3, severe symptoms were given based on following manifestation: erythema/haemorrhage, oedema, excoriation/erosion and dryness/scaling (13).

Histopathological studies

By the end of the study period, mice were sacrificed and their skin tissues were harvested for semi-quantitative immunoblotting and immunohistochemical studies. The 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 haematoxylin 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 (13).

Cytokine assay

By the end of the study period, blood specimens were collected from the retro-orbital sinus of every animal using a capillary tube.

Serum levels of mouse IL-4 and IFN γ were measured by enzyme-linked immunosorbent assay (ELISA) kits provided by thermoscientific Inc. (Meridian Rd, Rockford, IL USA) according to the manufacture's protocol.

Homogenization and fractionation of skin tissue

Briefly, 100 mg of skin was homogenized in 0.5 ml of buffer A (10 mM HEPES, pH 7.8, 10 mM KCl, 2 mM MgCl₂, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, 1 μM pepstatin and 1 mM p-aminobenzamidine) using a tissue homogenizer for 20 s and kept on ice for 15 min, and then, 125 μl of 10% Triton X-100 solution was added and mixed for 15 s. The mixture was centrifuged for 2 min at 13 000 g, and the supernatant containing cytosolic proteins was collected. The pelleted nuclei were washed once with 200 μl of 10% Triton X-100, centrifuged and then suspended in 50 μl of buffer B (50 mM HEPES, pH 7.8, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF and 10% v/v glycerol). The solution was mixed for 20 min and centrifuged for 5 min at 12 000 rpm. The supernatant containing nuclear proteins was stored at -80°C . The protein concentration of the resulting solutions was determined by the bicinchoninic acid method (15).

Protein analysis by western blotting

Protein samples were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes (16). 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, nuclear phospho (p)-NF κB , cyclooxygenase (COX)2, TNF α , TNF receptor (TNFR)1, TNFR2, IL-1 β , IL-2R α , p-extracellular signal-regulated kinases (ERK)1/2, Nrf2 and kelch-like ECH-associated protein (Keap)1. 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 for 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 Scion Image program (Epson GT-X700, Tokyo, Japan) or Image Studio Digits ver. 4 (Lincoln, NE, USA). Antibodies to Lamin A and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as control proteins for nuclear and cytosolic proteins, respectively.

Immunofluorescence determination of HMGB1

Formalin-fixed, paraffin-embedded skin tissue sections were used for immunofluorescence staining. Sections underwent microwave antigen retrieval were blocked with 10% goat serum in phosphate-buffered saline (PBS) and incubated with mouse polyclonal anti-HMGB1 antibody (Santa Cruz Biotechnology). Binding sites of the primary antibody were revealed with texas red-conjugated anti-mouse secondary antibody (Abcam Ltd., Cambridge, UK). Sections were visualized with a fluorescence microscope at 100 $\times$ magnification (AxioVs40v4.8.2; Carl Zeiss, Tokyo, Japan) (17).

Statistical analysis

Data are presented as mean $\pm$ standard error of mean (SEM) and were analysed using one-way analysis of variance (ANOVA) followed by Tukey's 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 (GraphPad Software, Inc., San Diego, CA, USA) was used.

Results

Effects of quercetin on AD severity

In the AD and quercetin treatment groups of NC/Nga mice, clinical signs and symptoms of dermatitis developed significantly during the 2 weeks of DfE cream application. During the course of treatment, the dermatitis score of the quercetin treatment group decreased and became significantly different from that of the group AD mice (Fig. 1a). In addition, we have measured the body weight of each mouse and its food intake, but no significant changes were observed in normal, AD and AD+quercetin group (data not shown).

Effects of quercetin on histopathological changes in DfE-induced AD

HE and toluidine blue staining of skin sections revealed hyperkeratosis, parakeratosis, acanthosis/spongiosis and infiltration of inflammatory cells and mast cells into the skin of AD mice. In contrast, quercetin-treated mice showed the reductions in these dermal changes compared with AD mice (Fig. 1b,c,c1).

Quercetin attenuated HMGB1 translocation in DfE-induced AD

Extracellular HMGB1 acts as pro-inflammatory cytokine and involved in many types of inflammatory diseases (18). To examine the effects of quercetin on HMGB1 translocation from nucleus to cytoplasm in the AD skin, we analysed the HMGB1 distribution in the skin tissues by immunofluorescence and Western blot. Immunofluorescence examination showed that HMGB1 staining was increased in DfE-induced AD mice. However, this overexpression was decreased by the treatment with quercetin (Fig. 1d). Data obtained from Western blot (Fig. 2a) were consistent with the immunofluorescence observation. AD mice showed significantly increased cytoplasmic levels of HMGB1 in the skin tissue. In contrast, quercetin treatment significantly inhibited the HMGB1 translocation from nucleus to cytoplasm in the AD skin in response to fulminating skin lesions.

Effect of quercetin on RAGE, TLR4, p-ERK1/2 and nuclear p-NFκB in DfE-induced AD mice

Binding of HMGB1 to RAGE, TLR2 and TLR4 activates cell migration-dependent pathways through NFκB and MAPK signalling (5). So, we performed Western blot analysis to measure the protein expressions of RAGE, TLR4, ERK1/2 and nuclear NFκB in the skin homogenates. Except TLR4, the protein levels of RAGE, p-ERK1/2 and nuclear p-NFκB were significantly increased in AD mice compared with those in normal mice and these changes were significantly attenuated by quercetin treatment (Fig. 2b–e).

Quercetin attenuates cytokine activities of HMGB1 in AD

Extracellular HMGB1 can activate several inflammatory cytokines related to AD. Skin TNFα, TNFR1, TNFR2, IL-1β, IL-2Rα and COX2 protein levels assessed by Western blotting were significantly upregulated in AD mice compared with those in normal mice, whereas treatment with quercetin significantly downregulated these cytokines in AD mice (Fig. 3a–f). In addition, the serum levels of Th1- and Th2-related cytokines (IFNγ, IL-4) were significantly decreased by quercetin treatment, as compared to that of AD mice (Fig. 4a,b).

Effects of quercetin on nuclear Nrf2 and cytoplasmic Keap1 expression

Western blotting analysis showed that the nuclear Nrf2 protein expression was significantly downregulated without significant change in the Keap1 expression in the skin of AD mice, but

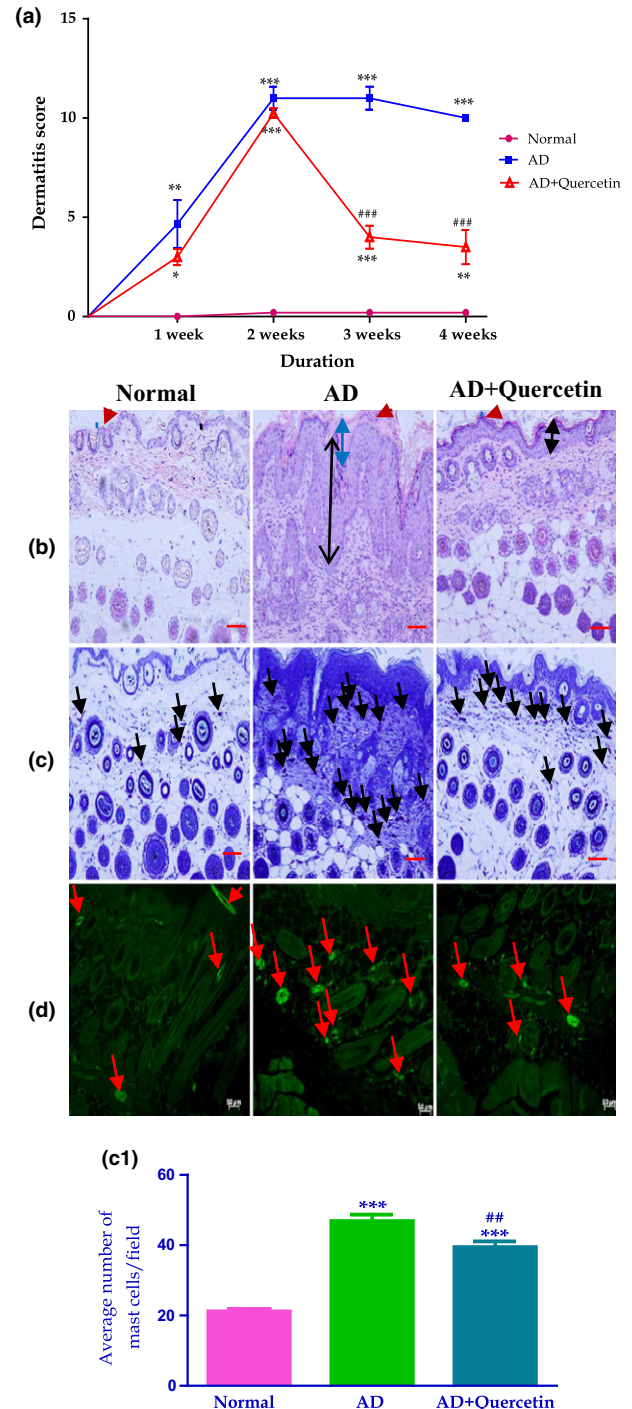


Figure 1. (a) Effect of quercetin on time-dependent dermatitis score of NC/Nga mice with atopic dermatitis (AD)-like skin lesions. (b) HE staining of the cross-sectional tissue slices of skin showing hyperkeratosis (blue arrow), parakeratosis (red arrow), acanthosis (black arrow), spongiosis (black arrow) and infiltration of inflammatory cells (200×). (c) Skin levels of mast cells (black arrow) by toluidine blue staining. (c1) Quantification of mast cells per field at 200× magnification. (d) Immunofluorescence determination of HMGB1 positive cells (red arrow), (100×). Each bar represents mean ± SEM. Normal, age-matched normal NC/Nga mice; AD, AD-induced NC/Nga mice; AD+quercetin, AD-induced mice administered with quercetin (50 mg/kg/day, per oral) (From 2 weeks to 4 weeks). *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$ vs Normal; ### $P < 0.001$ and ## $P < 0.01$ vs AD.

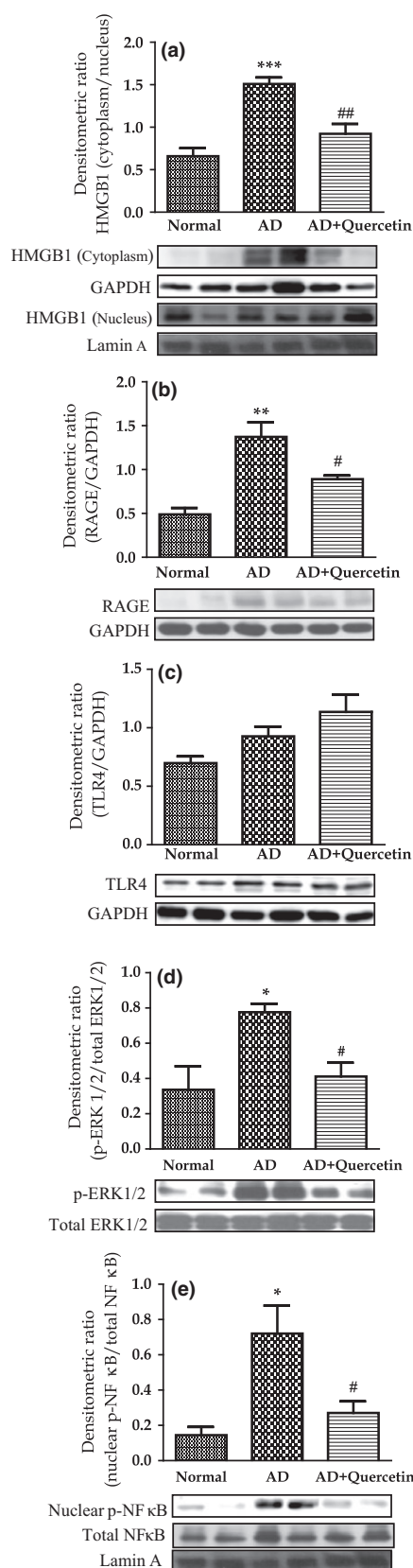


Figure 2. Quercetin effects on nuclear and cytoplasmic HMGB1, RAGE, TLR4, p-ERK1/2 and nuclear p-NFκB. Western blots show specific bands for (a) expression of nuclear and cytoplasmic HMGB1, expressed as a ratio relative to that of Lamin A and GAPDH, ratio of HMGB1 expression in cytoplasmic fraction to nuclear fraction was measured by relative optical density quantification, (b) expression of RAGE, expressed as a ratio relative to that of GAPDH, (c) expression of TLR4, expressed as a ratio relative to that of GAPDH, (d) expression of p-ERK1/2, expressed as a ratio relative to that of ERK1/2, (e) expression of nuclear p-NFκB, expressed as a ratio relative to that of NFκB. Each bar represents mean $\pm$ SEM. Normal, age-matched normal NC/Nga mice; AD, AD-induced NC/Nga mice; AD+quercetin, AD-induced mice administered with quercetin. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$ vs Normal; ## $P < 0.01$ and # $P < 0.05$ vs AD.

quercetin treatment has significantly increased the Nrf2 expression along with the reduced expression of Keap1 when compared with AD mice (Fig. 4c,d).

Discussion

In our study, the most important findings were as follows: (i) Quercetin treatment sharply suppresses skin HMGB1 release, RAGE cascade and inflammatory cytokines levels in DfE-induced AD in NC/Nga mice. (ii) Quercetin activates antioxidant responsive element-dependent gene expression by increasing nuclear Nrf2 in AD skin. (iii) As a result, quercetin attenuates AD symptoms, hyperkeratosis, parakeratosis, spongiosis, mast cells and infiltration of inflammatory cells.

Accumulating evidence indicates that quercetin (3,5,7,3',4'-pentahydroxy flavone), a polyphenol natural product, most widely distributed flavonoid, acts on a wide range of molecular targets controlling pro-inflammatory function and has anti-allergic and anticarcinogenic activities (9). In addition, quercetin prevented the formation of skin lesions abrogating various biochemical processes that cause epithelial loss and skin damage (19). AD mice received quercetin treatment showed that quercetin has anti-inflammatory action via suppressing angiogenesis and Th2-related cytokine expression (12). Quercetin also inhibits IL-6, histamine release and TNF production in murine mast cells (20). It has also been shown that quercetin attenuates lipopolysaccharide (LPS)-induced HMGB1 release and TNFα expression in sepsis mice (21). Furthermore, quercetin increases the Nrf2 levels and its downstream target along with the suppression of NFκB and MAPK expressions in mouse JB6 cells (22). Although several study data strongly suggest that quercetin could regulate pro-inflammatory cytokines levels and their function, the potential mechanism of quercetin in regulating HMGB1 release and RAGE cascade in AD has not been studied. In the present study, we show for the first time that quercetin protects skin inflammation in DfE-triggered AD in NC/Nga mice through the activation of Nrf2 and suppresses HMGB1 release and cytokines expression.

HMGB1 is expressed in almost all cells and is an evolutionarily conserved nuclear protein (5). Once HMGB1 released from cell into extracellular space, it acts as a damage-associated molecular pattern (DAMP) or alarmin (23). It is mainly secreted from immune cells such as dendritic cells (DC), monocytes, macrophages and damaged or necrotic cells (24). In addition to the role as a nuclear protein, HMGB1 is participating in lethal systemic inflammation, arthritis, meningitis, local inflammation, psoriasis and AD (13). We have observed in the present study that quercetin treatment significantly attenuates HMGB1 translocation in the AD skin. How quercetin modulated HMGB1 translocation in the skin during AD is unclear. Extracellular HMGB1 can bind with RAGE, TLR2 and TLR4 receptors, and also, there is a possibility that

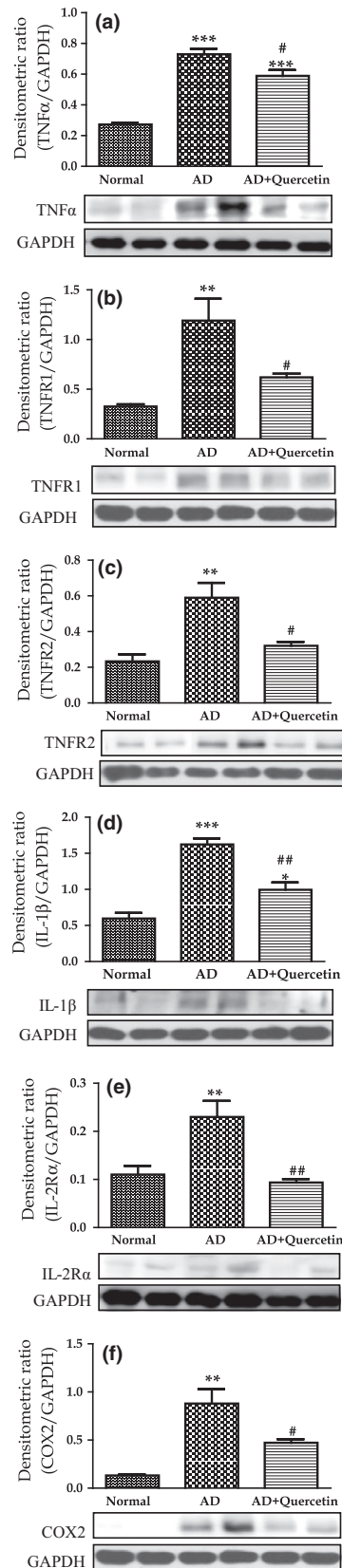


Figure 3. Effects of quercetin on TNF α , TNFR1, TNFR2, IL-1 β , IL-2R α and COX2. (a–f) Densitometric data of protein analysis. The mean density values of TNF α , TNFR1, TNFR2, IL-1 β , IL-2R α and COX2 (expressed as a ratio relative to that of GAPDH) with representative Western blots showing specific bands for TNF α , TNFR1, TNFR2, IL-1 β , IL-2R α and COX2. GAPDH was used as an internal control. Each bar represents mean $\pm$ SEM. Normal, age-matched normal NC/Nga mice; AD, AD-induced NC/Nga mice; AD+quercetin, AD-induced mice administered with quercetin. *** P < 0.001, ** P < 0.01 and * P < 0.05 vs Normal; ## P < 0.01 and # P < 0.05 vs AD.

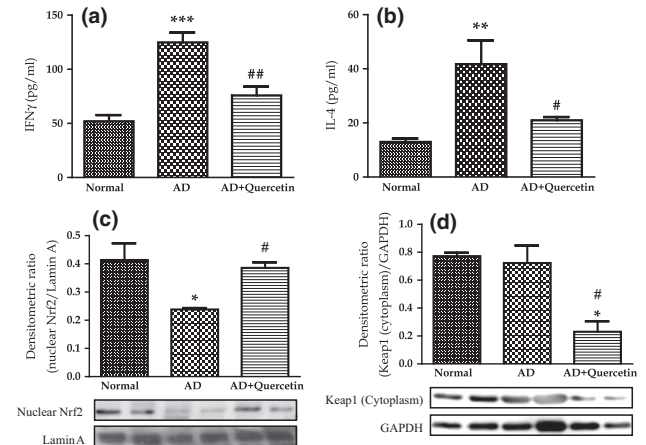


Figure 4. Effects of quercetin on IFN γ , IL-4, nuclear Nrf2 and cytoplasmic Keap1. The serum concentration of (a) IFN γ and (b) IL-4 in collected serum on day 28 was determined by ELISA. (c, d) Densitometric data of protein analysis. The mean density values of nuclear Nrf2 and cytoplasmic Keap1 (expressed as a ratio relative to that of Lamin A and GAPDH) with representative Western blots showing specific bands for Nrf2 and Keap1. Lamin A and GAPDH were used as an internal control. Each bar represents mean $\pm$ SEM. Normal, age-matched normal NC/Nga mice; AD, AD-induced NC/Nga mice; AD+quercetin, AD-induced mice administered with quercetin. *** P < 0.001, ** P < 0.01 and * P < 0.05 vs Normal; ## P < 0.01 and # P < 0.05 vs AD.

different regions in HMGB1 may interact with different receptors (25). Several studies demonstrated that the TLR4 interacts with extracellular HMGB1 to activate NF κ B pathway (26). TLR4 is regulated by pattern recognition receptors and prominently expressed in macrophages, DC and antigen-presenting cells (27). Other studies showed that RAGE is the primary binding receptor for HMGB1 and it mediates cytokine activity; interaction of HMGB1-RAGE is involved in inflammation and oxidative stress in endothelial dysfunction (28). Interestingly, in our study, quercetin modulated the RAGE signalling pathway, but did not affect the TLR4 signalling pathway in the skin of AD mice. It is plausible to speculate that HMGB1 might have interacted with RAGE to produce inflammation in AD mice.

Extracellular HMGB1 interaction with RAGE activates MAPK-NF κ B signalling, and it has an important role in inflammation (29). A recent report suggests that MAPK signalling is involved in inflammatory skin disease and inhibitors of this pathway ameliorate inflammation in various rodent models of human skin diseases (30). However, another similar study reported that in endothelial cells, ERK1/2 and NF κ B activations were suppressed by ERK1/2 inhibitor (18). Similar scenario has been described for NF κ B, and activation of RAGE can induce downstream signalling such as NF κ B expression in mouse inflamed skin (31). Moreover, quercetin treatment attenuates NF κ B phosphorylation in bone marrow-derived

macrophage (32). Here, we observed that quercetin treatment significantly attenuated the phosphorylation of ERK1/2 and nuclear NF κ B pathway in AD skin. This finding may be explained by HMGB1 activation, which induces the activation of ERK1/2 and NF κ B pathway, which were ameliorated by quercetin treatment.

Diverse cytokines are regulated by transcription factors, such as NF κ B, which is the centre of interest and promotes the transcription of Th2 (IL-4) and Th1 (IFN γ) cytokines (33). In addition, NF κ B activates potent pro-inflammatory cytokine TNF α , which can stimulate the secretion of other inflammatory cytokines, such as IL-1 β , IFN γ , IL-4 and secondary inflammatory mediators, including leucotriene and prostaglandins (34,35). In the present study, we demonstrated that the AD-induced upregulation of TNF α , TNFR1, TNFR2, IL-1 β , IL-4, IFN γ , IL-2R α and COX2 was inhibited by treatment with quercetin. Given the importance of these pro-inflammatory cytokines in AD, our present data suggest that quercetin attenuates inflammatory cytokines responses in inflammatory skin disease such as AD.

Nrf2, a member of the cap-N-collar family, is the major transcriptional regulator of antioxidant response element (ARE) sequence and detoxifying antioxidant enzymes (36). Nrf2 acts when released from its repressive cytosolic protein Keap1. Upon exposure of cells to oxidative stress or covalently transform thiol groups or electrophilic compounds, Nrf2 easily translocates from cytoplasmic Keap1 to nucleus, binds to ARE and transactivates the genes of detoxifying and antioxidant enzymes against oxidative stress and associated inflammation and damage (37). Activation of Nrf2 attenuates NF κ B signalling in cultured human keratinocytes (38). On other hand, the Nrf2-deficient mice exhibit greater induction of pro-inflammatory genes regulated by NF κ B such as IL-1 β , TNF α and COX2 pointing towards the fact that Nrf2 deficiency enhances NF κ B-mediated pro-inflammatory reaction (39). Moreover, some experimental studies also revealed the Nrf2–ARE pathway interaction with the inhibitory regulation of HMGB1 secretion from macrophages/monocytes cells (40). However, in another similar study,

treatment with quercetin activates Nrf2 levels and prevents the activation of MAPKs and NF κ B pathway (22). Here, we demonstrate that quercetin modulates nuclear Nrf2 levels in DfE-induced AD mice model. These results indicate that a possible mechanism behind the antioxidant and anti-inflammatory actions of quercetin may be via modulation of detoxifying enzymes.

In conclusion, our results clearly demonstrate that quercetin treatment in NC/Nga mice attenuates DfE-induced AD-like clinical symptoms. Furthermore, the quercetin effectively inhibits HMGB1 release, RAGE, as well as NF κ B and inflammatory cytokines such as IL-4, IFN γ , IL-1 β and TNF α , which may be possibly via the induction of Nrf2 signalling pathways in this model. Our findings suggest that quercetin may be an effective therapeutic agent that can be used to reduce the development of AD.

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Author contributions

VK, SA and KW involved in the study conception and design; VK and SA performed experiments and drafted the manuscript; VP, RS and RA performed cytokine assay and immunofluorescence staining; MH, MN1 and SM analysed and interpreted the data; RAT, SA, MN2 and KW revised the article, critically for important intellectual content; KS and HS carried out fluorescence microscopy and advised on inflammation signalling; All the authors, approved the final version to be published. All authors discussed the results and implication and commented on the manuscript at all stages.

Ethics approval for animal care

All experiments with mice were performed in accordance with the national guidelines and approved by the animal care committee of Niigata University of Pharmacy and Applied Life Sciences (Approval No. H26-03), Niigata, Japan.

Conflict of interest

The authors have declared no conflicting interests.

References

- Udompatikul M, Limpit-o-vart D. *J Drugs Dermatol* 2012; **11**: 366–374.
- Thomsen S F. *ISRN Allergy* 2014; **2014**: 1–7
- Spergel J M. *Ann Allergy Asthma Immunol* 2010; **105**: 99–106.
- Lim H S, Seo C S, Ha H *et al*. *Evid Based Complement Alternat Med* 2012; **2012**: 705167.
- Schaper F, Westra J, Bijl M. *Mol Med* 2014; **20**: 72–79.
- Voll R E, Urbonaviciute V, Herrmann M *et al*. *Isr Med Assoc J* 2008; **10**: 26–28.
- He Q, You H, Li X M *et al*. *Asian Pac J Cancer Prev* 2012; **13**: 1365–1370.
- Hollman P C, De Vries J H, van Leeuwen S D *et al*. *Am J Clin Nutr* 1995; **62**: 1276–1282.
- Weng Z, Zhang B, Asadi S *et al*. *PLoS One* 2012; **7**: e33805.
- Ma Z S, Huynh T H, Ng C P *et al*. *Int J Oncol* 2004; **24**: 1297–1304.
- Miyamoto N, Izumi H, Miyamoto R *et al*. *Invest Ophthalmol Vis Sci* 2011; **52**: 1055–1063.
- Jung M K, Hur D Y, Song S B *et al*. *J Invest Dermatol* 2010; **130**: 1459–1463.
- Karuppagounder V, Arumugam S, Thandavarayan R A *et al*. *Int Immunopharmacol* 2014; **23**: 617–623.
- Marcolin E, San-Miguel B, Vallejo D *et al*. *J Nutr* 2012; **142**: 1821–1828.
- Soetikno V, Sari F R, Lakshmanan A P *et al*. *Mol Nutr Food Res* 2013; **57**: 1649–1659.
- Arumugam S, Sreedhar R, Miyashita S *et al*. *Exp Mol Pathol* 2014; **97**: 137–143.
- Thandavarayan R A, Watanabe K, Sari F R *et al*. *Free Radic Biol Med* 2010; **49**: 1422–1431.
- Feng L, Zhu M, Zhang M *et al*. *Int Immunopharmacol* 2013; **15**: 206–216.
- Castangia I, Nácher A, Caddeo C *et al*. *Acta Biomater* 2014; **10**: 1292–1300.
- Kimata M, Shichijo M, Miura T *et al*. *Clin Exp Allergy* 2000; **30**: 501–508.
- Tang D, Kang R, Xiao W *et al*. *Am J Respir Cell Mol Biol* 2009; **41**: 651–660.
- Ding M, Zhao J, Bowman L *et al*. *Int J Oncol* 2010; **36**: 59–67.
- Kazama H, Ricci J E, Herndon J M *et al*. *Immunoty* 2008; **29**: 21–32.
- Lotze M T, Tracey K J. *Nat Rev Immunol* 2005; **5**: 331–342.
- Huang W, Tang Y, Li L. *Cytokine* 2010; **51**: 119–126.
- Tadie J M, Bae H B, Deshane J S *et al*. *Mol Med* 2012; **18**: 659–668.
- Weng H, Deng Y, Xie Y *et al*. *BMC Cancer* 2013; **13**: 311.
- Park J S, Svetkauskaite D, He Q *et al*. *J Biol Chem* 2004; **279**: 7370–7377.
- Nogueira-Machado J A, Volpe C M, Veloso C A *et al*. *Expert Opin Ther Targets* 2011; **15**: 1023–1035.
- Ipaktchi K, Mattar A, Niederbichler A D *et al*. *Shock* 2006; **26**: 201–209.
- Riehl A, Bauer T, Brors B *et al*. *BMC Genom* 2010; **11**: 537.
- Comalada M, Camuesco D, Sierra S *et al*. *Eur J Immunol* 2005; **35**: 584–592.
- Makarov S S. *Mol Med Today* 2000; **6**: 441–448.
- Croft M. *Nat Rev Immunol* 2009; **9**: 271–285.
- Strozyk E A, Desch A, Poeppelmann B *et al*. *Exp Dermatol* 2014; **23**: 670–676.
- Gounder V K, Arumugam S, Arozal W *et al*. *Int Immunopharmacol* 2013; **18**: 282–289.
- Palsamy P, Subramanian S. *Biochim Biophys Acta* 2011; **12**: 719–731.
- Tsuji G, Takahara M, Uchi H *et al*. *J Invest Dermatol* 2012; **132**: 59–68.
- Li W, Khor T O, Xu C *et al*. *Biochem Pharmacol* 2008; **76**: 1485–1489.
- Kawahara K, Hashiguchi T, Masuda K *et al*. *Int J Mol Med* 2009; **23**: 615–620.