Resveratrol ameliorates 2,4-dinitrofluorobenzene-induced atopic dermatitis-like lesions through effects on the epithelium

Sule Caglayan Sozmen, Meral Karaman, Serap Cilaker Micili, Sakine Isik, Zeynep Arikan Ayyildiz, Alper Bagriyanik, Nevin Uzuner, Ozkan Karaman

**Background:** Resveratrol is a natural polyphenol that exhibits anti-inflammatory effects. The aim of this study was to investigate the effects of resveratrol treatment on epithelium-derived cytokines and epithelial apoptosis in a murine model of atopic dermatitis-like lesions. **Material and methods:** Atopic dermatitis-like lesions were induced in BALB/c mice by repeated application of 2,4-dinitrofluorobenzene to shaved dorsal skin. Twenty-one BALB/c mice were divided into three groups: group I (control), group II (vehicle control), and group III (resveratrol). Systemic resveratrol (30 mg/kg/day) was administered repeatedly during the 6th week of the experiment. After the mice had been sacrificed, skin tissues were examined histologically for epithelial thickness. Epithelial apoptosis (caspase-3) and epithelium-derived cytokines [interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP)] were evaluated immunohistochemically. **Results:** Epithelial thickness and the numbers of IL-25, IL-33, TSLP and caspase-3-positive cells were significantly higher in group II compared to group I mice. There was significant improvement in epithelial thickness in group III compared with group II mice (p<0.05). The numbers of IL-25, IL-33, and TSLP-positive cells in the epithelium were lower in group III than in group II mice (p<0.05). The number of caspase-3-positive cells, as an indicator of apoptosis, in the epithelium was significantly lower in group III than in group II mice (p<0.05). **Conclusion:** Treatment with resveratrol was effective at ameliorating histological changes, inflammation by acting on epithelium-derived cytokines and epithelial apoptosis.
Resveratrol ameliorates 2,4-dinitrofluorobenzene-induced atopic dermatitis-like lesions through effects on the epithelium

Sule Caglayan Sozmen¹, Meral Karaman², Serap Cilaker Micili³, Sakine Isik¹, Zeynep Aryan Ayyildiz¹, Alper Bagriyanik³, Nevin Uzuner⁴, Ozkan Karaman¹

¹Dokuz Eylul University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Allergy and Immunology, Izmir, Turkey
²Dokuz Eylul University Faculty of Medicine, Department of Medical Microbiology, Izmir, Turkey
³Dokuz Eylul University Faculty of Medicine, Department of Histology and Embriology, Izmir, Turkey

Corresponding Author,
Sule Caglayan Sozmen¹

Dokuz Eylul University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Allergy and Immunology, 35340, Inciralti-Izmir

E-mail address: sulecaglayan07@yahoo.com
Resveratrol ameliorates 2,4-dinitrofluorobenzene-induced atopic dermatitis-like lesions through effects on the epithelium

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease primarily affecting children. Its prevalence has been steadily increasing during the last decade in both developed and developing countries (Bieber 2008). Although the etiology of AD remains obscure, interplay among immunological, environmental, and genetic factors leads to its development (Boguniewicz & Leung 2011). However, no current treatment for AD can ameliorate its pathogenesis permanently.

The pathogenesis of AD has not been clearly identified, but most information about its immunological features has been obtained in recent years. It was demonstrated that the epidermal cells in AD are unique in terms of both their barrier and immunological properties. The epidermis of patients with AD exhibits significant barrier disruption and prominent keratinocyte pathology. Keratinocytes are specialized epithelial cells in skin tissue that contribute to the initiation and maintenance of the inflammatory process in AD and are capable of producing, as well as responding to, various inflammatory mediators (Esche et al. 2004). Interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP) are mainly released from keratinocytes and promote the Th2-type immune response (Brandt & Sivaprasad 2011). Dysregulated apoptosis of keratinocytes plays a major role in the pathogenesis of AD by causing spongiosis and intercellular edema, leading to impaired epithelial integrity (Trautmann et al. 2000).

Resveratrol is a naturally occurring polyphenol found in various types of fruits and vegetables, most notably in the skin of red grapes. Several studies indicated that it exerts various pharmacological effects, such as anticancer, antioxidant, antiangiogenic, and anti-inflammatory properties (Harikumar & Aggarwal 2008). In this context, we investigated the effects of resveratrol treatment on keratinocyte-derived cytokines and keratinocyte apoptosis using a murine model of 2,4-dinitrophenylbenzene (DNFB)-induced AD-like lesions.
**Materials and Methods**

**Animals**

Twenty-one 6–8-week-old male BALB/c mice weighing 18–20 g, purchased from the Department of Multidisciplinary Animal Laboratory, Dokuz Eylul University (Izmir, Turkey), were used in this study. The animals were kept in hygienic macrolane cages in air-conditioned rooms under 12-h light/dark cycles for the experiment. Food and water were provided *ad libitum* in a pathogen-free laboratory in the same department. All experimental procedures complied with the requirements of the Dokuz Eylul University Animal Care and Ethics Committee (Registration number:92/2013).

**Induction of dermatitis**

The induction of AD by using DNFB was established based on previous research (Li et al. 2013). DNFB was purchased from Sigma Chemical (St. Louis, MO, USA) and dissolved in a mixture of acetone and olive oil (4:1). AD-like skin lesions were evoked by repeated application of 100 µL of 0.5% DNFB to the shaved backs of mice during the first week for sensitization (Figure 1). After the first week, 100 µL of 0.2% DNFB was applied twice a week for a further 4 weeks. The lesions developed at the end of the 5th week. During the 6th week, DNFB was applied once to maintain inflammation.

**Experimental Schedule**

The 21 BALB/c mice were randomly divided into three groups (n=7 per group), as follows: group I (control), group II (vehicle control), and group III (treatment with resveratrol) (Figure 1).

The acetone and olive oil mixture was applied to shaved back of group I (control) without DNFB in the same manner. Atopic dermatitis –like lesions were induced in Group II (vehicle control) and group III (treatment with resveratrol).

Resveratrol was given to group III (treatment with resveratrol) at a dose of 30 mg/kg/day for 7 days during the 6th week. Resveratrol was administered to each mice after dissolved in 100 µL dimethyl sulfoxide (DMSO) in group III (Lee et al. 2009; Sharma et al. 2014 (Johnson et al. 2011)). Resveratrol was purchased from Sigma-Chemical (St.Louis,MO,USA). Group II (vehicle control group) was treated with 100µl DMSO during the 6th week of experimental procedure Saline (0.9%...
NaCl) was administered to group I (control group) at a dose of 100µl during the 6th week. All drugs were administered via the orogastric route. The mice were weighed at the beginning of the experiment, at the end of the 5th and 6th week. Animals were sacrificed by an overdose of ketamine 24 h after the last drug administration, and dorsal skin samples were obtained for histomorphological analysis.

**Evaluation of dermatitis**

Severity of dermatitis was estimated macroscopically at the end of the 5th and 6th weeks. The following scoring procedure was applied: 0, no symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms. The dermatitis score was described as the sum of the scores for erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness (Hanifin et al. 2001).

**Histomorphological analysis**

Skin samples were placed in buffered formalin for light microscopic evaluation. After fixation, skin samples were embedded in paraffin for light microscopic evaluation and 5-µm serial sections were obtained with a rotary microtome (Leica RM2125; Leica Biosystems, Wetzlar, Germany). The samples were then stained with hematoxylin and eosin. Using these samples, general tissue features were examined and the thickness of the epithelium was measured. Photomicrographs were taken with an Olympus DP70 camera (Olympus, Tokyo, Japan), which was adapted on an Olympus BX51 model microscope (Olympus Optical, Tokyo, Japan). The photomicrographs were taken randomly from five fields of each section. A counting frame was randomly placed four times on the image analyzer system monitor, epithelial thickness was measured (UTHSCA Image Tool for Windows, version 3.0), and the average was taken.

**Immunohistochemical detection**

All sections were incubated in a solution of 3% H₂O₂ for 5 min to inhibit endogenous peroxidase activity and then in normal serum blocking solution. Sections were incubated in a humid chamber for 18 h at 4°C with IL-33 monoclonal antibody at 1:100 (anti-IL-33 mouse monoclonal antibody, NBP1-75516; Novus Biologicals, Littleton, CO, USA), IL-25 monoclonal antibody at 1:100 (anti-IL-25 mouse monoclonal antibody, NBP1-72027; Novus Biologicals), TSLP monoclonal antibody at 1:100 (anti-TSLP mouse monoclonal antibody, NBP1-76754; Novus Biologicals), and anti-caspase-3 antibody at 1:100 (AB3623; Millipore, Billerica, MA, USA). Sections were incubated with biotinylated IgG, followed by streptavidin conjugated to horseradish peroxidase for 15 min, each prepared in accordance with the manufacturer’s instructions (85-9043; Invitrogen...
Corporation, Camarillo, UK). Sections were finally stained with diaminobenzidine (1718096; Roche, Mannheim, Germany), counter-stained with Mayer’s hematoxylin, and analyzed using a light microscope. (Micili et al. 2013).

**Semi-quantification of immunostaining**

For each animal two adjacent sections were taken. Five images per section/animal were evaluated and the average immunoscopying of these images were calculated. Each section was graded by two blinded histologists to maintain consistency of the scoring system. A grading system was used to score the quantity of anti-IL-33, anti-IL-25, anti-TSLP, anti-caspase-3 positive staining in the sections. Semi-quantitative score was defined as follows: mild (+), moderate (++) , strong (+++) and very strong (++++) brown staining. Staining intensity was graded semiquantitatively using H-scores, which were calculated using the following equation: H-score = ΣPi (i + 1), where i was equal to the intensity of immunohistochemical staining with a value of 1-4, and Pi was the percentage of epithelial cells stained with each intensity, varying between 0-100% (Yuksel et al. 2008).

**Statistical analysis**

Values are presented as the mean ± standard deviation (SD). Normality of the distribution was assessed using the Kolmogorov-Smirnov test. The measurements followed a non-normal distribution, therefore non-parametric comparisons were made by the Kruskal–Wallis test. Pairwise comparisons were made using the Mann–Whitney U-test. A p value less than 0.05 was considered significant.

**Results**

**Dermatitis score and body weight**

All mice developed AD-like lesions with repeated DNFB challenge in both group II and III at the end of 5th week. The application of DNFB to the shaved back of mice firstly induced erythema and hemorrhage, then edema, erosion, excoriation, dryness and scaling appeared. Dermatitis scores
were not significantly different between groups. Treatment with resveratrol during 6th week resulted a decreased dermatitis score in group III that is significantly lower compared to group II (Table 1, Figure 2). There was no significant difference between groups in aspect of body weight gain (data not shown).

**Histological evaluation**

Epidermal thickness was significantly greater in group II (97.39±23.26 µm) than in group I (20.28±1.15µm) (p<0.05), indicating that the model for AD-like skin lesions had been successfully established(Figure 3, Figure 4). Epidermal thickness was significantly lower in group III (40.72±12.66µm) than in group II (p<0.05) (Figure 3,Figure 4).

**Immunohistochemical analysis**

The number of IL-25 positive cells per field in the skin biopsy were significantly higher in group II than in group I (p<0.05) (Table 2, Figure 5(A,B), Figure 6). The number of IL-33 positive cells in skin tissue were significantly higher in group II than in group I (p<0.05) (Table 2, Figure 5(D,E), Figure 6). The number of TSLP positive cells in skin tissue were significantly higher in group II compared to group I (Table 2,Figure 5(G,H), Figure 6). The number of caspase-3-positive cells, as an indicator of apoptosis, was significantly higher in group II than in group I in skin biopsy(p<0.05) (Table 1, Figure 5(J,K), Figure 6).

The number of IL-25 positive cells were significantly lower in group III compared to group II in skin biopsy (Table 2,Figure 5(B,C), Figure 6). IL-33 positive cells significantly lower in group III compared to group II (Table 2,Figure 5(E,F), Figure 6) in skin tissue. Number of TSLP positive cells in skin biopsy were lower in group III in compared to group II (Table 2,Figure 5(H,I), Figure 6). The number of caspase-3-positive cells in skin biopsy was lower in group III than in group II (Table 1, Figure 5(K,L), Figure 6).

**Discussion**

Atopic dermatitis is a relapsing, highly pruritic chronic inflammatory disease of the skin that is associated with significant morbidity and has deleterious effects on the quality of life of patients. It also places a substantial financial burden on both the patient’s family and society. The clinical presentation of AD includes erythematous, pruritic, and lichenified skin on some parts of the body.
(Lee & Detzel 2015). The early onset of AD in infancy often triggers the atopic march, which leads to the sequential development of asthma and allergic rhinitis. It is thus the initial step towards subsequent allergic diseases, therefore making an accurate diagnosis and providing appropriate treatment are critical. The pathophysiology of the disease is complex, as it involves impaired epidermal barrier function, a T-cell-mediated inflammatory skin reaction, and accompanying keratinocyte apoptosis (Werfel 2009). The mainstay therapies of AD are topical emollients to provide an effective epidermal barrier, the avoidance of triggers, and anti-inflammatory therapy with topical corticosteroids (TCSs) or topical calcineurin inhibitors (TCIs) (Weidinger & Novak 2015). However, regular long-term use of TCSs can lead to suppression of the hypothalamic-pituitary-adrenal axis, growth retardation in children, glaucoma, and skin atrophy, while the use of TCIs might increase the risk of lymphoma (Dhar et al. 2014; Hui et al. 2009). Besides these side effects, in a subgroup of patients with severe AD, these topical therapies are insufficient to control symptoms, therefore systemic treatment options become necessary. Immunosuppressant agents such as systemic corticosteroids, cyclosporine, and azathioprine should be considered in case the disease activity cannot be adequately controlled with conventional topical treatments. These systemic treatments can have serious, even life-threatening adverse effects, mainly due to immunosuppression (Ricci et al. 2009). However, there is still no cure for AD, therefore new systemic treatment options with minimal side effects are in demand.

In this study, we investigated the effects of resveratrol on epidermal thickness, keratinocyte apoptosis, and keratinocyte-derived cytokines on AD-like skin lesions. We examined the thickness of the epithelium and immunohistochemical staining of IL-25, IL-33, and TSLP antibodies to assess the severity of inflammation; we also used immunohistochemical analysis of caspase-3 to assess apoptosis. In this study, we found that resveratrol was effective at ameliorating AD-like lesions by controlling keratinocyte-derived inflammation and keratinocyte apoptosis.

In previous research, the role of resveratrol as a treatment modality for allergic diseases was investigated, and many important biological pathways were identified through animal studies. Resveratrol exerted anti-inflammatory effects on a murine model of eosinophilic chronic rhinosinusitis with nasal polyps by inhibiting lipoxygenase pathway and eosinophil recruitment (Kim et al. 2013). Treatment with systemic resveratrol improved chronic structural airway changes such as subepithelial extracellular matrix thickness and fibrosis, with decreased expression of...
transforming growth factor beta-1 (TGF-β1) (Royce et al. 2011). Resveratrol treatment also caused decreased mast cell degranulation and allergic inflammation by suppressing monocyte chemotactic protein-1 and macrophage inflammatory protein-2 in a mouse model of passive cutaneous anaphylaxis (Han et al. 2013). In a murine model of asthma, resveratrol treatment showed beneficial effects on mitochondrial function and attenuated oxidative stress (Reddy 2011). A recent experimental study investigated the effects of resveratrol on house-dust-mite-induced AD in mice, showing that resveratrol treatment down-regulated high-mobility group box (HMGB)1, which is secreted by various immune cells and acts as an important mediator in chronic inflammatory diseases. HMGB1 binds to its receptor, which in turn activates nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB). It was thus suggested that NFκB may play a role in the transformation of environmental insults into inflammation in skin tissue (Karuppagounder et al. 2014). Although all of these studies have shown that resveratrol has many favorable effects on allergic diseases, to the best of our knowledge, no studies have investigated its effects on keratinocyte-derived cytokines and keratinocyte apoptosis in experimental AD-like lesions.

Atopic dermatitis is elicited by the interplay between various cell types, including T-cells, Langerhans cells, basophils, eosinophils, and keratinocytes. Among these cell types, keratinocytes have attracted more attention in the pathogenesis of AD due to their roles in skin barrier function and their contribution to the initiation and maintenance of inflammation (Holgate 2007). Epidermal thickening is obvious in chronic skin lesions of AD, as demonstrated clinically with lichenified plaques and microscopically with acanthosis. Wu et al. found that resveratrol inhibited normal human epidermal keratinocytes by down-regulating aquaporin-3 in a concentration-dependent manner (Wu et al. 2014). In our study, resveratrol treatment led to smaller epidermal thickness, provided a regular epithelium, and produced clinical improvements in AD-like skin lesions.

There has been more supporting evidence that keratinocytes act as true innate immune cells. Keratinocytes secrete several inflammatory mediators that exert a variety of local and distant effects (Esche et al. 2004). IL-25, IL-33, and TSLP are generated by epithelial cells including keratinocytes as well as other cells, and considerable evidence has suggested that these cytokines play a crucial role in the induction of both innate and adaptive Th2 responses.

The pathologic mechanism behind AD begins with the processing of allergens by local dendritic cells (DCs), which migrate into draining lymph nodes. These DCs initiate the differentiation of
prime naive allergen-specific CD4 cells into Th2 lymphocytes, which secrete various cytokines (Leung et al. 2004). In this context, the following question arose: How do these DCs become activated to start inflammation in AD? It was shown that TSLP released from keratinocytes could be an activator of DCs. In addition, TSLP has been demonstrated to support the migration, maturation, and activation of DCs in AD skin lesions. Infectious agents and their products, allergens, trauma, and some cytokines, could induce TSLP expression in skin cells. TSLP receptors have been identified on cell types involved in immunological responses, such as T-cells, B-cells, monocytes, mast cells, and natural killer cells (Cianferoni & Spergel 2014). The role of TSLP in AD pathogenesis has been investigated in many studies. Yoo et al. found that overexpression of TSLP in the epidermis led to an AD-like disease in mice (Yoo et al. 2005). It was also reported that single nucleotide polymorphisms of TSLP and its receptors are associated with AD (Hunninghake et al. 2010). The role of TSLP in AD was demonstrated when increased TSLP levels were observed in lesional skin of AD patients, but not in either nickel-induced contact allergic dermatitis or in skin changes associated with lupus erythematosus (Soumelis et al. 2002). In our study, the number of cells that stained positively for the TSLP antibody was increased in AD-like lesions, demonstrating that these lesions resemble AD. In another mouse model, deficiency in notch signaling, which is an important regulator of skin epidermal integrity in keratinocytes, resulted in chronic skin changes and caused high levels of TSLP in keratinocytes (Dumortier et al. 2010). In addition to the many studies suggesting a critical role for TSLP in the immunopathogenesis of AD, it has attracted substantial attention as a therapeutic target. It was shown that a traditional Korean medicine known as Naju Jjok inhibited the expression of TSLP by blocking the caspase-1 signaling pathway in DNFB-induced AD-like lesions (Han et al. 2014a). Another natural anti-inflammatory agent, tryptanthrin, suppressed TSLP in 2,4-DNFB-induced AD-like skin lesions of NC/Nga mice and inhibited the mRNA expression of TSLP through blockade of the receptor-interacting protein 2/caspase-1/nuclear factor-κB pathway in an activated human mast cell line (Han et al. 2014b). Against this background, we hypothesized that resveratrol, which has been shown to be an anti-inflammatory molecule, might affect this key cytokine of AD. In our study, systemic resveratrol treatment was associated with lower expression of TSLP in AD-like skin lesions.

IL-33 is a member of the IL-1 cytokine family. Allergens, microbes, and pro-inflammatory cytokines can trigger the release of IL-33 from the epidermal barrier (Cevikbas & Steinhoff 2012).
Its receptor, ST2, presents on various cells including innate lymphoid cells, contributing to the initiation and maintenance of allergic inflammation. It has been shown that ST2 gene polymorphisms are related to the presence of AD and the IL-33-ST2 complex plays a crucial role in AD pathogenesis (Shimizu et al. 2005). Transgenic mice with increased skin-specific expression of IL-33 developed AD-like cutaneous manifestations through the activation of innate lymphoid cells in the skin and lymph nodes (Imai et al. 2013). In addition, Savinko et al. found increased expression of IL-33 in the epidermis of AD patients (Savinko et al. 2012). Although these previous studies clearly suggested a pivotal role for IL-33 in the pathogenesis of AD, to our knowledge, this is the first study to investigate the effects of resveratrol on IL-33 expression in AD-like lesions. In our study, resveratrol treatment resulted in lower immunohistochemical expression of IL-33 in the epidermis of AD-like skin lesions compared with that in a placebo group. This finding may provide a treatment option by suppressing one of the initiators of inflammation in AD.

IL-25 is a member of the IL-17 cytokine family that is expressed in epithelial cells in response to proteases such as allergen proteases, trypsin, and papain. It was reported that administration of IL-17 to mice promoted allergic inflammation by inducing IL-4, IL-5, and IL-13 gene expression (Fort et al. 2001). Moreover, DCs activated by TSLP enhance allergy-promoting Th2 memory cells by increasing the number of their receptors for IL-25. This could explain the potential role of IL-25 in the regulation of Th2 memory cells (Wang et al. 2007). IL-25 was shown to suppress filaggrin expression, resulting in epithelial barrier disruption. Conversely, an impaired epithelial barrier could induce the release of IL-25, which would further worsen epithelial barrier function due to its negative effects on filaggrin (Hvid et al. 2011). In terms of the results of this study, treatment with systemic resveratrol led to lower expression of IL-25 in the epithelium of AD-like lesions. Recent studies have indicated a central role for IL-25 in the immunopathogenesis of AD, but, to the best of our knowledge, this is the first study showing the beneficial effects of resveratrol on IL-25 expression in a mouse model of AD-like skin lesions.

Apoptosis is an essential physiologic process in the establishment and maintenance of both innate and adaptive immunity. However, it also actively participates in inflammatory and immunologic diseases such as asthma and AD (Trautmann et al. 2000). Keratinocyte apoptosis was found in situ in lesional eczematous skin and patch-test lesions of AD (Akdis et al. 2001). It was also reported that interferon-gamma (IFN-γ)-induced apoptosis in keratinocytes was increased in the skin of...
patients with AD compared with that in healthy subjects (Rebane et al. 2012). T-cell-mediated keratinocyte apoptosis via the Fas ligand decreased the expression of the adhesion molecule E-cadherin (Trautmann et al. 2000). This resulted in spongiosis, one of the histologic hallmarks of AD (Trautmann et al. 2001b). Keratinocyte apoptosis initiates the release of chemotactic factors and promotes T-cell infiltration into the epidermis. These T-cells increase the key elements of apoptosis, such as interferons and Fas (Klunker et al. 2003). The crucial role of keratinocyte apoptosis in inflammation makes it a highly attractive therapeutic target for the treatment of AD. Because of this, we hypothesized that systemic resveratrol treatment might exert anti-inflammatory effects by acting on keratinocytes.

The aspartate-specific cysteine protease (caspase) cascade is considered the main pathway by which apoptosis is orchestrated. The most prevalent protease in the cell is caspase-3. This caspase is the central executioner caspase, which is responsible for the majority of the effects in cellular death (Zimmermann & Green 2001). It was demonstrated that dexamethasone inhibited caspase-3 and caspase-7 and suppressed epithelial apoptosis. Blockage of apoptosis is one of the possible anti-inflammatory effects of steroids (Trautmann et al. 2001a). Keratinocytes are vulnerable to caspase-dependent apoptosis in response to IFN-γ when the Fas receptor levels increase to a certain threshold (Tian et al. 2014). In this study, resveratrol treatment showed beneficial effects on keratinocyte apoptosis, which was demonstrated with lower caspase expression in AD-like lesions. This study supports previous findings showing that apoptosis has an important role in the pathogenesis of AD and indicates its potential importance as a target for treatment.

There are some limitations to this study. First, although we demonstrated beneficial effects of resveratrol on inflammation and apoptosis, we could not reveal the molecular pathways by which resveratrol acts on the epithelium of AD-like lesions. Inhibition of the expression of NFκB is a possible common pathway because this transcription factor both activates the cytokines involved in Th2 inflammation and regulates the genes affecting apoptosis (Barkett & Gilmore 1999; Makarov 2000). In addition, the expression of NFκB has been found to be increased in the epithelium in chronic inflammatory diseases such as asthma (Donnelly et al. 2004). Ren et al. demonstrated the suppressor effects of resveratrol on NFκB signaling(Ren et al. 2013). Even we could not make a clear connection with T cell response and apoptosis, the inhibitor effects of resveratrol on NFκB expression might resulted anti-inflammatory and anti-apoptotic effects in this
AD-like murine model. Moreover, Yong-Hong et. al. showed that low dose resveratrol treatment led to a Th1 dominant immune response with enhanced expression of IL-2, IFN-γ and IL-12 (Feng et al. 2002). However, we could not show the effect of resveratrol on the Th-1 derived cytokines which should be taken into account in future experimental studies. Second, two blind histologists evaluated epithelial thickness in order to avoid a potential bias in our study but a marker for cell proliferation such as KI-67 protein could give a more conclusive data in this aspect (Scholzen et al. 2002). Third, this study was conducted on mice and the findings cannot be reliably extrapolated to AD in humans.

Conclusion

In conclusion, our data suggest that systemic resveratrol treatment exerts anti-inflammatory and antiapoptotic effects in a murine model of AD-like lesions. Although it is too early to draw definitive conclusions, our data indicate that resveratrol may be therapeutically beneficial to improve epithelium-derived allergic responses. Specifically, it may be effective at suppressing the very first step in inflammation.

References

Akdis M, Trautmann A, Klunker S, Blaser K, and Akdis CA. 2001. Cytokine network and dysregulated apoptosis in atopic dermatitis. Acta Odontol Scand 59:178-182.

Barkett M, and Gilmore TD. 1999. Control of apoptosis by Rel/NF-kappaB transcription factors. Oncogene 18:6910-6924. 10.1038/sj.onc.1203238

Bieber T. 2008. Atopic dermatitis. N Engl J Med 358:1483-1494. 10.1056/NEJMra074081

358/14/1483 [pii]

Boguniewicz M, and Leung DY. 2011. Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. Immunol Rev 242:233-246. 10.1111/j.1600-065X.2011.01027.x
Brandt EB, and Sivaprasad U. 2011. Th2 Cytokines and Atopic Dermatitis. J Clin Cell Immunol 2. 10.4172/2155-9899.1000110

Cevikbas F, and Steinhoff M. 2012. IL-33: a novel danger signal system in atopic dermatitis. J Invest Dermatol 132:1326-1329. 10.1038/jid.2012.66

Cianferoni A, and Spergel J. 2014. The importance of TSLP in allergic disease and its role as a potential therapeutic target. Expert Rev Clin Immunol 10:1463-1474. 10.1586/1744666X.2014.967684

Dhar S, Seth J, and Parikh D. 2014. Systemic side-effects of topical corticosteroids. Indian J Dermatol 59:460-464. 10.4103/0019-5154.139874

Donnelly LE, Newton R, Kennedy GE, Fenwick PS, Leung RH, Ito K, Russell RE, and Barnes PJ. 2004. Anti-inflammatory effects of resveratrol in lung epithelial cells: molecular mechanisms. Am J Physiol Lung Cell Mol Physiol 287:L774-783. 10.1152/ajplung.00110.2004

Dumortier A, Durham AD, Di Piazza M, Vauclair S, Koch U, Ferrand G, Ferrero I, Demehri S, Song LL, Farr AG, Leonard WJ, Kopan R, Miele L, Hohl D, Finke D, and Radtke F. 2010. Atopic dermatitis-like disease and associated lethal myeloproliferative disorder arise from loss of Notch signaling in the murine skin. PLoS One 5:e9258. 10.1371/journal.pone.0009258

Esche C, de Benedetto A, and Beck LA. 2004. Keratinocytes in atopic dermatitis: inflammatory signals. Curr Allergy Asthma Rep 4:276-284.

Feng YH, Zhou WL, Wu QL, Li XY, Zhao WM, and Zou JP. 2002. Low dose of resveratrol enhanced immune response of mice. Acta Pharmacol Sin 23:893-897.

Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, Menon S, Clifford T, Hunte B, Lesley R, Muchamuel T, Hurst SD, Zurawski G, Leach MW, Gorman DM, and Rennick DM. 2001. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity 15:985-995.

Han NR, Kang SW, Moon PD, Jang JB, Kim HM, and Jeong HJ. 2014a. Genuine traditional Korean medicine, Naju Jjok (Chung-Dae, Polygonum tinctorium) improves 2,4-dinitrofluorobenzene-induced atopic dermatitis-like lesional skin. Phytomedicine 21:453-460. 10.1016/j.phymed.2013.09.021
Han NR, Moon PD, Kim HM, and Jeong HJ. 2014b. Tryptanthrin ameliorates atopic dermatitis through down-regulation of TSLP. Arch Biochem Biophys 542:14-20. 10.1016/j.abb.2013.11.010

Han SY, Bae JY, Park SH, Kim YH, Park JH, and Kang YH. 2013. Resveratrol inhibits IgE-mediated basophilic mast cell degranulation and passive cutaneous anaphylaxis in mice. J Nutr 143:632-639. 10.3945/jn.112.173302

Hanifin JM, Thurston M, Omoto M, Cherill R, Tofte SJ, and Graeber M. 2001. The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. EASI Evaluator Group. Exp Dermatol 10:11-18.

Harikumar KB, and Aggarwal BB. 2008. Resveratrol: a multitargeted agent for age-associated chronic diseases. Cell Cycle 7:1020-1035. 5740 [pii]

Holgate ST. 2007. The epithelium takes centre stage in asthma and atopic dermatitis. Trends Immunol 28:248-251. 10.1016/j.it.2007.04.007

Hui RL, Lide W, Chan J, Schottinger J, Yoshinaga M, and Millares M. 2009. Association between exposure to topical tacrolimus or pimecrolimus and cancers. Ann Pharmacother 43:1956-1963. 10.1345/aph.1M278

Hunninghake GM, Soto-Quiros ME, Avila L, Kim HP, Lasky-Su J, Rafaeis N, Ruczinski I, Beatty TH, Mathias RA, Barnes KC, Wilk JB, O'Connor GT, Gauderman WJ, Vora H, Baurley JW, Gilliland F, Liang C, Sylvia JS, Klanderman BJ, Sharma SS, Himes BE, Bossley CJ, Israel E, Raby BA, Bush A, Choi AM, Weiss ST, and Celedon JC. 2010. TSLP polymorphisms are associated with asthma in a sex-specific fashion. Allergy 65:1566-1575. 10.1111/j.1398-9995.2010.02415.

Hvid M, Vestergaard C, Kemp K, Christensen GB, Deleuran B, and Deleuran M. 2011. IL-25 in atopic dermatitis: a possible link between inflammation and skin barrier dysfunction? J Invest Dermatol 131:150-157. 10.1038/jid.2010.277

Imai Y, Yasuda K, Sakaguchi Y, Haneda T, Mizutani H, Yoshimoto T, Nakanishi K, and Yamanishi K. 2013. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and...
elicits atopic dermatitis-like inflammation in mice. Proc Natl Acad Sci U S A 110:13921-13926.

Johnson JJ, Nihal M, Siddiqui IA, Scarlett CO, Bailey HH, Mukhtar H, and Ahmad N. 2011. Enhancing the bioavailability of resveratrol by combining it with piperine. Mol Nutr Food Res 55:1169-1176. 10.1002/mnfr.201100117

Karuppagounder V, Arumugam S, Thandavarayan RA, Pitchaimani V, Sreedhar R, Afrin R, Harima M, Suzuki H, Nomoto M, Miyashita S, Suzuki K, and Watanabe K. 2014. Resveratrol attenuates HMGB1 signaling and inflammation in house dust mite-induced atopic dermatitis in mice. Int Immunopharmacol 23:617-623. 10.1016/j.intimp.2014.10.014

Kim SW, Kim DW, Khammuratova R, Kim JH, Jung MH, Chang DY, Shin EC, Lee HK, Shin HW, Rhee CS, Jeon SY, and Min YG. 2013. Resveratrol prevents development of eosinophilic rhinosinusitis with nasal polyps in a mouse model. Allergy 68:862-869. 10.1111/all.12132

Klunker S, Trautmann A, Akdis M, Verhagen J, Schmid-Grendelmeier P, Blaser K, and Akdis CA. 2003. A second step of chemotaxis after transendothelial migration: keratinocytes undergoing apoptosis release IFN-gamma-inducible protein 10, monokine induced by IFN-gamma, and IFN-gamma-inducible alpha-chemoattractant for T cell chemotaxis toward epidermis in atopic dermatitis. J Immunol 171:1078-1084.

Lee BW, and Detzel PR. 2015. Treatment of childhood atopic dermatitis and economic burden of illness in Asia Pacific countries. Ann Nutr Metab 66 Suppl 1:18-24. 10.1159/000370221

Lee M, Kim S, Kwon OK, Oh SR, Lee HK, and Ahn K. 2009. Anti-inflammatory and anti-asthmatic effects of resveratrol, a polyphenolic stilbene, in a mouse model of allergic asthma. Int Immunopharmacol 9:418-424. 10.1016/j.intimp.2009.01.005

Leung DY, Boguniewicz M, Howell MD, Nomura I, and Hamid QA. 2004. New insights into atopic dermatitis. J Clin Invest 113:651-657. 10.1172/JCI21060
Li YZ, Lu XY, Jiang W, and Li LF. 2013. Anti-inflammatory effect of qingpeng ointment in atopic dermatitis-like murine model. Evid Based Complement Alternat Med 2013:907016. 10.1155/2013/907016

Makarov SS. 2000. NF-kappaB as a therapeutic target in chronic inflammation: recent advances. Mol Med Today 6:441-448.

Micili SC, Goker A, Sayin O, Akokay P, and Ergur BU. 2013. The effect of lipoic acid on wound healing in a full thickness uterine injury model in rats. J Mol Histol 44:339-345. 10.1007/s10735-013-9485-8

Rebane A, Zimmermann M, Aab A, Baurecht H, Koreck A, Karelson M, Abram K, Metsalu T, Pihlap M, Meyer N, Folster-Holst R, Nagy N, Kemeny L, Kingo K, Vilo J, Illig T, Akdis M, Franke A, Novak N, Weidinger S, and Akdis CA. 2012. Mechanisms of IFN-gamma-induced apoptosis of human skin keratinocytes in patients with atopic dermatitis. J Allergy Clin Immunol 129:1297-1306. 10.1016/j.jaci.2012.02.020

Reddy PH. 2011. Mitochondrial Dysfunction and Oxidative Stress in Asthma: Implications for Mitochondria-Targeted Antioxidant Therapeutics. Pharmaceuticals (Basel) 4:429-456. 10.3390/ph4030429

Ren Z, Wang L, Cui J, Huoc Z, Xue J, Cui H, Mao Q, and Yang R. 2013. Resveratrol inhibits NF-kB signaling through suppression of p65 and IккB kinase activities. Pharmazie 68:689-694.

Ricci G, Dondi A, Patrizi A, and Masi M. 2009. Systemic therapy of atopic dermatitis in children. Drugs 69:297-306. 10.2165/00003495-200969030-00005

Royce SG, Dang W, Yuan G, Tran J, El Osta A, Karagiannis TC, and Tang ML. 2011. Resveratrol has protective effects against airway remodeling and airway hyperreactivity in a murine model of allergic airways disease. Pathobiol Aging Age Relat Dis 1. 10.3402/PBA.v1i0.7134

Savinko T, Matikainen S, Saarialho-Kere U, Lehto M, Wang G, Lehtimaki S, Karisola P, Reunala T, Wolff H, Lauerma A, and Alenius H. 2012. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. J Invest Dermatol 132:1392-1400. 10.1038/jid.2011.446
Scholzen T, Endl E, Wohlenberg C, van der Sar S, Cowell IG, Gerdes J, and Singh PB. 2002. The Ki-67 protein interacts with members of the heterochromatin protein 1 (HP1) family: a potential role in the regulation of higher-order chromatin structure. J Pathol 196:135-144. 10.1002/path.1016

Sharma P, Huq AU, and Singh R. 2014. Cypermethrin-induced reproductive toxicity in the rat is prevented by resveratrol. J Hum Reprod Sci 7:99-106. 10.4103/0974-1208.138867

Shimizu M, Matsuda A, Yanagisawa K, Hirota T, Akahoshi M, Inomata N, Ebe K, Tanaka K, Sugiura H, Nakashima K, Tamari M, Takahashi N, Obara K, Enomoto T, Okayama Y, Gao PS, Huang SK, Tominaga S, Ikezawa Z, and Shirakawa T. 2005. Functional SNPs in the distal promoter of the ST2 gene are associated with atopic dermatitis. Hum Mol Genet 14:2919-2927. 10.1093/hmg/ddi323

Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A, Smith K, Gorman D, Zurawski S, Abrams J, Menon S, McClanahan T, de Waal-Malefyt Rd R, Bazan F, Kastelein RA, and Liu YJ. 2002. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol 3:673-680. 10.1038/ni805

Tian BP, Zhou HB, Xia LX, Shen HH, and Ying S. 2014. Balance of apoptotic cell death and survival in allergic diseases. Microbes Infect 16:811-821. 10.1016/j.micinf.2014.07.004

Trautmann A, Akdis M, Kleemann D, Altznauer F, Simon HU, Graeve T, Noll M, Brocker EB, Blaser K, and Akdis CA. 2000. T cell-mediated Fas-induced keratinocyte apoptosis plays a key pathogenetic role in eczematous dermatitis. J Clin Invest 106:25-35. 10.1172/JCI9199

Trautmann A, Akdis M, Schmid-Grendelmeier P, Disch R, Brocker EB, Blaser K, and Akdis CA. 2001a. Targeting keratinocyte apoptosis in the treatment of atopic dermatitis and allergic contact dermatitis. J Allergy Clin Immunol 108:839-846. 10.1067 mai.2001.118796

Trautmann A, Altznauer F, Akdis M, Simon HU, Disch R, Brocker EB, Blaser K, and Akdis CA. 2001b. The differential fate of cadherins during T-cell-induced keratinocyte apoptosis leads to spongiosis in eczematous dermatitis. J Invest Dermatol 117:927-934. 10.1046/j.0022-202x.2001.01474.x
Wang YH, Angkasekwinai P, Lu N, Voo KS, Arima K, Hanabuchi S, Hippe A, Corrigan CJ, Dong C, Homey B, Yao Z, Ying S, Huston DP, and Liu YJ. 2007. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. J Exp Med 204:1837-1847. 10.1084/jem.20070406

Weidinger S, and Novak N. 2015. Atopic dermatitis. Lancet. 10.1016/S0140-6736(15)00149-X

Werfel T. 2009. The role of leukocytes, keratinocytes, and allergen-specific IgE in the development of atopic dermatitis. J Invest Dermatol 129:1878-1891. 10.1038/jid.2009.71

Wu Z, Uchi H, Morino-Koga S, Shi W, and Furue M. 2014. Resveratrol inhibition of human keratinocyte proliferation via SIRT1/ARNT/ERK dependent downregulation of aquaporin 3. J Dermatol Sci 75:16-23. 10.1016/j.jdermsci.2014.03.004

Yoo J, Omori M, Gyarmati D, Zhou B, Aye T, Brewer A, Comeau MR, Campbell DJ, and Ziegler SF. 2005. Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. J Exp Med 202:541-549. 10.1084/jem.20041503

Yuksel H, Yilmaz O, Baytur YB, and Ozbilgin K. 2008. Prenatal administration of granulocyte-macrophage colony-stimulating factor increases mesenchymal vascular endothelial growth factor expression and maturation in fetal rat lung. Exp Lung Res 34:550-558. 10.1080/01902140802341736

Zimmermann KC, and Green DR. 2001. How cells die: apoptosis pathways. J Allergy Clin Immunol 108:S99-103.
Schematic presentation of experimental procedure

(A) Experimental procedure in control group (B) Experimental procedure in vehicle control group (C) Experimental procedure in resveratrol treatment group. DNFB, 2,4-Dinitrophenylbenzene; DMSO, dimethyl sulfoxide; IL, interleukin; TSLP, thymic stromal lymphopoietin
Representative pictures of dermatitis in groups after a 1-week treatment

(A) Control group  
1. Erythema/haemorrhage: 1/3  
2. Scaling/dryness: 0/3  
3. Edema: 0/3  
4. Excoriation/erosion: 0/3  
DS: 1

(B) Vehicle control group  
1. Erythema/haemorrhage: 1/3  
2. Scaling/dryness: 3/3  
3. Edema: 1/3  
4. Excoriation/erosion: 3/3  
DS: 8

(C) Resveratrol treatment  
1. Erythema/haemorrhage: 1/3  
2. Scaling/dryness: 1/3  
3. Edema: 0/3  
4. Excoriation/erosion: 2/3  
DS: 4  
DS; dermatitis score
3

Representative H&E staining of skin tissues in groups after 1-week treatment.

(A) Control group; Normal regular epithelium (B) Vehicle control group; Thickening of the epidermis and epidermal irregularity (C) Resveratrol treatment; A minimally irregular epithelium accompanying epithelial thickness
Figure 4 (on next page)

Boxplot of the epidermal thickness(µm) in groups.
Comparison of immunohistochemical analysis between groups

Comparison of immunohistochemical analysis between groups. (A,D,G,J) Control group; (B) Vehicle control group; yellow arrows shows prominent immunostaining for IL-25 (E) Vehicle control group; yellow arrows shows prominent immunostaining for IL-33 (H) Vehicle control group; yellow arrows shows prominent immunostaining for TSLP (K) Vehicle control group; yellow arrows shows prominent immunostaining for caspase-3 (C) Resveratrol treatment group; yellow arrows shows lower immunostaining for IL-25 (F) Resveratrol treatment group; yellow arrows shows lower immunostaining for IL-33 (I) Resveratrol treatment group; yellow arrows shows lower immunostaining for TSLP (L) Resveratrol treatment group; yellow arrows shows lower immunostaining for caspase-3. The dashed lines indicate the approximate location of the epidermal basement membrane. IL, interleukin; TSLP, thymic stromal lymphopoietin.
Figure 6 (on next page)

Boxplot of the IL-25, IL-33 and TSLP H scores in the various groups.

IL, interleukin; TSLP, thymic stromal lymphopoietin
Table 1 (on next page)

Comparison of dermatitis scores in study groups.

Values are expressed as the median (25 75 percentile). Two group comparisons were made using Mann Whitney U test; IQR, Interquartile Range; SD, Standard deviation.
### Table 1. Comparison of dermatitis scores in study groups

| Variables | Group I Control | Group II Vehicle Control | Group III Resveratrol | P value<sup>a</sup> |
|-----------|-----------------|--------------------------|-----------------------|-------------------|
|           | Mean±SD Median(IQR) | Mean±SD Median(IQR) | Mean±SD Median(IQR) |                   |
| 5<sup>th</sup> week | 0.57±0.53<sup>b</sup> 1.0 (0.0-1.0) | 8.29±0.49 8.0 (8.0-9.0) | 8.42±0.79 8.0 (8.0-9.0) | 0.001 |
| 6<sup>th</sup> week | 0.57±0.53 1.0 (0.0-1.0) | 8.86±0.69 8.0 (8.0-10.0) | 5.14±1.68<sup>c</sup> 4.0 (4.0-7.0) | 0.001 |

<sup>a</sup> P value was calculated by Kruskall Wallis H test

<sup>b</sup> Significantly lower compared to Group II and Group III

<sup>c</sup> Significantly lower compared to Group II

Values are expressed as the median (25-75 percentile). Two group comparisons were made using Mann Whitney U test; IQR, Interquartile Range; SD, Standard deviation
Table 2 (on next page)

Comparison of H scores in study groups.

Values are expressed as the median (25 75 percentile). P values were calculated using the Kruskal Wallis and Mann Whitney U test. IL, interleukin; TSLP, thymic stromal lymphopoietin.
| Variables | **Group I Control** | **Group II Vehicle Control** | **Group III Resveratrol** | **P value**<sup>a</sup> |
|-----------|-----------------|-----------------|-----------------|----------------|
| **Mean±SD** | **Median(IQR)** | **Mean±SD** | **Median(IQR)** | **Mean±SD** | **Median(IQR)** |
| IL-25     | 164.85±33.37    | 149 (138.5-206.5) | 297.50±28.83<sup>b</sup> | 26 (277.75-309.0) | 234.79±34.98<sup>bc</sup> | 226 (210-246) | 0.001 |
| IL-33     | 167.21±39.48    | 149 (138.5-206.5) | 311.71±60.13<sup>b</sup> | 312.5 (276.50-312.50) | 247.71±36.59<sup>bc</sup> | 230 (213.25-285.75) | 0.001 |
| TSLP      | 177.5±32.76     | 198 (141-206)    | 301.93±69.86<sup>b</sup> | 297.92 (229.75-367.25) | 247.5±45.30<sup>bc</sup> | 216.5 (207.75-296.25) | 0.001 |
| Caspase-3 | 157.07±36.43    | 134 (129-202.5)  | 282.42±37.41<sup>b</sup> | 288.5 (275-292.50) | 214.57±22.01<sup>bc</sup> | 206 (202.75-217.25) | 0.001 |

<sup>a</sup> P value was calculated by Kruskall Wallis H test

<sup>b</sup> Significantly higher compared to Group I

<sup>c</sup> Significantly lower compared to Group II

Values are expressed as the median (25-75 percentile). Two group comparisons were made using Mann Whitney U test. IL, interleukin; TSLP, thymic stromal lymphopoietin; IQR, Interquartile Range.