JRVM
Journal of Research in Veterinary Medicine

RESEARCH ARTICLE

J Res Vet Med. 2024: 43 (2) 93-101
DOI:10.30782/jrvm.1401217

Ameliorative Effect of Topical Clinoptilolite on 2,4- Dinitrofluorobenzene Induced Atopic Dermatitis Model in Mice

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Received 06-12-2023 Accepted 31-10-2024

Abstract

Atopic dermatitis is a multifactorial disease process. It is defined as "a genetically predisposed inflammatory and itchy allergic skin disease associated with the production of immunoglobulin E against environmental allergens". Experimental models are considered important in the evaluation of therapeutic agents for the treatment of atopic dermatitis. This study aimed to reveal the effects of clinoptilolite and tacrolimus on atopic dermatitis lesions in the atopic dermatitis model in mice induced with 2,4-dinitrofluorobenzene. For inducing the atopic dermatitis model, mice were administered topically on the back with 0.15% 2,4-dinitrofluorobenzene twice a week for 5 weeks. For the next 4 weeks, 0.15% 2,4-dinitrofluorobenzene was applied once a week to maintain inflammation. Afterward, topical tacrolimus cream (0.1%) and topical clinoptilolite powder were used for 4 weeks. Clinical score, serum thymus and activation-regulated chemokine, histopathology, and thymic stromal lymphopoietin (TSLP) immunostainings were evaluated between groups. While clinoptilolite treatment was found to be effective in the normalization of clinical scores, serum thymus and activation regulated chemokine levels were found to be variable and insignificant. Histopathologically, clinoptilolite had an ameliorative effect on epidermal thickness and inflammation yet there was no significant difference of mast cells and fibrosis between groups. Furthermore, clinoptilolite had an inhibitory effect of TSLP immunostaining on epidermal tissue. In conclusion, clinoptilolite could be an alternative treatment of atopic dermatitis with its effects similar to tacrolimus.

Keywords: Atopic dermatitis, clinoptilolite, tacrolimus, TARC, TSLP

Introduction

Atopic Dermatitis (AD), a prevalent chronic inflammatory skin condition, is marked by recurring eczema, dryness, and severe itching. Its global prevalence reaches up to 20% in children and 5% in adults. Notably, about 80% of cases start in early childhood or infancy, while the remaining 20% manifest during adulthood, as highlighted by Siegels et al. (1) and Bieber (2). The disruption of the skin's barrier function increases susceptibility to irritation and sensitization to various external stimuli. Inflammation and itchiness are key factors in the onset and exacerbation of AD (3). Alleviating itch is crucial for enhancing the quality of life of AD patients. Local or systemic corticosteroids have been the cornerstone of AD treatment, primarily due to their effectiveness in reducing inflammation. Despite their status as the primary treatment choice, the frequent occurrence of side effects with systemic steroids raises concerns (4). Consequently, there is an urgent need for the development of novel and more effective treatment modalities for AD.

Animal models play a pivotal role in understanding the etiopathogenesis of AD. These models have revealed that skin lesions in AD are linked with immunological shifts, notably Th2 immune responses and elevated serum IgE levels, making them crucial for evaluating potential AD treatments (5). In mice, it has been observed that a 0.15% concentration of 2,4-dinitrofluorobenzene in acetone, when applied topically and repeatedly, can cause eczema-

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tous changes. These changes include significant infiltration of neutrophils and eosinophils, as well as epidermal hypertrophy, leading to chronic itching (6).

Tacrolimus, an effective calcineurin inhibitor first isolated from Streptomyces tsukubaensis in Japan (7,8), was initially developed as an oral medication to prevent transplant rejection (9). It is believed to bind to the FK506 binding protein within cells and suppress calcineurin activity (6,9). Tacrolimus reduces inflammation by inhibiting T helper cell activity and the production of proinflammatory cytokines like IL-2, IL-3, and IL-4. It has also been demonstrated to lower the expression of high-affinity IgE receptors in epidermal cells from AD-affected skin (10). These properties have positioned topical calcineurin inhibitors, particularly tacrolimus, as primary anti-inflammatory agents in AD treatment (9). Additionally, tacrolimus cream has shown promise in animal models for mitigating inflammatory skin reactions in allergic contact dermatitis, acute irritant dermatitis, and delayed-type hypersensitivity (10). Clinoptilolite zeolite, a type of natural zeolite, is a microporous tuff stone composed of ions and crystalline water. Structurally, it is an aluminum silicate featuring channels in its crystal lattice that measure approximately 0.4 nanometers. The fundamental structure of clinoptilolite zeolites includes a crystal lattice with cavities approximately 4 Ångströms in size (1 Ångström = 10^-10 m = 0.1 nm). To date, over 34 minerals have been identified in natural zeolites, but only clinoptilolite zeolite is deemed suitable for human and animal treatments. No long-term adverse effects have been observed in humans or animals when administered in acceptable doses (11). Clinoptilolite powder is known for its antiviral, antibacterial, antifungal, and absorbent properties (12).

However, so far, there have been no reports on the effectiveness of clinoptilolite in treating atopic dermatitis-like lesions. Our study explores clinoptilolite's potential clinical and pathological benefits in 2,4-dinitrofluorobenzene (DNFB) induced dermatitis. The goal was to compare the efficacy of clinoptilolite with tacrolimus in a mouse model of AD induced by DNFB. To achieve this, we assessed effects of both clinoptilolite and tacrolimus clinically, biochemically, and pathologically.

Materials and methods

Animals

CD1 type female mice used for the research were obtained from Burdur Mehmet Akif Ersoy University Experimental Animal Production and Experimental Research Center. During the study, the principles of Burdur Mehmet Akif Ersoy University Experimental Animals Ethics Committee were followed. Thirty-two female CD1 mice, 24 weeks old, were kept in standard housing cages until the day of the experiment. There were 8 mice in each cage for each group (control, DNFB, Tacrolimus, and Clinoptilolite). Mice were provided with standard pellet feed and water ad libitum throughout the experiment. Drinking water was changed daily, and standard cage cleaning was performed throughout the experiment. Mice were housed at 21°C room temperature, in ventilated rooms with 12-hour light-dark cycles.

Chemicals

The lumbar regions of the mice in all groups were shaved, and the mice were kept for one day to allow for the amelioration of any microtraumas that might have occured. To create atopic dermatitis-like lesions, DNFB (Sigma, D1529) was used. DNFB was dissolved in a mixture of acetone and olive oil (3:1). For sensitization, 100 µL of 0.15% DNFB was applied to the shaved lumbal regions of mice (DNFB, Tacrolimus, Clinoptilolite group) twice a week for 5 weeks (13). Skin lesions formed after 5 weeks. The mice continued to be administered 100 µL of 0.15% DNFB once a week to maintain inflammation between weeks 6 and 9 (14). The mice in the control group were applied 100 µL of 3:1 acetone-olive oil solution to the lumbar regions once a week for 9 weeks. For treatment, topical tacrolimus (tacrolin 0.1% ointment) and clinoptilolite (froximun toxaprevent powder) were applied to the lumbar region once a day as a thin layer from weeks 6 to 9 weeks in the tacrolimus and clinoptilolite groups, respectively.

Measurement of clinical skin score

In the mouse atopic dermatitis model, clinical assessment and damage grading of skin lesions were performed for each mouse. According to this, lesions were graded as 0 (absent), 1 (mild), 2 (moderate), and 3 (severe) in terms of erythema, edema, abrasion, lichenification, oozing/scabbing, and dryness findings twice a week. The total score from the 6 symptoms of each mouse was counted as the score for that mouse (15). Evaluation was done by a researcher who was blinded to the grouping of the animals.

Pathological analyses

For histopathological and immunohistochemical examination, an area of 2 cm2 containing epidermis and dermis was excised from the lumbar regions of the animals with lesions. Skin samples were placed in 10% buffered formaldehyde and fixed for 24 hours. Following routine histolog-

ical procedures, tissues were embedded in paraffin blocks and 4 μm sections were taken. Sections were stained with hematoxylin & eosin (H&E) for histopathological analysis. Toluidine Blue staining was performed to reveal mast cells. In the histopathological examination, epidermis thickness and dermal inflammation levels were evaluated. Epidermal thickness level was determined by taking the average of 3 different measurements from a randomly selected area with 100x magnification. For dermal inflammation, a score of 0-3 was given by counting the inflammatory cells (mononuclear cells) in four 400x magnification fields. Accordingly, scores given the lesions similar to previous research (16,17) as follows: 0 - no inflammation, 1 - mild inflammation, 2 - moderate inflammation, 3 - severe inflammation. Additionally, in terms of fibrosis scores given in four 400x magnification field as follows: 0 - no fibrosis, 1 - mild fibrosis, 2 - moderate fibrosis and 3 - severe fibrosis. Mast cells were counted in four randomly selected 400x magnification fields from the slides stained with toluidine blue.

ImmPRESS® Excel Amplified Polymer Staining Kit (Vector Lab, Anti-Rabbit IgG, Peroxidase, MP-7601) was used for immunohistochemical analysis. All procedures were performed according to the manufacturer datasheet. Primary antibody for TSLP (Novus, NB110-55234) was diluted 1:1000 and applied overnight. DAB (ImmPACT EqV Reagent) was used as chromogen. The sections, which were counterstained with Mayer's hematoxylin for 20 seconds, were left to dry and covered with entellan. Mouse spleen and liver tissue were used for TSLP as positive control. For negative control, PBS was used instead of the primary antibody after protein blocking (Supp. Fig 1-3).

Quantitative Pathology & Bioimage Analysis (QuPath version 0.2.3) program was used to evaluate the staining intensity of immunohistochemically stained sections with anti-TSL*P.* For this purpose, DAB-positive cell numbers were determined with standard program settings by selecting areas of equal size at 100x microscopic magnification for each section.

Statistical analyses

Evaluation of clinical scores of mice in all groups was performed using the Repeated Anova test with the IBM SPSS program (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). Statistical evaluation of histopathological, histochemical, and immunohistochemical findings between groups was performed in the Minitab™ 16.1.1 program with One Way Anova and Tukey test following the Ryan-Joiner normality test.

Results

Effect of tacrolimus and clinoptilolite treatment on DNFB-induced AD-like skin lesions

To evaluate the efficacy of treatment, tacrolimus cream and clinoptilolite powder were applied topically once a day to mice with DNFB-induced atopic-like dermatitis between weeks 6 and 9. For clinical scoring, lesions were scored twice a week for 28 days. Repeated application of DNFB increased bleeding, crusting, edema, itching, and erosion on the dorsal skin of mice (Figure 1A-D). At the end of the treatment, a significant difference was found when the mean clinical scores of the mice in the DNFB group were compared with the mean clinical scores of the control, tacrolimus, and clinoptilolite groups ($p < 0.001$) (Figure 1E-H). However, there was no statistically significant difference between tacrolimus and clinoptilolite groups ($p > 0.05$) (Table 1).

Table 1. Clinical scores (between 5-9 weeks) and serum TARC levels of mice in groups

	Control	DNFB	Tacrolimus	Clinoptilolite
Clinical Score	0.0720±0.0294^{c}	15.048±0.212^{a}	9.07±1.55^{b}	9.77±1.25^{b}
TARC (ng/L)	265,9±15,9	230,3±10,0	252,1±13,5	249,9 ± 14,4

^{a,b,c}: The statistical difference between the means with different letters in the same row is significant. Each value reveals mean ± standard error.

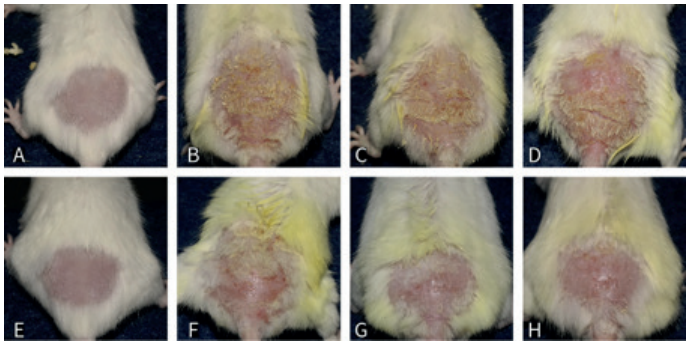

Figure 1. Atopic dermatitis-like lesions occurring at the end of the 5th week in the groups. Control group (A), DNFB group (B), clinoptilolite (C), tacrolimus (D). Appearance of skin lesions after 9th week in the groups. Control group (E), DNFB group (F), clinoptilolite (G), tacrolimus (H).

In the DNFB group, edema and erythema emerged at the application site in the first week. Itching and scratching due to skin irritation from the second week, and lichenification and crusting in the subsequent weeks were the most significant clinical observations. Three mice developed crusting in week five. While the group's mean clinical score at the end of the fifth week was 14.87±0.75, no significant increase or decrease in mean clinical scoring was seen with the lowering of the DNFB application dosage beginning in the sixth week. In the tacrolimus group, only two mice developed erythema at the application site in the first week. Scratch-

ing, abrasion due to irritation, and lichenification and crusting in the following weeks were the most significant clinical symptoms beginning in the second week. At week 5, eight mice exhibited crusting, but two had severe crusting. While the group's mean clinical score was 15.00±0.75 at the end of the fifth week, a substantial reduction in the weekly mean clinical score was observed with the lowering of the DNFB application dosage and the start of therapy in the sixth week. The group's mean clinical score at the end of the ninth week was 7.00±0.64. In the clioptilolite group, mice developed erythema at the experimental site in the first week, but only three animals developed edema. Scratching, abrasion due to itching, and lichenification and crusting in the following weeks were the most significant clinical symptoms beginning in the second week. Crust development was detected in 8 animals at week 5 and was more severe in 2 mice. While the group's mean clinical score was 14.37±0.75 at the end of the fifth week, a substantial drop in the weekly mean clinical score was noted with the lowering of the DNFB application dosage and the start of therapy in the sixth week. The group's mean clinical score at the end of the ninth week was 6.50±0.64 ($p < 0.05$).

Effect of Tacrolimus and Clinoptilolite on Serum TARC Level

At the end of the study, mean serum TARC levels in the treatment groups, tacrolimus and clinoptilolite, were 252.1±13.5 ng/L and 249.9 ± 14.4 ng/L, respectively. These values were lower than the mean serum TARC levels of the control group (265.9±15.9 ng/L) and higher than the mean serum TARC levels of the DNFB group (230.3±10.0 ng/L). In the statistical evaluation between the groups, no significant difference was found in terms of serum TARC levels ($p > 0.05$) (Table 1).

Effect of Tacrolimus and Clinoptilolite on Histopathological Changes

In the evaluations made in terms of epidermal thickness levels, a significant increase was observed in the DNFB group compared to the control group (18.75±0.52 μm) ($p < 0.001$). Epidermal thickness levels were significantly lower in the tacrolimus (107.25±6.470 μm) and clinoptilolite groups (83.25±4.95 μm) compared to the DNFB (179.00±9.02 μm) group ($p < 0.001$). In addition, epidermal thickness level was significantly lower in the clinoptilolite group compared to the tacrolimus group ($p < 0.001$) (Figure 2A-D).

In the evaluation of dermal inflammation, DNFB group (2.75±0.25) was found to be significantly higher than the control group (0.00±0.00) ($p < 0.001$). The dermal inflammation value of the tacrolimus group (2.25±0.25) was found to be lower numerically than the DNFB group, while it was found to be significantly lower in the clinoptilolite group (1.75±0.25) compared to the DNFB group ($p < 0.001$). While no significant difference was observed between the tacrolimus and clinoptilolite groups, a significant increase of inflammation was detected in these groups compared to the control group ($p < 0.001$).

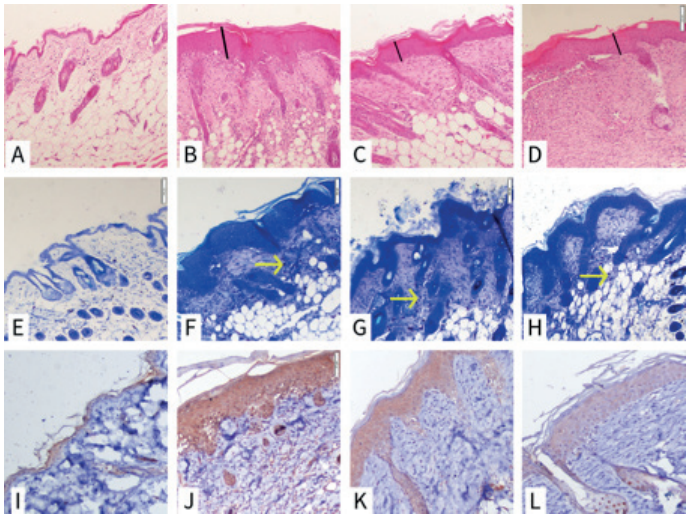

Figure 2. Comparative views of dermal lesions between groups. Compared to control (A) significant increase in epidermal thickness level in DNFB group (B), decrease in epidermal thickness levels (black line) in clinoptilolite (C) and tacrolimus (D) groups, 100x, H&E. Compared to control group (E), increased mast cells (yellow arrows) in DNFB (F), clinoptilolite (G) and tacrolimus (H) groups, 100x, Toluidine Blue. Immunohistochemically, low TSLP positivity in the control (I) group, high positivity in the DNFB (J) group and decreased TSLP positivity in the tacrolimus (K) and clinoptilolite (L) groups, 200x, DAB.

In terms of dermal fibrosis, a significant increase was observed DNFB (2.50±0.26), tacrolimus (2.37±0.37) and clinoptilolite (2.00±0.18) groups compared to the control (0.00±0.00) group ($p < 0.001$). There was no difference between the DNFB, tacrolimus and clinoptilolite groups themselves.

It was noted that mast cells showed a significant increase in the DNFB group (25.75±3.10) compared to the control group (7.88±1.32) in the counts performed on the sections stained with Toluidine Blue ($p < 0.001$). However, there was no significant difference between the tacrolimus (26.13±2.99), clinoptilolite (24.13±3.40) and DNFB groups ($p > 0.05$), while a significant increase was observed compared to the control group ($p < 0.001$) (Figure 2E-H). All histopathological score values are given in Table 2.

Table 2. Histopathological and immunohistochemical findings of mice in the groups.

Group	Epidermal thickness (µm)	Dermal inflammation	Dermal fibrosis	Number of mast cells	TSLP positivity
Control	18,75±0,52[a]	0,00±0,00[a]	0,00±0,00[a]	7,88±1,32[a]	77,80±14,40[b]
DNFB	179,00±9,02[b]	2,75±0,250[c]	2,50±0,260[b]	25,75±3,10[b]	291,6±45,60[a]
Tacrolimus	107,25±6,47[d]	2,25±0,25 [bc]	2,37±0,37[b]	26,13±2,99[b]	124,9±46,30[b]
Clinoptilolite	83,25±4,95[c]	1,75±0,25 [b]	2,00±0,18[b]	24,13±3,40[b]	132,40±42,20[b]

[a,b,c]: The statistical difference between the means with different letters in the same row is significant. Each value reveals mean ± standard error.

Effect of Tacrolimus and Clinoptilolite on Tissue TSLP in DNFB-Sensitized Mice

As a result of the statistical analyses performed on the positive cell numbers obtained from the immunohistochemically stained sections with anti-TSLP, a significant increase in positive cells was determined in the DNFB group (291.6±45.6) compared to the control group (77.80±14.4) ($p < 0.01$). Although TSLP positivity was found to be higher in both the tacrolimus (124.90±46.30) and clinoptilolite (132.4±42.2) groups compared to the control group, the difference was not statistically significant and it was significantly lower than the DNFB group ($p < 0.01$). The difference between tacrolimus and clinoptilolite groups was not significant (Figure 2I-L) (Table 2).

Discussion

Various factors play a role in the pathogenesis of AD, including immunological abnormalities (18). The spontaneous clinical symptoms of atopic dermatitis typically begin with itching, redness, bleeding, flaking, dryness, and hair loss (5). The severity of dermatitis induced by haptens can range widely, from mild and temporary skin inflammation characterized by itching and redness to more severe and lasting exudative dermatitis (19). Studies involving DNFB application in mice (15,20), have shown that the average clinical score was higher in the DNFB-treated group compared to control and vehicle groups. These mice exhibited symptoms including significant redness/bleeding, swelling, erosion, and dryness/scaling of the back skin. In similar experiments using DNCB on mice, the dermatitis score, based on factors like redness, dryness, swelling, and abrasion, was significantly higher than in control and vehicle groups (18,21). Our study, aligning with these findings, showed that the average clinical score was lower for mice that did not develop atopic dermatitis-like lesions after only vehicle administration. The clinical scores in the DNFB group were higher compared to the control and other groups. The clinical manifestations of dermatitis in mice, which include the inflammatory process marked by the infiltration of neutrophils and eosinophils, and the hyperplasia of the epidermis, lead to eczematous changes in the skin and itching, likely due to repeated topical applications of DNFB.

Inagaki and colleagues (22) assessed the effectiveness of tacrolimus and dexamethasone in a mouse model of AD using 0.15% DNFB in a 50 µl acetone solution. Histopathological evaluation of the untreated group's skin 24 hours post-final DNFB application showed pronounced inflammatory responses including epidermal and dermal thickening and an increase in neutrophils, eosinophils, lymphocytes, and mast cells. They also observed epidermal crusting, swelling, single-cell necrosis, and dermal fibrosis. Dexamethasone mitigated these alterations effectively, reducing lymphocyte and eosinophil levels significantly - effects not mirrored by tacrolimus, which did not influence mast cell counts or scratching behavior. Conversely, a 0.1% topical tacrolimus application, while not significantly anti-inflammatory, greatly reduced itching. Aksoy et al. (12) noted that clinoptilolite activates MHC class II and mononuclear phagocytes, elevating T cell numbers through the release of proinflammatory cytokines like IL-1 and TNF-alpha, and transcription factors, thereby promoting wound healing and granulation tissue formation. In our research, the DNFB group showed a significant increase in epidermal thickness compared to the control group, but this increase was markedly lower in the tacrolimus and clinoptilolite groups. The clinoptilolite group showed statistically lower epidermal thickness than the tacrolimus group. Furthermore, while the tacrolimus group did not show a significant reduction in dermal inflammation, a substantial decrease was observed in the clinoptilolite group, suggesting, in contrast to previous reports, that clinoptilolite may have an anti-inflammatory effect.

Mast cells, known as key players in allergic reactions, are involved in the formation of skin lesions in AD. During chronic skin inflammation, these cells release a variety of cytokines, contributing to allergic responses, as explained by Han et al. (23). In a study conducted by Yamashita et al. (6), the effectiveness of 0.1% tacrolimus, cyclosporine (Cys) -A at 30 mg/kg, and 0.05% dexamethasone was assessed in a mouse model of AD induced by 2,4-dinitrofluorobenzene (DNFB). The assessment included counting the number of mast cells and the rate of degranulated mast cells in three toluidine blue-stained areas chosen at random. The results indicated that neither tacrolimus nor Cys A had an impact on the mast cell count. In our own research, we effectively induced AD using DNFB. Following this, we treated the condition with tacrolimus as a control and observed that it mitigated some of the histopatholog-

ical features of AD, such as epidermal thickening and dermal inflammation. However, we noted that tacrolimus did not decrease the number of mast cells. Furthermore, the clinoptilolite group's mast cell count showed no significant difference compared to the DNFB group.

Despite the absence of histopathological markers exclusively identifying AD, skin biopsies often reveal acute, subacute, or chronic dermatitis patterns. Especially in chronic AD stages, a notable increase in collagen within healed and healing lesions has been observed. Lee et al. (24) highlighted that dermal fibrosis is a significant characteristic of AD in its chronic stage. While acute AD lesions histopathologically exhibit an increase in eosinophils and their dermal infiltration, chronic lesions are more defined by collagen accumulation and dermal thickening, as Jin et al. (25) reported. A study evaluating five different wound healing agents found that clinoptilolite enhanced the healing process notably. This research parallels ours in that clinoptilolite was shown to reduce inflammation microscopically, yet it also notably increased collagen formation and fibroblast proliferation, as noted by Uraloğlu et al. (26). In our research, clinoptilolite did not show effectiveness in reducing fibrosis; however, it seemed to primarily exert its healing effects by dampening inflammation. Şentürk Demirel et al. (27) conducted a study on rats, by adding varying doses of clinoptilolite to their diet. They reported that incorporating 6% clinoptilolite increased collagen density and epidermal thickness. Additionally, researchers like Hubner et al. (28) have found that gelatin-based clinoptilolite and silver-infused preparations, used as antimicrobial dressings, can aid in wound healing by inhibiting the growth of pathogens like Escherichia coli and Staphylococcus aureus in wounds. While our study did not assess antibacterial and antioxidative properties, the observed reduction in epithelial thickness, alongside clinoptilolite's anti-inflammatory action, might be attributed to these unexplored effects of the substance.

Tacrolimus acts on T cells in affected skin by binding to the cytosolic FK506 binding protein-12, thereby inhibiting calcineurin enzyme activity. This action prevents T cell activation and suppresses the release of proinflammatory cytokines, making tacrolimus particularly effective against immune cells involved in AD pathogenesis. It blocks cytokine production in mast cells, eosinophils, and basophils, inhibits T cell activation by antigen-presenting dendritic cells, and reduces the number of inflammatory dendritic epidermal cells, as detailed by Rustin (29). In our research, the average clinical score for the clinoptilolite-treated group was lower than that of the tacrolimus-treated group from the first week of treatment, though the difference was not statistically significant. Upon histopathological and immunohistochemical examination, we observed that both clinoptilolite and tacrolimus reduced epidermal thickness, with clinoptilolite showing a more significant reduction than tacrolimus. Only the clinoptilolite group demonstrated a considerable reduction in dermal inflammation compared to the DNFB group. However, no difference was observed in fibrosis and mast cell count between the clinoptilolite and tacrolimus groups relative to the DNFB group. Regarding the count of TSLP-positive cells, both the tacrolimus and clinoptilolite groups had lower counts than the DNFB group, but no significant difference was noted between the two treatment groups.

TSLP, a cytokine resembling interleukin 7, is produced by activated mast cells and plays a crucial role in triggering allergic inflammation, through dendritic cell-mediated Th2 responses. TSLP is overexpressed in both acute and chronic AD skin lesions, as observed by Han et al. (23). This cytokine, produced by epithelial cells located on barrier surfaces such as the skin, lungs, and intestines in both mice and humans shows significantly elevated expression AD-affected epidermis compared to non-allergic dermatitis or healthy skin. Genetic mutations that compromise the barrier functions of skin in mouse models have been linked to increased TSLP expression in the epidermis, contributing to Th2-type inflammation and AD as noted by Indra (30). Additionally, Yoou et al. (31) found elevated levels of TSLP, IL-6, and TNF-α, alongside a higher count of inflammatory cells in DNFB-induced AD mice compared to controls. In our study, TSLP expression was notably higher in the DNFB group versus the control group. However, in both the tacrolimus and clinoptilolite groups, TSLP positivity was found to be similar to the control and significantly lower than in the DNFB group.

TARC, part of the CC chemokine family, found naturally in the thymus, is produced by dendritic cells, endothelial cells, keratinocytes, bronchial epithelial cells, and fibroblasts. Acting as a ligand for CCR4, which is primarily found in Th2 lymphocytes, basophils, and natural killer cells, TARC plays a pivotal role in AD, particularly in its acute phase where Th2 cells are predominant (32,33). Using the NC/Nga mouse model, similar to human AD, Vestergaard et al. (34) revealed that TARC significantly expressed in lesioned skin but not in healthy skin. Further studies demonstrated that NC/Nga mice with DNFB-induced AD-like lesions had higher serum TARC levels compared to controls (18), a pattern also seen in humans where AD patients show markedly higher serum TARC levels, especially in severe

cases. Notably, TARC levels decreased following treatment, correlating with clinical improvement (33). Thijs et al. (35) proposed serum TARC as a reliable marker for AD. Similarly, tissue TARC levels were also significantly higher in AD-like lesion groups (36,37).

However, our study found no significant difference in serum TARC levels between AD-like lesion groups and controls, diverging from established literature. It's known that serum TARC levels are considerably higher in infants than adults and decrease significantly with age (38). Additionally, thymus cellularity in mice notably decreases with age, almost halving by 12 months (39). Given that our mice were, on average, 24 weeks old at the start and around 33 weeks or 8 months old at the end, this age-related reduction in thymic size and TARC level could explain the lack of significant increase in TARC in our AD mice, suggesting that TARC may not be a reliable marker for diagnosing AD in adult mice.

Conclusion

This study aimed to evaluate the efficacy of tacrolimus cream and clinoptilolite, which has been used for a long time in the treatment of atopic dermatitis model in mice. Although it seems to contribute to the improvement of some local clinical and histopathological parameters, it has been observed that it is better in terms of correcting some parameters when compared to tacrolimus. It is thought that this mouse model can contribute to atopic dermatitis and its treatment, which is an important problem in both human and veterinary medicine. As a result, it was concluded that clinoptilolite may be a safe alternative in the treatment of atopic dermatitis with local lesions. However, further studies are needed to elucidate the therapeutic potential and molecular mechanism of clinoptilolite in the treatment of AD.

Ethics statements

Before the study, the ethics committee approval was obtained of Burdur Mehmet Akif Ersoy University Experimental Animals Local Ethics Committee (17.02.2021; decision number:727).

Funding

This research was supported by the "Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordinatorship" with the project 0668-YL-20.

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