Original Article

Low-dose Apremilast Versus Low-dose Cyclosporine: Antipruritic Efficacy and Reversal of Epidermal Pathology in a Mouse Model of Atopic Dermatitis

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Abstract

Background: Itch control is important in improving the atopic dermatitis patients' quality of life, reducing the damage to the skin barrier, and, thereby, adding to the downregulation of skin inflammation. We aimed to investigate the efficacy of calcineurin inhibition by cyclosporine versus phosphodiesterase-4 inhibition by apremilast in controlling pruritus and reversing skin pathology in an experimental model of atopic dermatitis (AD) induced by oxazolone in mice. **Materials and Methods:** Forty BALB/c female mice were randomly assigned to four groups. AD-like lesions were induced in groups 2, 3, and 4 by repetitive application of oxazolone to the mouse skin. Group 2 mice were left untreated receiving vehicle placebo, whereas those in groups 3 and 4 received cyclosporine (2 mg/kg PO daily) and apremilast (2.5 mg/kg PO twice daily), respectively. Studied mice were subjected to weekly assessment of skin inflammation and scratching behavior for 6 weeks. The oxazolone-treated right ear thickness and skin hydration were measured at the end of the study. Serum immunoglobulin E (IgE) and interleukin (IL)-31 were measured, and biopsies of lesional back skin were obtained for histopathologic evaluation. **Results:** Both cyclosporine and apremilast significantly reduced scratching behavior in treated mice, accompanied by a significant decrease in the elevated levels of IL-31 and IgE by both drugs. IL-31 and IgE suppressions were significantly greater with apremilast. A significant reduction of mean itching started earlier at week 3 with apremilast versus week 4 with cyclosporine. **Conclusion:** We propose that the earlier control of itch observed with apremilast is clinically significant as this will lead to less epidermal damage and that will interrupt the itch-scratch cycle and progression of dermatitis.

Keywords: Atopic dermatitis, cyclosporine, itch, phosphodiesterase-4 inhibitor

Introduction

Atopic dermatitis (AD) is a chronic pruritic inflammatory dermatosis associated with an impaired skin barrier function.[1] Itching is a hallmark of AD to the extent that the disease has been described as an "itch that rashes." Chronic pruritus not only affects the patients' psychological well-being and quality of life but also injures epithelial keratinocytes promoting the release of inflammatory alarmins that activate Th2 cells to release inflammatory and pruritogenic cytokines that augment skin inflammation and pruritus.[2] Controlling AD-related itch is, therefore, considered to be a cornerstone in the management of AD.[3]

AD pruritus is believed to be mediated by the action of nonhistaminergic pathways and, thereby, does not respond to conventional antihistamines. Pruritogens including keratinocyte-derived products, mast cell factors,

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environmental allergens, pathogen-derived molecules, and inflammatory cytokines act on pruritogenic receptors.[2] Immune cells involved in the pathogenesis of AD such as T-helper cell 2 (Th2) lymphocytes, eosinophils, neutrophils, and mast cells activate the pruriceptive pathways through the release of cytokines and neurogenic peptides. The AD-associated interleukin (IL)-31 "itch cytokine" stimulates itch by activation of the receptors on pruriceptive neurons. IL-4 further sensitizes pruriceptive-sensory neurons to direct pruritogens as IL-31.[2] IL-31 also binds to its receptor IL-31RA on keratinocytes maintaining the chronicity of inflammation and atopic itch.[2]

Cyclosporine A (CsA) is a calcineurin inhibitor that acts primarily on T cells to inhibit signal transduction mediated by T-cell receptor activation.[4] It is a commonly used drug for systemic treatment of moderate-to-severe AD unresponsive to topical therapy and oral antihistamines.[5] Phosphodiesterase-4 (PDE4) is involved in the regulation of proinflammatory cytokines through the degradation of cyclic adenosine monophosphate. PDE4 activity was reported to be increased in the inflammatory cells of patients with AD leading to increased production of proinflammatory cytokines and chemokines. Inhibition of PDE4 will, therefore, lead to the reduction of the production of proinflammatory mediators in AD.[6] Apremilast is a PDE4 inhibitor (PDE4I) that is better tolerated, with a more favorable safety profile than cyclosporine.[7] The most commonly reported side effects of apremilast are mild as diarrhea, nausea, upper respiratory infection, and headache with no known end-organ damage.[8] It has been approved by The United States Food and Drug Administration (FDA) for the treatment of plaque psoriasis and psoriatic arthritis. Apremilast has demonstrated a potential as a treatment option for AD.[6,9] In the current study, we compared the potential antipruritic effects of cyclosporine and apremilast in an experimental chronic AD mouse model induced by oxazolone.

Materials and Methods

Animal care measures and experimental procedures were all conducted in accordance with the National Institute of Health Animal Care Guidelines.[10] The research protocol was approved by the institutional ethics committee (IRB 00012098).

Based on the reported mean ear thickness of 0.27 mm in cyclosporine-treated AD mice,[11] 0.39 mm in apremilast-treated AD mice,[12] 0.43 mm in vehicle-administered AD mice,[12] and 0.21 mm in normal mice,[12] the sample size was calculated using the G-power software (Heine University Dusseldorf, Dusseldorf, Germany) using a one-way analysis of variance (ANOVA) analysis, adjusting a power at 80%, level of confidence at 95, and effect size 0.6. The minimum sample size needed to investigate the efficacy of cyclosporine versus apremilast in controlling pruritus and reversing epidermal pathology in oxazolone-induced AD mouse model is 36 female BALB/c female mice (nine per group).

Forty BALB/c 5-week-old female mice were purchased from the animal house of the medical physiology department and housed in clean polypropylene cages at a room temperature of 22–25°C and a 12 h dark/12 h light cycle with free access to food and tap water throughout the experiments. Mice were allowed a period of 1 week for adaptation after which they were randomly assigned to four groups.

Group 1 (normal control mice)

Ten mice in which distilled water was painted to the right ear and shaved rostral back as a single application and continued from day 8 every other day for 6 weeks instead of 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone).

Group 2 (untreated atopic dermatitis mice)

Ten mice were exposed to a single application of 20 µL of 5% oxazolone (Sigma-Aldrich, St. Louis, Missouri) dissolved in a mixture of acetone and olive oil (4:1),[13] which was painted to the right ear and shaved rostral back, to develop acute dermatitis. Starting at day 8, mice were rechallenged by 0.1% oxazolone solution (20 µL applied to the right ear and 40 µL applied to the shaved rostral back) every other day for 6 weeks to develop chronic dermatitis and received vehicle (placebo) daily by gavage feeding for 6 weeks.

Group 3 (cyclosporine-treated atopic dermatitis mice)

Ten mice were similarly challenged as group 2. Starting at day 8, the mice received cyclosporine in a dose of 2 mg/kg/day by gavage in 200 µL of water for 6 weeks (Neoral, Novartis, Switzerland). Three low-dose cyclosporine regimens (2, 5, and 10 mg/kg/day) were initially tested in a pilot study (five oxazolone-induced AD mice per group) and the lowest effective dose with no renal toxicity was chosen, which was 2 mg/kg/day. The three doses were initially tested against a vehicle-treated mouse as regards oxazolone-treated ear thickness. All three doses were associated with decreased ear thickness compared with the control group. Serum creatinine levels were measured. We observed that two mice of the 5-mg/kg/day treated mice developed diarrhea and two of the 10-mg/kg/day treated group demonstrated gingival hyperplasia. None of the mice showed increase serum creatinine levels. All three groups showed decreased ear thickness relative to the control group. We, therefore, selected a low-dose cyclosporine of 2 mg/kg/day.[14] It is known that the risk of chronic cyclosporine nephropathy is minimal with doses less than 5 mg/kg/day,[15] and serum creatinine in

mice receiving 2 mg/kg of CsA was reported to be similar to that of non-CsA-treated mice.[16] In the present study, the oral route for cyclosporine administration was chosen because of its clinical relevance in patient treatment.

Group 4 (apremilast-treated atopic dermatitis mice)

Ten mice were similarly challenged as group 2. Starting at day 8, the mice received apremilast 2.5 mg/kg (Otezla, Amgen, California) dissolved in vehicle and administered in a volume of 5 mL/kg twice daily by gavage feeding for 6 weeks. Similarly, for apremilast, we tested 2.5, 5, and 25 mg/kg twice daily doses in a pilot study (five oxazolone-induced AD mice each). All three doses were associated with decreased oxazolone-treated ear thickness compared with the vehicle-treated group. The 2.5- and 5-mg/kg twice daily treated mice demonstrated no side effects. Three of the 25-mg/kg/day treated mice suffered from vomiting. The 2.5 mg/kg twice daily dose was chosen for the study by virtue of the absence of observed side effects and decreased ear thickness. Furthermore, in a preclinical toxicology study conducted on mice (CC-10004-TOX-004) receiving 10, 100, and 1000 mg/kg/day of apremilast daily, the no-observed adverse effect level (NOAEL) was demonstrated to be 10 mg/kg/gay. Hence, the 2.5 mg/kg twice daily dose employed in our study represents 50% of NOAEL.[17]

All behavioral tests and study measurements were performed by an experimenter blinded to experimental conditions. The following parameters were evaluated:

a) Scratching behavior: Mice were placed individually in acrylic cages. A camcorder (HDR-SR11; Sony, Tokyo, Japan) was positioned above the observation chambers to record the behavior of the mice. Mice were allowed an acclimation period of 1 h, after which a challenge with oxazolone was done, and the mice were quickly returned to the observation chamber. Mice could not see each other during an experiment. The behavior of mice was recorded on video for 40 min with no experimenters present in the observation room, and the number of scratching bouts was assessed by monitoring and counting the replays of each video. A scratching bout was defined as the raising to lowering of a leg, scratching behind the ears was counted, whereas scratching episodes on the face were not counted. One scratching bout was defined as a single or uninterrupted scratching actions of the hindpaws to the neck area that ended with the animals putting the hindpaws back on the floor or licking the hindpaws. Scratching behavior was observed weekly for 6 weeks and expressed as the number of scratching bouts/40 min.
b) Skin hydration was evaluated by EnviroDerm Services Tewameter (Dermal Measurement System EDS12, UK) at the end of the 6th week as an indicator of the epidermal barrier function.[18]
c) Skin inflammation severity scoring was assessed weekly by the Matsuoka scoring system.[19] The severity of the macroscopic clinical signs of dermatitis was measured by the extent of (1) erythema/hemorrhage, (2) scarring/dryness, (3) edema, and (4) excoriation/erosion. The score for each criterion was graded as follows: 0 (none), 1 (mild), 2 (moderate), and 3 (severe).
d) The right ear thickness was measured at the end of the 6th week by using a micrometer (Mitutoyo Corp, Kawasaki, Japan). The micrometer was applied to the right ear edge immediately adjacent to the cartilage bulge, and thickness was recorded. Each measurement was taken twice, and the mean of the two readings was calculated. Measurements were made by a single independent blinded observer to ensure similar pressure and placement of the micrometer.
e) Serologic evaluation: Blood samples from the abdominal aorta of mice were obtained after sacrificing the mice at the end of the 6th week. Serum was collected immediately from the blood by centrifugation and stored at −80°C till laboratory measurements. Serum immunoglobulin E (IgE) and IL-31 concentrations were measured using mouse solid phase standard sandwich IgE enzyme-linked immunosorbent assay (ELISA) (Chongqing Biopsies Co, Ltd, Chongquing, China) and IL-31 ELISA kits (Innova Biotech Co Limited, Chai Wan, Hong Kong) following the manufacturer's instructions. Samples were analyzed in duplicate and expressed in ng/L.[18,20] Blood samples were also obtained after sacrificing the animals for serum creatinine assessment to confirm the absence of renal toxicity.
f) Histopathological examination.

Following scarification, the liver and kidneys were fixed in 10% neutral-buffered formalin for the histopathological examination to confirm the absence of toxicity, and skin specimens were collected from the rostral back skin and ear skin of mice in all groups, then fixed in 10% neutral-buffered formalin solution. After a minimum of 24 h, specimens were subjected to dehydration in ascending grades of ethanol, then cleared in xylene and embedded in paraffin wax. Tissue sections (3–5-μ thick) were cut and stained with hematoxylin and eosin and Masson's trichrome stain according to Bancroft and Stevens,[21] and histopathologically evaluated at (×400).

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0 (IBM Corp., Armonk, New York). Categorical data were represented as numbers and percentages. The Chi-square test was applied to investigate the association between the categorical variables. Alternatively, the Monte Carlo correction test was applied when more than 20% of the cells had expected counts of less than 5. For continuous data, they were tested for normality by the Shapiro–Wilk test. Quantitative data were expressed as range (minimum and maximum), mean, standard deviation, and median.

The ANOVA test was used for comparing the four studied groups and followed by the post hoc test (Tukey) for pairwise comparison. Pearson coefficient was used to correlate between normally distributed quantitative variables. Significance of the obtained results was judged at the 5% level.

Results

Thickness of the oxazolone-treated ear

The mean thickness of the oxazolone-treated ear in normal control mice was 0.41 ± 0.05 mm compared with 1.0 ± 0.09 mm in untreated AD mice ($P < 0.001$). The thickness of the oxazolone-treated ear was lowest in the apremilast-treated AD mice (0.61 ± 0.07), followed by cyclosporine-treated AD mice (0.64 ± 0.05), and highest in untreated AD mice (1.0 ± 0.09). Both cyclosporine- and PDE4I-treated mice groups had lower ear thickness (0.64 ± 0.05 mm, 0.61 ± 0.07 mm) than the untreated AD mice ($P < 0.001$). The difference between the cyclosporine- and PDE4I-treated mice groups was statistically insignificant ($P = 0.784$) [Table 1 and Figure 1].

Skin inflammation scoring at the 6th week

The mean Matsuoka score was 0 ± 0 and 8.0 ± 0.67 in normal controls and AD-untreated mice, respectively ($P < 0.001$). The mean Matsuoka score was reduced in both cyclosporine-treated mice and apremilast-treated AD mice (2.60 ± 0.52 and 2.20 ± 0.42, respectively) compared with untreated AD mice (8.0 ± 0.67). This difference was statistically significant ($P < 0.001$). The mean Matsuoka score was lower in the apremilast group than in the cyclosporine treatment group, but the difference was statistically insignificant ($P = 0.247$) [Table 1 and Figure 2].

Mice scratching behavior at the 6th week

The mean scratching score was 9.7 ± 1.25 and 75.8 ± 4.49 in normal controls and AD-untreated mice, respectively ($P < 0.001$). The mean scratching score was reduced in

Table 1: Clinical and laboratory parameters in the studied groups at the 6th week

Clinical/lab parameter	Normal negative control ($n = 10$)	Untreated atopic dermatitis mice ($n = 10$)	Cyclosporine-treated mice ($n = 10$)	PDE4 inhibitor-treated mice ($n = 10$)	P[a]
Thickness of oxazolone-treated ear (mm)					
Mean ± SD (range)	0.41 ± 0.05 (0.34–0.50)	1.0 ± 0.09 (0.88–1.12)	0.64 ± 0.05 (0.58–0.74)	0.61 ± 0.07 (0.53–0.74)	<0.001*
P_0		<0.001*	<0.001*	<0.001*	
		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 = 0.784$			
Matsuoka score					
Mean ± SD (range)	0 ± 0 (0–0)	8.0 ± 0.67 (7–9)	2.60 ± 0.52 (2–3)	2.20 ± 0.42 (2–3)	<0.001*
P_0		<0.001*	<0.001*	<0.001*	
		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 = 0.247$			
Scratching score					
Mean ± SD (range)	9.7 ± 1.25 (8–12)	75.8 ± 4.49 (65–81)	21.4 ± 2.41 (18–26)	19.6 ± 2.17 (17–23)	<0.001*
P_0		<0.001*	<0.001*	<0.001*	
		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 = 0.497$			
Hydration					
Mean ± SD (range)	3.80 ± 0.79 (3–5)	1.0 ± 0 (1–1)	2.0 ± 0.67 (1–3)	2.30 ± 0.67 (1–3)	<0.001*
P_0		<0.001*	<0.001*	<0.001*	
		$P_1 = 0.005$*, $P_2 < 0.001$*, $P_3 = 0.699$			
Ig E (ng/mL)					
Mean ± SD (range)	34.2 ± 9.3 (23–49.5)	231 ± 69.2 (168–350)	119 ± 19.4 (94.5–160)	67.95 ± 20.97 (45– 110)	<0.001*
P_0		<0.001*	<0.001*	0.206	
		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 = 0.025$*			
IL-31 (ng/L)					
Mean ± SD (range)	6.90 ± 1.17(5–9)	24.40 ± 0.66(23–25)	15.65 ± 1.03(14–17)	11.85 ± 1.06(10–13)	<0.001*
P_0		<0.001*	<0.001*	<0.001*	
		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 < 0.001$*			

SD = standard deviation.

P: P value for comparing between the four studied groups.

P_0: P value for comparing between Negative control and each of the other groups.

P_1: P value for comparing between untreated atopic dermatitis mice and Cyclosporine-treated mice.

P_2: P value for comparing between untreated atopic dermatitis mice and PDE4 inhibitor-treated mice.

P_3: P value for comparing between Cyclosporine-treated mice and PDE4 inhibitor-treated mice.

[a]One way ANOVA test, pairwise comparison between each two groups was done using post hoc test (Tukey).

*Statistically significant at $P \leq 0.05$

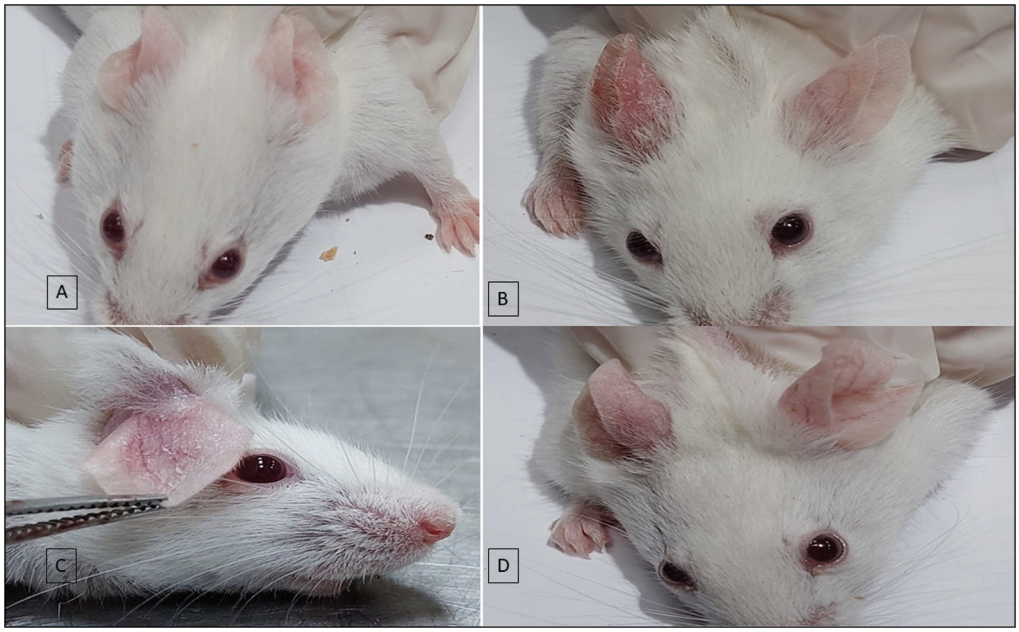

Figure 1: Right ear at the 6th week in (A) normal mice, (B) untreated AD mice, (C) cyclosporine-treated mice, and (D) apremilast-treated mice

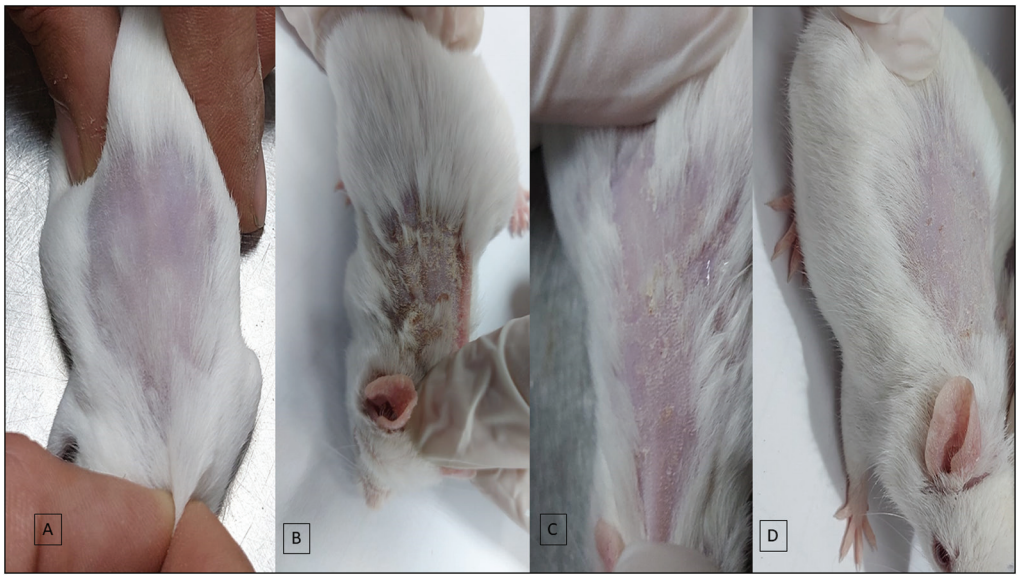

Figure 2: Rostral back skin at the sixth week in (A) normal mice, (B) untreated AD mice, (C) cyclosporine-treated mice, and (D) apremilast-treated mice

cyclosporine-treated AD mice and PDE4I-treated AD mice (21.4 ± 2.41 and 19.6 ± 2.17, respectively) compared with untreated AD mice (75.8 ± 4.49). This difference was statistically significant ($P < 0.001$). The mean scratching score was lower in the PDE4I-treated group compared with the cyclosporine-treated group, but the difference was statistically insignificant ($P = 0.497$) [Table 1].

Scratching scores during different time periods in the three groups

At the end of week 1, the mean scratching scores were 8.0 ± 1.05, 39.70 ± 1.57, 38.40 ± 3.17, and 38.60 ± 2.22 in the normal control, untreated AD, cyclosporine-treated, and apremilasttreated AD mice, respectively. At the of the 4th week, the mean scores were 8.20 ± 0.92, 57.70 ± 3.74, 30.0 ± 3.37, and 27.60 ± 3.06 in normal controls, untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the end of the 5th week, the mean Matsuoka scores were 8.50 ± 0.53, 66.40 ± 3.86, 25.30 ± 3.06, and 22.90 ± 2.69 in normal controls, untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the end of the study, the mean scores were 9.7 ± 1.25, 75.8 ± 4.49, 21.4 ± 2.41, and 19.6 ± 2.17 in normal controls, untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively.

Compared with week 1, the apremilast-treated group showed a significant reduction of the scratching score starting at week 3, which significantly decreased further at weeks 4, 5, and 6. However, the cyclosporine-treated group demonstrated a significant reduction of the scratching behavior starting at week 4 and decreased significantly further at weeks 5 and 6 [Figure 3].

Matsuoka scores during different time periods in the three groups

At the end of week 1, the mean Matsuoka scores were 6.10 ± 0.74, 6.30 ± 0.48, and 6.40 ± 0.52 in the untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the end of the 2nd week, the mean Matsuoka scores were 6.80 ± 0.63, 5.10 ± 0.74, and 5.30 ± 0.48 in untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. The mean scores at the end of the 3rd week were 7.10 ± 0.74, 4.50 ± 0.71, and 4.50 ± 0.53 in untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the of the 4th week, the mean scores were 7.50 ± 0.71, 3.50 ± 0.71, and 3.70 ± 0.48 in untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the end of the 5th week, the mean Matsuoka scores were 7.70 ± 0.48, 2.80 ± 0.42, and 2.80 ± 0.42 in untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. At the end of the study, the mean scores were 8.0 ± 0.67, 2.60 ± 0.52, and 2.20 ± 0.42 in untreated AD, cyclosporine-treated, and apremilast-treated AD mice, respectively. Both the cyclosporine-treated and apremilast-treated mice groups showed a significant reduction of the Matsuoka scores starting at week 2 until the end of the study at the 6th week.

Skin hydration at the 6th week as an indicator of skin barrier function

The mean hydration at the 6th week in normal control mice was 3.80 ± 0.79 compared with 1.0 ± 0 in AD-untreated mice ($P < 0.001$). The mean hydration levels were 2.0 ± 0.67 and 2.30 ± 0.67 in the cyclosporine and apremilast-treated groups, respectively, which were significantly higher than AD-untreated mice ($P = 0.005$ and $P < 0.001$, respectively). The difference between cyclosporine- and PDE4I-treated AD mice groups regarding skin hydration at the 6th week was statistically insignificant ($P = 0.699$) [Table 1].

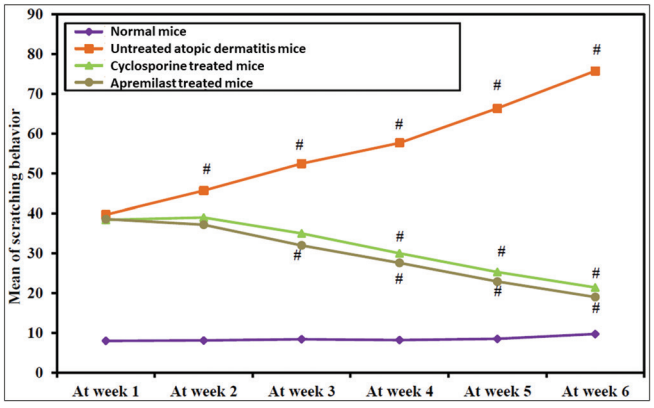

Figure 3: Weekly scratching scores in the studied groups

Serum IL-31 and IgE levels

The mean serum IL-31 was 6.90 ± 1.17 ng/L and 24.40 ± 0.66 ng/L in normal control mice and AD-untreated mice, respectively. This difference was statistically significant ($P < 0.001$). Either group receiving cyclosporine and apremilast had significantly lower mean serum IL-31 (15.65 ± 1.03 ng/L and 11.85 ± 1.06 ng/L, respectively) than untreated AD mice (24.40 ± 0.66 ng/L). This difference was statistically significant ($P < 0.001$). The mean serum IL-31 was significantly lower in AD mice receiving apremilast than in AD mice receiving cyclosporine ($P < 0.001$) [Table 1].

Both the apremilast-treated group and cyclosporine-treated group had significantly lower serum IgE level (67.95 ± 20.97 and 119 ± 19.4, respectively) than AD-untreated mice (231 ± 69.2). The mean serum IgE was significantly lower in AD mice receiving apremilast than in AD mice receiving cyclosporine ($P < 0.001$) [Table 1].

Histopathologic evaluation

Rostral back lesional skin

The mean thickness of the epidermis was significantly lower in normal controls (141.8 ± 47.41 µm) than in the lesional skin in the AD model group (507.3 ± 197.0 µm) ($P = 0.003$). The mean epidermal thickness in the cyclosporine-treated group (303.4 ± 93.15 µm) and apremilast-treated AD mice (134.3 ± 19.87 µm) were significantly lower than the epidermal thickness in untreated AD mice ($P = 0.008$ and $P = 0.002$, respectively). The difference between cyclosporine- and apremilast-treated mice was statistically insignificant ($P = 0.197$) [Table 2 and Figure 4].

The mean number of dermal cell infiltrate was 27.25 ± 4.57 cells/×400 in the normal controls versus 78.0 ± 10.68 cells/×400 in the untreated AD mice group ($P < 0.001$). The mean number of cell infiltrate in the cyclosporine-treated mice (36.75 ± 3.10 cells/×400) and the apremilast-treated mice (49.25 ± 7.76 cells/×400) were significantly lower than the untreated AD mice ($P < 0.001$ and $P = 0.001$, respectively). The difference between cyclosporine- and apremilast-treated mice was statistically insignificant ($P = 0.116$) [Table 2 and Figure 4].

Oxazolone-treated ear skin

The mean epidermal thickness of the right ear skin in the normal control mice was 49.27 ± 0.06 µm and 204.0 ± 4.86 µm in the untreated AD mice ($P < 0.001$). The mean epidermal thickness of the ear skin in each of the cyclosporine-treated (158.7 ± 36.33 µm)

Table 2: Histopathological scores of the lesional back and right ear skin

Histopathologic parameter	Normal negative control (n = 10)	Untreated atopic dermatitis mice (n = 10)	Cyclosporine-treated mice (n = 10)	PDE4 inhibitor-treated mice (n = 10)	P[a]
Lesional back skin					
Thickness of skin epidermis (μm)	141.8 ± 47.41	507.3 ± 197.0	303.4 ± 93.15	134.3 ± 19.87	0.001*
P_0		0.003*			
			P_1 = 0.008, P_2 = 0.002*, P_3 = 0.197		
Number of cell infiltrates (cells/×400)	27.25 ± 4.57	78.0 ± 10.68	36.75 ± 3.10	49.25 ± 7.76	<0.001*
P_0		<0.001*			
			P_1 < 0.001*, P_2 = 0.001*, P_3 = 0.116		
Oxazolone-treated ear skin					
Thickness of skin epidermis (μm)	49.27 ± 0.06	204.0 ± 4.86	158.7 ± 36.33	121.0 ± 18.01	<0.001*
P_0		<0.001*			
			P_1 = 0.037*, P_2 < 0.001*, P_3 = 0.091		
Thickness of skin dermis (μm)	231.8 ± 28.82	874.8 ± 131.4	426.7 ± 40.01	355.9 ± 138.7	<0.001*
P_0		<0.001*			
			P_1 < 0.001*, P_2 < 0.001*, P_3 = 0.745		
Number of cell infiltrates (cells/×400)	20.25 ± 0.96	89.50 ± 5.0	72.75 ± 4.50	46.75 ± 2.06	<0.001*
P_0		<0.001*			
			P_1 < 0.001*, P_2 < 0.001*, P_3 < 0.001*		

SD = standard deviation.

Data were expressed by using mean ± SD.

P: P value for comparing between the four studied groups.

P_0: P value for comparing between negative controls.

P_1: P value for comparing between untreated atopic dermatitis mice and cyclosporine-treated mice.

P_2: P value for comparing between untreated atopic dermatitis mice and PDE4 inhibitor-treated mice.

P_3: P value for comparing between cyclosporine-treated mice and PDE4 inhibitor-treated mice.

[a]One way ANOVA test, pairwise comparison between each 2 groups was done using the post hoc test (Tukey).

*Statistically significant at $P \leq 0.05$

and apremilast-treated mice (121.0 ± 18.01 μm) were significantly lower than in the untreated AD group (P = 0.037 and P < 0.001, respectively). The difference between cyclosporine- and apremilast-treated mice was statistically insignificant (P = 0.091) [Table 2 and Figure 5].

The mean dermal thickness of the ear skin was significantly lower in normal control mice (231.8 ± 28.82 μm) versus untreated AD mice (874.8 ± 131.4 μm) (P < 0.001). The mean dermal thickness of the ear skin in the cyclosporine-treated group (426.7 ± 40.01 μm) and the apremilast-treated group (355.9 ± 138.7 μm) was significantly lower than in the untreated AD mice (P < 0.001). The difference in ear dermal thickness between cyclosporine- and apremilast-treated mice was statistically insignificant (P = 0.745) [Table 2 and Figure 5].

The mean number of cell infiltrate in the normal controls (20.25 ± 0.96 cells/×400) was significantly lower than the untreated AD group cells (89.50 ± 5.0 cells/×400). The mean number of cell infiltrate in the cyclosporine-treated mice (72.75 ± 4.50 cells/×400) and the apremilast-treated mice (46.75 ± 2.06 cells/×400) was significantly lower than in the untreated AD mice (P < 0.001). The mean number of cell infiltrate in the two treated groups was statistically significant (P < 0.001) [Table 2 and Figure 5].

Discussion

AD is a common dermatologic disease with a worldwide prevalence of about 34% in children and 10% in adults.[22] The disease is characterized by an impaired barrier function, eczematous dermatitis, and chronic itching.[23] Reduction of pruritus contributes to barrier repair and suppression of cutaneous inflammation and is, therefore, considered a cornerstone in the AD management.[24,25]

Interestingly, itching in AD does not respond to systemic antihistamines. It has been postulated that the atopic itch is conveyed through nonhistaminergic sensory nerves. These nonhistaminergic sensory nerves are believed to be stimulated primarily by inflammatory mediators central to AD pathogenesis. Released alarmins TSLP, IL-33, and IL-25 stimulate itch and activate both innate and adaptive immune responses that accentuate the predominant Th2 inflammatory

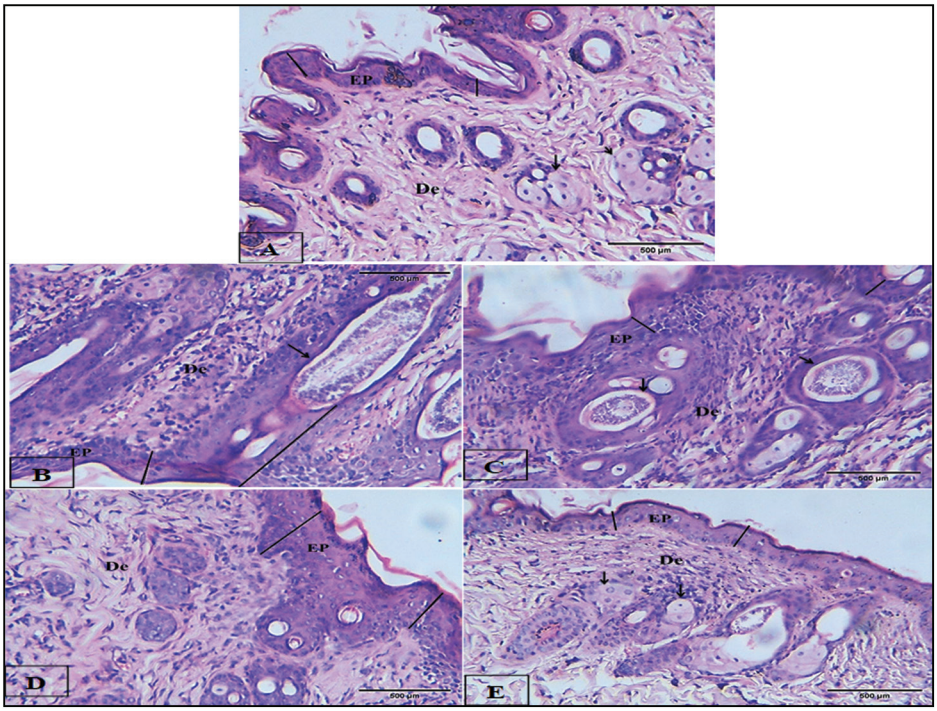

Figure 4: Rostral back skin sections in (A) normal mice showing external thin layer of epithelium (EP) over the dermis (De) cell layer and the sebaceous glands are fully developed (black arrows). (B and C) untreated AD mice showing loss of normal structure with marked hyperplasia of the epidermis and epidermal thickening (EP), and dermal infiltrate (De) consists of neutrophils, eosinophils, and lymphocytes and spongiosis (intraepidermal edema) are seen and most glandular tissue is seen as a cystic structure (black arrows). (D) Cyclosporine-treated mice showing almost normal structure with hyperplasia of the epidermis and epidermal thickening (EP) still present and dermal infiltrate (De) consists of neutrophils, eosinophils, and lymphocytes and spongiosis (intraepidermal edema) are seen. (E) Apremilast-treated mice showing almost normal structure with thin layer of EP over the De cell layer with mild dermal infiltrate (De) consists of neutrophils, eosinophils, and lymphocytes and the sebaceous glands are fully developed (black arrows)

immune response in AD and stimulate the generation of pruritus.[24] IL-31, also known as the itch cytokine, is believed to play an important role in the pathogenesis of atopic itch. It is produced by Th2 cells and acts on IL-31 receptors on sensory nerves generating itch sensation. The binding of IL-31 to its receptors on sensory nerves also stimulates the branching of the sensory nerves and also decreases the stimulatory threshold to IL-31 and other pruritogens. This increased sensitivity of sensory nerves is believed to be responsible for the chronic itch and perpetuation of the itch-scratch cycle.[24]

We employed an AD mouse model in BALB/c 5-week-old female mice based on outside–inside theory and compared the efficacy of cyclosporine and apremilast in the inhibition of pruritus and cutaneous inflammation. Our untreated AD mice demonstrated increased scratching behavior and decreased skin hydration compared with normal control mice. Mice in the employed AD mouse model demonstrated skin inflammation evidenced clinically by significantly higher Matsuoka score, and histopathologically by increased epidermal and dermal thickness, significant dermal inflammatory infiltrate of the oxazolone-treated ear skin and increased epidermal thickness and evident dermal cellular inflammatory infiltrate of the lesional back skin. These observations are in agreement support that repetitive extracutaneous application of the haptenoxazolone induces sensitization. This repetitive exposure provokes a Th2 immune response with several AD-like features such as scratching behavior and eczematous dermatitis. It also induces increased epidermal and dermal thickness and an inflammatory dermal infiltrate with several ultrastructural changes of decreased expression of skin differentiation proteins, decreased stratum corneum ceramide content leading to decreased stratum corneum hydration, and increased transepidermal water loss.[26]

The untreated AD mice also demonstrated significantly higher mean serum IgE and IL-31 levels than the normal control mice. The Th2 inflammatory response induced by repetitive oxazolone application AD stimulates B cells to produce IgE that binds with IgE receptors on several immune cells such as mast cells, basophils, and eosinophils inducing further production of cytokines, chemokines, histamine, and leukotrienes, maintaining and exacerbating the inflammatory response and clinical manifestations of AD. In fact, elevated IgE is regarded as a key immunologic feature of AD.[27-29] IL-31 is also known to be predominantly produced by Th2 cells.[25] A meta-analysis by Lu *et al*.[30] reported that serum IL-31

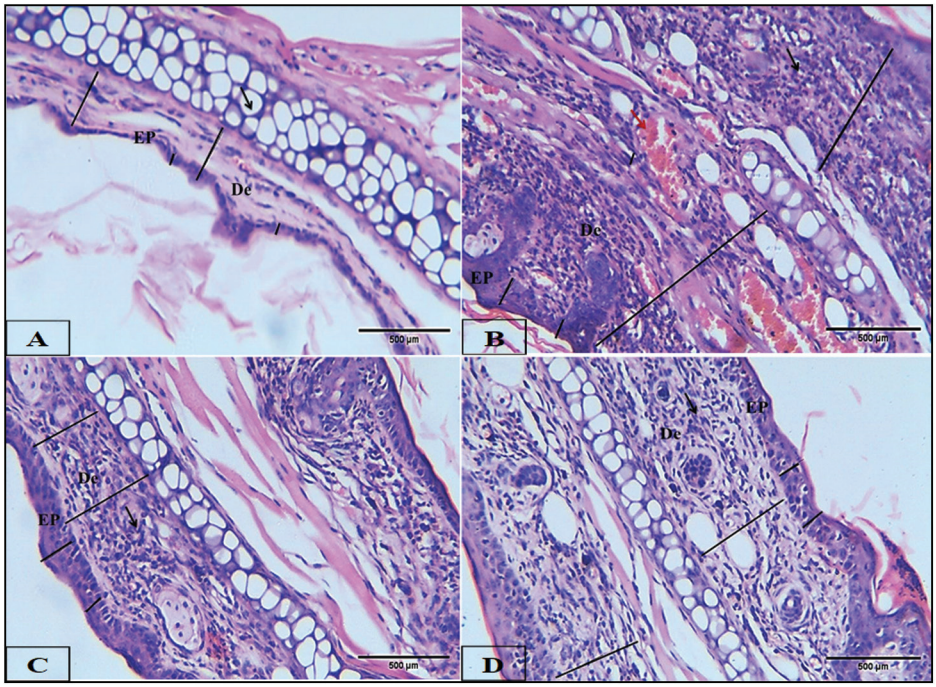

Figure 5: Ear skin sections in (A) normal mice showing normal thin epidermal layer of epithelium (EP) over the dermal layer (De) cell layer and cartilage (black arrow). (B) Untreated AD mice showing loss of normal structure with marked hyperplasia of the epidermis, epidermal (EP) and subepidermal thickening, and dermal dense cellular infiltrates dermis (De) of primarily mononuclear and some polymorphonuclear cells and spongiosis (black arrow) and sever hemorrhage (red arrow). (C) Cyclosporine-treated mice showing almost normal structure with hyperplasia of the epidermis and epidermal thickening (EP) still present and dermal infiltrate (De) consists of mononuclear and some polymorphonuclear cells and spongiosis (black arrow). (D) Apremilast-treated mice showing almost normal structure with thin epidermal layer of EP over the De cell layer with mild dermal infiltrate (De) consists of mononuclear and some polymorphonuclear cells and spongiosis (black arrow) and hyperplasia of the dermal cell layer

is significantly higher in AD patients than in normal controls. Available data suggests that IL-31 plays a possible role in AD pathogenesis and generation of itch.[25,31]

The cyclosporine-treated AD mice in this study demonstrated a significant reduction of dermatitis severity and increased skin hydration compared with untreated mice as evidenced by a significantly lower mean thickness of oxazolone-treated ear skin, mean Matsuoka score, and a higher mean epidermal hydration score. In agreement with our observations, Ko *et al*.[32] reported that intraperitoneal injection of CsA (5 mg/kg) significantly reduced dermatitis severity and transepidermal water loss in the AD mice model. These effects are the result of CsA-mediated T-lymphocyte activation and transcription of IL-2 and other cytokines involved in AD.

Our results show that cyclosporine-treated AD mice had significantly lower mean serum IgE and IL-31 levels than untreated AD mice. Lucae *et al*.[33] suggested that serum IgE levels in AD patients parallel the degree of skin inflammation, which explains the reduction of serum IgE following the reduction of skin inflammation with cyclosporine treatment. Cyclosporine is a calcineurin inhibitor that inhibits the activation of nuclear factor of activated T cells, decreasing T-lymphocyte activation and cytokine transcription of interferon-gamma (IFN-γ)/TH1– and IL-4/IL-13/IL-5/TH2–producing T cells and associated products including IL-31.[4,34]

The cyclosporine-treated AD mice in our study also demonstrated significantly lower epidermal and dermal thickness and lower dermal inflammatory infiltrate of the oxazolone-treated ear skin. The rostral back skin of cyclosporine-treated mice also showed a significantly lower epidermal thickness and significantly less dermal inflammatory infiltrate compared with AD mice. Ko *et al*.[32] reported that intraperitoneal injection of CsA (5 mg/kg) significantly reduced the epidermal thickness of treated mice. Similarly, Khattri *et al*.[4] reported that regenerative hyperplasia of the epidermis of AD skin was reversed with CsA as evidenced by reductions in epidermal proliferation and differentiation markers. This might be secondary to the CsA-mediated reduction of factors regulating epidermal hyperplasia (IL-19, IL-22, fibroblast growth factor, and vascular endothelial growth factor) and TH2/IL-13–, IL-19–, and IL-22/IL-17–modulated genes (S100A7-9 and PI3/elafin).

We reported a significantly lower scratching score in cyclosporine-treated mice than in untreated AD mice. Ko *et al*.[32] similarly reported that intraperitoneal injection of

CsA (5 mg/kg) significantly reduced scratching behavior and a number of scratching bouts. The inhibition of itch-related cytokines, such as IL-31, improved skin barrier function, reduction of acanthosis, and dermal inflammatory cell infiltrate to explain the antipruritic effects of cyclosporine treatment.

We reported a significantly lower thickness of oxazolone-treated ear skin and mean disease severity scores (Matsuoka scores) and improved barrier function (skin hydration) in apremilast-treated mice compared with AD mice. Schafer *et al*.[12] showed that apremilast of 2.5 mg/kg twice daily significantly reduced ear swelling in two models of dermatitis. Bissonnette *et al*.[35] showed that topical PDE4I reversed improved skin barrier function in terms of decreased transepidermal water loss.[35] Apremilast inhibits T-helper 1 and T-helper 17 cells through inhibition of IL-12 and IL-23 release from monocytes, respectively. Furthermore, it decreases prostaglandin E2-suppressing Th2 cell response. Inflammatory cytokines such as IFN-γ and tumor necrosis factor-alpha released from Th1 cells, IL-4 and IL-13 released from Th2 cells, and IL-17 and IL-22 released from Th17 cells are, thereby, decreased.[8] This inhibition of T-cell immune responses explains the observed reduction of clinical signs of inflammation.

We demonstrated significantly lower epidermal and dermal thickness and less dense dermal inflammatory infiltrate of the oxazolone-treated ear skin of apremilast-treated mice compared with the untreated AD group. The rostral back skin of apremilast-treated mice also showed a significantly lower epidermal thickness and dermal inflammatory infiltrate than AD mice. It was shown that mice ears topically treated with apremilast microemulsion exhibited less inflammatory cell infiltrate and a normal stratum corneum comparable with normal skin were observed.[36] The reduction of epidermal hyperplasia supports a role of apremilast in normalizing epidermal homeostasis and integrity regulation of epidermal keratinocytes.

We demonstrated that apremilast-treated AD mice had significantly lower serum mean IgE and IL-31 levels than untreated AD mice. Expression of PDE4 isoforms in the AD skin was found to be three-fold greater than in healthy skin,[12] and elevated PDE activity has been demonstrated in leukocytes from patients with AD.[6] The reduction of serum IgE probably reflects the reduction of skin inflammation. Mohan *et al*.[37] reported that apremilast treatment normalized IL-31 production. Apremilast inhibits T-helper 2 and 17 immune responses. Therefore, IL-4– and IL-17–dependent IL-31 production from keratinocytes is subsequently decreased.[8]

We reported a significantly lower mean scratching score in apremilast-treated mice than in untreated AD mice. Recent clinical trials highlighted the potential for apremilast in the treatment of AD and AD-related itch.[6,8] This can be explained by the inhibition of IL-4– and IL-17–dependent IL-31 production from keratinocytes contributing to the relief of pruritus,[8] in addition to decreased skin inflammation, improved barrier function, and the reduction of inflammatory cells that directly release itch-related mediators, such as NGF, cytokines, and proteases.

To the best of our knowledge, this is the first study to compare the efficacy of itch control between the commonly used low-dose cyclosporine and apremilast. Apremilast treatment was associated with significantly lower mean serum IgE and IL-31 levels than cyclosporine treatment. There was also a significantly less dermal inflammatory infiltrate in the ear skin of apremilast-treated mice compared with cyclosporine-treated mice. We observed that the dermatitis severity scores (mean Matsuoka scores and thickness of oxazolone-treated ear skin) were lower with apremilast; however, the difference was not statistically significant. Skin barrier function as assessed by hydration despite being higher with apremilast than cyclosporine treatment, the difference was not statistically significant. The histopathologic assessment showed no significant difference regarding epidermal, dermal thickness, or dermal infiltrate of the back skin. We suggest that both apremilast and cyclosporine showed comparable efficacy in reducing the severity of skin inflammation and decreasing epidermal and dermal hyperplasias. However, the apremilast-treated group showed a more rapid significant reduction of the scratching score starting earlier at week 3 after treatment. The cyclosporine-treated group demonstrated a significant reduction of the scratching behavior starting later at week 4. This might be secondary to a greater reduction of mean serum IL-31 levels and a greater reduction of the dermal inflammatory infiltrate that interacts with sensory nerve fibers in the atopic skin as reported in our study. This early control of pruritus was similarly reported by a post hoc analysis of phase 3 clinical trials of a topical PDE4I (crisaborole), which demonstrated an early improvement of pruritus.

The study is limited by the use of low-dose cyclosporine with minimal renal risk and a known apremilast dose representing 50% of the no-observed side defect dose. Higher doses are expected to exhibit more clinical efficacy. We believe that the earlier control of itch observed with apremilast is clinically significant as this will lead to less epidermal damage and that will interrupt the itch-scratch cycle and progression of dermatitis.[32,38,39] We suggest that apremilast is promising for the control of pruritus, reducing inflammation, and improving the skin barrier function. Studies employing different doses of apremilast owing to its favorable safety profile may help optimize dosing to reduce pruritus in AD patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Furue M, Yamamura K, Kido-Nakahara M, Nakahara T, Fukui Y. Emerging role of interleukin-31 and interleukin-31 receptor in pruritus in atopic dermatitis. Allergy 2018;73:29-36.
2. Yosipovitch G, Berger T, Fassett MS. Neuroimmune interactions in chronic itch of atopic dermatitis. J Eur Acad Dermatol Venereol 2020;34:239-50.
3. Heimall J, Spergel JM. New pathways for itching in patients with atopic dermatitis? J Allergy Clin Immunol 2017;140:393-4.
4. Khattri S, Shemer A, Rozenblit M, Dhingra N, Czarnowicki T, Finney R, *et al*. Cyclosporine in patients with atopic dermatitis modulates activated inflammatory pathways and reverses epidermal pathology. J Allergy Clin Immunol 2014;133:1626-34.
5. Kim JE, Kim HJ, Lew BL, Lee KH, Hong SP, Jang YH, *et al*. Consensus guidelines for the treatment of atopic dermatitis in Korea (part II): Systemic treatment. Ann Dermatol 2015;27:578-92.
6. Deleanu D, Nedelea I. Biological therapies for atopic dermatitis: An update. Exper Ther Med 2019;17:1061-7.
7. Krishnamoorthy G, Kotecha A, Pimentel J. Complete resolution of erythrodermic psoriasis with first-line apremilast monotherapy. BMJ Case Rep 2019;12:e226959.
8. Saporito RC, Cohen DJ. Apremilast use for moderate-to-severe atopic dermatitis in pediatric patients. Case Rep Dermatol 2016;8:179-84.
9. Simpson EL, Imafuku S, Poulin Y, Ungar B, Zhou L, Malik K, *et al*. A phase 2 randomized trial of apremilast in patients with atopic dermatitis. J Invest Dermatol 2019;139:1063-72.
10. Guide for the Care and Use of Laboratory Animals. The National Academies Collection: Reports Funded by National Institutes of Health. Washington, DC: National Academies Press; 2011.
11. Lee HJ, Cho SH. Therapeutic effects of Korean Red Ginseng extract in a murine model of atopic dermatitis: Anti-pruritic and anti-inflammatory mechanism. J Korean Med Sci 2017;32:679-87.
12. Schafer PH, Adams M, Horan G, Truzzi F, Marconi A, Pincelli C. Apremilast normalizes gene expression of inflammatory mediators in human keratinocytes and reduces antigen-induced atopic dermatitis in mice. Drugs R D 2019;19:329-38.
13. Lee S, Park NJ, Bong SK, Jegal J, Park SA, Kim SN, *et al*. Ameliorative effects of Juniperus rigida fruit on oxazolone- and 2,4-dinitrochlorobenzene-induced atopic dermatitis in mice. J Ethnopharmacol 2018;214:160-7.
14. Kim CH, Choi YS, Cheong KA, Lee AY. Mechanism underlying the effect of combined therapy using glucosamine and low-dose cyclosporine A on the development of atopic dermatitis-like skin lesions in NC/Nga mice. Int Immunopharmacol 2013;15:424-32.
15. Isnard Bagnis C, Tezenas du Montcel S, Beaufils H, Jouanneau C, Jaudon MC, Maksud P, *et al*. Long-term renal effects of low-dose cyclosporine in uveitis-treated patients: Follow-up study. J Am Soc Nephrol 2002;13:2962-8.
16. Kholmukhamedov A, Logdon C, Hu J, McKinney RA, Spinale FG, Lemasters JJ, *et al*. Cyclosporin A in left ventricular remodeling after myocardial infarction. Am J Physiol Heart Circ Physiol 2014;306:H53-9.
17. European Medicines Agency (EMA) Committee for Medicinal Products for Human Use. Assessment Report. Otezla. London: EMA; 2015.
18. Xu J, Zanvit P, Hu L, Tseng PY, Liu N, Wang F, *et al*. The cytokine TGF-beta induces interleukin-31 expression from dermal dendritic cells to activate sensory neurons and stimulate wound itching. Immunity 2020;53:371-383.e5.
19. Matsuoka H, Maki N, Yoshida S, Arai M, Wang J, Oikawa Y, *et al*. A mouse model of the atopic eczema/dermatitis syndrome by repeated application of a crude extract of house-dust mite Dermatophagoides farinae. Allergy 2003;58:139-45.
20. Duarte J, Deshpande P, Guiyedi V, Mecheri S, Fesel C, Cazenave PA, *et al*. Total and functional parasite specific IgE responses in Plasmodium falciparum-infected patients exhibiting different clinical status. Malar J 2007;6:1.
21. Bancroft JD, Stevens A. Theory and Practice of Histological Techniques. 4th ed. New York, Edinburgh, London: Churchill Livingston; 1996.
22. Hadi HA, Tarmizi AI, Khalid KA, Gajdacs M, Aslam A, Jamshed S. The epidemiology and global burden of atopic dermatitis: A narrative review. Life (Basel) 2021;11:936-55.
23. Kim D, Kobayashi T, Nagao K. Research techniques made simple: Mouse models of atopic dermatitis. J Invest Dermatol 2019;139:984-990.e1.
24. Legat FJ. Itch in atopic dermatitis—What is new? Front Med 2021;8.
25. Kim S, Kim HJ, Yang HS, Kim E, Huh IS, Yang JM. IL-31 serum protein and tissue mRNA levels in patients with atopic dermatitis. Ann Dermatol 2011;23:468-73.
26. Jin H, He R, Oyoshi M, Geha RS. Animal models of atopic dermatitis. J Invest Dermatol 2009;129:31-40.
27. Shi L, Liu C, Xiong H, Shi D. Elevation of IgE in patients with psoriasis: Is it a paradoxical phenomenon? Front Med (Lausanne) 2022;9:1007892.
28. Kitamura A, Takata R, Aizawa S, Watanabe H, Wada T. A murine model of atopic dermatitis can be generated by painting the dorsal skin with hapten twice 14 days apart. Sci Rep 2018;8:5988.
29. Furue M, Chiba T, Tsuji G, Ulzii D, Kido-Nakahara M, Nakahara T, *et al*. Atopic dermatitis: Immune deviation, barrier dysfunction, IgE autoreactivity and new therapies. Allergol Int 2017;66:398-403.
30. Lu J, Wu K, Zeng Q, Xiang Y, Gao L, Huang J. Serum interleukin-31 level and pruritus in atopic dermatitis: A meta-analysis. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2018;43:124-30.
31. Council for International Organizations of Medical Sciences (CIOMS). International Ethical Guidelines for Epidemiological Studies. Geneva: CIOMS; 2008. Available from: http://www.ufrgs.br/bioetica/cioms2008.pdf.
32. Ko KC, Tominaga M, Kamata Y, Umehara Y, Matsuda H, Takahashi N, *et al*. Possible antipruritic mechanism of cyclosporine A in atopic dermatitis. Acta Derm Venereol 2016;96:624-9.
33. Lucae S, Schmid-Grendelmeier P, Wuthrich B, Kraft D, Valenta R, Linhart B. IgE responses to exogenous and endogenous allergens in atopic dermatitis patients under long-term systemic cyclosporine A treatment. Allergy 2016;71:115-8.
34. Brandt C, Pavlovic V, Radbruch A, Worm M, Baumgrass R. Low-dose cyclosporine A therapy increases the regulatory T cell population in patients with atopic dermatitis. Allergy 2009;64:1588-96.
35. Bissonnette R, Pavel AB, Diaz A, Werth JL, Zang C, Vranic I, *et al*. Crisaborole and atopic dermatitis skin biomarkers: An intrapatient randomized trial. J Allergy Clin Immunol 2019;144:1274-89.
36. Sarango-Granda P, Silva-Abreu M, Calpena AC, Halbaut L, Fabrega MJ, Rodriguez-Lagunas MJ, *et al*. Apremilast microemulsion as topical therapy for local inflammation: Design, characterization and efficacy evaluation. Pharmaceuticals (Basel) 2020;13:484- 509.
37. Mohan G, Bao L, Shen K, Chan L. Apremilast inhibits proinflammatory effects of IL-4 on primary human keratinocytes: Molecular mechanism for atopic dermatitis treatment. J Am Acad Dermatol 2015;72:AB73.
38. Hashimoto Y, Arai I, Nakanishi Y, Sakurai T, Nakamura A, Nakaike S. Scratching of their skin by NC/Nga mice leads to development of dermatitis. Life Sci 2004;76:783-94.
39. Nakamizo S, Egawa G, Doi H, Natsuaki Y, Miyachi Y, Kabashima K. Topical treatment with basic fibroblast growth factor promotes wound healing and barrier recovery induced by skin abrasion. Skin Pharmacol Physiol 2013;26:22-9.