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## Is pimecrolimus cream (1%) an appropriate therapeutic agent for the treatment of external ear atopic dermatitis?

### Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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### Summary

#### Background:

In recent years, pimecrolimus 1% cream has been demonstrated to reduce symptoms of atopic dermatitis in patients when applied topically.

#### Material/Methods:

In our study we compared the therapeutic effects of local 1% pimecrolimus to 1% hydrocortisone, and to a control group in a mouse model with atopic dermatitis in the external ear canals. Atopic dermatitis was created by application of Dinitrochlorobenzene in the external ear canals of mice. The development of atopic dermatitis was detected by clinical observation score and determination of total serum IgE levels. Pimecrolimus and hydrocortisone cream were topically applied to the external ear canal skin once a day for 14 days.

#### Results:

There was no significant difference between the hydrocortisone and the pimecrolimus therapy groups, while there was a statistically significant difference between these 2 groups and the control group ( $p < 0.05$ ). Assessment of the clinical observation scoring carried out on the 14th day of therapy revealed that there was no difference between the hydrocortisone and pimecrolimus groups. Biopsies were taken on the 14th day following treatment. Tissue samples were histologically evaluated; contact dermatitis was observed microscopically in the control group, but in the therapy groups only minimal evidence of contact dermatitis was found.

#### Conclusions:

The results of our study reveal that the therapeutic efficacy of 1% pimecrolimus was equivalent to 1% hydrocortisone treatment in the artificially developed atopic dermatitis model in external ear canals of mice. These results clearly demonstrate that 1% pimecrolimus cream can be an effective alternative therapeutic agent in cases where steroid treatment proves to be insufficient or in cases where treatment must be discontinued due to its adverse effects.

#### key words:

external auditory canal • pimecrolimus • hydrocortisone • atopic dermatitis • albino mice

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## BACKGROUND

Atopic dermatitis (AD) is a complex eczematous, inflammatory, chronically relapsing, non-contagious, and pruritic skin disease [1,2]. The elevated allergen-specific serum Ig E levels are the most important biochemical parameters in many patients [3]. AD may occur due to various genetic and environmental factors; however, its precise cause is unclear [4,5]. Environmental factors, nutrition, and hygiene play a role in AD [6]. AD now affects 10–20% of children and 1–3% of adults, and its prevalence has increased in recent years in developed countries [7].

The skin of a patient with atopic dermatitis reacts abnormally and easily to irritants, food and environmental allergens. Itching and skin lesions like rash, discharge, desquamation, hyperpigmentation, and sometimes fissures are generally observed in patients with atopic dermatitis. These symptoms significantly impair the patient's quality of life.

Patients with isolated external auditory canal atopic dermatitis (EACAD) are common in otolaryngologic practice. Otolaryngologists may treat EACADs with low density hydrocortisone creams and ointments, topical acetic acid solutions, or dilute aluminum acetate solutions (Burrow's solution). Although the use of these types of medication is efficacious in some patients, there are other patients who relapse or continue to have symptoms.

Long-term use of topical steroids is contraindicated because they are associated with thinning of the epidermis, decreased microvasculature and decreased keratinocyte size [8], especially in the external auditory canal (EAC) and auricular skin because of its very thin nature. Furthermore, higher-potency steroid creams must not be used on EAC skin where the skin is naturally thin; usually a lower-potency steroid is prescribed for this area. Due to inadequate therapeutic effects and common adverse effects, new treatment options are being investigated.

Pimecrolimus belongs to a new class of topical immune modulators and was approved by the Food and Drug Administration (FDA) in 2001. It is a calcineurin inhibitor developed for the topical treatment of atopic dermatitis. Pimecrolimus inhibits T cell activation by inhibiting the synthesis and release of cytokines from T cells. It has been observed that pimecrolimus binds to macrophilin-12 and inhibits calcineurin. Therefore, it inhibits T cell activation by blocking the transcription of early cytokines, including that of interleukin IL-2, IL-4, IL-10 and interferon gamma. Pimecrolimus also prevents the release of inflammatory cytokines and mediators from mast cells after stimulation by antigens or Ig E [9,10].

Topical pimecrolimus has been recommended in the treatment of atopic dermatitis by various authors [9,11,12]. We wanted to explore the probable success of this drug on EACAD. For this purpose the present study evaluated the therapeutic efficacy of 1% topical pimecrolimus compared with 1% topical hydrocortisone on mice with artificially induced EACAD.

## MATERIAL AND METHOD

### Animals

Gazi University's Animal Research Ethics Committee approved our experimental animal protocol. All procedures

performed on the mice were in accordance with the guidelines of the Gazi University Animal Research Ethics Committee.

Thirty female albino mice, 25–30 grams in weight, obtained from Gazi University Experimental Researches Animal Laboratory (GUERAL), were used. The mice were divided into 3 groups: hydrocortisone treatment group (Group 1), pimecrolimus treatment group (Group 2) and control group (Group 3), each comprising ten animals.

All mice were kept in a pathogen-free environment. They were housed in an air-conditioned animal room with a temperature of  $22\pm 2^\circ\text{C}$  and a humidity of  $60\pm 5\%$ . Food and tap water were provided to the animals ad libitum.

### Sensitization

First, all hair on the backs of auriculae of the mice was shaved with an electric clipper and then removed with depilatory cream. 2,4-Dinitrochlorobenzene powder (DNCB, Merck Chemical Company, Germany) was used to induce controlled dermatitis. DNCB was dissolved in acetone at a concentration of 10%. We painted the auricula and the EAC with 10% DNCB and acetone/olive oil (1:3) solution repeatedly 3 times a day for 28 days.

### Drugs

Elidel cream (1% Pimecrolimus, Novartis), Eurax hydrocortisone cream (1% hydrocortisone, Novartis), Ketalar Vial (Ketamine HCl 50 mg/ml, Pfizer), and Xylazine Hydrochloride Vial (2% Xylazine hydrochloride solution, Rompun) were used. No therapeutic agent or vehicle has been administered to the control group.

Pimecrolimus and hydrocortisone creams were used for the EACAD therapy topically on the EAC and auricular skin once a day for 14 days. Twenty-five mg/kg ketamine and 2 mg/kg xylazine were injected intramuscularly into the biceps femoris muscle for general anesthesia before the biopsies. A disposable sterile insulin injector (1 ml, AYSET) was used for the intramuscular injection.

### Macroscopic observation

Macroscopic observing inflammation scale (MOIS) was used and erythema, edema, and ulceration were graded on a scale of 0 to 5. Clinical skin condition was defined in terms of cutaneous lesions that consisted of edema, erythema, erosion and ulceration. The scoring method used in the present study (MOIS) was transformed through the modification of the method described by Hikita et al. [13].

These evaluation parameters were assessed by determining the total area of lesions on the shaved auricular area. The scoring system was as follows: 0, not detectable; 1, 0–25% of total shaved auricular skin surface; 2, 25–50% of total shaved auricular skin surface; and 3, 50–75% of total auricular shaved skin surface; 4, 75–100% of total auricular shaved skin surface; and 5, 100% of total shaved auricular skin surface.

## Biopsies

Biopsies from each of the 3 groups were performed only once at the end of the treatment. Ketamine and xylazine hydrochloride were administered for general anesthesia, applied intramuscularly in the leg prior to the biopsies. The biopsies were performed with a size 11 scalpel in the shape of a full thickness triangle through 2 intersecting incisions at the external ear canal that widened outwards to the free edge from the center of the auricula at the external auditory canal. Intraoperative bleeding during the biopsies was controlled with a bipolar cautery and covered with sterile strips.

Skin changes formed in atopic dermatitis take place in the area where allergenic materials come into contact with the skin. Hence, tissue changes may not be the same in all areas. In addition, performing repetitive biopsies from the same area may cause additional tissue damage and tissue changes. All these factors result in difficult follow-up of the disease through obtaining biopsy samples from the same area. Consequently, follow-up of the disease in our study by repetitive biopsies was avoided.

## Histopathological observation

Specimens were obtained from the skin of the patched area, fixed with 10% neutral formalin and embedded in white paraffin for the histological observation of the skin. Serial paraffin sections, 3  $\mu$ m thick, were prepared and stained with hematoxylin and eosin. For histological evaluation, the removed tissues were fixed in buffered (pH=7.4) 10% formaldehyde solution for 2 days at room temperature. Specimens were dehydrated in increasing concentrations of ethanol and were immersed in xylene until they had a clear appearance, followed by incubation in 3 changes of liquid paraffin at 60°C and were embedded in paraffin blocks. Sections of 3  $\mu$ m were obtained by Leitz-1512 microtome (Leitz, Germany) and stained with hematoxylin and eosin. Specimens were examined under a Nikon Eclipse E600 (Nikon, Tokyo, Japan) bright field microscope and images were captured using a Nikon Coolpix 5000 (Nikon) digital camera attachment.

The histological evaluation was performed by 2 different histologists who did not know to which group the preparations belonged. The histologists assessed tissue integrity and inflammatory findings during their analyses. They observed only inter-group differences whether or not there was any difference between samples.

## Determination of total IgE in mouse serum

Blood was collected from the tail vein following DNCB sensitization period on days 1, 14 and 28, and during the therapy period on days 14 and 28. Serum was obtained by centrifugation 500 $\times$ g for 10 min at 4°C and stored at 20°C until use. Total IgE levels in serum were measured by enzyme immunoassay. The concentration of total IgE in the sera was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) as previously described [14]. Total IgE levels in sera were measured by using an ELISA kit (Yamasa Shoyu Co., Ltd., Chiba, Japan) at the Turkish Atomic Energy Authority, Chemical Engineering Laboratories, Ankara.

## Statistical analysis

### Statistical analysis of total IgE levels

All the results are presented as means  $\pm$  standard deviations for all groups. Differences were assessed using variance analysis (daily total IgE levels between the groups). Post-hoc comparisons were assessed with Tukey's test. The differences between the days of each group were assessed using the paired t test.  $P < 0.05$  was considered to be statistically significant.

### Statistical analysis of MOIS scores

All results are presented as means  $\pm$  standard deviations for all groups. Differences between the groups were assessed by the Kruskal Wallis test. Paired comparisons were assessed with the Mann Whitney test. Bonferroni correction was applied in order to take multiple testing into account. Differences between the days of each group were assessed using the Friedman test. Paired comparisons were assessed using the Wilcoxon Signed Ranks test after Bonferroni correction for a separate analysis. The power analysis of the study revealed 100% rate.

## RESULTS

### Total IgE levels

It was observed that the mean levels gradually increased during the sensitization period in all 3 groups. With the initiation of the treatment they started to go down in all groups and that this decrease continued until the last day of the treatment, day 14 (Figure 1, Table 1).

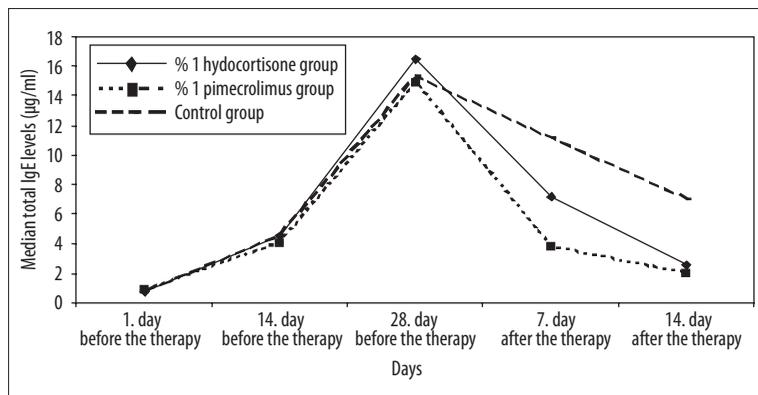
When the total IgE levels were analyzed it was seen that there were no differences among the 3 groups during the DNCB sensitization period.

The assessment carried out on day 7 after treatment revealed that there was a statistically significant difference among the results of the 3 groups ( $P < 0.05$ ). The groups' mean levels of total IgE were, from the least to the most, Group 2 < Group 1 < Group 3. When the total IgE results were assessed on day 14 after treatment, there was no significant difference between Group 1 and Group 2, while there was a statistically significant difference between these 2 groups and Group 3 ( $P < 0.05$ ).

### MOIS scores

When the MOIS results were assessed, it was seen that there was no difference among the groups for day 1, while there were statistically significant differences for the other days among the groups ( $P < 0.05$ ). The assessments carried out on days 3, 5, 7, 10, and 14 revealed that there was no difference between Group 1 and Group 2, while there were differences between Groups 1 and 3, and 2 and 3 ( $P < 0.017$ ).

Differences were detected among the groups regarding the days ( $P < 0.05$ ). There was a significant difference between Groups 1 and 2 within the framework of binary comparisons for all days ( $P < 0.003$ ). As for Group 3, there was no difference among the days 1–3, 3–5, and 7–10 ( $P < 0.003$ ) (Table 2).



**Figure 1.** The mean ± standard deviation (µg/ml) values for the total IgE values of the hydrocortisone, pimecrolimus therapies, and control groups during the DNCB sensitization period (days 1, 14, and 28) and the therapy period (days 7 and 14) are displayed graphically.

**Table 1.** The mean ± standard deviation (µg/ml) values for the total IgE values of the hydrocortisone, pimecrolimus therapies, and control groups during the DNCB sensitization period (days 1, 14, and 28) and the therapy period (days 7 and 14) are displayed.

Groups	Day 1, DNCB sensitization, total IgE levels (µg/ml)	Day 14, DNCB sensitization, total IgE levels (µg/ml)	Day 28, DNCB sensitization, total IgE levels (µg/ml)	Day 7, therapy period, total IgE levels (µg/ml)	Day 14, therapy period, total IgE levels (µg/ml)
Hydrocortisone group	0.72±0.26	4.50±0.83	16.51±2.17	7.20±1.35	2.57±0.75
Pimecrolimus group	0.88±0.34	4.03±0.80	14.85±1.96	3.73±1.06	1.96±0.60
Control group	0.80±0.24	4.51±1.13	15.25±3.17	11.14±3.00	7.00±1.94

**Table 2.** The mean ± standard deviation macroscopic evaluation scores (MOIS) for the hydrocortisone, pimecrolimus, and the control groups during the 14 days of treatment are shown together.

Groups	Day1 (MOIS, mean ± standard deviation)	Day 3 (MOIS, mean ± standard deviation)	Day 5 (MOIS, mean ± standard deviation)	Day 7 (MOIS, mean ± standard deviation)	Day 10 (MOIS, mean ± standard deviation)	Day 14 (MOIS, mean ± standard deviation)
Hydrocortisone group	4.30±0.57	3.75±0.79	2.85±0.81	2.10±0.72	1.35±0.59	0.40±0.50
Pimecrolimus group	4.50±0.51	3.40±0.50	2.60±0.50	2.15±0.75	1.35±0.67	0.70±0.57
Control group	4.40±0.50	4.35±0.49	4.00±0.56	3.40±0.50	3.10±0.55	2.40±0.50

**Microscopic evaluation**

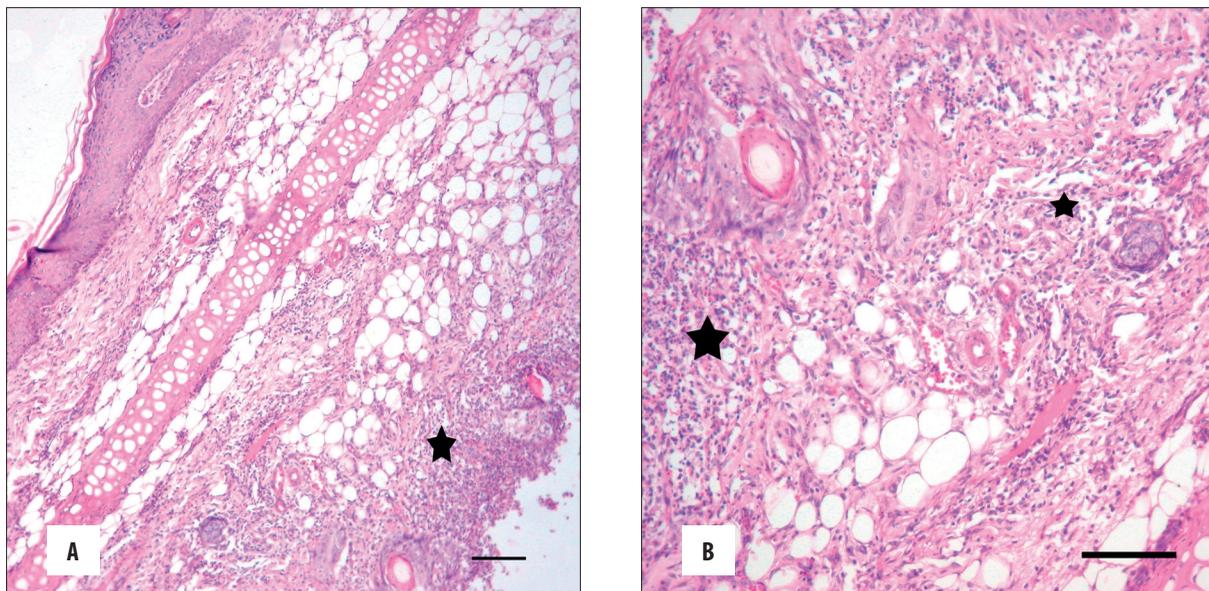
Tissue samples were taken from all animal groups at the end of the therapy and were evaluated by 2 histologists with light microscopy.

Very few inflammatory cells (mostly macrophages and eosinophiles) were detected in the connective tissue under the epithelium. Epithelium and cartilage tissues were also intact in the hydrocortisone treatment group (Figures 2A,B).

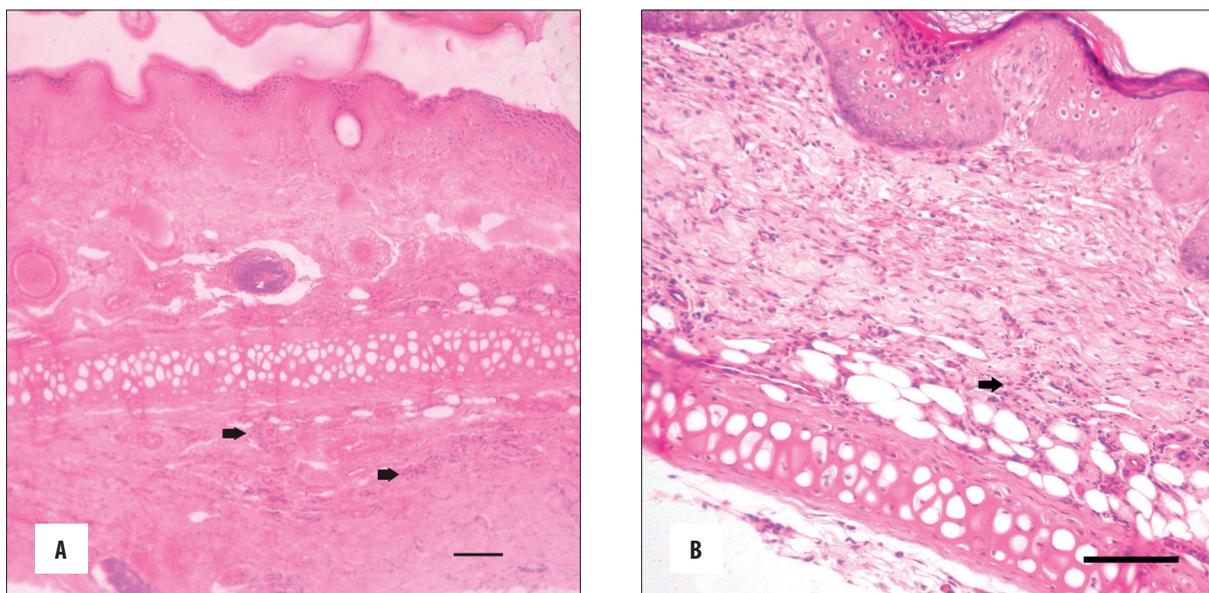
In the pimecrolimus treatment group, inflammation improved. Very few inflammatory cells (mostly eosinophiles) were detected in the connective tissue under the epithelium. The number of eosinophiles in this group was insignificantly higher in comparison to the hydrocortisone treatment group. Epithelium and cartilage tissues were also intact in this group (Figures 3A,B).

Contact dermatitis was sought microscopically in Group 3. A large number of inflammatory cells (mostly neutrophils, lymphocytes, macrophages and eosinophiles) were detected in the connective tissue under the epithelium. Epithelium and cartilage tissues were intact (Figures 4A,B). No statistical data on the number of immune cells are shown.

Since pre-treatment histopathologic examination was not undertaken, it was not possible to make pre- and post-treatment comparison in this regard. However, although post-treatment abundant and dense eosinophils and inflammatory cells were seen in the dermis in the control group, eosinophils were scarce in the hydrocortisone and pimecrolimus treatment groups. Apart from this, histologic parameters such as eosinophilic spongiosis, papillary dermal edema and epidermal thickening, which are all specific for allergic atopic dermatitis, were not observed. This result



**Figures 2A,B.** Control group samples. A lot of inflammatory cells are seen (\*). H.E. Bar 100 µm.



**Figures 3A,B.** Hydrocortisone treatment group samples. A few inflammatory cells (arrows) are seen. H.E. Bar 100 µm.

demonstrates that the disease regressed in accordance with the evaluation parameters in all 3 groups.

**DISCUSSION**

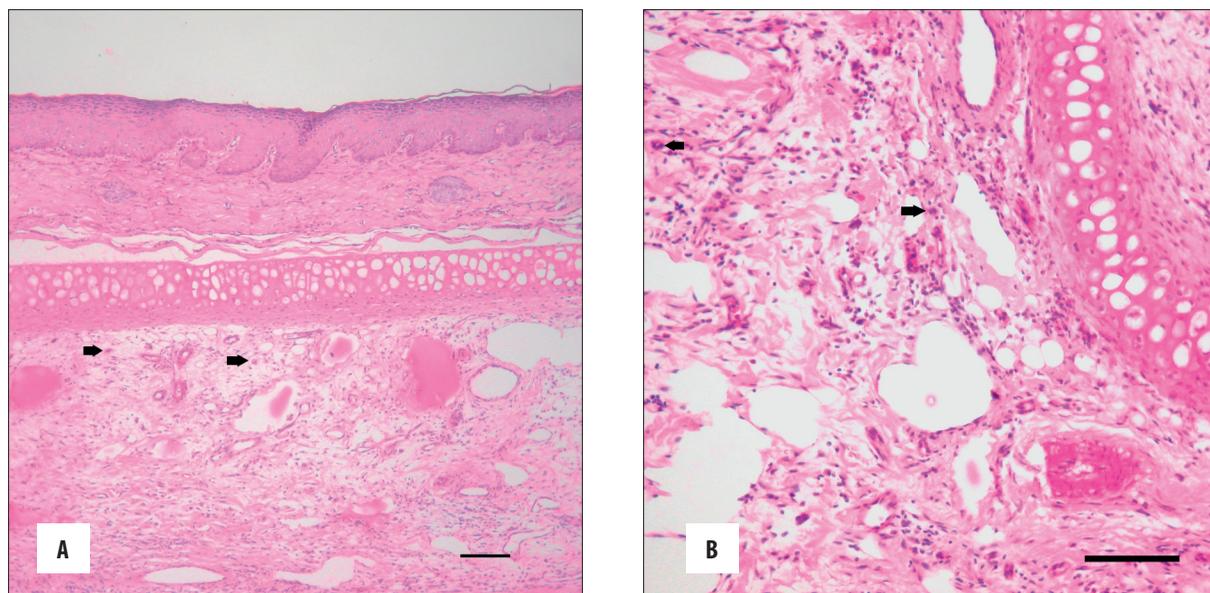
The thin skin of the EAC is normally protected by the natural oils and cerumen produced by the glands in the EAC skin. When the protective layers of the EAC skin are removed by the use of cotton-tipped (Q-tip) swabs or by other means, the thin EAC skin is vulnerable to the penetration of haptens.

With multiple sensitizations an allergic dermatitis occurs, which leads to an inflammatory process in the EAC skin. The inflammation in the EAC, in turn, may lead to a reduction or a halt in the secretion of the natural oils and cerumen.

This gives way to a vicious cycle in patients with a lack of cerumen, a dry EAC, and continuous contact dermatitis [15].

Allergic contact dermatitis is caused by small molecules that can penetrate the skin, weighing less than 500 Da (haptens). Haptens are weakly allergenic, requiring multiple exposures before they cause sensitization. Allergic contact dermatitis requires the presentation of the antigens to T cells by epidermal Langerhans cells. The principal antigen presenting cells of the skin, the Langerhans cells, in turn up-regulate IL-1 and tumor necrosis factor. Keratinocytes are also activated by haptens to secrete immunomodulatory cytokines. The clinical result is itching and irritation of the skin [16].

Preservatives are another large class of molecules that are among the common causes of contact dermatitis and are



**Figures 4A,B.** Pimecrolimus treatment group samples. A few inflammatory cells especially eosinophils (arrows) are seen H.E. Bar 100  $\mu$ m.

found in almost all hair care products. Considering the fact that shampoos and conditioners come in contact with the EAC, contact dermatitis can occur in the EAC. Surfactants such as cocamidopropyl betaine in shampoos and its purported allergen amidoamine are among the top 20 most frequent patch test positive allergens. Moreover, fragrances containing protein, lanolin, parabens or formaldehyde are all among the causes of contact dermatitis [17].

Absence or altered pH of the cerumen, high humidity or remaining moisture in the auditory canal, the presence of a foreign material (eg, ear plugs, hearing aid ear molds), local trauma caused while using certain instruments for cleaning the auditory canal, increased susceptibility to contact allergens (eg, nickel ear rings, piercing) and secondary sensitization to ototopic medications, congenital, or acquired narrowing of the auditory canal (eg, exostosis), and systemic diseases (eg, diabetes mellitus) provide the basis for the development of chronic eczematous changes of the auricular skin [18].

In the present study, we established an allergic dermatitis model in mice using a contact sensitizer, 2,4-dinitrochlorobenzene (DNCB). DNCB is a contact sensitizer that triggers the Th-1 and Th-2 responses [19–22]. We painted the EAC dermis with DNCB acetone solution repeatedly once a day for 28 days. We selected this skin area because the mouse external ear atopic dermatitis model could be used as an imitated human external ear AD.

We used DNCB (1-Chloro-2,4-Dinitrobenzene) in the formation of the disease. DNCB is an agent that was previously used in many studies to form induced allergic dermatitis in mice [23,24]. In our study we successfully formed atopic dermatitis through the use of local DNCB in all the trial subjects. Our study revealed that there was an increase in the total IgE levels with the formation and exacerbation of the clinical findings of the disease, and there was a continuous increase in the serum IgE levels and the clinical findings with the progression of sensitization (Figure 1). These results

indicate that DNCB is an effective agent in inducing atopic dermatitis as was also put forward by previous studies [25].

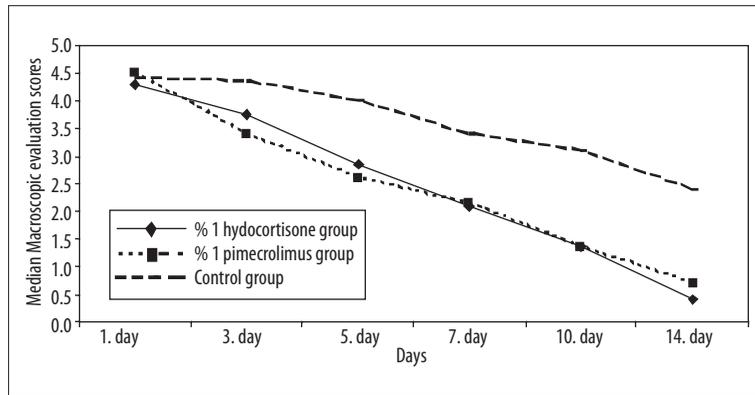
The treatment of contact dermatitis depends on avoidance of the allergen and the treatment of the associated inflammation in the skin. There are multiple medications used in the treatment of contact dermatitis.

Topical glucocorticoids are the most frequently used first-line medications for this indication and provide an effective treatment choice for AD. They are very beneficial during acute disease flares, but their prolonged use is generally associated with adverse effects [26].

Since longer durations of corticosteroid exposure can lead to permanent skin atrophy and telangiectasia, its chronic use in EAC is contraindicated [27]. A recent *in vivo* study has shown that 1 week of glucocorticoid application increased the scratching triggered by an irritant chemical [28].

In recent years pimecrolimus has been introduced for the treatment of AD. Pimecrolimus is an ascomycin macrolactam derivative. Pimecrolimus acts through the inhibition of T cell activation by the calcineurin pathway and inhibition of the release of different inflammatory cytokines from T cells and mast cells [29].

Pimecrolimus 1% cream has been approved by the FDA for the treatment of AD. It has been proven to be effective in various inflammatory skin diseases such as seborrheic dermatitis [30], cutaneous lupus erythematosus [31], oral lichen planus [32], vitiligo [33] and psoriasis [34,35]. It has lower permeation to the skin than topical steroids or topical tacrolimus. Their skin penetration and absorption are 70 to 110 times lower than corticosteroids [36,37]. Furthermore, in contrast to topical steroids, pimecrolimus does not produce skin atrophy [38]. These topical macrolide immunosuppressive agents can be used in areas with thin skin for prolonged periods of time without the adverse effects of corticosteroids [39].



**Figure 5.** The mean macroscopic evaluation scores for the hydrocortisone, pimecrolimus, and the control groups during the 14 days of treatment are shown together.

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Due to the low adverse effects profile of these agents compared to corticosteroids, we examined and compared the therapeutic effects of pimecrolimus and dexamethasone on the mouse auricular atopic dermatitis model.

The drugs were applied topically to the auricular skin lesions once a day for 14 days. During the treatment period, the effectiveness of both therapies was evaluated by the total IgE levels and MOIS scores. A perpetual decrease in serum IgE levels, which was initiated by the treatment, was seen in Groups 1 and 2, which received both treatment methods. This result shows that both types of medication are effective in the treatment of atopic dermatitis. This is also in agreement with the results of other studies [40–42].

The total IgE measurements carried out on day 7 following treatment revealed that there was a significant difference among the results of the 3 groups ( $P < 0.05$ ). The groups' mean values of total IgE were: Group 2 < Group 1 < Group 3. This result suggests that the pimecrolimus therapeutic activity during the first 7 days was more characteristic than the hydrocortisone and the control groups. This result supports the other result derived from the total IgE results in favor of pimecrolimus. When the total IgE results of day 14 were evaluated, we saw that there was no significant difference between the results of Groups 1 and 2, but there was a statistically significant difference between these 2 groups and Group 3 ( $P < 0.05$ ). In other words, both medications have therapeutic activity, but none is superior to the other.

In MOIS scoring results based on clinical observation, however, there was no significant difference between Groups 1 and 2 while there were differences between the Groups 1 and 3, and 2 and 3 ( $P < 0.05$ ). The macroscopic evaluation done on days 3, 5, 7, 10, and 14 revealed no difference between Groups 1 and 2, while there were differences between Groups 1 and 3, and 2 and 3 ( $p < 0.017$ ) (Figure 5). These results also implied that there was no difference between the therapeutic activities of pimecrolimus and hydrocortisone during the therapy period. This result supports the thesis that both medications have therapeutic activity, but their effectiveness is the same.

There was a significant difference between Groups 1 and 2 according to the results of the binary comparison among the days ( $P < 0.003$ ). This result showed that both medications have a gradually increasing therapeutic activity. As for Group 3, there were no differences among the days 1–3, 3–7,

and 7–10, while there was a difference in binary comparisons among the days. This result reveals the fact that MOIS scores also decreased within the control group that received no treatment with the halting of DNCB sensitization, but this took place much more slowly than in the medicated groups.

The histological evaluation of the mice after sacrificing them at the end of the treatment revealed minimal inflammation results for Groups 1 (Figure 2A,B) and 2 (Figure 3A,B) in correlation, while it also showed a continuous inflammation histologically for the control group (Figure 4A,B) that received no treatment. Histological evaluation of all groups displayed intact cartilage tissue and intact epithelial structure overlying the cartilage. We found that all the inflammatory cells located in the subepithelial area and in the connective tissue were intensive in the control group (Group 3) (Figure 2). The tissue analysis of the hydrocortisone treated subjects (Group 1) revealed that macrophages and eosinophiles were especially dominant, whereas microscopic assessment of the pimecrolimus treated subjects (Group 2) showed that eosinophiles were dominant. This result supports the idea that both medications have an inflammation suppressive effect in atopic dermatitis. The fact that we found fewer macrophages, which are cellular immunity elements, in the pimecrolimus treated group as compared to the hydrocortisone treated group supports the suppressive effect of pimecrolimus on the known cellular immune system [9,10].

There was no difference in the improvement rates between the medications. Treatment was achieved in all animals at the end of therapy, and no complications were observed in any of them. Treatment activity with both medications was satisfactory in the short-term.

When all these findings are assessed together, we see that the clinical, total IgE, and histological findings support one another. The results indicate that local pimecrolimus 1% treatment is as effective as local hydrocortisone 1% treatment in the artificially induced mouse auricular atopic dermatitis model with DNCB. It is seen as a good alternative, especially for patients who cannot receive adequate benefit from hydrocortisone use or for those who had to quit the medication because of its adverse effects.

## CONCLUSIONS

Topical pimecrolimus can be used only as a short-term, second-line agent and as an intermittent treatment of atopic

dermatitis in patients unresponsive to other treatments. On the other hand, the FDA has issued a warning about topical pimecrolimus being linked with a possible risk of cancer found in studies associated with high doses and prolonged use.

The use of these medications are to be avoided in children younger than 2 years and in immune-compromised patients, especially in consideration of a comprehensive review of all the animal studies related to risk of cancer with topical calcineurin inhibitors [43]. The cancers and malign lymphoblastic disorders were found in humans treated with a higher dose of the drug than is routinely used [43–47].

According to our results, local pimecrolimus may be an alternative therapeutic choice for EACAD. However, we believe that topical pimecrolimus should only be used as a short-term, second-line agent in EACAD.

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