European Review for Medical and Pharmacological Sciences 2022; 26: 6827-6836

Pharmacological basis of the putative therapeutic effect of Topical Vitamin D3 on the experimental model of atopic dermatitis in mice

N.S. ALOSAIMI[1,2], M.A.A. SATTAR AHMAD[1], H.M. ALKREATHY[1], A.S. ALI[1,3], L.M. KHAN[1]

[1]Department of Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Taif University, Taif, Saudi Arabia
[3]Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt

Abstract. – OBJECTIVE: The aim of the study was to explore the effect of topical vitamin D3 in atopic dermatitis (AD) induced by ovalbumin (OVA) in contrast with topical betamethasone in mice.

MATERIALS AND METHODS: 35 BALB/c adult male mice, weighing between 25-30 gm were used to induce AD by topically sensitizing the dorsal surface of the skin with the OVA patch. Subsequently, treatments were performed in each group by application of vitamin D3 cream (0.0003%), betamethasone cream (0.1%), or vehicles (QV cream) on the skin.

RESULTS: Remarkably, vitamin D3 had a marked improvement in the skin of OVA-induced AD mice. Additionally, vitamin D3 revealed a considerable diminution in the levels of IgE, IL-5, filaggrin, and epidermal thickness, whereas a significant augmentation in the levels of IL-4 and IL-13 was observed when compared with the control group, and histopathological studies had further confirmed these findings.

CONCLUSIONS: This study essentially highlighted the anti-inflammatory effect of vitamin D3 by effective alteration in the immunological components responsible for AD. Moreover, this pioneer experimental work represents a new paradigm and sheds a light on the importance of vitamin D3 in the implications of AD. A comprehensive creative approach is crucial to concretely establish and further corroborate vitamin D3 for this therapeutic role.

Key Words:
Atopic dermatitis, Vitamin D3, OVA-induced mice model for AD, Cytokines.

Abbreviations

AD: Atopic dermatitis, OVA: Chicken ovalbumin, VDTC: vitamin D_3 cream treated, BET: betamethasone cream treated, NC: Normal control.

Introduction

One of the common persistent and often relapsing skin disorders is recognized as Atopic Dermatitis (AD). The basic essence of its etiology is characterized by the intricate interaction between the inherent genetic and immunological components of the patient and certain environmental factors. Basically, AD is exemplified by chronic inflammation, interruption of epithelial barrier, and aberration of the immunological status invariably with escalated levels of serum IgE[1].

Accumulated evidence revealed that a remarkable skin barrier defect is displayed in the epidermal layer with a basic defect, leading to trans-epidermal water loss; this in turn alters the skin sensitivity. Furthermore, allergen penetration and microbial colonization with imminent inflammation ensue. The resultant skin barrier defect is primarily attributed to certain vital factors like a deficiency in structural proteins, such as filaggrin and keratin, changes in the pH of stratum corneum, diminution of skin ceramide, which is responsible for lipid barrier and water retention[2]. Nevertheless, the most remarkable genetic factor responsible for the disposition to AD seems to be the mutation in the filaggrin gene. Interestingly, 50% of the population studies[3] have revealed filaggrin mutations.

The contemporary acquaintance with the function of vitamin D in the last few decades unlocked a new vista for its extra-skeletal effect. Seemingly, it became quite apparent to portray its vital facets in cell proliferation regulation and their differentiation, in addition to the modulation of the immune system[4].

This regulatory action of vitamin D is typically recognized as its genomic action. Fascinatingly, vitamin D has been implicated in several stud-

Corresponding Author: Lateef Mohiuddin Khan, MD; e-mail: lmkhan00@hotmail.com

ies[5], regarding its contributory effect on maintaining appropriate epidermal barrier which is disrupted by the application of corticosteroids. Furthermore, the mediation of this effect of vitamin D was attributed to its effect on the enhancement of the synthesis of the cornified envelope's structural protein. In addition, it modifies the ultimate synthesis of the long chain glucosylceramide, which is quite necessary for the formation of lipid barrier[6].

Moreover, in many observational studies and meta-analysis[7-9], there is a reciprocal and inadvertent relationship between AD and deficiency of vitamin D in children, as well as adults. Besides, the severity of AD has been observed to be negatively correlated with vitamin D levels. Notwithstanding it was categorically proposed that restoration of the transformed Th1 and Th2 cytokines, the upregulation of IL-2, IL-4, IL-6 and IFN-γ in the patients affected with AD can explicitly be successfully reduced by the supplementation of vitamin D[10]. Conversely, the Th2 cytokines decrease the expression of FLG protein and antimicrobial peptides (AMPs), and these AMPs have an essential role against infections[11].

The contemporary therapeutic remedy for AD includes topical corticosteroids and topical calcineurin inhibitors (immunosuppressive agents), nevertheless, their chronic therapy leads to numerous adverse effects. In contrast, newer drug therapy for AD approved by FDA, e.g., crisaborole and dupilumab, demonstrated to be highly expensive in terms of cost effectiveness[12].

This study essentially aims at scrutinizing the potential curative effect of vitamin D_3 against the therapeutic impact of corticosteroids in the mice model of AD. Moreover, to offer a concept of IgE, Th2- cytokines (IL-5, IL-4 and IL-13), and filaggrin implication, with the supportive role of histological study to confirm the effectiveness of vitamin D.

Materials and Methods

Chemical and Reagents

1α, 25-dihydroxy vitamin D_3, and chicken ovalbumin egg white (OVA) (Sigma Aldrich CO., Saint Louis, MO, USA) were used. Betamethasone cream (15 g), 0.1% w/w betamethasone valerate, (Betasone®, JULPHAR, UAE). QV® cream (Ego Pharmaceuticals, Austria) was applied. ELISA kits for immunological parameters IgE, IL-4, IL-5, and IL-13 (MyBioSource Inc., USA). All other chemicals are of analytical grade.

Formulations

Vitamin D_3 cream was daily prepared. An appropriate amount of QV® cream (vehicle) was added in geometric dilution to Vitamin D_3 solution in accordance with the protocol observed in Chauhan et al[13]. Furthermore, to prepare 1 gram of the medicated cream of final concentration, 3 µg/g (0.0003% w/w) were prepared according to the validated technique[14].

Animal and Housing

BALB/c adult male mice, weighing between 25-30 gm, were obtained from KFCMR, KAU, Jeddah, Saudi Arabia. The animals were divided in groups of seven and were kept in a stable environment at room temperature of 24°C with a 12-hour natural light/dark cycle. Free access to food and water was provided *ad libitum* during the entire span of the experiment.

Study Design

In this contemporary study, 35 mice were acquired and divided randomly in five groups (n=7) as follows: 1-Normal control group (NC); 2-OVA-induced AD group (OVA); 3-OVA-induced AD + QV vehicle group (OVA+QV); 4-OVA-induced AD + vitamin D3 cream treated group (VDTC), 5-OVA-induced AD + betamethasone cream treated group (BET).

Allergic Dermatitis Induction in Mice

AD was induced topically in the mice as follows: the dorsal skin of all mice was shaved and sensitized topically with the OVA patch. Then the OVA patch was attached to the shaved dorsal skin using a transparent dressing, then fixed with silk tape. The patches were renewed every other day for 14 days (from day 14 to 27). After the AD induction, the mice in each group were treated according to the protocol: from day 30 to 34, 3 µg/g (0.0003%) of Vitamin D3 cream, betamethasone cream (0.1%), or vehicles (QV cream) were applied directly on the dorsal skin.

Sample Collection

All mice were subjected to anesthesia by diethyl ether and samples of the blood were subsequently obtained from the orbital sinus in the heparinized capillary tubes. Finally, the mice were sacrificed

by decapitation[15], and the dorsal skin was dissected out and divided into two parts. One part was frozen at -80°C for the measurement of immunological parameters, and the other part was stored in 10% of buffered formalin for the histopathological studies.

Assessment of AD Appearance and its Severity

It needs to be emphasized that the development of AD was confirmed by the Three-Item Severity Score (TIS), which is the scoring system to determine the severity of AD. Its significance well corresponds with the scoring for the atopic dermatitis assessment[16]. The common parameters for the TIS scoring are erythema (redness), edema (swelling), and excoriations (scratched abrasion). Each sign is scored as: 0 = clear; 1 = mild; 2 = moderate; 3 = severe[16,17].

Serum Samples

The samples of blood obtained were duly centrifuged at 3,000 rpm for a period of 20 minutes. Consequently, the resultant serum was collected in Eppendorf tubes and then stored at -80°C until the assays were accomplished.

Skin Tissue Homogenate Preparation

To accomplish this task, 10 mg of tissues were minced to small pieces and homogenized in 100 µl of PBS. Then, the subsequent suspension obtained was exposed to ultra-sonification for the purpose of breaking the cell membrane. This was followed by centrifugation of the homogenate at 5,000 rpm for 15 minutes duration. Finally, the supernatants were carefully obtained and stored at -80°C till the assays were done.

Biochemical Estimations

The levels of IgE, IL-5, IL-4, IL-13, and filaggrin in serum and tissue homogenate were prepared by using ELISA kits manufactured by MyBioSource (San Diego, CA, USA) following the manufacturer's recommended procedures. It needs to be emphasized that all the kits acquired belong to the sandwich type, except for IgE ELISA kit, which is designated as a competitive type. Moreover, the absorbance was measured at λ= 450 nm, by using the BIO-TEK ELx 800 plate reader (VT, USA).

Histopathological Examination

The harvested skin was spread on a plastic cassette fixed in 10% buffered formalin. This was further processed by an automatic paraffin processing machine to prepare the blocks. Subsequently, for general structure and morphometric studies, the blocks were sectioned into 5 µm thick slices and mounted on slides. This was followed by Hematoxylin and Eosin (H&E) staining. Then, the slides were photographed using an Olympus Microscope BX-51 at the magnification of 4X with a digital camera connected to the computer, in accordance with the established method[18].

Statistical Analysis

The data analysis was accomplished by the utilization of the Social Package of Social Science (SPSS), version 23 (IBM Corp., Armonk, NY, USA). Typically, the data were expressed as mean ± SEM. Moreover, we used one-way analysis of variance (ANOVA) to examine statistical comparisons between more than two independent groups, followed by the Least significance difference (LSD) test. However, we used an independent sample *t*-test to compare the two groups. Finally, consideration of the significance of differences was expressed as $p < 0.05$.

Results

Effect of Daily Topical Administration (TA) of Vitamin D3 Cream (3 µg/g) over Betamethasone Cream for Five Days on OVA-Induced AD Mice

The VDTC and BET groups showed a marked improvement in the skin appearance compared to the OVA group. Vitamin D3 treatment ameliorated AD in the VDTC group and scored 0 (clear) for erythema and edema, while it scored 1 (mild) for excoriations (Figure 1).

Effect of Daily TA of Vitamin D3 Cream (3 µg/g) over Betamethasone Cream for Five Days on Serum and Skin Homogenate IgE Levels (ng/ml) in OVA-Induced AD in Mice

The result in Figure 2 illustrates a significant increase ($p < 0.05$) in the serum and skin homogenate IgE levels for the OVA and vehicle OVA+QV group in comparison with the control NC group. However, a significant reduction ($p < 0.05$) was shown in the serum and skin homogenate IgE levels for the vitamin D_3 treated VDTC and betamethasone treated BET group compared to the OVA group (Figure 2).

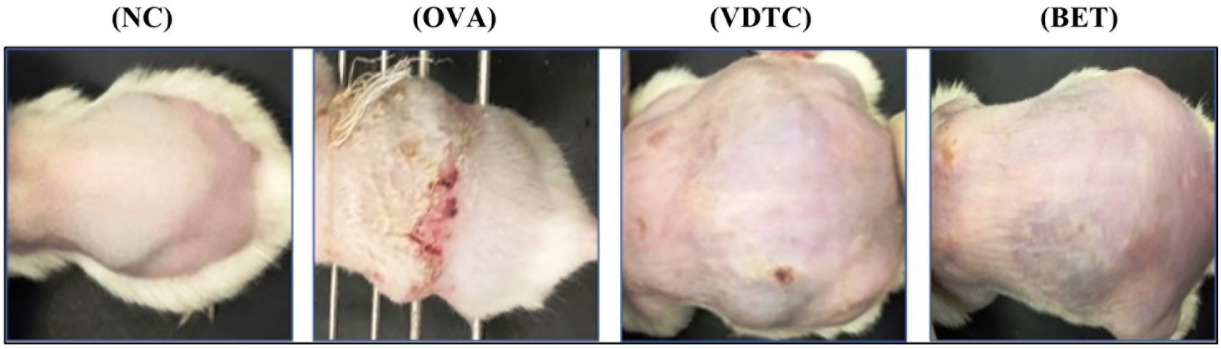

Figure 1. Scheme of AD induction and vitamin D3 cream treatment over betamethasone cream treatment. Dorsal skin lesions in each group of mice were depicted as follows: NC = normal control, OVA = AD induced by OVA, VDTC = AD induced by OVA +treated group of vitamin D3 cream, BET = AD induced by OVA+treated with betamethasone.

Effect of Daily TA of Vitamin D3 Cream (3 µg/g) vs. Betamethasone Cream for Five Days on Serum and Skin Homogenate IL-5 Levels (pg/ml) in OVA-Induced AD in Mice

As shown in Figure 3, there was a significant increase ($p < 0.05$) in the serum and skin homogenate IL-5 levels for the OVA and vehicle OVA+QV group relative to the control NC group. However, a significant decrease ($p < 0.05$) in the serum and skin homogenate IL-5 levels was observed for the vitamin D_3 cream treated VDTC and betamethasone cream treated BET group compared to the OVA group. Moreover, the VDTC group showed a significant increase ($p < 0.05$) in the serum and skin homogenate IL-5 levels compared to the BET group (Figure 3).

Effect of Daily TA of Vitamin D3 Cream (3 µg/g) vs. Betamethasone Cream for Five Days on Serum and Skin Homogenate IL-4 Levels (pg/ml) in OVA-Induced AD in Mice

The data in Figure 4 display a significant decrease ($p < 0.05$) in the serum and skin homogenate IL-4 levels relative to the control NC group for the OVA and vehicle OVA+QV group. Moreover, VDTC and BET group demonstrated

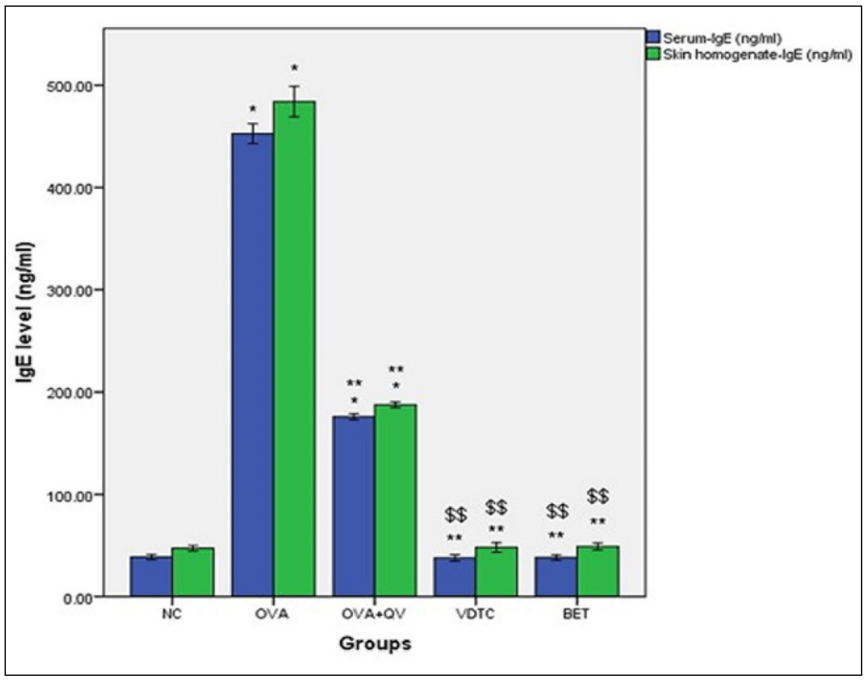

Figure 2. Effect of daily TA of vitamin D3 cream (3 µg/g) *vs.* betamethasone cream for five days on serum and skin homogenate IgE levels (ng/ml) in OVA-induced AD in mice. *Significant *p*-value < 0.05 *vs.* (NC) group, **Significant *p*-value < 0.05 *vs.* (OVA) group, $^{\$\$}$Significant *p*-value < 0.05 *vs.* (OVA+QV) group.

Table I. Effect of vitamin TA of vitamin D3 cream over betamethasone cream on the OVA-induced increased skin homogenate filaggrin (ng/ml).

Groups (n = 7)	Skin homogenate filaggrin (ng/ml) mean ± SEM
Normal control group (NC)	2.50±0.16
OVA-induced AD group (OVA)	8.15±0.27*
OVA-induced AD + QV vehicle group (OVA+QV)	5.92±0.37*,**
OVA-induced AD + vitamin D3 cream treated group (VDTC)	2.52±0.16$^{**,\$\$}$
OVA-induced AD + betamethasone cream treated group (BET)	3.21±0.24$^{**,\$\$}$

*Significant p-value < 0.05 *vs.* NC group, **Significant p-value < 0.05 *vs.* OVA group, $^{\$\$}$Significant p-value < 0.05 *vs.* OVA+QV group.

a significant increase ($p < 0.05$) in the serum and skin homogenate IL-4 levels in comparison to the OVA group (Figure 4).

Effect of Daily TA of Vitamin D3 Cream (3 µg/g) vs. Betamethasone Cream for Five Days on Serum and Skin Homogenate IL-13 Levels (pg/ml) in OVA-Induced AD in Mice

A significant augmentation ($p < 0.05$) was demonstrated in the serum and skin homogenate IL-13 levels for the Vitamin D_3 cream treated VDTC and betamethasone cream treated BET group compared to the OVA group. Additionally, the VDTC group showed a significant decrease ($p < 0.05$) in the serum and skin homogenate IL-13 levels *vs.* the BET group (Figure 5).

Effect of Daily TA of Vitamin D3 Cream (3 µg/g) vs. Betamethasone Cream for Five Days on Skin Homogenate Filaggrin Level (ng/ml) in OVA-Induced AD in Mice

The data provided in Table I revealed quite significant augmentation ($p < 0.05$) in the skin homogenate filaggrin level induced for the OVA and vehicle OVA+QV group relative to the control NC group. On the other hand, VDTC and BET group, a significant decrease ($p < 0.05$) in the level of skin homogenate filaggrin was registered in comparison to the OVA group.

Effect of TA of Vitamin D3 Cream (3 µg/g) and Betamethasone Cream for Five Days in OVA-Induced AD in Sections of the Mice Skin

The NC control group has a typical appearance (double head arrow) and thickness of both

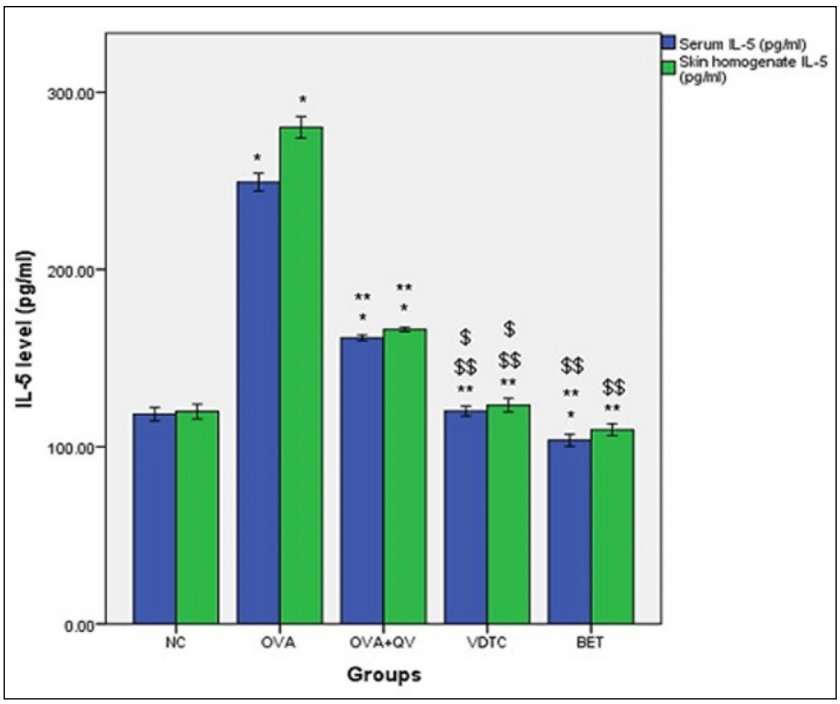

Figure 3. Effect of daily TA of vitamin D3 cream (3 µg/g) *vs.* betamethasone cream for five days on serum and skin homogenate IL-5 levels (pg/ml) in OVA-induced AD in mice. Significant p-value < 0.05 *vs.* (NC) group, **Significant p-value < 0.05 *vs.* (OVA) group, $$ Significant p-value < 0.05 *vs.* (OVA+QV) group, $^{\$}$Significant p-value < 0.05 *vs.* (BET) group.

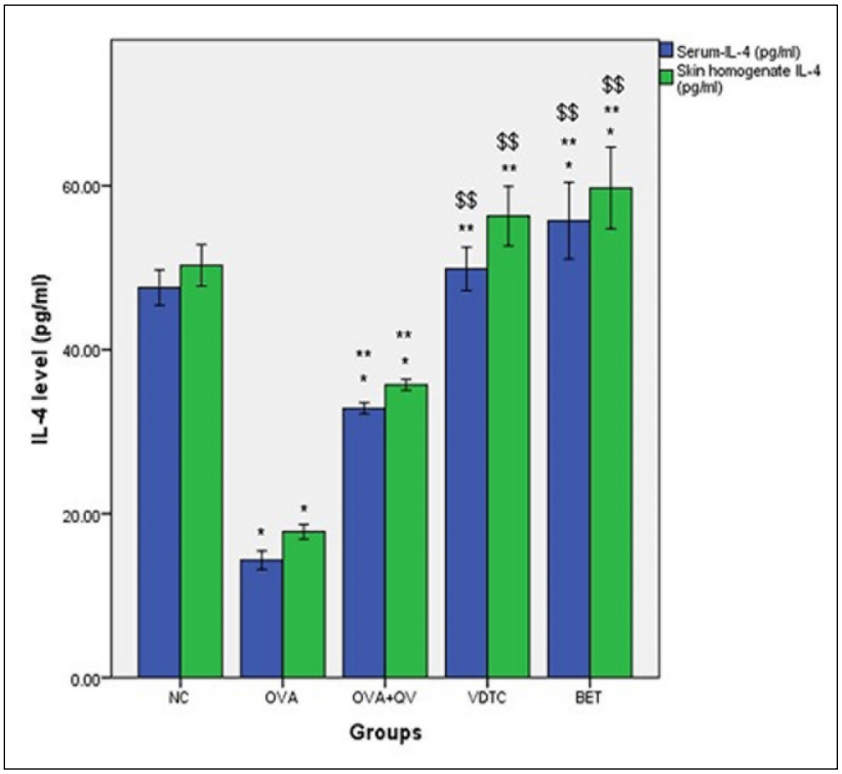

Figure 4. Effect of daily TA of vitamin D3 cream (3 µg/g) *vs.* betamethasone cream for five days on serum and skin homogenate IL-4 levels (pg/ml) in OVA-induced AD in mice. *Significant *p*-value < 0.05 *vs.* (NC) group, **Significant *p*-value < 0.05 *vs.* (OVA) group, $$Significant *p*-value < 0.05 *vs.* (OVA+QV) group.

the upper epidermis (thick black arrows) and deep dermis (black stars). While the OVA group has shown patchy epidermal thickening (thick black arrows), some epidermal regions showed hyaline degeneration (acidophilic stained, dotted arrow). The OVA+QV group has shown similar changes as seen in the OVA group (epidermal thickening and dermal fibrosis and inflammation). However, the VDTC group has shown marked curative effects against AD-associated changes with only a few residual patches of epidermal thickening (thick black arrow) and dermal fibrosis (black star) with a marked decrease in inflammatory and fibroblasts cellular density (thin black arrows). Moreover, the BET group has shown potential curative effects with the epidermal thickness (thick black arrows), which have been more registered in some areas and less in others, compared to the NC group (Figure 6).

Discussion

A noteworthy and realistic fact designates that atopic dermatitis is a widespread and recurrent inflammatory skin disorder. Furthermore, it is exemplified by the key symptoms of severe pruritis, dehydrated dry skin, swelling and eczematous lesions.

The fundamental hallmark of this condition is its association with an increased serum-IgE level[19]. It needs to be emphasized that the characteristic features of OVA-induced AD are: epidermal thickening, immune-dominated responses with upregulated expression of Th2 cytokines (IL-5, IL-4, and IL-13), elevated IgE level and infiltration of inflammatory cells[14]. To reiterate that, this model was preferred for the current study because the OVA-induced AD model displays many human AD features and seemingly BALB/c mice are more suitable for immunological studies[13].

In the present study, regarding the AD appearance and skin condition, the group treated with vitamin D_3 cream demonstrated a marked healing enhancement in skin condition as compared to OVA group. On the other hand, before dissection, the OVA group appeared to have recovered, but they still displayed moderate AD symptoms according to the TIS score. The OVA group mice underwent the subacute or interphase stage of AD (4-5 weeks) with moderate symptoms compared to the NC group (Figure 1). Concerning the effect of vehicles on AD, the skin of the vehicle group (OVA+QV) showed a marked improvement in skin appearance compared to the OVA group. At the same time, they had AD symptoms compared to the NC group. This pertinent finding agreed

with the study of Kim et al[14], where authors documented that the AD mice underwent a stage called "interphase" or "subacute condition" after 4-5 weeks of repeated epicutaneous AD-induction. The mice in this stage seemed to recover, but the AD condition had still progressed, and the inflammation was detected[20]. Hence, in the present study, the OVA group was observed to be in the interphase stage, between acute and chronic AD phases. The interphase stage of AD has its unique features (discussed later). Furthermore, the enhancement of the AD condition for the OVA+QV group perhaps is because of the vehicle (Figure 1), or it is only the time-dependent AD progression.

Moreover, in the present study, the OVA and OVA+QV groups demonstrated a significant augmentation in the serum and skin homogenate IgE levels in comparison with the NC group (Figure 2). On the other hand, a significant decrease in the serum and skin homogenate IgE levels was observed for the VDTC, and BET groups compared to the OVA group. These findings agree with a previous study[21], which demonstrated that vitamin D treatment significantly suppresses the IgE levels. The authors found that vitamin D regulates the serum-IgE level *in vivo*; besides, vitamin D deficiency was reported in mice with a higher IgE level. Their data suggested that vitamin D potentially controls the IgE response through direct and indirect regulation. The direct effect of vitamin D was demonstrated, where the B cells significantly produce IL-10 following OVA immunization, which in turn suppresses the IgE synthesis. In addition, the supportive role of vitamin D was also identified as a potent anti-inflammatory agent due to its action on the immune system's innate and adaptive cells[22,23]. Apparently, in the adaptive immune system, vitamin D_3 treatment decreases the activation, differentiation, and proliferation of B cells leading to reduced immunoglobulin (Ig) production from B cells. This control of B cell activation by vitamin D plays a significant role in inflammatory diseases[23]. These results strongly indicate that vitamin D has a regulatory role in IgE production.

We wish to enlighten that, in the contemporary study the effect of vitamin D_3 on the inflammatory cytokines IL-5, IL-4, and IL-13 levels was also investigated (Figure 3, 4 and 5). The data obtained demonstrated that the OVA and OVA+QV groups showed a significant augmentation in the serum and skin homogenate IL-5 levels (Figure3), while

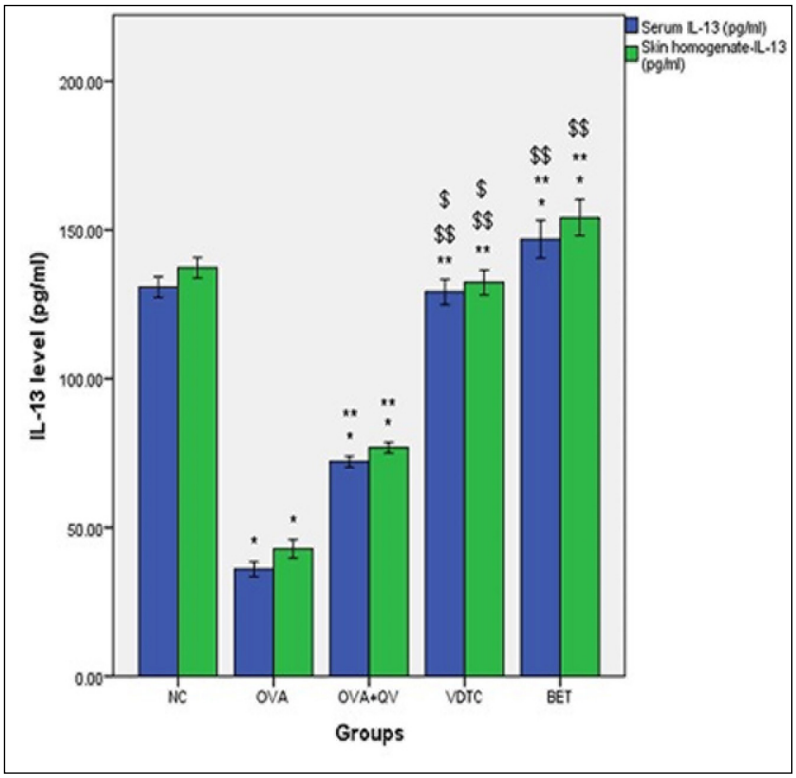

Figure 5. Effect of daily TA of vitamin D3 cream (3 µg/g) *vs.* betamethasone cream for five days on serum and skin homogenate IL-13 levels (pg/ml) in OVA-induced AD in mice. *Significant *p*-value < 0.05 *vs.* (NC) group, **Significant *p*-value < 0.05 *vs.* (OVA) group, $$Significant *p*-value < 0.05 *vs.* (OVA+QV) group, $Significant *p*-value < 0.05 *vs.* (BET) group.

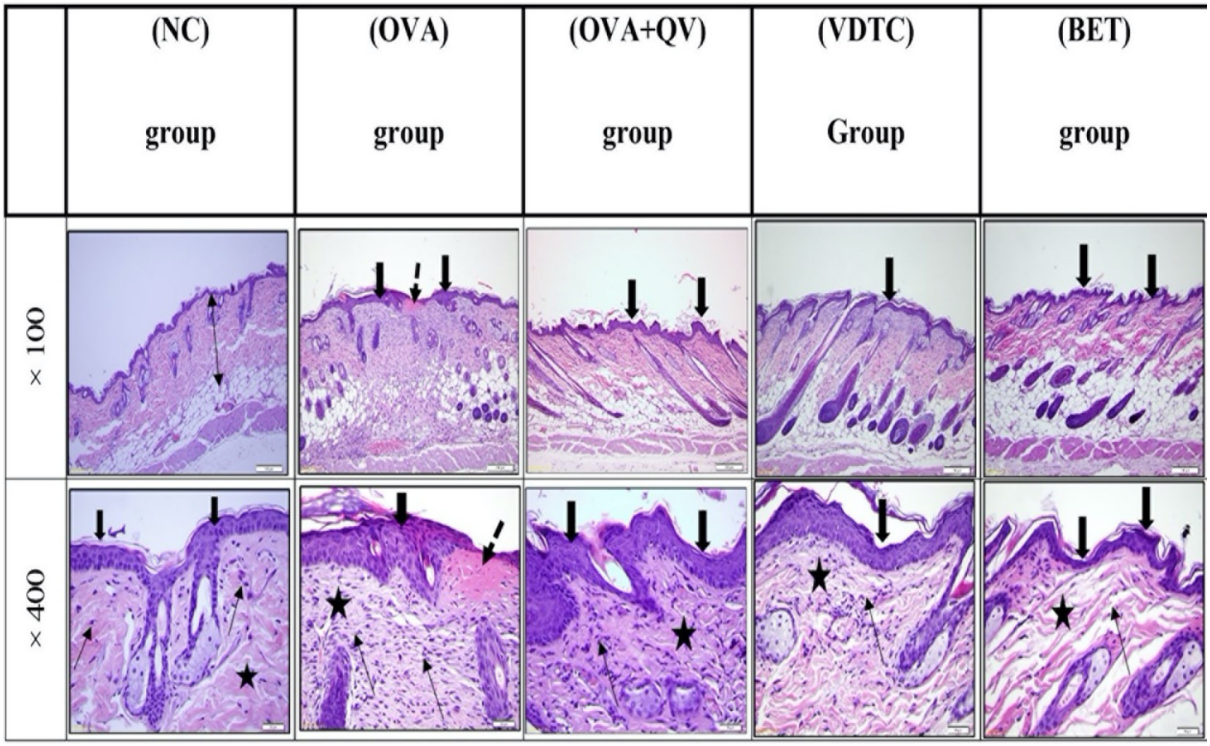

Figure 6. Effect of daily TA of vitamin D3 cream (3 µg/g) *vs.* betamethasone cream for five days in OVA-induced AD in sections of the mice skin. (× 100 for the first row and × 400 for the second row). NC = normal control, OVA= AD induced by OVA, OVA-induced AD + QV vehicle group VDTC= AD induced by OVA +treated group of vitamin D3 cream, BET= AD induced by OVA+ treated with betamethasone.

significant diminutions in the IL-4 and IL-13 levels (Figure 4 and 5) were observed as compared to the NC group. These results are consistent with other studies[14] that dealt with the OVA-induced AD mice model, where an increase in the IL-5, IL-4, and IL-13 levels were reported.

We wish to emphasize that in our study a significant upsurge in the IgE and IL-5 levels in serum and skin homogenate (Figure 3) was registered, concomitantly with a significant reduction in the serum and skin homogenate IL-4 and IL-13 levels (Figure 4 and 5), and the time-dependent progression suggested that the mice might have been in the interphase or subacute phase of AD.

The underlying details of VDTC and BET groups demonstrated a significant decrease in the serum and skin homogenate IL-5 levels (Figure 3), and a significant increase in the serum and skin homogenate IL-4 and IL-13 levels compared to the OVA group (Figure 4 and 5). Concurrently, a non-significant difference was also observed in the serum and skin homogenate IL-4 levels, only in the VDTC group *vs.* BET group comparison (Figure 4). Intriguingly, this is in accordance with a recent study by Szymczak and Pawliczak[23], affirming that treatment with the active form of vitamin D_3 (calcitriol) inhibits the secretion of pro-inflammatory cells Th1, such as IL-2, INF-γ, and TNF-α, and restores the balance between Th1 and Th2 by increasing the anti-inflammatory effect *via* promoting the production of T helper Type 2 induced cytokines, such as IL-3, IL-4, IL-5 and IL-10.

Another longstanding focus of interest displayed in this study comprises vital parameters and filaggrin level, which is a protein component of epithelial cells of the skin; interestingly, the skin homogenate filaggrin level was significantly increased in the OVA and OVA+QV groups, compared to the NC group (Table I). These findings are in conformity with previous studies[12,24] that have documented that the filaggrin levels are reduced in the AD. It has been reported in other studies[19,25] that filaggrin expression is modulated by the atopic inflammatory responses, specifically Th2 cytokines like IL-4 and IL-13, which modify the expression of filaggrin when differentiation of keratinocyte occurs during the acute phase

of AD. Moreover, in the presence of the cytokines like IL-4 and IL-13, the expression of filaggrin is substantially decreased.

In contrast, the absence of Th2 cytokines and the presence of IFN-γ, which is a Th1 cytokine control, increase the filaggrin expression[25]. Unequivocally, these results have a positive correlation with the results of our present study and further confirm that the mice were in the subacute phase of AD.

Notably, with the intention of developing targeted base drug therapy, several studies[23,26] have demonstrated that vitamin D can modulate the proliferation and differentiation of keratinocytes. Interestingly, vitamin D is used to normalize the hyperproliferation present in psoriasis skin, and many studies observed that vitamin D is essential for the generation and maintenance of the skin barrier. In an experimental study by Hong et al[5], it was shown that filaggrin was higher in the histological levels of mice treated with narrowband UVB phototherapy. This study further extended the approach to substantiate that the vitamin D_3 treated groups (Table I) showed decreased filaggrin levels when compared to the OVA control. Furthermore, the histological study (Figure 6) illustrated a significant increase in the epidermal thickness in the OVA and OVA+QV groups compared to the NC group. This has been confirmed in a recent study by Kim et al[14], demonstrating that the epidermal thickness is increased in mice presenting OVA-induced AD.

The VDTC and BET groups in this study (Figure 6) resulted in a significant decrease in the epidermal thickness compared to the OVA group. These findings agree with the Hong et al[5] study, which demonstrated that the topical administration of the active form vitamin D_3 showed an improvement in the stratum corneum integrity and barrier recovery. In contrast, the vitamin D_3 analogs have shown interruption in the permeability barrier of the epidermis by virtue of producing the proliferation of epidermal cells and inducing premature keratinization with no morphological signs of inflammation[27].

Realistically, AD upsets the routine life of the patients, and its economic burden is quite high. Additionally, the contemporary well- acquainted corticosteroid therapy shows several unacceptable adverse effects. In contrast, vitamin D is quite safe and affordable. Seemingly, it may serve as a novel anti-inflammatory agent, as evident in our present study which revealed almost equivalent results to corticosteroids. It needs to be emphasized that more extensive studies seem to be a pre-requisite requirement for topical vitamin D_3, to assess its safety and pharmaceutical stability.

Conclusions

The present study effectively demonstrated the target-based anti-inflammatory effect of vitamin D_3 by effective and favorable alteration in the immunological components responsible for AD. Moreover, this pioneer experimental work represents a new paradigm and sheds a light on the importance of vitamin D_3 in the treatment of AD. Hence, vitamin D_3 may provide a promising future agent for AD treatment.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

None.

Funding

None.

Ethics Approval

This experimental study was performed at King Fahd Center for Medical Research (KFCMR), King Abdulaziz University (KAU), Jeddah, Saudi Arabia, and the animal study protocol was approved by the KAU research ethics committee (Approval number: 306-18).

Authors' Contributions

NSA: formal analysis, experimental work, investigation, MAASA: Conceptualization, data curation, supervision, HMK: visualization, original draft preparation ASA: methodology, LMK: writing–review and editing, validation, and software. "All authors have read and agreed to the published version of the manuscript.

References

1) Umar M, Sastry KS, Al Ali F, Al-Khulaifi M, Wang E, Chouchane AI. Vitamin D and the pathophysiology of inflammatory skin diseases. Skin Pharmacol Physiol 2018; 31: 74-86.

2) Bieber T. Atopic dermatitis. N Engl J Med 2008; 358: 1483-1494.
3) Le Lamer M, Pellerin L, Reynier M, Cau L, Pendaries V, Leprince C, Méchin M-C, Serre G, Paul C, Simon M. Defects of corneocyte structural proteins and epidermal barrier in atopic dermatitis. Biol Chem 2015; 396: 1163-1179.
4) Zittermann A, Gummert JF. Nonclassical vitamin D actions. Nutrients 2010; 2: 408-425.
5) Hong S, Oh Y, Jung M, Lee S, Jeon H, Cho MY, Lee S, Choi E. Topical calcitriol restores the impairment of epidermal permeability and antimicrobial barriers induced by corticosteroids. Br J Dermatol 2010; 162: 1251-1260.
6) Oda Y, Uchida Y, Moradian S, Crumrine D, Elias PM, Bikle DD. Vitamin D receptor and coactivators SRC2 and 3 regulate epidermis-specific sphingolipid production and permeability barrier formation. J Invest Dermatol 2009; 129: 1367-1378.
7) El Taieb MA, Fayed HM, Aly SS, Ibrahim AK. Assessment of serum 25-hydroxyvitamin d levels in children with atopic dermatitis: correlation with SCORAD index. Dermatitis 2013; 24: 296-301.
8) Kim MJ, Kim S-N, Lee YW, Choe YB, Ahn KJ. Vitamin D status and efficacy of vitamin D supplementation in atopic dermatitis: a systematic review and meta-analysis. Nutrients 2016; 8: 789.
9) Cheon BR, Shin JE, Kim YJ, Shim JW, Kim DS, Jung HL, Park MS, Shim JY. Relationship between serum 25-hydroxyvitamin D and interleukin-31 levels, and the severity of atopic dermatitis in children. Korean J Pediatr 2015; 58: 96.
10) Di Filippo P, Scaparrotta A, Rapino D, Cingolani A, Attanasi M, Petrosino MI, Chuang K, Di Pillo S, Chiarelli F. Vitamin D supplementation modulates the immune system and improves atopic dermatitis in children. Int Arch Allergy Immunol 2015; 166: 91-96.
11) Fujii M. Current understanding of pathophysiological mechanisms of atopic dermatitis: Interactions among skin barrier dysfunction, immune abnormalities and pruritus. Biol Pharm Bull 2020; 43: 12-19.
12) Frazier WT, Bhardwaj N. Atopic dermatitis: diagnosis and treatment. AFP 2020; 101: 590-598.
13) Chauhan L, Gupta S. Creams: A Review on Classification, Preparation Methods, Evaluation and its Applications. JDDT 2020; 10: 281-289.
14) Kim WH, An HJ, Kim JY, Gwon MG, Gu H, Jeon M, Sung WJ, Han SM, Pak SC, Kim MK. Beneficial effects of melittin on ovalbumin-induced atopic dermatitis in mouse. Sci Rep 2017; 7: 1-12.
15) Association AVM. AVMA guidelines on euthanasia. Available at: http://www.avma org/issues/animal_welfare/euthanasia pdf 2007.
16) Van Oosterhout M, Janmohamed SR, Spierings M, Hiddinga J, Oranje AP. Correlation between Objective SCORAD and Three-Item Severity Score used by physicians and Objective PO-SCORAD used by parents/patients in children with atopic dermatitis. Dermatology 2015; 230: 105-112.
17) Chopra R, Silverberg JI. Assessing the severity of atopic dermatitis in clinical trials and practice. Clin Dermatol 2018; 36: 606-615.
18) Bancroft JD, Gamble M. Theory and practice of histological techniques. Elsevier health sciences, 2008.
19) Kim J, Kim BE, Leung DY. Pathophysiology of atopic dermatitis: clinical implications. In: Allergy Asthma Proc 2019, p.84. OceanSide Publications.
20) Kim JY, Jeong MS, Park MK, Lee MK, Seo SJ. Time-dependent progression from the acute to chronic phases in atopic dermatitis induced by epicutaneous allergen stimulation in NC/N ga mice. Exp Dermatol 2014; 23: 53-57.
21) James J, Weaver V, Cantorna MT. Control of circulating IgE by the vitamin D receptor in vivo involves B cell intrinsic and extrinsic mechanisms. J Immunol 2017; 198: 1164-1171.
22) Hartmann B, Riedel R, Jörß K, Loddenkemper C, Steinmeyer A, Zügel U, Babina M, Radbruch A , Worm M. Vitamin D receptor activation improves allergen-triggered eczema in mice. J Invest Dermatol 2012; 132: 330-336.
23) Szymczak I, Pawliczak R. The active metabolite of vitamin D3 as a potential immunomodulator. Scand J Immunol 2016; 83: 83-91.
24) Drislane C, Irvine AD. The role of filaggrin in atopic dermatitis and allergic disease. Ann Allergy Asthma Immunol 2020; 124: 36-43.
25) Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, DeBenedetto A, Schneider L, Beck LA, Barnes KC, Leung DY. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol 2009; 124: R7-R12.
26) Mesquita KdC, Igreja ACdSM, Costa IMC. Atopic dermatitis and vitamin D: facts and controversies. An Bras Dermatol 2013; 88: 945-953.
27) Von Brenken S, Jensen J-M, Fartasch M, Proksch E. Topical vitamin D3 derivatives impair the epidermal permeability barrier in normal mouse skin. Dermatology 1997; 194: 151-156.