ORIGINAL ARTICLE

Barrier abnormalities and keratinocyte-derived cytokine cascade after cessation of long-term topical glucocorticosteroid on hairless mouse skin

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ABSTRACT

Background: Previous studies have shown that topical corticosteroid (TCS) use induces structural abnormalities of the stratum corneum (SC), resulting in permeability barrier disruption. It is well-known that epidermal barrier perturbation induces a cytokine cascade, leading to cutaneous inflammation. Accordingly, we hypothesize that barrier disruption caused by long-term TCS therapy may trigger a cutaneous cytokine cascade, which plays an important role in withdrawal dermatitis (WD) following discontinuation of TCS. The objective of this study was to elucidate the possible mechanism of WD.
Methods: Hairless mice were treated once daily with 0.064% betamethasone dipropionate ointment for 6 weeks. After discontinuation of TCS, we examined the transepidermal water loss (TEWL), SC lipids and expression of the cytokines interleukin 1-alpha (IL1-α) and tumor necrosis factor-alpha (TNF-α) and their downstream signaling pathway in the following 2 weeks.
Results: We observed upregulation of IL1-α, TNF-α, inhibitor of nuclear factor kappa-B kinase subunits alpha and beta (IKK1, IKK2) and nuclear factor kappa-B (NF-κB) in the epidermis, accompanied by a significantly higher TEWL after TCS cessation. These cytokines gradually disappeared with concomitant normalization of TEWL after 1 week. Only negligible amounts of the aforementioned cytokines were observed in the dermis. Furthermore, concurrent application of petrolatum during TCS treatment decreased barrier impairment and production of cytokines.
Conclusion: An epidermis-derived cytokine cascade was observed following TCS-induced barrier disruption, which is similar to that from permeability barrier insults by acetone or tape stripping. The study suggests that concurrent application of skin care products during TCS treatment improves barrier homeostasis, and should become a standard practice to alleviate TCS-induced WD.
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Introduction

Topical corticosteroid (TCS) use is one of the most efficient therapeutic anti-inflammatory modalities in dermatology. However, cessation of TCS after long-term treatment may induce rebound flare, which is a troublesome, recalcitrant, and adverse event frequently observed in clinical practice. Rebound flare takes the form of a rapidly evolving dermatitis with intense redness, scaling, crusting, pustulation, and dryness of skin, occurring approximately

4–10 days after the termination of all TCS.[1–5] Rebound flare occurs not only in patients with predisposing cutaneous disorders,[2,6] but also in normal skin after cessation of long-term TCS treatment.[1,7]

Many confusing names describe such types of TCS-induced flare-up inflammation, including rebound phenomenon, steroid rosacea,[8] steroid dermatitis resembling rosacea,[5] steroid-induced rosacea-like dermatitis,[4,9] steroid-withdrawal rosacea-like dermatitis,[10] steroid-induced rosacea,[11] and steroid addiction.[12] To our understanding, "rebound flare" describes the exacerbation of the original unresolved inflammatory diseases (for example, psoriasis, atopic dermatitis, and chronic hand eczema), which are temporarily suppressed but recur after cessation of therapy. However, long-term application of TCS induces a different type of skin inflammation in both lesion and even the normal skin. This inflammation is an entirely new manifestation not associated with the original disease. To distinguish this distinct syndrome from "rebound flare", we propose to use the term "TCS-induced withdrawal dermatitis" (WD), which is induced by TCS and not associated with the original diseases. In the present work, we focus on WD, instead of the rebound flare of the original disease.

The mechanism of WD remains uncharacterized. Rapaport et al[13,14] believed that its clinical presentations are caused by compensatory vessel dilatation, because prolonged vessel constriction is induced by unbalanced nitric oxide, which is triggered by TCS. However, the treatments for dilated blood vessels are not effective. In addition, topical calcineurin inhibitors have been reported to ameliorate symptoms of WD.[15–17] Controversially, recent studies showed that application of topical calcineurin inhibitor may be a potential cause of rosaceiform dermatitis.[18–20] To date, the gold-standard treatment for WD has not been established.

TCS use, both short-term and long-term, profoundly interferes with barrier maturation processes (for examples, DNA synthesis, mitotic rate, lipid synthesis, keratinocyte differentiation, and lamellar body formation), thereby resulting in permeability barrier disruption and decrease in water content of the stratum corneum (SC), especially with mid- or high-strength TCSs such as betamethasone dipropionate.[2,3,21–23] Inhibition of sebocytes by TCS also contributes to the decreased water content (WC) of the SC.[24]

Elias et al[25–27] provided clear concepts of a linkage between disturbances in epidermal barrier function and a cytokine cascade causing cutaneous inflammation. Cutaneous barrier perturbation by tape stripping or acetone treatment induces upregulation of mRNA and protein levels of interleukin 1-alpha (IL1-α), IL1-β, tumor necrosis factor-alpha (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in epidermis, aimed at normalizing SC function.[25,28] Minor barrier perturbations remain localized to the epidermis and modulate the repair process, while repeated or severe barrier disruption results not only in the desired barrier repair, but also in inappropriate responses in the subjacent dermis and endothelium. The barrier disruption-related cytokine cascade further stimulates chemokine and intracellular adhesion molecule (ICAM) formation and Langerhans cell activation, which in turn produce downstream paracrine effects, leading to trapping of circulating inflammatory cells in the dermis, melanocyte activation, angiogenesis, and fibroplasias.[26] The elicitation of inflammation induces epidermal hyperplasia and abnormal keratinization, leading invariably to the production of an intrinsically inferior SC, thus creating a vicious cycle of events unless the barrier function is competent.[26,29] Furthermore, the extent of the enhanced cytokine synthesis is proportional to the degree of barrier disruption. None of these changes have been observed in individuals who have undergone limited tape stripping, which has not perturbed the barrier function.[30]

It is known that skin barrier perturbation independently induces an epidermis-initiated inflammation.[25,27,28] TCS-induced skin barrier perturbation may trigger an inflammatory cytokine change, which could be masked by the anti-inflammatory action of TCS but becomes obvious after stopping TCS. Therefore we hypothesize that the barrier disruption caused by long-term TCS therapy triggers a cutaneous cytokine cascade, which plays a vital role in WD following discontinuation of TCS. In this study, we developed an animal model of WD using hairless mice to observe pathologic and molecular changes after cessation of long-term TCS. We further investigated whether early intervention with concurrent application of skin barrier replenishment might ameliorate WD.

Materials and methods

Animals

All experiments were performed on hairless SKH-hr1 mice (6–8 weeks old, female, purchased from Charles River Laboratories, Wilmington, MA, USA). An approved protocol for animal use from the Institutional Animal Care and Use Committee (IACUC) of the National Cheng-Kung University (Tainan, Taiwan) was followed.

Treatment protocol

In two groups of animals, 25 mg of 0.064% betamethasone dipropionate ointment (BDO; Septon, Shionogi & Co., Ltd., Taiwan) or the vehicle ointment alone was applied to the lower back of each mouse once a day for 6 weeks. Another group was assigned petrolatum co-application treatment, where the mice were topically treated at the same site with petrolatum 12 hours after each treatment of BDO.

Measurement of transepidermal water loss

Transepidermal water loss (TEWL) was measured by a commercial Tewameter TM210 (Courage + Khazaka GmbH, Cologne, Germany), in an air-conditioned room with the relative humidity varying from 40% to 60% and the temperature kept constant at $20 \pm 2°C$. The mice were anesthetized by means of intraperitoneal injection of 4% chloral hydrate. No topical agent was applied to the area of measurement for 24 hours prior to the measurement.

Tissue examination

The full-thickness skin from the lower one-third of the back was harvested immediately after the mice were sacrificed at indicated times following cessation of application of BDO. Two skin strips from the central area of each specimen were taken. One strip was snap-frozen for Nile red staining. The other strip was fixed in 4% paraformaldehyde solution and embedded in paraffin for further examination.

Acute barrier perturbation by tape stripping

Acute barrier disruption of the skin of a group of mice was carried out by repeated applications of cellophane tape (5–8 times) to remove layers of the SC to serve as a positive control. The procedure was terminated when the TEWL reached a 2- to 3-fold increase (or 30 mg/cm²/h). The full thickness of skin was harvested 24 hours after tape stripping.

Nile red staining for SC neutral lipids

Nile red fluorescence staining was used to quantify and determine the localization of SC neutral lipids.[31] A stock solution of Nile red (500 μg/mL) in acetone was prepared and stored at −20°C,

protected from light. A fresh staining solution of Nile red was made by the addition of 20 μL of the stock solution to 1 mL of glycerol (75%), followed by a brief vortex to mix. To stain frozen sections of tissue (5 μm thickness), one drop of the glycerol staining solution was added to each section. After 10 minutes at room temperature in darkness, the sections were examined using a fluorescence microscope (Olympus BH-2; Olympus, Tokyo, Japan), utilizing 455 nm and 530 nm excitation and emission frequencies, respectively. Neutral lipids were visualized as yellow-gold fluorescent structures.

Generation of complementary RNA probes and in situ hybridization

Total RNA was isolated from the epidermis of hairless mice 24 hours after tape-stripping, by using TRIzol reagent (Invitrogen, Life Technologies, Austin, TX, USA). Two micrograms of total RNA was reverse-transcribed. Single-stranded, digoxigenin-labeled TNF-α, IL-1α and inhibitor of nuclear factor kappa-B kinase subunit beta (IKK2) cRNA probes were generated using two-step PCR amplification and *in vitro* RNA transcription. The primer pairs used are listed in Table S1. Briefly, 38 cycles of amplification under standard conditions were performed using the primer pairs (sense and T7+antisense). The purified PCR product was further amplified using the sense primer and composite T7 universal primer to extend and complete the full-length 23 bp T7 promoter. One microgram of the purified secondary PCR products served as a template for RNA transcription using a DIG RNA Labeling Kit (Roche, Basel, Switzerland). A sense cRNA probe was generated for negative controls. Paraffin-embedded specimen sections on microscope slides were deparaffinized and rehydrated in xylene and serial concentrations of ethanol. After prehybridizing with DIG Easy Hyb buffer (Roche) at 42°C for 1 hour, the slides were incubated with DIG-labeled cRNA probes at 42°C overnight. After hybridization, the slides were washed with 0.1× saline sodium citrate buffer (SSC) at 42°C for 1 hour to remove the unhybridized probes. The sections were incubated with anti-digoxigenin antibody conjugated with alkaline phosphatase (Roche) for 1 hour at room temperature and the signal was detected with nitro-blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP) stock solution (Roche).

Immunohistochemical staining for TNF-α, IL-1α, IKK-1, IKK-2, NF-κB and Ki-67

Cryostat sections (5 μm thickness) were mounted on poly l-lysine (Sigma-Aldrich, St. Louis, MO, USA)-treated glass slides. After quenching the endogenous peroxidase activity by using 3% H_2O_2 in methanol for 5 minutes, the tissue sections were blocked with antibody diluents (Dako Cytomation, Carpinteria, CA, USA) and incubated with appropriate dilutions of anti-TNF-α, anti-IL-1α (Santa Cruz Biotechnology, Inc., Dallas, TX, USA), anti-IKK-1, anti-IKK-2, anti-nuclear factor kappa B (NFκB p65; Abcam, Cambridge, UK) and anti-Ki-67 (Thermo Fisher Scientific, Waltham, MA, USA) antibodies at room temperature for 1 hour. The slides were then incubated with biotinylated secondary antibody (Dako Cytomation) for 30 minutes at room temperature. After incubation with streptavidin-horseradish peroxidase (HRP) reagent (Dako Cytomation) for 30 minutes, the skin sections were incubated with aminoethylcarbazole solution (Dako Cytomation) for 15–30 minutes at room temperature. An Olympus DP50 light microscope was used to examine the skin sections.

Western blot analysis

Skin sections were separated into epidermal and dermal layers by heating to 60°C for 55 seconds and the layers were individually examined. Protein was extracted using T-PER solution (Pierce Biotechnology, Waltham, MA, USA) and the protein concentration was measured using a Micro-BCA protein assay kit (Pierce). Electrophoresis of 200 μg of total protein was carried out on a 10–15% gradient sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel and was transferred onto polyvinylidene difluoride (PVDF) membrane (PerkinElmer, Waltham, MA, USA). After blocking in 5% nonfat milk, the membrane was incubated with anti-TNF-α (1:100; Santa Cruz Biotechnology, Inc.), anti-IL-1α (1:500; R&D Systems, Inc., Minneapolis, MN, USA), anti-NFκB p65 (1:1000; Abcam) and anti-α-tubulin (1:20,000, Sigma-Aldrich) antibodies at 4°C overnight. Antibody binding was probed with peroxidase-coupled secondary antibodies and then detected by enhanced chemiluminescence (Amersham, Buckinghamshire, UK).

Statistical analyses

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed by using paired and unpaired Student *t*-test measures between two groups. A *p* value < 0.05 was considered statistically significant.

Results

Skin permeability was disrupted by long-term TCS and gradually recovered after cessation of TCS

TEWL, representing an inside–outside permeability function, was significantly increased by a 6-week treatment of 0.064 % BDO, and was not affected by the vehicle (Figure 1A). TCS application was then stopped, and TEWL continued increasing until PS5D (post-steroids 5th day), and subsequently returned to normal by PS7D.

Neutral lipids existing in the intercellular space of the SC provide a permeability barrier to prevent water loss. Nile red is a sensitive method to visualize and semi-quantify the lipid deposition in both physiological and pathological SC.[31] Normal skin showed abundant fluorescent, horizontal staining in SC, and the TCS treatment decreased the fluorescence density in SC (Figure 1B). Neutral lipids in SC gradually increased and then recovered to normal level 7 days after cessation of TCS (Figure 1B).

Cessation of long-term TCS initiated upregulation of IL1-α and TNF-α, in the epidermis (keratinocytes), but not in the dermis, and activated their downstream effecter signals (IKK1 and IKK2)

Long-term TCS caused significant epidermal atrophy (Figure 2). After cessation of TCS, expression of Ki-67, a marker of cells undergoing proliferation, increased as early as PS1D. The thickness of the epidermis increased and became morphologically similar to rebound hyperplasia without obvious dermal inflammatory infiltrate up to PS7D. Both the epidermal thickness and Ki-67 expression returned to normal on PS14D.

In situ hybridization of mRNA and immunohistochemical (IHC) staining of protein were performed to examine barrier disruption-related cytokine changes. The mRNAs of TNF-α, IL1-α and IKK-2 were markedly upregulated in the epidermis on PS3D and subsequently declined to normal range by PS14D (Figure 3). Meanwhile, IHC staining of TNF-α, IL1-α and their downstream effectors, IKK-1 and IKK-2, showed significant upregulation in the epidermis on PS3D, which gradually disappeared by PS7D (Figure 4). Only negligible amounts of cytokine mRNAs and inflammatory cells were present in the dermis.

Thereafter, Western blot analysis for IL1-α and TNF-α protein expression in the epidermis and dermis were performed separately.
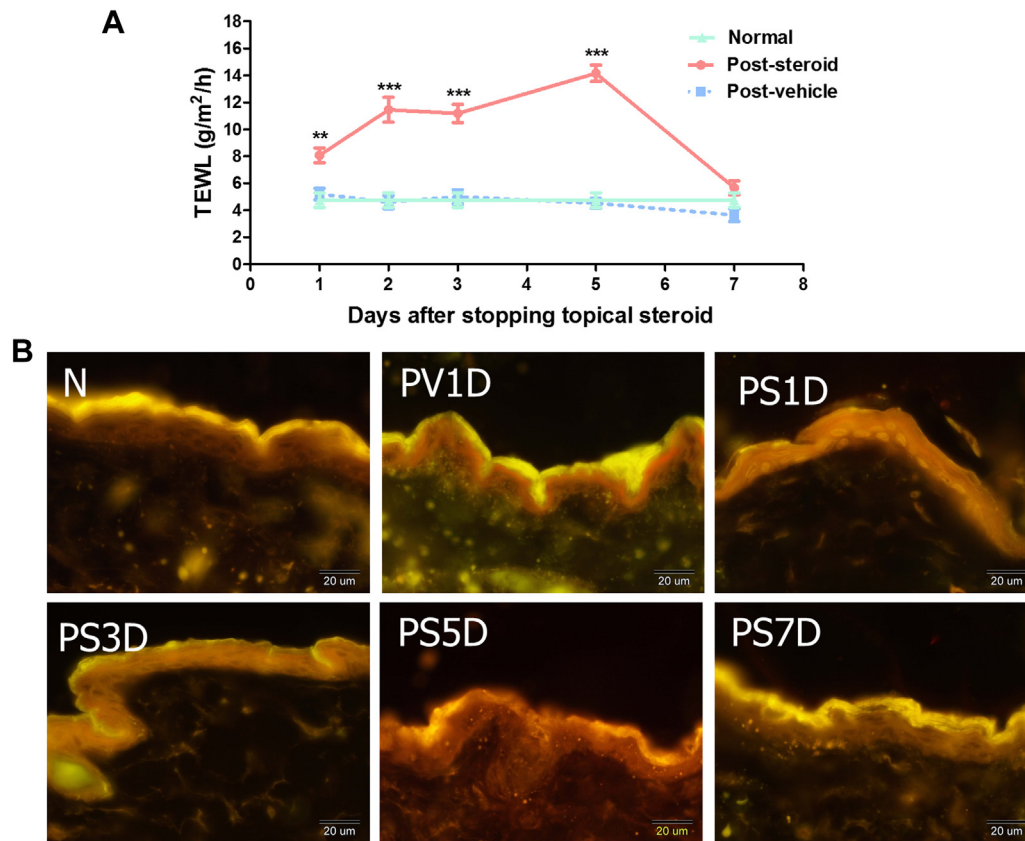
Figure 1 (A) Cutaneous permeability barrier (represented by TEWL) was impaired after 6 weeks of topical 0.064% betamethasone dipropionate ointment (BDO) treatment. The barrier function gradually recovered 7 days (PS7D) after stopping topical corticosteroid (TCS) treatment. (B) As demonstrated by Nile red staining, the neutral lipid expression in the stratum corneum (SC) diminished after long-term TCS treatment and recovered after cessation of TCS. $^{**}p < 0.05$, $n = 5$. $^{***}p < 0.01$, $n = 5$. N = normal skin; PS1–7D = post-steroids 1st to 7th days; PV1D = 1 day after stopping topical vehicle; TEWL = transepidermal water loss. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

No inflammatory cytokine presentation was detected in the dermis (data not shown). In contrast, IL1-α and TNF-α proteins in the epidermis increased by PS2D, were significantly upregulated at PS3D, subsequently peaked at PS5D and declined by PS7D (Figure 5). These sequential results corroborated the IHC findings (Figure 4). Based on these results, it is concluded that the cytokine cascade was mainly derived from keratinocytes, but not from inflammatory cells.

Translocation and activation of NF-κB were significantly enhanced after cessation of TCS

In normal mouse skin, only weak expression of NF-κB appeared in scattered basal keratinocytes. Acute barrier disruption by tape stripping resulted in prominent activation and translocation of NF-κB from the cytosol to the nucleus in both basal and suprabasal keratinocytes of the epidermis (Figure 5A). After cessation of TCS, NF-κB was significantly activated in the keratinocytes of all layers of the epidermis by PS3D and subsequently declined. It is known that TCSs exert their anti-inflammatory effects by interfering with NF-κB. However, NF-κB was activated early in WD. Western blot analyses revealed a similar sequential expression of NF-κB (Figure 5B).

Co-application of petrolatum diminished barrier disruption during TCS treatment, and decreased cytokine cascade after cessation of TCS

After 6 weeks of TCS treatment, the increase in TEWL was less when co-applied with petrolatum than in the TCS-only group (Figure 6A).

Three days after cessation of all topical agents, neutral lipids of SC were more abundant in the petrolatum co-application group (Figure 6B). Moreover, IHC staining revealed that co-application of petrolatum significantly reduced the expression of TNF-α, IL-1α, and IKK2 on PS3D (Figure 6C).

Discussion

It is intriguing that cessation of TCS triggers an entirely new and distinct type of inflammation, WD, while TCS use suppresses the original inflammatory process. In this study, we demonstrated that long-term application of TCS caused barrier defects in hairless mice. Moreover, discontinuation of TCS after long-term treatment resulted in a keratinocyte-derived cytokine cascade, which was similar to the characteristic reactions of acute barrier disruption.[25] Disappearance of these cytokines in the epidermis is accompanied by the normalization of TEWL one week after stopping TCS.

TCS use inevitably causes skin barrier dysfunction in both short-term and long-term applications, by profoundly affecting epidermal terminal differentiation and SC formation.[2,3,21–23,32,33] The disrupted skin barrier, in turn, causes a cytokine cascade derived from keratinocytes and subsequently induces clinical inflammation.

We propose a possible mechanism of TCS-related withdrawal dermatitis and rebound phenomenon (Figure 7). TCSs exert anti-inflammatory effects to suppress the inflammatory skin dermatoses. Some of the inflammatory skin diseases (for examples: psoriasis vulgaris, atopic dermatitis) are only temporarily suppressed by TCS and relapse after cessation of TCS. Conversely, many of the
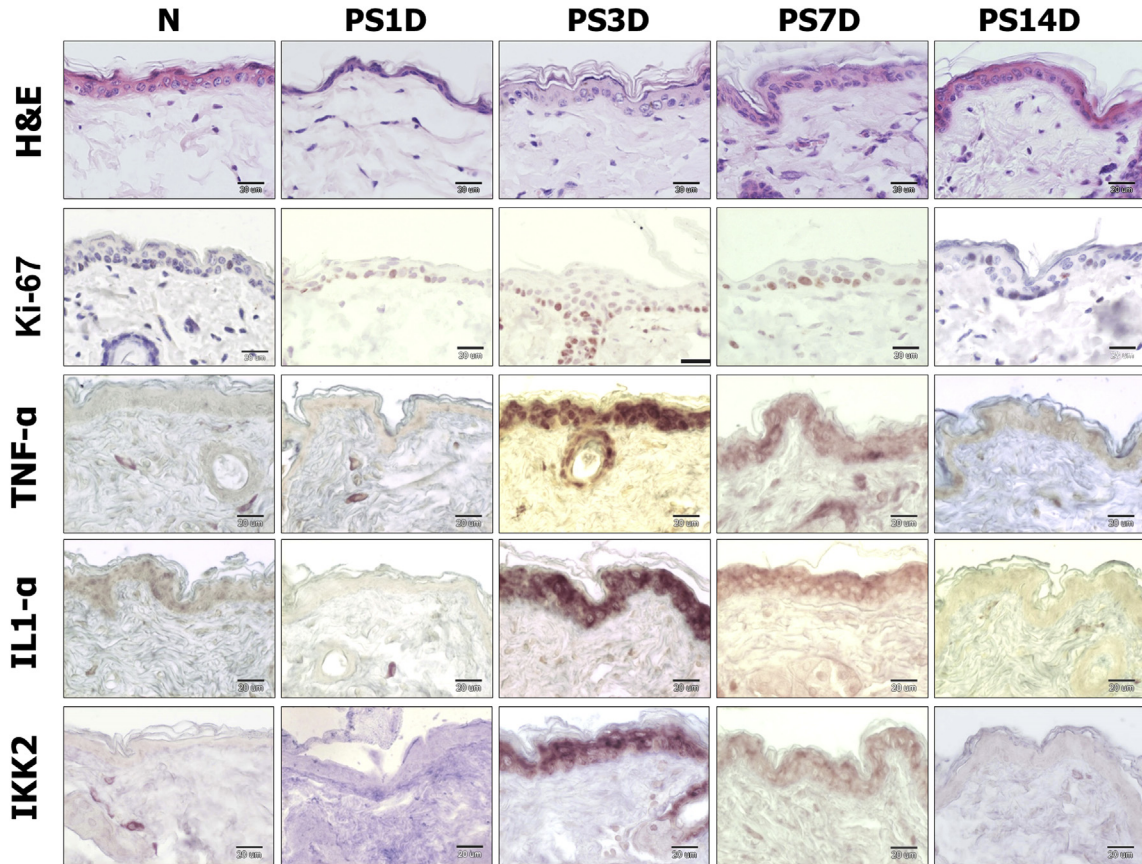
Figure 2 The epidermis appeared atrophic after 6 weeks of 0.064% betamethasone dipropionate ointment (BDO) application, became hyperplastic on PS7D, and returned to baseline on PS14D. Increase in proliferation marker (Ki-67) was noted as early as on PS1D. *In-situ* mRNA hybridization demonstrated increased expression of TNF-α, IL1-α and IKK2 in the epidermis on PS3D and subsequently normalization on PS14D. H&E = hematoxylin and eosin; IKK2 = inhibitor of nuclear factor kappa-B kinase subunit beta; IL1-α = interleukin 1-alpha; N = normal skin; PS1–14D = poststeroids 1st to 14th days; TNF-α = tumor necrosis factor-alpha.
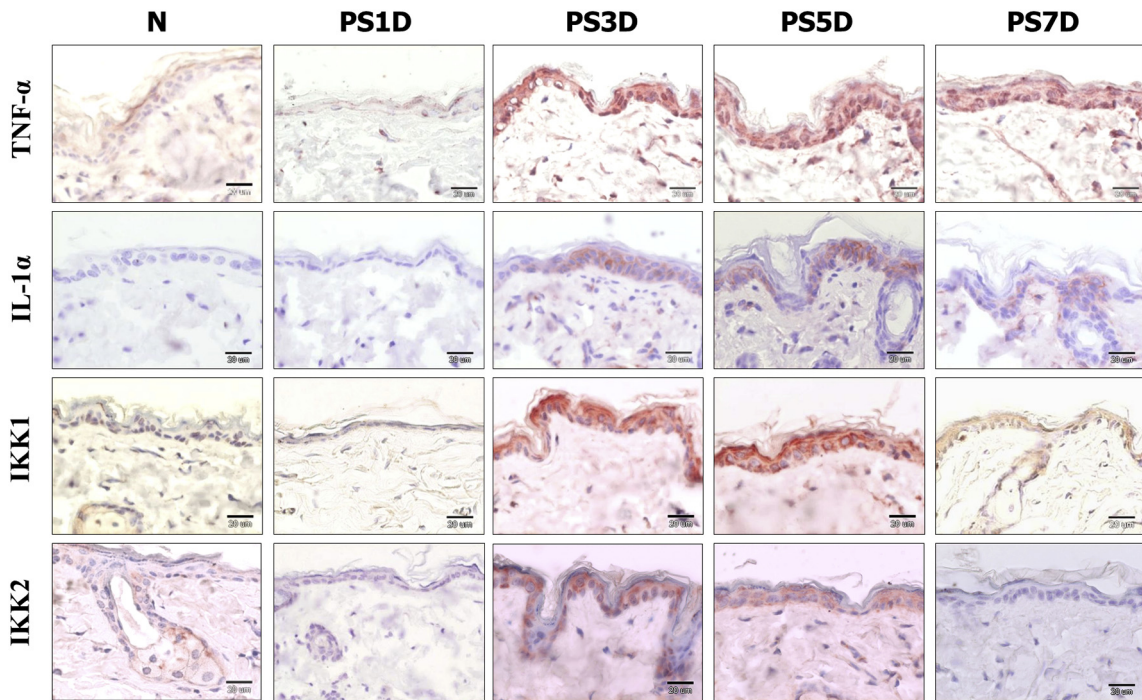

Figure 3 Immunohistochemical staining demonstrated increased TNF-α, IL1-α, IKK1 and IKK2 expressions in the epidermis on PS3D and PS5D, and normalization on PS7D. IKK1/2 = inhibitor of nuclear factor kappa-B kinase subunit alpha/beta; IL1-α = interleukin 1-alpha; N = normal skin; PS1–7D = poststeroids 1st to 7th days; TNF-α = tumor necrosis factor-alpha.

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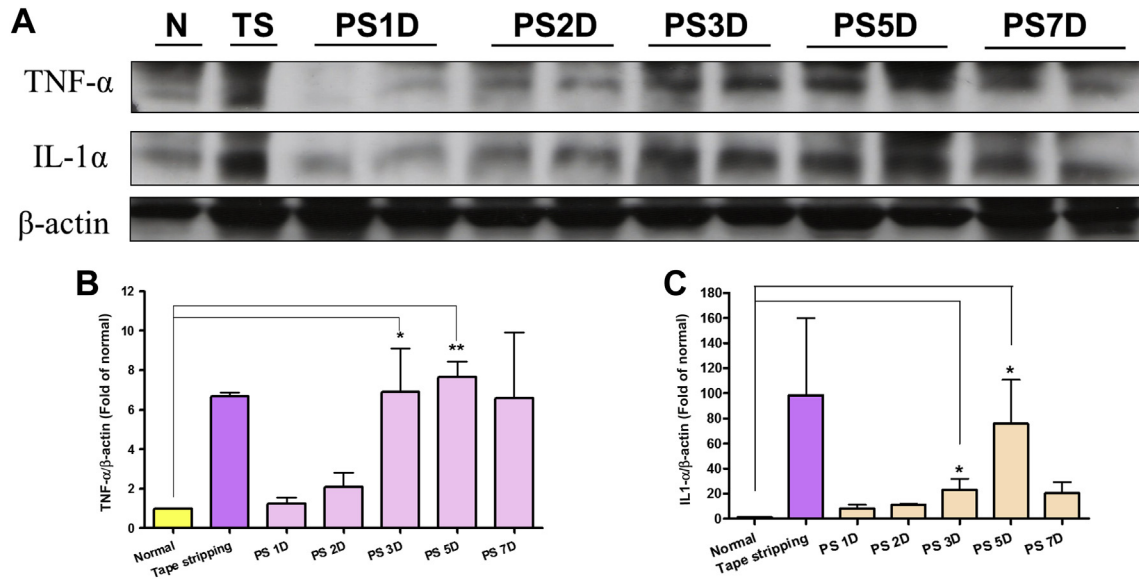
Figure 4 In accordance with the results obtained from immunohistochemical staining, Western blot analyses also demonstrated significant upregulation of epidermal TNF-α and IL-1α expression on PS3D and PS5D following 6 weeks of 0.064% betamethasone dipropionate ointment (BDO) treatment. The epidermis after tape-stripping served as a positive control. *p < 0.05, n = 4. **p < 0.01, n = 4. IL1-α = interleukin 1-alpha; N = normal epidermis protein; PS1–7D = poststeroids 1st to 7th days; TS = tape-stripped epidermis; TNF-α = tumor necrosis factor-alpha.

original diseases have been cured and their rebound phenomenon is caused by a different process. TCSs profoundly interfere with the differentiation processes of keratinocytes and the maturation of the skin permeability barrier, thereby resulting in barrier perturbation. The disrupted barrier tends to initiate a keratinocyte-derived inflammatory cascade to compensate for and repair the defected barrier function, while TCSs mask and temporarily suppress this type of inflammation. After cessation of TCS, the barrier disruption induces the release of many kinds of cytokines, including TNF-α and IL-1α, and promotes subsequent activation of the IKK complex which includes IKK1/2. The downstream signaling further activates

the NF-κB pathway. If the barrier disruption exceeds the threshold below which the early cytokine cascade fails to repair barrier disruption promptly, the increased epidermal TNF-α and IL-1α will result in recruitment of inflammatory cells to the dermis.[26] The subsequent mononuclear cell infiltrate in the dermis is likely to produce more inflammatory cytokines which further aggravate the WD, compromise the barrier repair process, and consequently cause the clinical presentations of WD. In our experiments, we demonstrated the increased epidermal TNF-α, IL-1α and IKK1/2 and concurrent elevated TEWL after cessation of long-term TCS. By PS3D, while TEWL had increased significantly, dermal expression of
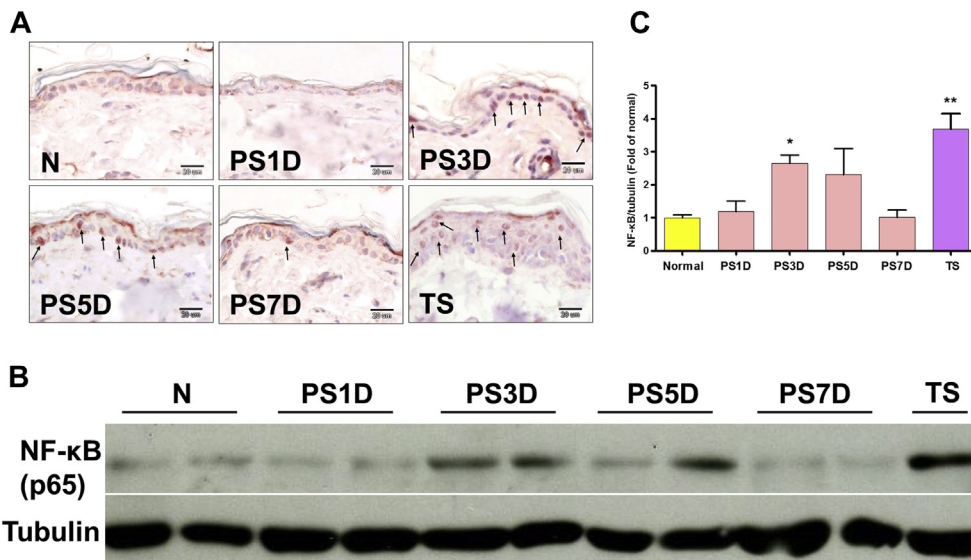

Figure 5 Skin barrier disruption induced translocation of NF-κB from cytosol to nucleus in epidermal keratinocytes. (A) After 6 weeks of 0.064% betamethasone dipropionate ointment (BDO) treatment, positive immunohistochemical nuclear staining were observed in the epidermal keratinocytes as early as on PS1D and became more prominent on PS3D. The arrow indicates the translocation of NF-κB into the nucleus. (B, C) The relative expression of NF-κB by Western blot examinations showed similar serial changes. The epidermis after tape stripping served as a positive control. *p < 0.05, n = 3. N = normal skin; NF-κB = nuclear factor-kappa B p65 subunit; PS1–7D = poststeroids 1st to 7th days; TS = tape-stripped epidermis.
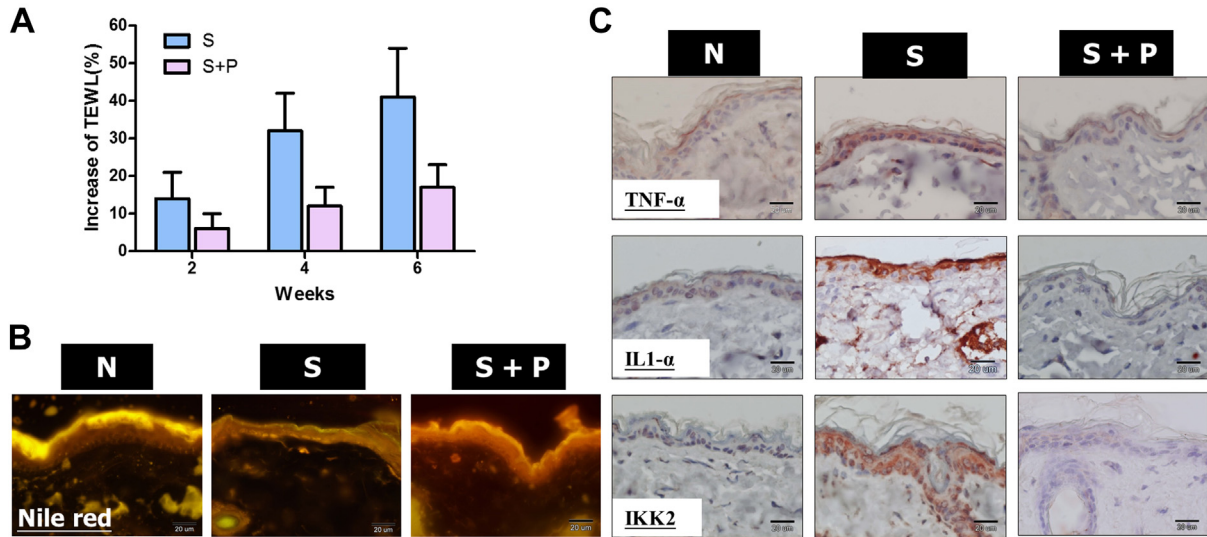
Figure 6 Co-application of petrolatum during topical corticosteroid (TCS) treatment improved the epidermal permeability barrier as demonstrated by the examination of (A) transepidermal water loss (TEWL) and (B) stratum corneum (SC) neutral lipids by Nile red stain on PS3D. (C) Immunohistochemical staining also demonstrated attenuation of the barrier disruption-related cytokines cascade in the epidermis with petrolatum and TCS combined treatment on PS3D. $^*p < 0.05$, S versus S+P, $n = 3$. IKK2 = inhibitor of nuclear factor kappa-B kinase subunit beta; IL1-α = interleukin 1-alpha; N = normal control; S = TCS once daily for 6 weeks; S + P = Alternating application of TCS and petrolatum in the interval of 12 hours for 6 weeks; TNF-α = tumor necrosis factor-alpha.
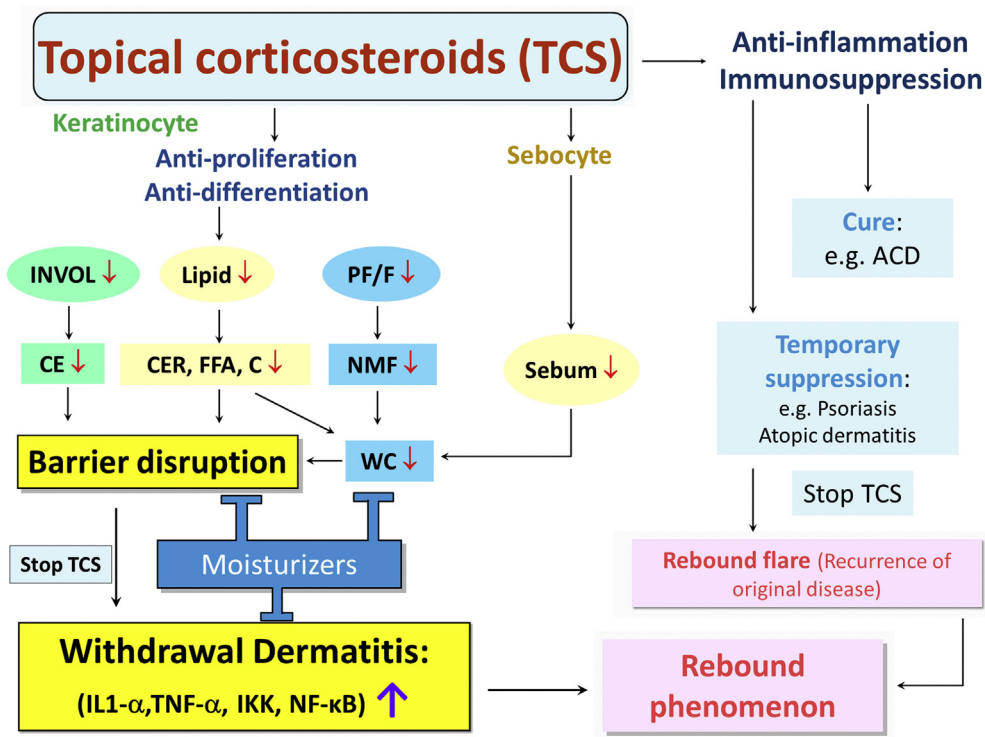
Figure 7 Proposed mechanisms of topical corticosteroid (TCS)-related withdrawal dermatitis (WD) and rebound phenomenon (RP). Besides the therapeutic effect of TCS use in reducing cutaneous inflammation, it also inhibits the proliferation and differentiation of keratinocytes, thus interfering with the terminal differentiation processes of the epidermis. This results in decreased physiological lipids (ceramide, free fatty acid and cholesterol), profilaggrin/filaggrin (PF/F), and cornified envelope (CE) formation, and finally perturbs the epidermal barrier function. Inhibition of sebocytes also contributes to the decreased water content (WC) of stratum corneum (SC). The disrupted barrier tends to initiate a keratinocyte-derived cytokine cascade and is relevant to the inflammatory cutaneous reaction of WD during and after discontinuation of TCS treatment. However, RP following discontinuation of TCS treatment may be the combination of WD with recurrence of the original cutaneous disorders, such as psoriasis vulgaris and atopic dermatitis. As a result, TCS acts as a double-edged sword in chronic inflammatory cutaneous disorders. It reduces cutaneous inflammation, but at the same time, through barrier disruption, TCS induces another dermatitis (WD). The severity of WD is proportional to the degree of barrier disruption. Since the barrier defects play an important role in the TCS-induced WD, co-application of petrolatum during TCS treatment or after discontinuation of TCS improves the barrier function and decreases the severity of WD and RP. ACD = allergic contact dermatitis; C = cholesterol; CER = ceramide; FFA = free fatty acid; IKK = inhibitor of nuclear factor kappa-B kinase; IL1-α = interleukin 1-alpha; INVOL = involucrin; NF-κB = nuclear factor kappa B; NMF = natural moisturizing factor; TCS = topical corticosteroid; TNF-α = tumor necrosis factor alpha; WC = water content.

TNF-α and IL-1α had not significantly increased, as compared with epidermal expression of TNF-α and IL-1α. The results suggest that the impaired barrier function of WD is more related to increased expression of TNF-α and IL-1α in the epidermis rather than in the dermis. In the murine model, the WD skin recovered within one week of cessation of TCS. However, in the clinical observation, it seemed to take a longer time for human skin to recover from WD, the prolonged recovery time in humans probably related to the complicated daily exposure of human skin or simply species difference between human and murine, which has not yet been investigated thoroughly.

However, it is worth noting that the detailed signaling pathways underlying epidermal barrier defects and acute cutaneous inflammation following TCS treatment are still not fully understood. Recently, studies have demonstrated that local glucocorticoid production (such as cortisol) and glucocorticoid receptor (GR) signaling play important roles in skin barrier homeostasis in normal or stressed conditions.[34—37] It is possible that TCS application will alter local expression of cortisol, 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), or GR in the epidermis, and ultimately trigger skin barrier defects and acute inflammatory responses of the epidermis. Therefore, in order to answer these questions, future study should target the complex interactions of GR, 11β-HSD1, and signaling pathways during application and after cessation of TCS.

So far, efforts to dissociate the side effect of skin barrier perturbation from anti-inflammatory activity during TCS treatment by modification of the drug structure or formulation has not been successful. Moreover, it is noteworthy that many inflammatory skin diseases, such as psoriasis,[38] atopic dermatitis,[39] and irritant contact dermatitis, are associated with underlying skin barrier dysfunction.

Treatment for WD requires discontinuation of all TCS; however, avoidance of TCS use usually leads to further aggravation of symptoms. Although WD spontaneously resolves several weeks later without treatment,[40] many patients resume TCS treatment to suppress this undesirable syndrome and thus become virtually dependent on the application of corticosteroids. This type of flare remains a recalcitrant problem.

To prevent WD as well as disease flare-up, early replenishment of topical agents to improve barrier function should become a standard practice during and after TCS treatment, both for treating the defective barrier due to the original dermatitis and for preventing possible new barrier abnormalities induced by TCS application. It has been demonstrated that petrolatum can be absorbed into the outer layer of the SC, reducing the barrier defects and accelerating barrier repair after disruption by tape stripping and detergents.[41] The barrier repair effect of petrolatum is attributed to enhancement of SC lipid formation. Our results showed early application of petrolatum reduces skin barrier disruption, as well as the inflammatory changes after discontinuation of TCS, suggesting the beneficial role of petrolatum in preventing or decreasing the barrier disruption due to long-term TCS treatment. Furthermore, during WD, petrolatum can also improve the recovery of the barrier function and reduce disease severity.[6] It was also reported that physiological SC lipids supplemented at an optimal combination (cholesterol, ceramide, and fatty acid in the ratio of 3:1:1) can reverse the TCS-induced abnormalities in permeability barrier homeostasis.[21] Recent studies have also demonstrated that co-application of pseudoceramide-containing physiological lipid mixture (multi-lamellar emulsion) minimizes TCS-induced barrier impairment.[42—44] Moreover, topical treatment with PPARs or LXR activators improve the TCS-induced abnormalities in proliferation, differentiation, and permeability barrier function in murine epidermis.[45] The beneficial effects of optimal combination therapy, and prophylactic effects to WD by these compounds (i.e., petrolatum, physiologic SC lipids, or PPARs / LXR activators) are worthy of further investigation.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.dsi.2015.05.002.