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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.

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

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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 ± 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/cm^2/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,