

Differential effects of topical corticosteroid and calcineurin inhibitor on the epidermal tight junction

Sang E. Lee^{1*}, Yuri Choi^{2*}, Song-Ee Kim^{2,3}, Eun B. Noh² and Soo-Chan Kim^{2,3}

¹Department of Dermatology, CHA Bundang Medical Center, CHA University, Seongnam, Korea; ²Department of Dermatology and Cutaneous Biology Research Institute, Yonsei University College of Medicine, Seoul, Korea; ³BK21 Project for Medical Science, Yonsei University, Seoul, Korea

Correspondence: Soo-Chan Kim, MD, PhD, Department of Dermatology, Yonsei University College of Medicine, Gangnam Severance Hospital, 712 Eonjuro, Gangnam-gu, Seoul 135-720, Korea, Tel.: +82 2 2019 3362, Fax: +82 2 3463 6136, e-mail: kimsc@yuhs.ac

*These two authors contributed equally to this work.

Abstract: Tight junction (TJ) is one of the functional barriers present in the skin. Although topical corticosteroids and calcineurin inhibitors are used widely for atopic dermatitis, the effect of these agents on TJs has not been reported. We investigated the structural changes of TJs in mice skin after application of 0.05% clobetasol propionate or 0.1% tacrolimus ointment for 10 days. Clobetasol caused epidermal thinning and decreased collagen density. Basal transepidermal water loss was significantly increased in clobetasol-treated versus vehicle- or tacrolimus-treated skin. Confocal immunofluorescence showed that clobetasol altered the structure of claudin-1, -4 and occludin. Tacrolimus also caused morphological

alteration of occludin. Western blot and real-time PCR revealed that clobetasol significantly decreased claudin-1, -4 and occludin, whereas tacrolimus did not significantly affect claudin-1 and -4 but downregulated occludin to a lesser extent compared to clobetasol. In conclusion, we suggest that downregulation of TJ proteins expression is another pathomechanism of corticosteroid-induced permeability barrier disruption.

Key words: calcineurin inhibitor – epidermal tight junction – permeability barrier – topical corticosteroid

Accepted for publication 22 October 2012

Background

Topical corticosteroids and topical calcineurin inhibitors (TCIs), the two main topical medications for atopic dermatitis (AD), have different effects on the epidermal permeability and antimicrobial barriers (1–6). The functional barrier of the skin resides mainly in the stratum corneum (SC), but there is evidence that tight junction (TJ)s also contribute to the permeability barrier (7–10). Moreover, TJs function as an immunological barrier by regulating antigen uptake (11). TJs are regulated by numerous stimuli, such as pathogens, UV irradiation and wounding (12–14). While the regulation of TJs in response to external stimuli is important, the effect of topical corticosteroids and TCIs on the TJ proteins expression remains uninvestigated.

Question addressed

We investigated whether topical corticosteroids and TCIs affect epidermal TJ protein expression and structure. The different effects of topical corticosteroids and TCIs on barrier function and morphology of mouse skin were also investigated.

Experimental design

For experimental procedures, see Data S1.

Results

Application of clobetasol for 10 days led to skin atrophy, telangiectasia (Fig. S1), epidermal thinning (Fig. S2) and a decrease in collagen density (not shown) compared with untreated controls or tacrolimus-treated mice. Basal transepidermal water loss (TEWL) increased significantly in clobetasol-treated versus vehicle- or tacrolimus-treated area on day 7 and 11, suggesting that topical corticosteroid impairs permeability barrier function (Fig. S3). Basal TEWL does not represent SC lipid composition, hydration or capacity for barrier recovery. Therefore, although we showed

that tacrolimus did not increase basal TEWL, it remains to be ascertained whether tacrolimus does not affect the permeability barrier homeostasis. Next, we investigated whether topical corticosteroid or tacrolimus affects TJ protein expression using immunofluorescence staining. In untreated skin, claudin (Cldn)-1 was found in suprabasal keratinocytes, while Cldn-4 was restricted to the stratum granulosum (SG) and the upper stratum spinosum (Fig. 1a,e). Cldn-1 and -4 expression was found markedly reduced in clobetasol-treated versus untreated or vehicle-treated skin (Fig. 1b,c,f,g). In contrast, there were no obvious changes after tacrolimus compared to vehicle treatment (Fig. 1d,h). The immunoreactivity of occludin (Ocln), which is normally found to be restricted in the SG, was reduced and distributed as dots in both clobetasol- and tacrolimus-treated versus vehicle-treated skin (Fig. 1i–l). By western blot analysis, Cldn-1 and -4 protein levels were significantly decreased in clobetasol-treated versus skin treated with vehicle, whereas no alterations were seen in tacrolimus-treated skin (Fig. 2a). In contrast, we observed a similar trend towards decreased Ocln in both clobetasol- and tacrolimus-treated versus vehicle-treated skin (Fig. 2a). By real-time PCR, clobetasol significantly decreased mRNA expression of Cldn-1, Cldn-4 and Ocln (Fig. 2b–d). In contrast, Cldn-1 and -4 mRNA levels did not change significantly in control versus tacrolimus-treated skin (Fig. 2b,c). The mRNA level of Ocln significantly decreased after tacrolimus treatment compared to control skin (Fig. 2d). These gene expression results were consistent with the protein expression data.

Discussion

In this study, we demonstrated a different effect of topical corticosteroid and TCIs on the expression of TJ proteins, permeability

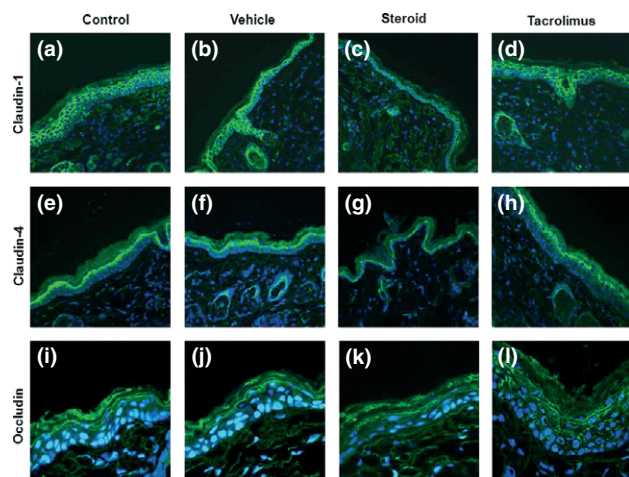


Figure 1. The effect of topical corticosteroid (0.05% clobetasol propionate ointment) and calcineurin inhibitor (0.1% tacrolimus ointment) on the expression of immunoreactive tight junction (TJ) proteins in mouse skin. The dorsal skin of mouse was treated with clobetasol propionate, tacrolimus or hydrophilic base ointment (vehicle) as a vehicle, twice daily for 10 days ($n = 4$ in each group). At 24 h after last treatment, treated skin samples from steroid (c, g, k), tacrolimus (d, h, l) or vehicle-treated skin (b, f, j) were collected and immunostained for claudin (Cldn)-1, Cldn-4 and occludin (Ocln). (Cldn-1, Cldn-4: $\times 400$ original magnification, Ocln: $\times 600$ original magnification).

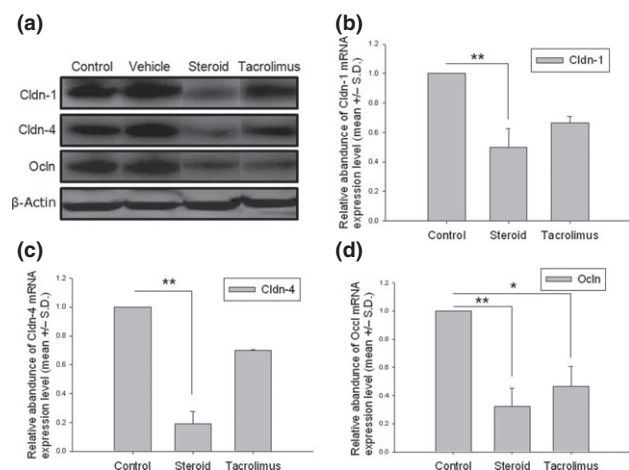


Figure 2. The effect of topical corticosteroid (0.05% clobetasol propionate ointment) and calcineurin inhibitor (0.1% tacrolimus ointment) on the protein and mRNA levels of tight junction (TJ) proteins in mouse skin. (a) The levels of protein for claudin (Cldn)-1, Cldn-4 and occludin (Ocln) were determined using Western blot analysis. Epidermal lysates from the biopsy specimens from steroid, tacrolimus and vehicle-treated area were incubated with monoclonal antibodies for Cldn-1, Cldn-4 and Ocln. Equal amounts of proteins were separated, and β -actin was used as a loading control. (b–d) The levels of mRNA for Cldn-1, Cldn-4 and Ocln were determined using real-time PCR and normalized to that of β -actin. Results are expressed as mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.001$ (Student's t -test).

barrier function and morphology of mouse skin. Topical corticosteroid downregulated gene and protein expressions of TJ components, including Cldn-1, -4 and Ocln and altered TJ structures. TCI did not alter the gene and protein expression of Cldn-1 and -4,

but downregulated the expression of Ocln. Immunofluorescence analysis showed that TCI did not alter the structure of Cldn-1 and -4, but altered the immunolocalization of Ocln, suggesting that TCI also alters TJ structure and composition. Among TJ proteins, Cldn-1 and -4 have a critical role in cutaneous barrier function (7–10,15). Cldn-1 null-mutant mice showed normal SC, but died after birth with increased TEWL (7). Moreover, Cldn-1 gene mutations are associated with neonatal sclerosing cholangitis with ichthyosis and AD (8,9). Cldn-4 was shown to play a role in forming an inside–out barrier for 550 Da tracer in organotypic skin culture (15). Unlike Cldn-1 and -4, Ocln was found to be not essential for barrier function (16). Taken together, our results suggest that topical corticosteroids could negatively impact the TJ permeability barrier function by decreasing the expression of Cldn-1 and -4, meanwhile tacrolimus may protect TJ barrier function by preserving Cldn-1 and -4. However, the evidence that lipid mixture co-application can reverse topical glucocorticoid-induced barrier dysfunction (2) implicates that SC acts a primary water barrier in skin and raises the issue regarding the barrier function of TJs for water. Previous studies found that TJs form a functional barrier for small molecules such as 557 Da tracers (7) and lanthanum; however, TJ barrier function for water permeability still remains uncertain. In this context, TJs may represent a second line of defense in epidermal permeability barrier or a compensatory barrier which functions especially when SC barrier is disrupted. In addition, further studies are necessary for confirming the functional significance of the downregulation of TJ proteins caused by topical corticosteroids. Our results also suggest that Ocln expression might be somewhat more sensitive than that of Cldn-1 and -4 in response to topical corticosteroids and tacrolimus.

In conclusion, topical corticosteroids and TCIs have different effects on TJ proteins Cldn-1 and Cldn-4 expression. Recent evidence suggests that TJs influence on keratinocyte differentiation and proliferation (17) and the permeability barrier homeostasis by controlling calcium ion gradient (18,19). Moreover, the associations between the reduced Cldn-1 and enhanced susceptibility to herpes simplex virus 1 infections and impetigo contagiosa implicate that TJ functions as an antimicrobial barrier (12,20). Together, our results suggest that suppression of TJ proteins expression may be another mechanism to explain the topical corticosteroid-induced impairment of permeability and antimicrobial barrier function of the skin. The impact of topical corticosteroids and TCIs on TJ barrier functions and the effect of systemic glucocorticoids on the expression of TJ structural proteins require further investigation.

Acknowledgements

This study was supported by a faculty research grant of Yonsei University College of Medicine for 2011 (6-2010-0033).

Author contributions

SEL and YC designed and performed the research and wrote the paper. S-CK designed and supervised the project. S-EK and EBN performed the research and analysed the data.

Conflict of interests

The authors have declared no conflicting interests.

References

- Hengge U R, Ruzicka T, Schwartz R A *et al.* *J Am Acad Dermatol* 2006; **54**: 1–15.
- Kao J S, Fluhr J W, Man M Q *et al.* *J Invest Dermatol* 2003; **120**: 456–464.
- Nghiem P, Pearson G, Langley R G. *J Am Acad Dermatol* 2002; **46**: 228–241.

- 4 Stütz A, Baumann K, Grassberger M *et al.* *Int Arch Allergy Immunol* 2006; **141**: 199–212.
- 5 Jensen J M, Pfeiffer S, Witt M *et al.* *J Allergy Clin Immunol* 2009; **123**: 1124–1133.
- 6 Jensen J M, Ahrens K, Meingassner J *et al.* *Exp Dermatol* 2011; **20**: 783–788.
- 7 Furuse M, Hata M, Furuse K *et al.* *J Cell Biol* 2002; **156**: 1099–1111.
- 8 Hadj-Rabia S, Baala L, Vabres P *et al.* *Gastroenterology* 2004; **127**: 1386–1390.
- 9 De Benedetto A, Rafaels N M, McGirt L Y *et al.* *J Allergy Clin Immunol* 2011; **127**: 773–786.
- 10 Yuki T, Haratake A, Koishikawa H *et al.* *Exp Dermatol* 2007; **16**: 324–330.
- 11 Kubo A, Nagao K, Yokouchi M *et al.* *J Exp Med* 2009; **206**: 2937–2946.
- 12 Ohnemus U, Kohrmeyer K, Houdek P *et al.* *J Invest Dermatol* 2008; **128**: 906–916.
- 13 Yamamoto T, Kurasawa M, Hattori T *et al.* *Arch Dermatol Res* 2008; **300**: 61–68.
- 14 Brandner J M, Kief S, Grund C *et al.* *Eur J Cell Biol* 2002; **81**: 253–263.
- 15 Yuki T, Hachiya A, Kusaka A *et al.* *J Invest Dermatol* 2011; **131**: 744–752.
- 16 Saitou M, Furuse M, Sasaki H *et al.* *Mol Biol Cell* 2000; **11**: 4131–4142.
- 17 Kirschner N, Rosenthal R, Günzel D *et al.* *Exp Dermatol* 2012; **21**: 171–175.
- 18 Kirschner N, Bohner C, Rachow S *et al.* *Arch Dermatol Res* 2010; **302**: 483–493.
- 19 Kurasawa M, Maeda T, Oba A *et al.* *Biochem Biophys Res Commun* 2011; **406**: 506–511.
- 20 De Benedetto A, Slifka M K, Rafaels N M *et al.* *J Allergy Clin Immunol* 2011; **128**: 242–246.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Experimental design.

Figure S1. Phenotype changes observed in mice treated with 0.05% clobetasol propionate ointment or 0.1% tacrolimus twice daily for 10 days.

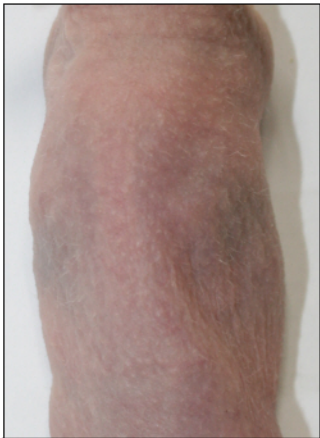
Figure S2. Topical application of 0.05% clobetasol propionate ointment for 10 days caused epidermal thinning compared to vehicle- or tacrolimus-treated skin (H&E stain $\times 200$).

Figure S3. Topical steroid impaired epidermal permeability barrier function in murine skin.

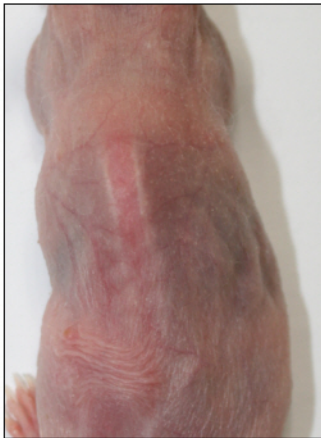
Data S1. Experimental design

Female hairless mice, aged 6–8 weeks, were treated topically on the back with 0.05% clobetasol propionate ointment, 0.1% tacrolimus ointment or hydrophilic base ointment (Samapharm Co, Seoul, Korea) as a vehicle (n=4 in each group) twice daily for 10 days, or left untreated (control, n=3). Basal transepidermal water loss (TEWL) was measured on days 1 (before the treatment), 4, 7 and at 24 hours after the last treatment, using a Tewameter TM 210 (Courage & Khazaka, Cologne, Germany). Skin samples were taken 24 hours after the last application. Hematoxylin and eosin (H&E) and Masson's trichrome stains were performed to analyze morphologic changes. The expression of TJ proteins, including claudin (Cldn)-1, Cldn-4, and occludin (Ocln) was examined in frozen sections by immunofluorescence staining with monoclonal antibodies (Zymed Laboratories, San Francisco, CA) and examined by confocal microscopy (Nikon, Tokyo). Protein levels of Cldn-1, Cldn-4, and Ocln were determined by Western blot analysis using the same antibodies. Quantitative real-time PCR was performed using the Tagman gene expression assay and the ABI StepOne real-time PCR machine (Applied Biosystems).

Untreated control



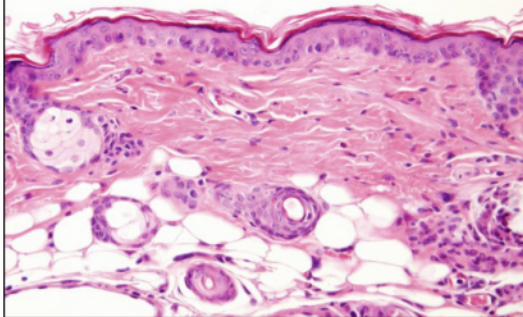
Steroid



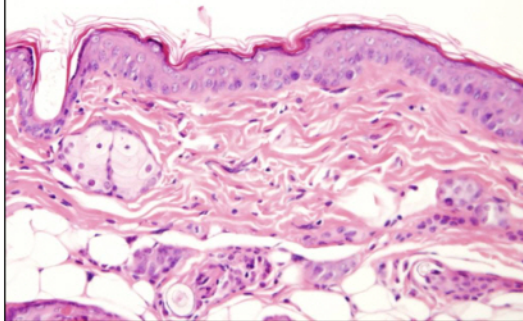
Tacrolimus



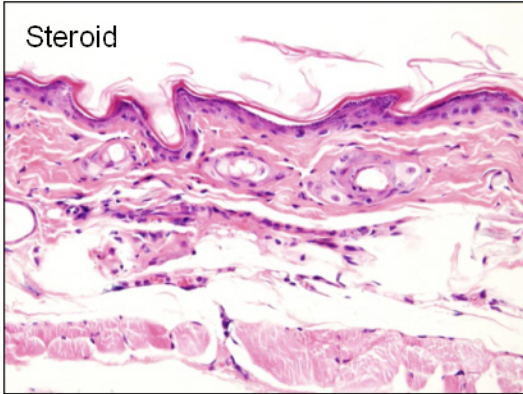
Untreated control



Vehicle



Steroid



Tacrolimus

