Abstract

Background: The skin produces cortisol by itself and regulates its own proliferation and differentiation. There is a possibility that topical corticosteroids (TCSs) influence the cortisol homeostasis in the skin. Aims and Objectives: The author described the density and distribution of cortisol and its parties in the epidermis after application of topical steroids immunohistologically. Materials and Methods: The forearm skin was biopsied before and after 2 weeks’ application of clobetasol propionate 0.05% two times a day in one healthy volunteer. The biopsied skin was stained immunohistologically by ant-MLN64, StAR, CPY11A1, cortisol, HSD11B1, HSD11B2, glucocorticoid receptor alpha, glucocorticoid receptor beta (GRB), and mineralocorticoid receptor (MCR) antibodies. The skin biopsy was performed similarly in 19 adult patients with atopic dermatitis who had used TCS for a considerable period. They were 4 TCS present users (TCS+), 12 TCS nonusers with skin manifestation on the biopsied site (TCS-E+), and 3 TCS nonusers without skin manifestation on the biopsied site (TCS-E−). Results: The staining density increased during TCS application in MLN64, cortisol and HSD11B2 in a healthy volunteer. The staining density was stronger in HSD11B2 of the basal layer and MCR of the spinous layer in the TCS-E+ patients than in the TCS+ and TCS-E− patients. The staining density was weaker in MLN64 of the basal and granular layers, HSD11B1 of the basal layer and GRB of the whole layer in the TCS-E+ patients than in the TCS+ and TCS-E− patients. Conclusion: The hypertrophy of the epidermis and insufficient keratinization recognized in the TCS-E+ patients might be caused by the decreased cortisol synthesis regulated by MLN64 and the increased cortisol inactivation by HSD11B2. Decreased GRB and increased MCR might enhance the reactivity of cortisol in the keratinocytes.

Key Words: Cortisol, rebound, red skin syndrome, topical steroid addiction

Introduction

Topical corticosteroids (TCSs) are useful and common medication in the dermatological field. Various dermatoses are successfully treated in many cases. However, they have side effects also. The so-called rebound phenomenon after the prolonged use of TCS is sometimes discussed though its mechanism is unknown. In the previous study, applying TCS for several weeks on the skin of healthy volunteers, the epidermis after discontinuation of TCS was revealed to become temporarily hypertrophic. If TCS do not leave any effect after discontinuation, the epidermis must only come back to the normal thickness. As TCS has anti-proliferating effect to the keratinocyte and the keratinocyte itself has an ability to synthesize cortisol, it is hypothesized that TCS work to impair the cortisol synthesis of keratinocytes after the prolonged use and cause the temporary hypertrophy of the epidermis.

Cortisol works by combining to receptors. Glucocorticoid receptor alpha (GRA) is the main one, and mineralocorticoid receptor (MCR) seems to work secondarily. Glucocorticoid receptor beta (GRB) is the decoy of GRA and has no function. In addition to impairing the ability of cortisol production, there are possibilities that TCS induce suppression of GRA and MCR or argumentation of GRB.
The process of cortisol production initiates from cholesterol intake into the mitochondria. StAR and MLN64 are necessary proteins for the intake.\(^6\) Cholesterol is metabolized by various enzymes including CPY11A1, CPY17, CPY21, CPY3B1, and HSD3B1 to produce steroids including cortisol. Cortisol is inactivated by HSD11B2 to cortisone in the cytoplasm and cortisone can be re-activated by HSD11B1 to cortisol.\(^7\)

The illustrated scheme of cortisol and its parties is shown in Figure 1.

Materials and Methods

The forearm skin was biopsied before and after 2 weeks’ application of clobetasol propionate 0.05% two times a day in one healthy volunteer (56-year-old, male). The biopsied skin was stained immunohistologically by ant-MLN64, StAR, CPY11A1, CPY17, CPY19, CPY21, CPY3B1, HSD3B1, cortisol, HSD11B1, HSD11B2, GRA, GRB, and MCR antibodies. The staining conditions were determined beforehand using other organ specimens including the adrenal gland as Table 1. The skin biopsy was performed similarly in 19 adult patients with atopic dermatitis (AD) who had used TCS for a considerable period at the biopsied site. They were 4 TCS present users (TCS+), 12 TCS nonusers with skin manifestation on the biopsied site (TCS-E+) and 3 TCS nonusers without skin manifestation on the biopsied site (TCS-E−). Two of TCS+ patients had apparent skin manifestation which was considered TC resistant. The skin was stained by ant-MLN64, StAR, HSD11B1, HSD11B2, cortisol, GRA, GRB, and MCR antibodies. The study was approved by the ethical committee in Okuma Hospital and the informed consent was obtained from all individuals.

Results

The transition of staining densities in the healthy volunteer is shown in Figure 2. The top; before application of TCS, the upper middle; 2 days after initiation of TCS application, the lower middle; 15 days after initiation of TCS application (1 day after discontinuation) and the bottom; 30 days after initiation of TCS application (16 days after discontinuation).

Cortisol in the epidermis increased in density by TCS application and decreased after discontinuation. AtAR, CPY11A1 and other enzymes related to steroid synthesis did not change so much while MLN64 increased markedly in accordance with cortisol. HSD11B2 increased also, and MCR increased mildly. GRB decreased slightly after TCS application.

![Figure 1: The process of cortisol synthesis and receptors of cortisol](image1)

![Figure 2: Immune-histological stainings of a healthy individual using anti-PCNA, cortisol, MLN64, HSD11B1, HSD11B2, CPY11A1, CPY17, glucocorticoid receptor alpha, glucocorticoid receptor beta, and mineralocorticoid receptor antibodies](image2)
The homeostasis of cortisol is influenced by topical corticosteroids

Skin biopsy was performed only one time in patients with AD except two patients who experienced skin biopsy two times because their stages of illness changed obviously. Patient A had developed rebound after prolonged use of TCS, and the first skin biopsy was performed at that time [Figure 3, lower]. After 2 years, the rebound eruption subsided and the second biopsy was performed [Figure 3, lower]. Patient B had used TCS for years and felt ineffectiveness compared to the past. As there was an intractable eczematous lesion in the apparently normal skin on his forearm, the first skin biopsy was performed from both sites [Figure 4, upper and middle]. He had withdrawn from TCS after the first biopsy and experienced rebound flare. One month after when the rebound subsided a little, the second biopsy was performed [Figure 4, lower].

When the patient A developed rebound flare, MLN64 stained only in the middle of the epidermis (spinous layer) and HSD11B2 stained markedly on the basal layer. After the improvement, MLN64 stained throughout the whole layer, and HSD11B2 decreased. GRB staining was stronger and MCR staining disappeared in the improved skin.

Table 1: The conditions of immunohistological staining

| Antibody | MLN64 | STAR | CPY11A1 | CPY11B1 | CPY17 | CPY19 | CPY21 | HSD3B1 | cortisol | GRA | GRB | MCR | HSD11B1 | HSD11B2 | PCNA |
|----------|-------|------|---------|---------|-------|-------|-------|--------|----------|-----|-----|-----|---------|---------|------|
| Brand    | Proteintech: 20292-1-AP | Abcam: ab133657 | ATLAS: HPA016436 | Santa Cruz: sc-374096 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 |
| Dilution rate | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 |
| Reaction time | 4°C overnight | 4°C overnight | 4°C overnight | 4°C overnight | 4°C overnight | 4°C overnight | 4°C overnight | Room temperature, 60 min | Room temperature, 60 min | Room temperature, 60 min | Room temperature, 60 min | Room temperature, 60 min | Room temperature, 60 min | Room temperature, 60 min |
| Antigen activating method | Heat treatment, pH 6.0, citrate buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer |
| Staining method | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 |
| Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer | Heat treatment, pH 9.0, EDTA buffer |
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Figure 3: The clinical appearance of the patient A and immune-histological staining using MLN64, HSD11B1, HSD11B2, cortisol, glucocorticoid receptor alpha, beta, and mineralocorticoid receptor.
Fukaya: The homeostasis of cortisol is influenced by topical corticosteroids

In the patient B, before withdrawal, MLN64 and GRB were stronger in the apparently normal skin than in the lesion with intractable eczema. HSD11B2 staining in the basal layer was strong in the lesion. After withdrawal, HSD11B1 in the basal layer is as strong as in the lesion with intractable eczema before withdrawal, but MLN64 increased.

The results in other 17 patients are shown in Figure 5. Although the sample size is small, there seemed to be some tendencies. They are (1) MLN64 in the basal and granular layers is weaker in the TCS-E+ group (Green highlighted), (2) HSD11B2 staining is stronger than HSD11B1 staining in the basal layer in the TCS-E+ group, and it is contrary in the TCS+ and TCS-E- groups (Magenta highlighted), (3) GRB staining is weaker in the TCS-E+ group (Red highlighted), and (4) MCR staining is stronger in the spinous layer in the TCS-E+ group (blue highlighted). It is worth mentioning that the maximum vertical number of Malpighi layer cells is obviously large in the TCS-E+ group and insufficient keratinization simply coincide with epidermal thickness (yellow highlighted).

**Discussion**

It is not inconceivable phenomenon that cortisol synthesis in the epidermis is accelerated by application of TCS. There is a report that fibroblasts in the dermis also have the similar positive feedback mechanism in synthesizing cortisol.\(^7\)

Among the elements which constitute the pathway of cortisol synthesis, MLN64 seemed to regulate the process most dynamically. As other protein or enzymes did not show a change of staining by TCS application in a healthy control, they were omitted in the patients. There is a report that the staining of HSD11B2 was strong in the granular layer in healthy controls.\(^8\) On the other hand, there is also a report that HSD11B2 staining was strong in the basal layer in cases of skin tumor.\(^9\) In cases of AD, HSD11B2 seems to increase mainly in the basal layer especially after discontinuation of prolonged TCS use when rebound flare is developed. Considering that HSD11B2 increases in the healthy control by TCS application, the increase of HSD11B2 may work as the inactivation mechanism of the excessed corticosteroids. If the mechanism is amplified by the prolonged application of TCS and left even after discontinuation of TCS, the epidermis becomes short of cortisol and hyperplasia, and insufficient keratinization can occur. It is because cortisol in the epidermis works for its self-regulation of proliferation and differentiation.

The staining of cortisol itself did not differ markedly among patients. The author considers that this staining procedure is not sensitive enough. The difference of MLN64 and HSD11B2 was dynamic and considered as they are maintaining homeostasis of cortisol which must be very important for the epidermis.

As for receptors, GRA density seems not to differ so much. It is unexpected that GRB density slightly decreases after TCS application in the healthy control because GRB has been considered as the competent of GRA and therefore it may increase after TCS application.

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*Figure 4: The clinical appearance of the patient B and immune-histological staining using MLN64, HSD11B1, HSD11B2, cortisol, glucocorticoid receptor alpha, glucocorticoid receptor beta, and mineralocorticoid receptor.*
There are several reports that GRB is associated with corticosteroid resistance. There is a possibility that GRB clarifies cortisol signal more specifically. On and off message could become more definite by the decrease of GRB when corticosteroids increased.

Decreased GRB staining in TCS-E+ patients can be interpreted as the ameliorating mechanism of cortisol signal by increasing binding possibility between cortisol and GRA.

MCR is known to be the secondarily receptor of cortisol and could appear in the condition that cortisol density is not enough.

Taken together, decrease of MLN64, increase of HSD11B2, decrease of GRB, and increase of MCR in TCS-E+ patients can all be interpreted as the aftereffects of prolonged TCS application and the ameliorating mechanism for the cortisol homeostasis.

The rebound phenomenon, also known as red skin syndrome (RSS) or topical steroid addiction, is a distressful problem in the dermatological field. It is easy to occur especially when the original disease is AD, or the patient has the predisposing atopic factor. As the skin manifestation of the rebound is very similar to the original disease, the illness has not been universally recognized yet as an independent clinical entity, and the study about the rebound phenomenon is sparse. This study might give some clues to investigate the illness.

If the disturbed homeostasis of cortisol in the epidermis causes the rebound, the facilitation of cortisol synthesis may lead the patients to recovery. Ultraviolet B (UVB) therapy increases the cortisol concentration in the epidermis and so does the low humidity in the environment also. In the clinical practice of RSS, UVB, and withdrawal from whole emollients are both useful treatments empirically. There is a possibility that topical tar ointment works by facilitating cortisol synthesis because some components of tar are catalyzed by CPY11A1. Glycyrrhizin is the HSD11B2 antagonist and some physicians treat RSS patients by the medication.

To the last, the author would like to admit that the sample size of this study is small and the immune-histological method is less quantitative. However, as the study about aftereffects of TCS or RSS is sparse, the author believes this study has some suggestion for the advanced study in the future.

**Conclusion**

The hypertrophy of the epidermis and insufficient keratinization recognized in the TCS-E+ patients might be caused by the decreased cortisol synthesis regulated by MLN64 and the increased cortisol inactivation by HSD11B2. Decreased GRB and increased MCR might enhance the reactivity of cortisol in the keratinocytes.

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Nil.
Conflicts of interest

There are no conflicts of interest.

What is new?

Cortisol homeostasis in the epidermis is influenced by TCS in patients with AD.

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