의학박사 학위논문

Effect of cutaneous steroidogenesis and related protein expression on rosacea pathogenesis

피부의 스테로이드 합성 및 관련 단백질의 발현이 주사의 병태 생리에 미치는 영향

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홍 종 수

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이 논문을 의학박사 학위논문으로 제출함

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Effect of cutaneous steroidogenesis and related protein expression on rosacea pathogenesis

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ABSTRACT

Effect of cutaneous steroidogenesis and related protein expression on rosacea pathogenesis

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Rosacea is a chronic inflammatory disorder usually affecting the central face. Chronic use of topical steroids on the face causes lesions similar to rosacea. Synthesis and function of glucocorticoid (GC) have been established in healthy skin and various cutaneous diseases but not in rosacea. In most skin disorders, increased inflammation is usually coupled with a decline in the production of cutaneous GC. We designed this study to investigate the *de novo* GC synthesis in the skin and GC receptor expression in the lesional and the nonlesional skin of patients with erythematotelangiectatic rosacea (ETR). The key steroidogenic enzymes, CYP11A1, 3βHSD2 and CYP21A2, were significantly increased in the lesional skin at the gene and

protein levels. CYP17A1 and steroidogenic acute regulatory protein (StAR) tended to be higher in the lesional skin. GC receptor was also significantly increased in the lesional skin. Simultaneously, lesional p65/p50 subunits of nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) were increased significantly at the gene and protein levels and matrix metalloproteinases (MMP) gene expressions were significantly increased in the lesional skin. Of MMP genes, increased gene expression of MMP-2 and MMP-9, which is observed in angiogenesis, was confirmed. These concurrent increments demonstrate that crosstalk between GC receptor and NFκB may be hampered in the lesional skin of ETR and may contribute to rosacea pathogenesis. We demonstrate that ETR is an inherently inflammatory disorder mediated by NFκB and that lesional GC synthesis and GC receptor expression is increased in ETR in contrast to other cutaneous inflammatory diseases.

Keyword

Rosacea, Steroids, Endocrine Regulation

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INTRODUCTION

Rosacea is a chronic inflammatory skin disorder that usually affects the central face. Two to ten percent of the general population is thought to have rosacea.[1] In addition, due to the characteristics that occur mainly on the central face, it greatly affects the daily life and the quality of life of the patient. Telangiectasia, vasodilation, inflammatory papules and later fibrosis are common manifestations of rosacea. However, in practice, the phenotypes of rosacea are heterogenous and clinical manifestation can be diverse. We usually classify rosacea into four subtypes, erythematotelangiectatic rosacea, papulopustular rosacea, rhinophyma and ocular rosacea.[2] However, we cannot distinguish these subtypes clearly in practice. Not only are there overlapping gray zones among subtypes but also one subtype can change into another subtype.[3] Due to this clinical heterogeneity, the pathophysiology of rosacea is believed to be ‘unknown’ and is still under debate.

Topical glucocorticoids (GC) are the primary treatment for

various skin diseases. Whereas GC application is beneficial in acute phase dermatoses, the chronic use of GC can lead to various side effects. One of these is the so-called 'rosacea-like dermatitis', which is a paradoxical inflammatory reaction, such as the dilatation of the blood vessels of the skin, and the development of papules and pustules.[4] After these side effects have appeared, the more GC is applied, the more likely the symptoms will become severe. In view of the occurrence of these side effects, the GC seems capable of demonstrating pro-inflammatory effects. GC is synthesized through the hypothalamic-pituitary-adrenal (HPA) axis systemically. Healthy skin has its own cutaneous HPA axis, producing all the elements for synthesizing and regulating GC.[5-7] In major skin disorders, such as psoriasis and atopic dermatitis, increased inflammation is usually coupled with a decline in the production of cutaneous GC.[8,9] The fact that symptoms are improved when topical GC is applied in patients with psoriasis and atopic dermatitis supports the reduction of these GC production. Based on the similarity between rosacea and rosacea-like dermatitis caused by chronic topical GC use, we hypothesized that dysregulation of primary cutaneous *de novo* GC synthesis

would play a role in the pathogenic mechanism of rosacea. Rosacea patients have primarily altered cutaneous innate immunity. Increased Toll-like receptor 2 (TLR2) expression in the epidermis of patients with rosacea was confirmed.[3] When TLR2 is activated by various triggering factors, epidermal keratinocytes in rosacea produce proinflammatory cytokines. TLR2 is also known to boost the expression of the serine protease kallikrein(KLK)-5, the enzyme that cleaves cathelicidin into its effective form, LL-37. Increased amount of LL-37 plays a critical role in the abnormal cutaneous innate immunity in rosacea. However, why the environmental stress triggers activation of innate immune system is not well established. Thus, demonstrating abnormal GC synthesis and GC receptor expression in rosacea skin may provide additional information to explain uncontrolled redness within the affected skin.

MATERIALS AND METHODS

Human skin samples

To investigate *de novo* glucocorticoid synthesis in the lesional skin of erythematotelangiectatic rosacea (ETR), both lesional and non-lesional facial skin samples from the same individuals were provided by twelve volunteers with rosacea (mean age, 40.1 years; range, 30 to 55 years). The volunteers who had used topical GC were excluded by scrupulous review of the past history. Skin biopsies were immediately stored in −80 ° C to analyze for mRNA expression levels or fixed in 10% formalin and stored as paraffin block in room temperature for staining. This study was approved by the Institutional Review Board of Seoul National University Boramae Medical Center, and all human subjects gave informed consent according to the Declaration of Helsinki Principles.

Quantitative real-time reverse transcriptase (RT)-PCR

Total RNA was isolated from the skin samples using RNAiso (Takara Bio, Shiga, Japan), and converted to cDNA using First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania)

according to the manufacturer’ s instructions. To quantitatively analyze mRNA expression, PCR was performed on a 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA) using SYBR Premix Ex Taq (Takara Bio) according to the manufacturer’ s instructions, with the human primer pairs in Table 1.

The PCR conditions were 50 ° C for 2 min, 95 ° C for 2 min, followed by 40 cycles at 95 ° C for 15 s and 60 ° C for 1 min. The data were analyzed using the comparative $\Delta\Delta$Ct method and presented as mean ± standard error of relative mRNA expressions against corresponding controls and normalized to 36B4.

Immunohistochemical (IHC) staining

Paraffin-embedded skin samples were sectioned 6 μm thick and mounted onto silane-coated slides (Dako, Glostrup, Denmark). The slides were dewaxed in xylene substitutes (Thermo Fisher Scientific, Waltham, MA, USA), rehydrated in ethanol and washed with water. Then, samples were autoclaved in Target Retrieval Solution (Dako) for antigen retrieval. Endogenous blocking by 3% hydrogen peroxide and exogenous

blocking by pre-blocking solution (GBI Labs, Mukilteo, WA, USA) were performed to reduce the non-specific staining. Primary antibodies were diluted in Diluent Buffer (Dako) and used to probe each antigen (Table 2). After washing with phosphate-buffered saline, the slides were incubated with secondary antibody and streptavidin-horse radish peroxidase consecutively for 15min each and visualized using AEC kit (GBI Labs).

Liquid chromatography-mass spectrometry (LC-MS)

Measurement of cortisol and its precursors and metabolites in paraffin-embedded skin tissues was performed using LC-MS. Briefly, tissue samples were washed with 2 mL of n-hexane at 60 ° C in three times and pulverized with 2 mL phosphate buffer (pH 7.2) twice using a TissueLyser (Qiagen; Hilden, Germany). Homogenized sample was purified with solid-phase extraction cartridge after enzymatic hydrolysis with β-glucuronidase, and the sample was quantitatively estimated by LC-MS. The LC-MS system (LC/MS 8050, Shimadzu Corp., Japan) was composed of a Shimadzu Nexera ultra-performance liquid chromatograph and 8050 triple quadrupole mass

spectrometer. All steroids were separated through a 1.9 μm particle C18 column (50 × 2.1 mm) and gradient elution with a mobile phase consisting of 0.1% formic acid in 5% acetonitrile (solvent A) and 0.1% formic acid in 95% acetonitrile (solvent B) at a flow rate of 250 μL/min. In method validation, the linearity (r2) was > 0.992 within 0.1−20 ng/mL dynamic range, which was defined as the lowest concentration with accuracy and precision of less than 20%.

Visual grading analysis

The degree of IHC staining intensities was evaluated using a 6−point scale, from 0 (unstained) to 6 (very intensively stained) by three independent dermatologists. With this score, the significance was evaluated.

Statistical analysis

Data are presented as mean ± standard error. Significance was determined using the Wilcoxon matched−pairs signed rank test. *P*−values less than 0.05 were considered statistically significant. The correlations between genes were determined by Pearson correlation analysis.

Table 1. Human and mouse primer sequences for quantitative real-time RT-PCR

Gene Symbol	5' primer sequence	3' primer sequence
StAR	GAGAAGTCTTGCTTTATGGGCTCAAGAATG	GGTGCCTATGAAAGCAATAGGGAAACATGT
CYP11A1	GAGATGGCACGCAACCTGAAG	CTTAGTGTCTCCTTGATGCTGGC
CYP17A1	TGAGTTTGCTGTGGACAAGG	TCCGAAGGGCAAATAGCTTA
CYP21A2	TCCCAGCACTCAACCAACCT	CAGCTCAGAATTAAGCCTCAATCC
3β-HSD1	AGAAGAGCCTCTGGAAAACACATG	TAAGGCACAAGTGTACAGGGTGC
3β-HSD2	AGAAGAGCCTCTGGAAAACACATG	CGCACAAGTGTACAAGGTATCACCA
17β-HSD5	GTCATCCGTATTTCAACCGGAGTAAATTGC	CATCGTTTGTCTCGTTGAGATCCCAGA

11β-HSD1	TTGCTTTGGATGGGTTCTTC	AGAGCTCCCCCTTTGATGAT
11β-HSD2	GACCTGACCAAACCAGGAGA	GCCAAAGAAATTCACCTCCA
5α-red1	CCAATGGCGCTTCTCTATGGACTTTGTAAA	CCCAGAGCTTGAAATTCTGACCTGTTACA
5α-red2	GGCGACAGTTGCTTCTTAGCTATTGCT	GTGGGTATGAAGCCACACATGTACTTGGAT
GRα	CATTGTCAAG AGGGAAGGAA ACTC	GATTTTCAAC CACTTCATGC ATAGAA
IκB	GCAAAATCCTGACCTGGTGT	GCTCGTCCTCTGTGAACTCC
p65	GCGAGAGGAGCACAGATACC	CTGATAGCCTGCTCCAGGTC
p105	CCTGGATGACTCTTGGGAAA	TCAGCCAGCTGTTTCATGTC
p38	GCCCCAGTAGTCAGAAGCAG	TAGGGGCTGAAGAGAGGTGA
MMP-2	GGCCAAGTGGTCCGTGTG	GAGGCCCCATAGAGCTCC

MMP-9	TTGACAGCGACAAGAAGTGG	GCCATTCACGTCGTCCTTAT
36B4	TGGGCTCCAAGCAGATGC	GGCTTCGCTGGCTCCCAC

Table 2. Characteristics of the primary antibodies used in IHC staining

Primary antibody	Source	Dilution	Incubation time	Antigen retrieval
CYP11A1	Abcam	400:1	1h 30m	Autoclaved in Target Retrieval Solution (pH 6.0)
3 β – HSD2	Abcam	100:1	2h	Autoclaved in Target Retrieval Solution (pH 9.0)
17 β – HSD5	Abcam	100:1	2h	Autoclaved in Target Retrieval Solution (pH 9.0)
GR α	Cell Signaling Technology	100:1	1h 30m	Autoclaved in Target Retrieval Solution (pH 6.0)
pGR α	Cell Signaling Technology	100:1	2h	Autoclaved in Target Retrieval Solution (pH 9.0)
p65	Cell Signaling Technology	100:1	20h at 4℃	Autoclaved in Target Retrieval Solution (pH 6.0)
p105	Abcam	100:1	20h at 4℃	Autoclaved in Target Retrieval Solution (pH 6.0)

RESULTS

Relative expressions of corticosteroid synthesizing enzymes and GRα in rosacea

To investigate the expression levels of enzymes involved in the corticosteroid synthesis, non-lesional and lesional facial skin samples from the same individuals with rosacea were analyzed by quantitative real-time RT-PCR. Amongst the factors known to regulate corticosteroid synthesis, CYP11A1, CYP21A2, 17β-HSD5, 11β-HSD2, and 5α-red2 were significantly increased in the lesional skin while StAR, CYP17A1, 3β-HSD1, 11β-HSD1, and 5α-red1 were not increased significantly. Additionally, when we checked the level of glucocorticoid receptor α (GRα), it was upregulated in the lesional skin (**Figure 1**), suggesting that both *de novo* synthesis of glucocorticoid and the expression level of its receptor are elevated simultaneously in rosacea.

Expression patterns of corticosteroid synthesizing enzymes and GRα in sebaceous gland and epidermis of the rosacea lesion

To further confirm the protein expression patterns of genes

that were significantly increased in the lesional skin of rosacea, IHC staining was performed. Of all the elevated genes regulating corticosteroid synthesis, CYP11A1, which initiates corticosteroid synthesis by converting cholesterol to pregnenolone,[10] was increased the most in both sebaceous gland and epidermis of rosacea lesion. 3β-HSD2 and 17β-HSD5, which play pivotal roles in GC synthesis, were also increased. Furthermore, not only the expression but also the phosphorylation of GRα was notably increased in the nuclei of sebocytes and epidermal keratinocytes. The degree of IHC staining intensities was evaluated using a 6-point scale, from 0 (unstained) to 6 (very intensively stained), by three independent dermatologists. Aforementioned enzymes and receptor were increased significantly when ranked by visual grading analysis (**Figure 2 and 3**).

Measurement of cortisol and its precursors and metabolites

To find out the level of cortisol and its precursors and metabolites, non-lesional and lesional facial skin samples from the same individuals with rosacea were analyzed by liquid chromatography-mass spectrometry. Progesterone, which is

produced from pregnenolone by 3β-HSD and also the precursor of cortisol, was found to be increased significantly in the lesional skin of rosacea compared to that in the non-lesional skin while the level of cortisol was unmeasurable (Figure 4).

Relative expressions of NF-κB subunits and downstream target genes in the rosacea lesion

The phosphorylation of GRα induces its translocation into the nucleus where GRα binds to glucocorticoid response element (GRE) of DNA. The binding induces the transcription of IκB, the inhibitor of NF-κB signaling pathway.[11] Hence, we checked the mRNA levels of IκB as well as NF-κB subunits and its downstream target genes such as IL-8, matrix metalloproteinase-2 (MMP-2), and MMP-9. We observed that the level of IκB was significantly increased; however, p65, p105, IL-8, MMP-2, and MMP-9 were also increased concurrently (Figure 5).

Expression patterns of NF-κB subunits in epidermis of the rosacea lesion

To further confirm the protein expression patterns of NF-κB subunits that were significantly increased in the lesional skin of rosacea, IHC staining was performed. NF-κB subunits, p65 and p105, were increased in the nuclei of epidermal keratinocytes. The degree of IHC staining intensities was also evaluated using a 6-point scale. Significant increments were observed (**Figure 6**).

Correlation between the mRNA expression levels of corticosteroid synthesizing enzymes and MMP-2

To investigate the relationship between corticosteroid synthesizing enzymes and MMP-2, which plays a role in the pathogenesis of rosacea by triggering cutaneous angiogenesis,[12] Pearson correlation analysis was performed using the mRNA ratio (lesion/non-lesion) of each gene. CYP11A1 and 11β-HSD1 showed weak correlation coefficient ($0 < r < 0.3$), whereas 3β-HSD2, 17β-HSD5, 11β-HSD2, and 5α-red2 showed positive correlation with MMP-2 ($0.5 < r < 1$) (**Figure 7**). This suggests that *de novo* synthesis of corticosteroid may be related to the upregulation of genes that contribute to the pathogenesis of rosacea.

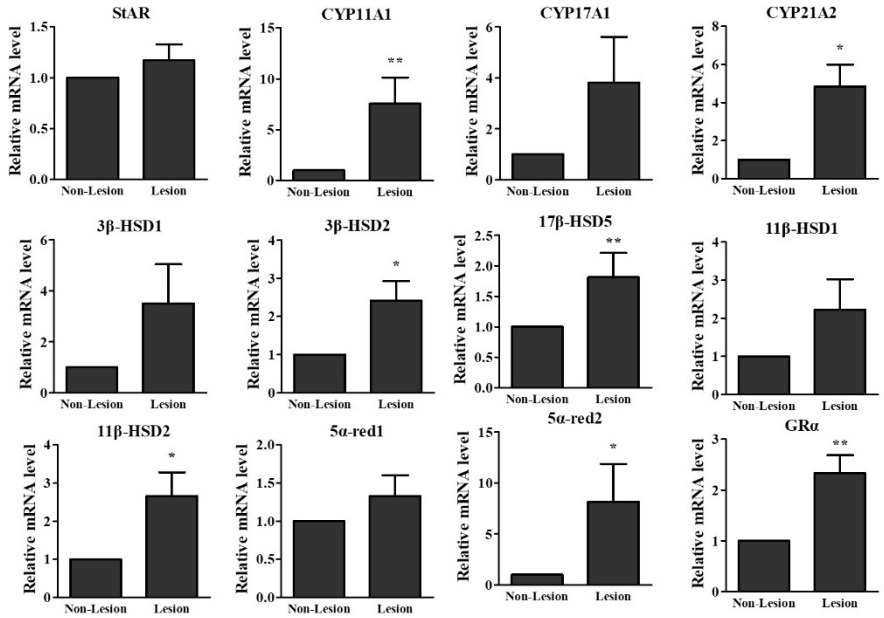

Figure 1. Relative mRNA levels of glucocorticoid synthesizing enzymes and glucocorticoid receptor.

Relative mRNA levels of enzymes synthesizing corticosteroid and GR α were analyzed from rosacea patients. Non-lesional and lesional skin samples from the same individuals with rosacea were analyzed by quantitative real-time RT-PCR (n=12). Data represent mean $\pm$ standard error of relative mRNA expressions, normalized to 36B4. $*P<0.05$ and $**P<0.01$ versus non-lesional skin.

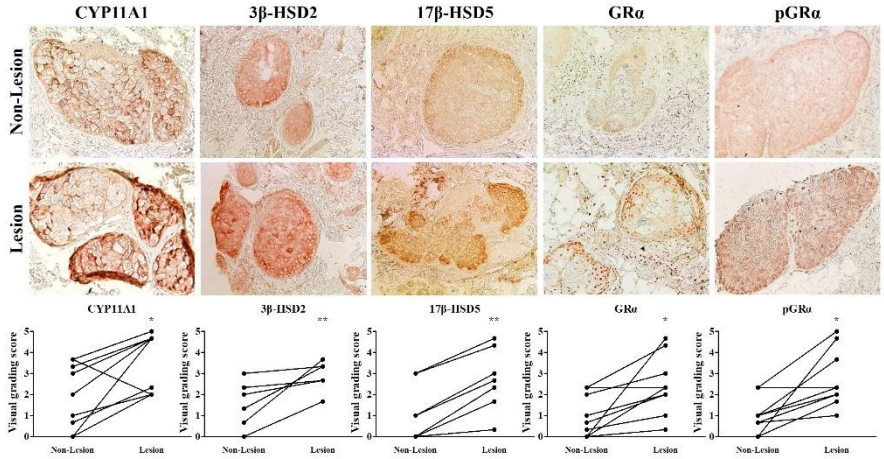

Figure 2. Expression of glucocorticoid synthesizing enzymes and glucocorticoid receptor in the sebaceous gland.

The expression patterns of CYP11A1, 3β-HSD2, 17β-HSD5, and GRα in the sebaceous gland were analyzed. Non-lesional and lesional skin samples from the same individuals with rosacea were analyzed by immunohistochemical staining (original magnification x 200). The data shown are representative of independent experiments (n=12). Staining intensity of the sebaceous gland was graded using a visual grading score (0-5). The scores are presented as dot plots. *P<0.05 and **P<0.01 versus non-lesional skin.

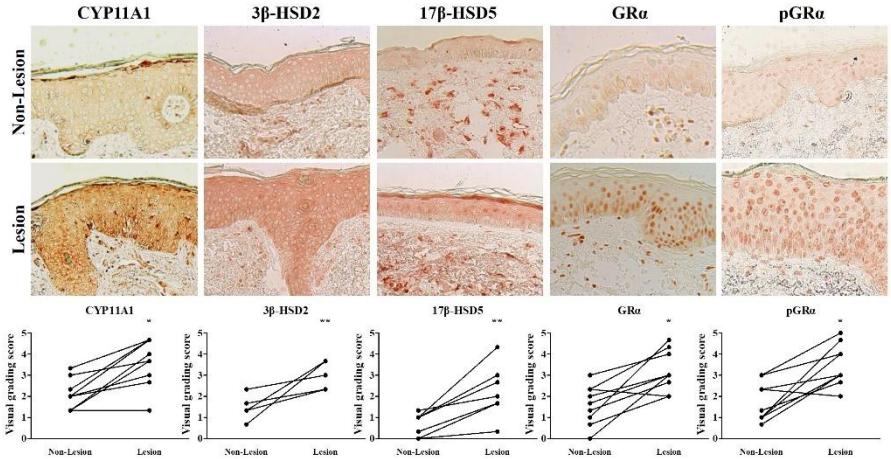

Figure 3. Expression of glucocorticoid synthesizing enzymes and glucocorticoid receptor in the epidermis.

The expression patterns of CYP11A1, 3β-HSD2, 17β-HSD5, and GRα in the epidermis were analyzed. Non-lesional and lesional skin samples from the same individuals with rosacea were analyzed by immunohistochemistry staining (original magnification x 400). The data shown are representative of independent experiments (n=12). Staining intensity of the epidermis was graded using a visual grading score (0-5). The scores are presented as dot plots. *P<0.05 and **P<0.01 versus non-lesional skin.

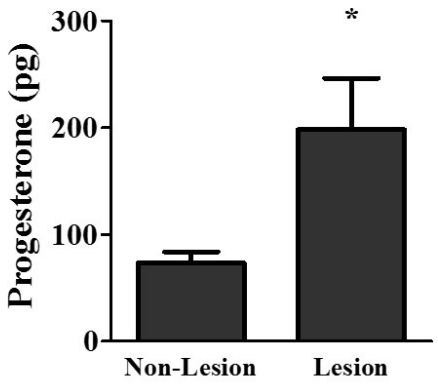

Figure 4. The level of progesterone in the lesional skin of rosacea.

The amount of progesterone was analyzed from rosacea patients. Non-lesional and lesional skin samples from the same individuals with rosacea were analyzed by liquid chromatography-mass spectrometry (n=10). Data represent mean ± standard error of progesterone level. *P<0.05 versus non-lesional skin.

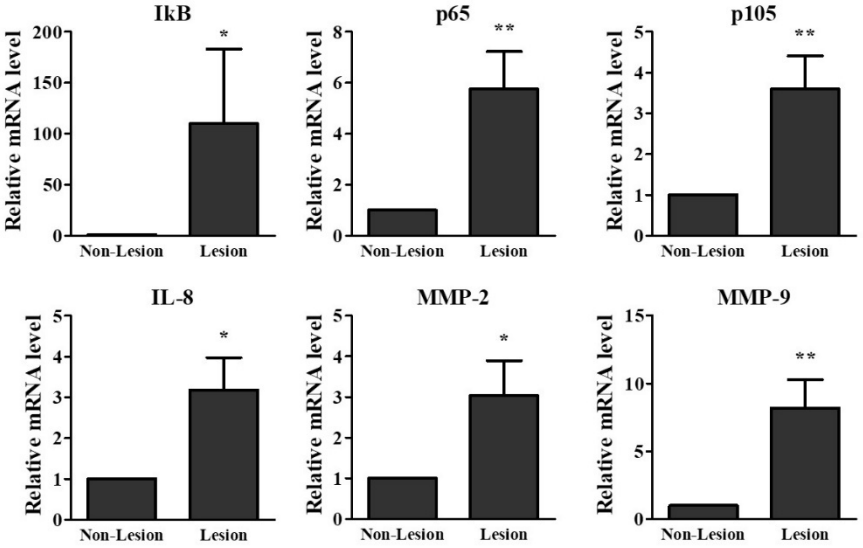

Figure 5. Relative mRNA levels of NF-κB subunits and related genes.

Relative mRNA levels of NF-κB subunits and related genes were analyzed from rosacea patients. Non-lesional and lesional skin samples from the same individuals with rosacea were analyzed by quantitative real-time RT-PCR (n=12). Data represent mean ± standard error of relative mRNA expressions, normalized to 36B4. *P<0.05 and **P<0.01 versus non-lesional skin.

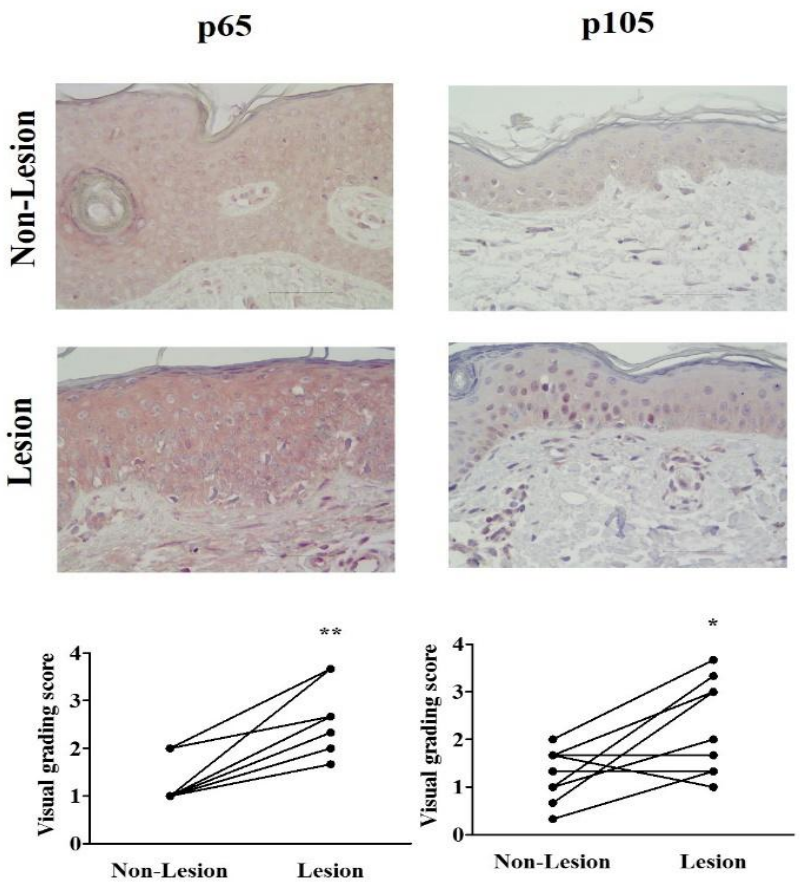

Figure 6. Expression of NF-κB subunits in the epidermis.

The expression patterns of p65 and p105 in the epidermis were analyzed. Non-lesional and lesional skin samples from the same individuals with rosacea were analyzed by immunohistochemical staining (original magnification x 400). The data shown are representative of independent experiments (n=12). Staining intensity of the sebaceous gland was graded using a visual grading score (0-5). The scores are presented as dot plots. *P<0.05 and **P<0.01 versus non-lesional skin.

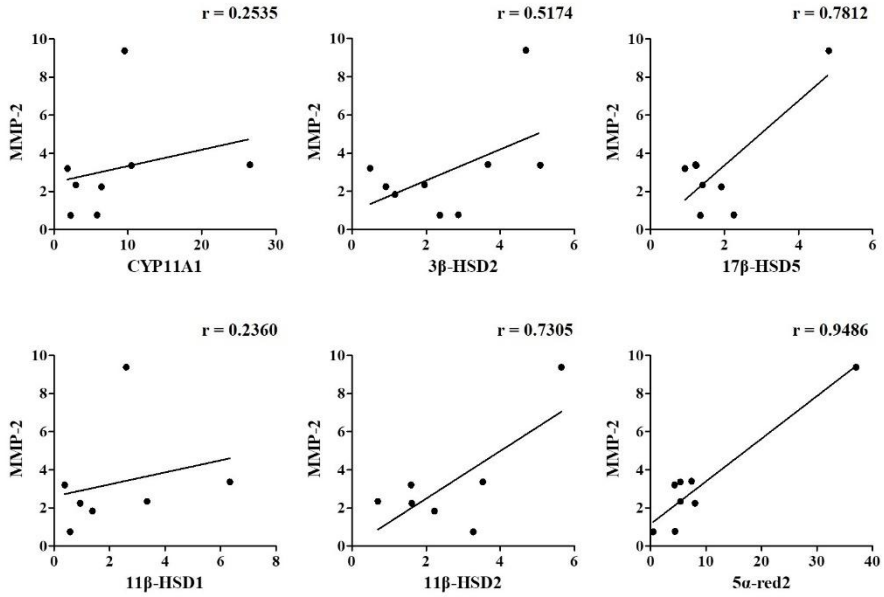

Figure 7. The correlation between corticosteroid synthesizing enzymes and MMP-2.

Correlation between corticosteroid synthesizing enzymes and MMP-2 were analyzed by Pearson correlation analysis. The mRNA ratios (lesion/non-lesion) of genes obtained from quantitative real-time RT-PCR were used as variants (n=12). Coefficients are presented on the graph.

DISCUSSION

Rosacea is a chronic inflammatory dermatosis which usually affects the central face. Rosacea mainly manifests as inflamed papules and facial telangiectasia. These lesions are very similar to the cutaneous phenomenon shown in chronic topical GC usage.[4] GC has very strong anti-inflammatory effect, thus it is used as a primary therapeutic agent for dermatological diseases such as psoriasis and atopic dermatitis. However, it is not used in the treatment of rosacea. This is because the use of topical GC in patients with rosacea worsens the signs and symptoms of the disease in most patients. Otherwise the role of cutaneous GC synthesis in individual skin diseases is poorly understood. *De novo* cutaneous synthesis of GC in healthy individuals has been well recognized.[6,7,13-15] Steroidogenesis begins with the upstream CYP11A1, which converts cholesterol to pregnenolone. 3βHSD, which converts pregnenolone to progesterone, and CYP17A1, which converts pregnenolone to 17OH-pregnenolone, are enzymes that act early on. Finally, 11βHSD1 and 11βHSD2 act to transform cortisone to cortisol

and vice versa.[16] Normal epidermis and cultured primary human keratinocytes express all of the components in cortisol steroidogenesis including CYP11A1, CYP21A2, CYP17A1, 3β HSD and 11β HSD.[13] Primary cultured human keratinocytes can metabolize pregnenolone through each intermediate steroid to terminal cortisol. On the other hand, decreased and dysfunctional local cutaneous GC synthesis was observed in psoriatic skin.[9] Impaired nuclear localization of GR, which means GR did not function properly, was also observed in psoriatic skin.[17] The aberrant synthesis of elements of steroidogenesis was also proven in atopic dermatitis skin. The cholesterol transporter, steroidogenic acute regulatory protein (StAR), regarded as the rate-limiting step of steroidogenesis, was unusually down regulated in atopic dermatitis skin.[13] In epidermal GR knock-out mice, degranulating mast cells and infiltration of macrophages were observed. These findings are consistent with the characteristics of atopic dermatitis.[8] This observation of impaired steroidogenic protein synthesis and GR expression can explain why the above two skin diseases can usually be treated with topical GC. In contrast, the fact that rosacea is exacerbated by topical GC enables the hypothesis

that there is a local abnormality of GC in patients with rosacea. In order to eliminate the influence of the topical GC usage, patients with rosacea who have previously used topical GC were thoroughly excluded in this investigation.

In our study, cutaneous steroidogenic systems were significantly more upregulated in the lesional skin of rosacea than those in the non-lesional skin. This was confirmed both at mRNA level and protein level. Particularly, increases in key steroidogenic enzymes from the beginning to the end were observed in our study. Both 11βHSD1 and 11βHSD2, which play a crucial role upstream of the terminal receptor level, showed an increasing tendency. We could confirm that cortisol synthesis occurs actively in the lesional skin of rosacea. Aforementioned changes were observed not only in the epidermis, but also in the sebaceous gland. The autocrine effect of *de novo* steroid synthesis in the keratinocyte may interact with the paracrine effect of steroids from the sebaceous gland, and together they may exert a synergistic effect on rosacea lesions.

When ultraviolet (UV) was radiated to the *ex vivo* skin, the production of cortisol was increased and the GR was

downregulated simultaneously.[18] This decline of GR may countervail the induction of steroidogenesis by UV irradiation. In patients with rosacea, the increment in the synthesis of steroid enzymes and the simultaneous increase in the expression of GR were observed. The homeostatic function, i.e., downregulation of GR, observed in normal skin may not work properly in the lesional skin of rosacea. Moreover, considering that the major affected site of rosacea is usually the facial convex area which is the most UV-irradiated area, we speculate that UV plays a cardinal role in increasing the steroidogenesis observed in patients with rosacea.

It was observed in this study that NF-κB pathway-related molecules were increased in the lesional skin of rosacea patients. Since this study included only the ETR patients, we can confirm that inflammatory reactions mediated by NF-κB pathway exist even in the ETR subtype which has no papules and pustules. It was confirmed that the inflammatory response existed even in the lesional skin of mild ETR patients with only slight telangiectasia. Among the molecules related to the inflammatory reaction, MMPs are also important. In the present study, MMP-2 and MMP-9 were upregulated significantly at

the gene level, but MMP-1 did not show any significant difference between lesional skin and non-lesional skin (data not shown). Enhanced expression of MMP-2 and MMP-9 in the skin of rosacea was also observed in a previous investigation.[19] MMP-1 plays a major collagenase role in the skin while MMP-2 and MMP-9 are known to be involved in angiogenesis of cutaneous wounds and in neo-vascularization of tumors.[12,20] MMP-2 and MMP-9 enable endothelial cell migration during angiogenesis.[21] This facilitated angiogenic process can be one cause of telangiectasia in rosacea. The fact that there is no difference in MMP-1 between lesional and non-lesional skin while there are significant increases in MMP-2 and MMP-9 in the lesional skin is in good agreement with the manifestation of ETR which only manifests telangiectasia. Furthermore, our research shows that the increase of steroid biosynthetic enzymes and the increase in GR activity have a strong correlation with the increase of MMP-2. These results support the hypothesis that the activation of the GC system is responsible for the increased inflammation of rosacea and the development of telangiectasia.

When GR is activated, it usually inhibits NF-κB and its

associated pathway through IκB, which will act in a direction to lower inflammation.[22] Cortisol binding to GR results in dissociation of heat shock proteins and ensuing phosphorylation. Cortisol-GR complex then translocates to the nucleus and binds to glucocorticoid response elements upstream of GR-associated genes. As a result, generated IκB inhibits the function of NF-κB and brings anti-inflammatory effects of cortisol. In our study, we observed the opposite phenomenon. There are cases in which steroids act paradoxically in the direction of increasing the inflammatory response. In the case of chronic exposure to GC, the anti-inflammatory ability of GC is mainly reduced by a mechanism of decreasing the activity of GR.[23,24] Moreover, not only does GR not adequately suppress NF-κB, but also excessively formed NF-κB further disturbs the function of GR, resulting in a vicious circle.[25] Simultaneous increases of GR, NF-κB and IκB were observed in the leukocytes from patients with mood disorder such as major depressive disorder or chronic stress disorder.[26-28] It is also known that the use of GC in post-stroke edema patients can increase inflammation and exacerbate the patient's condition.[29] Activation of GR and increase of p65 subunit of NF-κB were

at the same time observed in myeloid cells under acute injury condition.[30] Although it is unknown under which circumstances GC shows pro-inflammatory effects, the timing of GC release can be an important determinant factor. There are studies showing that GC release after immune activation has an anti-inflammatory effect, whereas the presence of GC before immune activation has a stimulatory effect in inflammation.[31,32] GC synthesis before immune activation may show a pro-inflammatory effect in ETR.

It is well known that abnormal function of cutaneous innate immunity is the main mechanism of rosacea pathogenesis. The epidermis of patients with rosacea expressed more Toll-like receptor 2 (TLR2) than normal controls. When TLR2 is activated in rosacea, keratinocytes produce proinflammatory cytokines such as IL-1β and IL-8. IL-8, which were confirmed to increase in our study, and this leads to chemotaxis of neutrophils in the skin and subsequently affects the release of inflammatory proteases including elastase and cathepsin G.[33] TLR2 is also known to increase the expression of the serine protease kallikrein(KLK)-5, the enzyme responsible for processing cathelicidin into its active form, LL-37.[34] Increased

amount of LL-37 plays a pivotal role in the dysfunctional cutaneous innate immunity in rosacea. Notably, this increase of TLR2 in rosacea was not observed in other inflammatory dermatoses.[35] In another experiment, TLR2 expression in the human keratinocytes was enhanced by GC. TLR2 gene induction was mediated by mitogen-activated protein kinase (MAPK) phosphatase-1.[36] TLR2 and TLR4 signaling in microglial cells can also be important factors in the immune-priming effect of GC. Upon exposure to an acute stressor, GC induction primes the innate immunity of central nervous system through a pathway that activates TLR2 and TLR4.[37,38] Via this signal, GC can create a pro-inflammatory effect in neuroimmune microenvironment. All these findings suggest that GC-induced innate immune system enhancement may play an important role in the pathogenesis of rosacea.

It is not yet established whether increase of cutaneous steroid synthesis and activation of GR directly affects the pathomechanism of ETR or not. The present study demonstrates that *de novo* cutaneous steroidogenesis is associated with ETR development, although causal relationships and precise mechanisms could not be elucidated. Further

studies are warranted to determine the effects of chronic increase of *de novo* cutaneous steroid synthesis on the skin and keratinocytes. Taken together, our results suggest that ETR is an inherently inflammatory disorder mediated by NF-κB and that localized GC synthesis and GC receptor expression is increased in ETR unlike in other cutaneous inflammatory diseases, thereby impairing the crosstalk between GC receptor and NF-κB and thus contributing to ETR pathogenesis.

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국 문 초 록

피부의 스테로이드 합성 및 관련 단백질의 발현 이 주사의 병태 생리에 미치는 영향

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주사는 만성염증성 질환으로 주로 얼굴의 중앙부위를 침범한다. 스테로이드 국소도포제의 만성적 사용은 주사와 비슷한 병변을 유발시킨다. 글루코코르티코이드의 기능과 합성이 건강한 피부와 여러 피부질환에서 잘 밝혀져 있으나, 주사에서는 알려진 바가 없다. 대부분의 피부질환에서 염증의 증가는 보통 피부의 글루코코르티코이드의 생산의 감소와 연결되어 있다. 우리는 홍반혈관확장성 주사환자의 병변부와 비병변부의 피부에서 자체적인 글루코코르티코이드의 합성과 글루코코르티코이드 수용체의 발현정도에 차이가 있는지 알아보기 위하여 이 연구를 기획하였다. 주요한 스테로이드 합성 효소인 CYP11A1, 3βHSD2 그리고 CYP21A2가 주사환자의 병변부 피부에서 유전자와 단백질 수준에서 통계적으로 유의하게 증가되어 있었다. CYP17A1과

steroidogenic acute regulatory protein (StAR)은 병변부 피부에서 증가되어 있는 경향을 보였다. 글루코코르티코이드 수용체도 병변 피부에서 유의하게 증가되어 있었다. 이와 동시에, nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB)의 하부단위인 p65/p50 도 유전자와 단백질 수준 모두에서 유의하게 병변부에서 증가됨이 관찰되었다. Matrix metalloproteinases (MMP) 유전자 발현의 증가도 병변부에서 관찰되었는데, 주로 혈관생성에 관여하는 MMP-2와 MMP-9의 발현 증가가 확인되었다. 글루코코르티코이드 수용체의 증가와 NF-κB의 증가가 동시에 관찰되는 현상은, 이 두 물질 간의 상호작용의 감소가 주사환자의 병변 발현의 기전에 기여할 수 있음을 보여준다. 우리는 이 연구에서 주사가 NF-κB에 의해 매개되는 염증성 질환임을 확인하였고, 다른 피부질환과는 다르게 주사환자 병변부 피부에서 글루코코르티코이드 생합성의 증가와 글루코코르티코이드 수용체 발현의 증가가 동시에 관찰됨을 확인하였다.

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