Effects of α-melanocyte stimulating hormone and 11β-hydroxysteroid dehydrogenase type 1 on sebogenesis in SZ95 sebocytes

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Effects of α-melanocyte stimulating hormone and 11β-hydroxysteroid dehydrogenase type 1 on sebogenesis in SZ95 sebocytes

Directed by Professor Seung Hun Lee

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ABSTRACT

Effects of α-melanocyte stimulating hormone and 11β-hydroxysteroid dehydrogenase type 1 on sebogenesis in SZ95 sebocytes

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A causative link between psychological stress and acne has long been postulated. When the activity of the hypothalamic-pituitary-adrenal (HPA) axis system is increased due to stress, it results to the secretion of various hormones, such as the melanocortins and glucocorticoids. It is well known that α-melanocyte stimulating hormone (α-MSH) causes lipogenesis in cultured human sebocytes. Although corticosteroids cause immunosuppressive and anti-inflammatory effects, it is clinically well known that systemic or topical glucocorticoid treatment aggravates an acneiform reaction. The 11β-hydroxysteroid dehydrogenase type 1 (HSD11β1) regenerates active glucocorticoids from inactive forms and plays a central role in regulating intracellular glucocorticoid concentration.

First of all, the effect of α-MSH was investigated on the expression of its receptors, MC1R and MC5R, according to the differentiated status of sebocytes and the expression of pro-inflammatory cytokines and toll-like receptor (TLR) 2. Additionally, different effects of α-MSH on the lipogenesis were investigated. Second, it was investigated whether HSD11β1 is expressed in sebocytes and cytokines affect on the expression of HSD11β1. Similarly, whether HSD11β1 affects sebogenesis on sebocytes was also investigated.

Consequently, MC5R was only observed in differentiated SZ95 sebocytes. Lipid droplets were increased by α-MSH in differentiated SZ95 sebocytes. Moreover, SREBP-1c mRNA was increased by α-MSH, and SREBP-1c mRNA was more

increased in differentiated SZ95 sebocytes. These results suggest that the sebotrophic effect of α-MSH in SZ95 sebocytes via MC5R is superior to that of the sebotrophic effect happening via MC1R. The IP_3 receptor antagonist and the PLC inhibitor suppressed the expression of SREBP-1c mRNA which was induced by α-MSH in both undifferentiated and differentiated SZ95 sebocytes. These results indicate that α-MSH induces sebogenesis in SZ95 sebocytes by induction of SREBP-1c via IP_3/PLC pathway. The expression of HSD11β1 in SZ95 sebocytes was increased by dexamethasone. An increment of HSD11β1 expression was inhibited by RU-486, glucocorticoid receptor (GR) antagonist, PF-915275, HSD11β1 specific inhibitor, and pioglitazone, PPAR-γ agonist. Similarly, dexamethasone increased sebogenesis in SZ95 sebocytes. In addition, RU-486, PF-915275, and pioglitazone inhibited sebogenesis induced by dexamethasone in SZ95 sebocytes. Moreover, dexamethasone increased the expression of SREBP-1a and 1c mRNA. The increment of SREBP-1a and 1c mRNA expression was inhibited by RU486, PF-915275, and pioglitazone. These data demonstrate that HSD11β1 involves dexamethasone-induced lipid synthesis, which is induced by SREBP-1a and 1c via GR. α-MSH induced the gene expression level of pro-inflammatory cytokines, such as IL-1α, IL-6 and TNF-α in both undifferentiated and differentiated SZ95 sebocytes. IL-1α, IL-6 and TNF-α induced HSD11β1 gene expression level in SZ95 sebocytes and α-MSH induced TLR-2 gene expression in differentiated SZ95 sebocytes. Dexamethasone also induced TLR-2 gene expression in SZ95 sebocytes. These results suggest that α-MSH and dexamethasone may involve inflammatory and immune reaction in acne vulgaris lesions.

To conclude, psychological stress induced α-MSH induced not only sebum secretion but also inflammation in acne vulgaris. Furthermore, the following findings suggest that receptors of α-MSH and HSD11β1 could be critical factors for the medical treatment of acne vulgaris.

Key words : acne, sebogenesis, α-MSH, HSD11β1, TLR-2

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I. INTRODUCTION

Acne vulgaris is a disease that affects the infundibulum and sebaceous glands of the human pilosebaceous unit and of the gland itself. This condition develops during puberty, appearing usually at the site of acne prone lesions, mainly the chest, back, and face. Although it is not life-threatening, it can cause psychological stress and permanent scars. The earlier forms of acne are characterized by microcomedones. As the disease progresses into severity, comedogenesis and inflammation develop. Duct rupture is a late event in the development of most inflammatory lesions[1-5]. Pathogenesis of acne vulgaris that has so far been elucidated includes excessive sebum secretion, abnormal keratinization of follicular infundibulum, microbial overgrowth including *Propionibacterium acnes*, and perifollicular inflammation. In addition to these factors, psychological stress is also considered as an important pathophysiologic factor[6].

The possibility of a causative influence of emotional stress, especially of stressful life events, on the course of acne has long been postulated[7,8]. Chiu *et al*., in a prospective study of 22 university students, found that patients with acne may experience a worsening of their disease during examinations[9]. The endocrine mediators controlling the activity of hypothalamus-pituitary-adrenal (HPA) axis including the corticotropin-releasing hormone (CRH) and the proopiomelanocortin (POMC) peptides and their receptors, are known to be expressed in the skin and sebaceous gland[6, 10-13]. CRH, its binding protein (CRH-BP) and corticotropin receptors (CRH-Rs) act as a central regulatory system of the HPA axis[11]. Recent

studies have confirmed the presence of a complete CRH/CRH-BP/CRH-R system in human sebocytes. It was also confirmed that CRH directly induces lipid synthesis[13-14]. Melanocortins, the products of the POMC gene, include adrenocorticotropin hormone (ACTH), and α-, β- and γ-melanocyte stimulating hormone (MSH)[15]. MSHs derive their functions by binding and activating their respective receptors, which are termed as melanocortin receptor (MCR). Thus far, five MCRs—MC1R to MC5R—have been identified. In sebaceous glands and cultured human sebocytes, the expression of MC1R and MC5R was reported[16-17]. It was also reported that α-MSH treatment can induce lipogenesis in cultured human sebocytes[18]. Moreover, a recent study demonstrated that α-MSH in human sebocytes controls a key cellular signaling pathway—the calcium ion response—which may coordinate MC1R-mediated sebum secretion[19].

Stress activates the HPA axis and the increased activity of the HPA axis results in the increase of corticosteroids, especially cortisol[20]. Endogenous glucocorticoids regulate a host of critical physiological functions, such as stress and inflammatory responses, cellular growth and differentiation, and a variety of metabolic processes[21]. Glucocorticoid action on target tissues depends not only on circulating levels but also on their intracellular concentration. Both active glucocorticoids (cortisol in human and corticosterone in rodents) and their inactive 11-keto forms (cortisone in human and 11- deoxycorticosterone) circulate in similar concentrations in blood[22]. The major fraction of active glucocorticoids exists as complex with binding proteins, cortisol binding protein (CBG) and albumin, and a small fraction is biologically active. Inactive forms circulate without binding proteins, which readily enter the cells where they are converted to active forms in a tissue-specific manner[22]. The 11β-hydroxysteroid dehydrogenase type 1 and 2 (HSD11β1 and HSD11β2) are tissue-specific glucocorticoid deactivating enzyme. HSD11β1 functions predominantly as oxoreductase, which converts inactive cortisone into active cortisol[23]. Located in the endoplasmic reticulum membrane, it requires the cofactor NADPH for the oxoreductase activity[24-25]. It also functions as dehydrogenase, which converts cortisol into cortisone. HSD11β1 is widely

distributed in most tissues including the liver, adipose, skeletal muscles, vascular smooth muscles, brain, eye, synovium and bone[26-33]. HSD11β2 functions as a potent dehydrogenase. It is expressed in the kidney and in the colon[34-35]. It is well known that HSD11β1 is associated with the pathogenesis of many metabolic diseases[33, 36-38]. Recently, it was reported that HSD11β1 is located in keratinocytes and fibroblastes in humans, and in mouse skin[39]. It is demonstrated that glucocorticoid increases HSD11β1 mRNA expression in human fibroblastes[39].

Psychological stress induces a variety of neuropeptides and stress hormones. Some of these neuropeptides and hormones, including α-MSH and glucocorticoid, increase the lipogenesis of sebocytes. In this study, the mechanism of lipogenesis induced by α-MSH or glucocorticoid and the biological effects of α-MSH or glucocorticoid on sebocytes were investigated.

Although it is well known that the human sebocyte is a direct cellular target of α-MSH, which appears to regulate lipogenesis, current knowledge about the exact mechanism involved in the regulation of lipogenesis by α-MSH and the biological effects of melanocortins on the human sebaceous gland besides lipogenesis is still incomplete. No report has yet been made on the effect of gluccorticoid on sebogenesis in sebocytes. Moreover, the effect of HSD11β1 on lipogenesis in sebocytes has not yet been investigated. This study investigated the effect of α-MSH on the expression of its receptors, MC1R and MC5R, according to the differentiation status of sebocytes and the expression of proinflammatory cytokines and toll-like receptor (TLR) 2. This study also investigated the differential effect of α-MSH on lipogenesis. The expression of HSD11β1 on sebocytes and their regulation by cytokines were investigated as well. Further, this study investigated whether HSD11β1 affects sebogenesis in sebocytes.

II. MATERIALS AND METHODS

1. Cell lines and cell culture

Immortalized human sebocyte cell line SZ95, which expresses the major characteristics of normal sebocytes[40] were maintained in Dubecco's modified Eagle medium (DMEM)/F-12 containing 10% heat-inactivated fetal bovine serum, 2.5 mM glutamax I, 50 μg/ml gentamicin, 2.5 ng/ml human epidermal growth factor (hEGF) and 10 mM 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES) (Gibco Invitrogen Corporation, Pasley, UK). Before the treatment of reagents, SZ95 sebocytes were maintained for 24 h in phenol red-free DMEM/F12 containing 50 μg/ml bovine pituitary extract, 1mg/ml fatty acid free bovine serum albumin, 2.5 mM glutamax I, 50 μg/ml gentamicin, 2.5 ng/ml hEGF and 10 mM HEPES. Cells were maintained in humidified atmosphere of 5% CO_2 at 37℃ and medium was replaced every two days.

2. Reagents

α-MSH, 2-aminoethoxydipheryl borate (2-APB), u-73122, dexamethasone, RU486, and pioglitazone were purchased from Sigma Aldrich (Sigma Aldrich, St.Louis, MI, USA). To induce the differentiation of SZ95 sebocytes, 20 nM testosterone (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and 100 μM linoleic acid (Sigma Aldrich) were simultaneously treated on SZ95 sebocytes for 24 h[41].

3. Immunofluorescent staining

The cell were cultured in Lab-Tek 4-chamber slides (Nunc, Roskilde, Denmark) at a seeding density of $1x10^5$ per well for two days before reagent

treatment. After reagent treatment, the wells were washed twice with PBS and fixed with 4% paraformaldehyde in PBS for 5 min. Then, the chamber slides were washed three times with PBS and 500 μl of 0.1% Triton X-100 in PBS was added to each well for 5 min for cell permeabilization. After washing with 0.1% Triton X-100 in PBS, the cells were stained with the goat anti-MC1R antibody or goat anti-MC5R antibody (1:100 respectively, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in PBS with 30 mg/ml BSA for 30 min at 37℃. After rinsing in PBS, the cells were stained with a donkey anti-goat antibody conjugated with FITC (1:100 Santa Cruz Biotechnology) as a secondary antibody for 30 min at room temperature. After counterstaining with propidium iodide (1:100, Invitrogen Life Technologies, Carlsbad, CA, USA) for 3 min at room temperature, the slides were mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA). Cells were examined and imaged with confocal microscope equipped with digital camera (Nikon ECLIPSE Ti, Nikon, Tokyo, Japan).

4. Immunohistochemistry

A 2 mm punch biopsy specimen was obtained from the acne lesional skin. Biopsy specimens from three patients were embedded in paraffin. Paraffin-embedded punch biopsy tissues were cut into 4-μm sections. Deparaffinized paraffin sections were processed for rehydration and air dried. Later the sections were incubated with a peroxidase-blocking reagent for 10 min in order to prevent endogenous peroxidase activity. After blocking the non-specific antibody binding by incubation with a serum free protein for 10 min at room temperature, the primary antibodies (1:100 dilution) of HSD11β1 (Cayman Chemical, Ann Arbor, MI, USA) and GR (Cell Signaling Technology, INC., Danverse, MA, USA) were applied for 30 min at 37℃. Sections were then soaked with horseradish peroxidase (HRP)-conjugated secondary antibody for 30 min at room temperature. Staining for these

proteins was detected using NovaRed (Vector Laboratories) as a substrate. Between steps, the slides were rinsed for 10 min in 0.05 M Tri-HCl buffer with 0.01% Triton-X-100. All sections were lightly counterstained with hematoxylin.

5. Measurement of lipid droplets in cytoplasm

Detection of lipid contents was performed by Nile-red staining as previously described[18-19]. Briefly, SZ95 sebocytes were cultured in 96-well tissue culture plates at a seeding density of 2,500 cells per well for two days. After the treatment of reagents, the wells were washed with PBS, and 100 μl of a 10 μg/ml nile red solution in PBS was added to each well. The plates were then incubated at 37℃ for 30 min and were read on a Fluostar Optima (BMG Labtechnologies Pty. Ltd, Australia). The results are presented as percentages of the absolute fluorescence units in comparison with the controls, using 485 nm excitation and 565 nm emission filter for neutral lipids and 540 nm excitation and 620 nm emission filters for polar lipids. Experiments were carried out in triplicate.

6. Oil-red O staining

The detection of lipid contents in SZ95 sebocytes was performed by Oil-red O staining. SZ95 sebocytes were cultured in Lab-Tek 4-chamber slides (Nunc) at a seeding density of $1x10^5$ per well for two days before reagent treatment. After the reagent treatment, the wells were washed twice with PBS and fixed with 4% paraformaldehyde in PBS for 5 min. Fixed cells were stained with the Oil red O solution, a mixture of 1% Oil red O (Sigma Aldrich) and dH_2O in a ratio of 6:4 (vol/vol), for 15 min. These were then, washed with PBS. Stained cells were visualized by microscopy.

7. Real-time quantitative RT-PCR

Total RNA was extracted from cells using TRIzol reagent (Invitrogen Life Technologies) and 1 μg of total RNA was converted to cDNA using TaKaRa RNA PCR Kit Ver.2.1 (TaKaRa BIO INC. Shiga, Japan), as following reaction condition : 45℃ for 45 min and 95℃ for 5 min. Gene expression signals were quantified with real-time PCR (TaqMan, Applied Biosystems, Foster City, CA, USA). Probes and primers were obtained from Applied Biosystems as Assays-on-Demand Gene Expression Assays (HSD11β1: Hs00194153_m1 and β-actin: TaqMan β-actin Control Reagents). Reactions were carried out on the ABI 7300 and relative transcript levels were determined using β-actin as a reference gene. The data were analyzed using of 7300 system software (Applied Biosystems). Real-time PCR amplifications were performed using Express SYBR GreenER Supermix with Premixed ROX (Invitrogen Life Technologies) in an ABI 7300 (Applied Biosystems) following the manufacturer's protocol. Gene-specific primers were designed by using Primer Blast of NCBI. The primers for β-actin, MC1R, MC5R, sterol response element-binding protein (SREBP)-1a and SREBP-1c are showed in Table 1. Reaction condition was as follows: initiated at 50℃ for 2 min, 95℃ for 10 min, followed by a cycling condition of 95℃ for 10 sec and 60℃ for 1 min for 40 cycles. The expression of β-actin was used as internal standard. The data were analyzed using of 7300 system software (Applied Biosystems).

8. Western blot analysis

Reagents-treated SZ95 sebocytes were washed with PBS. Then cells were lysed with RIPA buffer (GenDepot, barker, TX, USA) with proteinase inhibitor cocktail (Thermo Fisher Scientific Inc. Rockford, IL, USA) and centrifuged at 13200 rpm for 30 min at 4℃. Supernatants were collected, and the protein concentration was measured with the use of a BCA Protein Assay

Reagent (Thermo Fisher Scientific Inc). After blocking with 5% skim milk, the blots were washed with TBS that contained 0.01% tween 20 (Amresco Inc. Branded Products Group, Cochran Solon, OH, USA) (0.01% TBS-T) and incubated with an appropriate primary antibody at 4℃ overnight. Antibodies against keratin 7 (Abcam plc, Cambridge, MA, USA), epithelial membrane antigen (EMA) (Abcam plc), and human β-actin (Santa Cruz Biotechnology) were used at 1:1000 dilution. After washing with 0.01% TBS-T three times, the membranes were incubated with HRP-conjugated secondary antibody (1:3000, Santa Cruz Biotechnology) for 1 h at room temperature and then washed again. The membranes were visualized with the use of ECL Plus Western Blotting Detection System (GE Healthcare, Amersham, UK). The images were recorded using JP-33 X-ray Filmprocessor (Jungwon Precision IND. Co., Ltd. Seoul, Korea).

9. RNA interference

Three HSD11β1-specific siRNAs (HSD11β1-siRNA-1, 2, 3 groups) and negative control (Scramble group) with no significant homology with human gene sequences were purchased from Invitrogen (Invitrogen Life Technologies). Cells were seeded at a density of 5 x 10^4 into a six-well plate 24 h before transfection, resulting in approximately 50% confluency. In each well, 50 nM HSD11β1-siRNA-1, 2, 3 or scramble and 5 μl Lipofectamine RNAiMAX (Invitrogen Life Technologies) were added to 250 μl Opti-MEM (Invitrogen Life Technologies), mixed gently and then added to the plates. The plate was incubated for 48 h at 37℃ until it was ready for further assay.

10. Statistical analysis

The results are expressed as means ± standard deviation (S.D.). P values less than 0.05 were considered as statistically significant. On-way ANOVA with

Dunnett's post test was performed using GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA, USA, www.graphpad.com)

Table 1. Primer sequences for various human genes for the real-time quantitative PCR

Gene	Foward	Reverse	Gene number
MC1R	TGC AAA AGG AGG TGA AAT CC	AGT GCC CAG TCT GAG CCT TA	NM-002386.3
MC5R	TGA TAG CAG ACG CCT TTG TG	CGT CAT GAT GTG GTG GTA GC	NM-005913.2
SREBP-1a	GCT GCT GAC CGA CAT CGA A	TCA AAT AGG CCA GGG AAG TCA	NM-001005291.2
SREBP-1c	GGA GCC ATG GAT TGC ACT TT	TCA AAT AGG CCA GGG AAG TCA	NM-001005291.2
β-actin	GAT GAG ATT GGC ATG GCT TT	CAC CTT CAC CGT TCC AGT TT	NM-001101.3

III. RESULTS

1. Differentiation of cultured sebocytes SZ95 was induced by testosterone and linoleic acid co-treatment.

In order to examine the differentiation status of cultured sebocyte, the expression of cytokeratin 7 and EMA were observed through Western blotting as markers for undifferentiated and differentiated sebocytes[42], respectively. The induction of sebocyte differentiation by simultaneous treatment with testosterone (20 nM) and linoleic acid (100 μM) (T/LA)[41] showed significant increase in EMA expression but no significant difference in cytokeratin 7 expression (Figure 1). These results suggest that T/LA induces the differentiation of cultured SZ95 sebocytes.

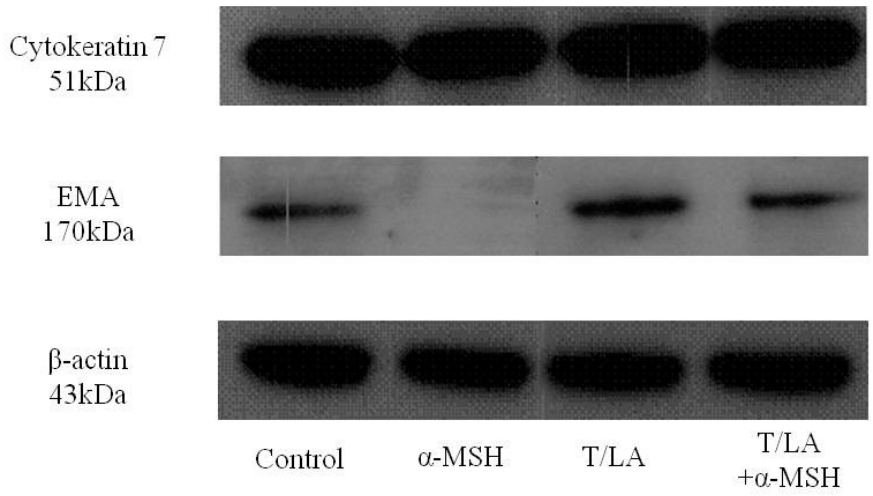


Figure 1. EMA expression was increased by testosterone and linoleic acid co-treatment. The expression of proliferation or differentiation markers in sebocytes in response to α-MSH was analyzed by Western blotting analysis. Expression of cytokeratin 7, a marker for proliferating sebocytes was not changed. However, the marker for differentiated sebocytes—epithelial membrane antigen (EMA) expression—was increased by T/LA.

T/LA : testosterone and linoleic acid co-treatment

2. α-MSH-induced MC1R and MC5R expression in sebocytes was dependent on the differentiation status.

Previously, it was reported that MC1R is constitutively expressed in sebocytes, irrespective of its differentiation status. The MC5R expression was affected by differentiation status[41]. In order to investigate the effects of α-MSH on the expression of both receptors, 0.1 μM of α-MSH was treated on either undifferentiated or T/LA treated sebocytes. Similar to the previous results, T/LA treatment did not affect the protein expression of MC1R but increased the expression of MC5R along with sebocyte differentiation (Figure 2). When α-MSH alone was treated to SZ95 sebocytes, MC1R expression was increased while MC5R expression remained the same (Figure 2, Figure 3 A). In contrast, sequential treatment of α-MSH after T/LA treatment showed increments of MC5R expression but not MC1R expression (Figure 2, Figure 3 B).

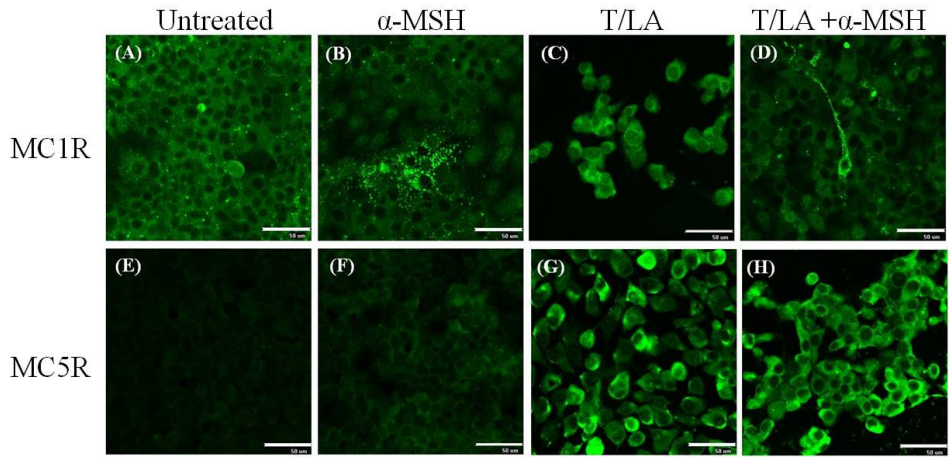


Figure 2. Effects of α-MSH treatment on expression of MC1R and MC5R in SZ95 sebocytes SZ95 sebocytes were fixed and then stained with MC1R or MC5R as described in the text. MC1R was detected in untreated SZ95 sebocytes (A). α-MSH induced the increase of MC1R expression in the undifferentiated SZ95 sebocytes (B). However, T/LA did not affect MC1R expression in T/LA-treated SZ95 sebocytes (C). In addition, α-MSH did not affect expression of MC1R in T/LA-treated SZ95 sebocytes (D). MC5R was not detected in untreated SZ95 sebocytes (E). Similarly, α-MSH did not induce the expression of MC5R in the undifferentiated SZ95 sebocytes (F). On the other hand, T/LA induced MC5R expression in T/LA-treated SZ95 sebocytes (G). Interestingly, α-MSH significantly increased MC5R expression in the T/LA-induced differentiated SZ95 cells (H).

T/LA : testosterone and linoleic acid co-treatment

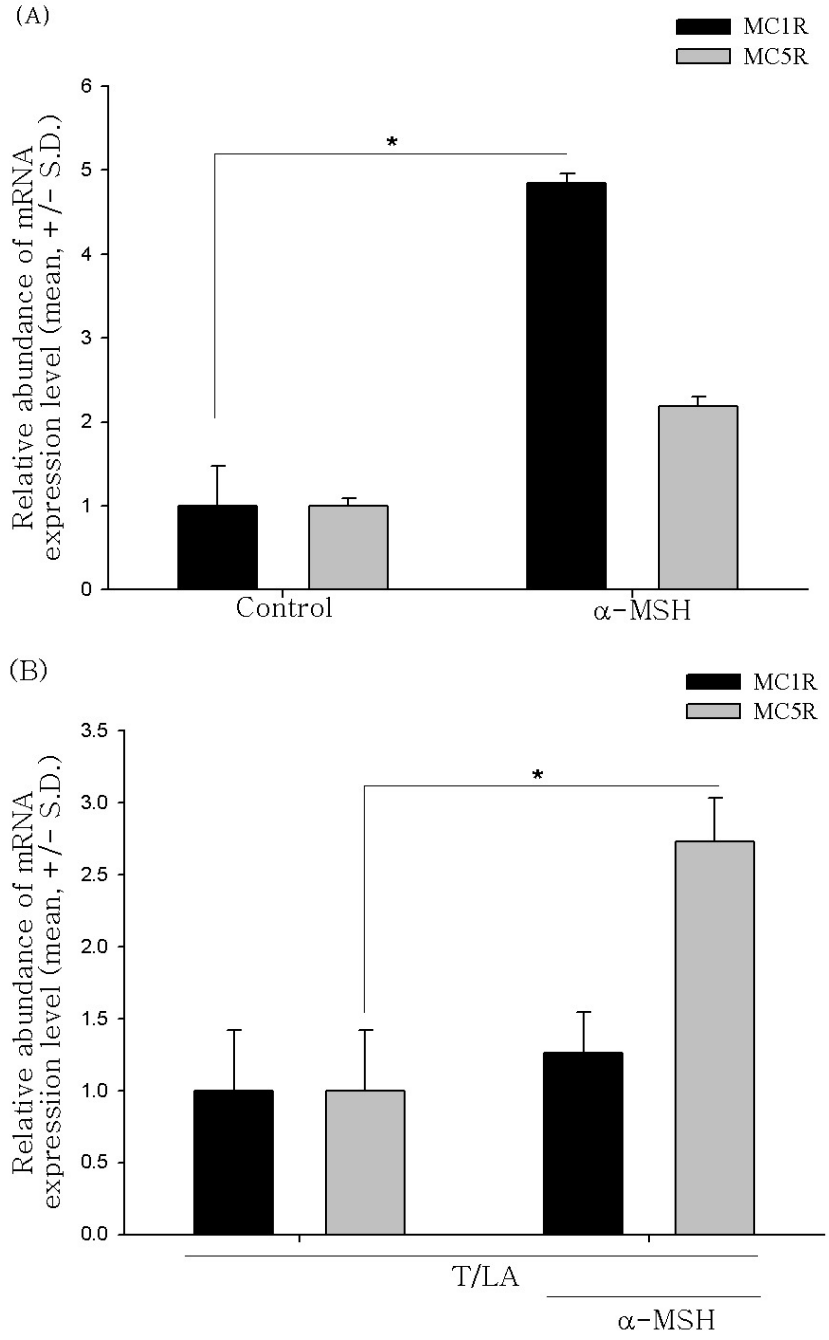


Figure 3. Effects of α-MSH treatment on mRNA expression of MC1R and MC5R in SZ95 sebocytes While α-MSH treatment significantly increased the

expression of MC1R in the undifferentiated SZ95 sebocytes (A), increase of MC5R mRNA was observed in T/LA induced differentiated SZ95 cells (B). T/LA : testosterone and linoleic acid co-treatment, *: $p<0.05$

3. α-MSH induced lipid droplets in cytoplasm of SZ95 sebocytes.

In order to examine the lipogenetic effects of α-MSH on cultured sebocytes, nile red staining was performed. In undifferentiated SZ95 ssebocytes, nile red stained lipid droplets in the cytoplasm was not detected (Fgiure 4 A). While a slight increase of lipid droplets was detected in α-MSH treated cells, the increment was not so significant, compared to the T/LA treated cells (Figure 4 B).Along with the differentiation, T/LA treatment also significantly increased the number of nile red stained lipid droplets in the cytoplasm of sebocytes (Figure 4 C). Moreover, during the sequential treatment of α-MSH following T/LA, the significant increase of lipid droplets was observed (Figure 4 D). The combination of testosterone and linoleic acid showed synergistic effect on the amplification of polar and neutral lipids (Figure 4 E and F). α-MSH increased polar and neutral lipids in undifferentiated and differentiated SZ95 sebocytes (Figure 4 E and F).

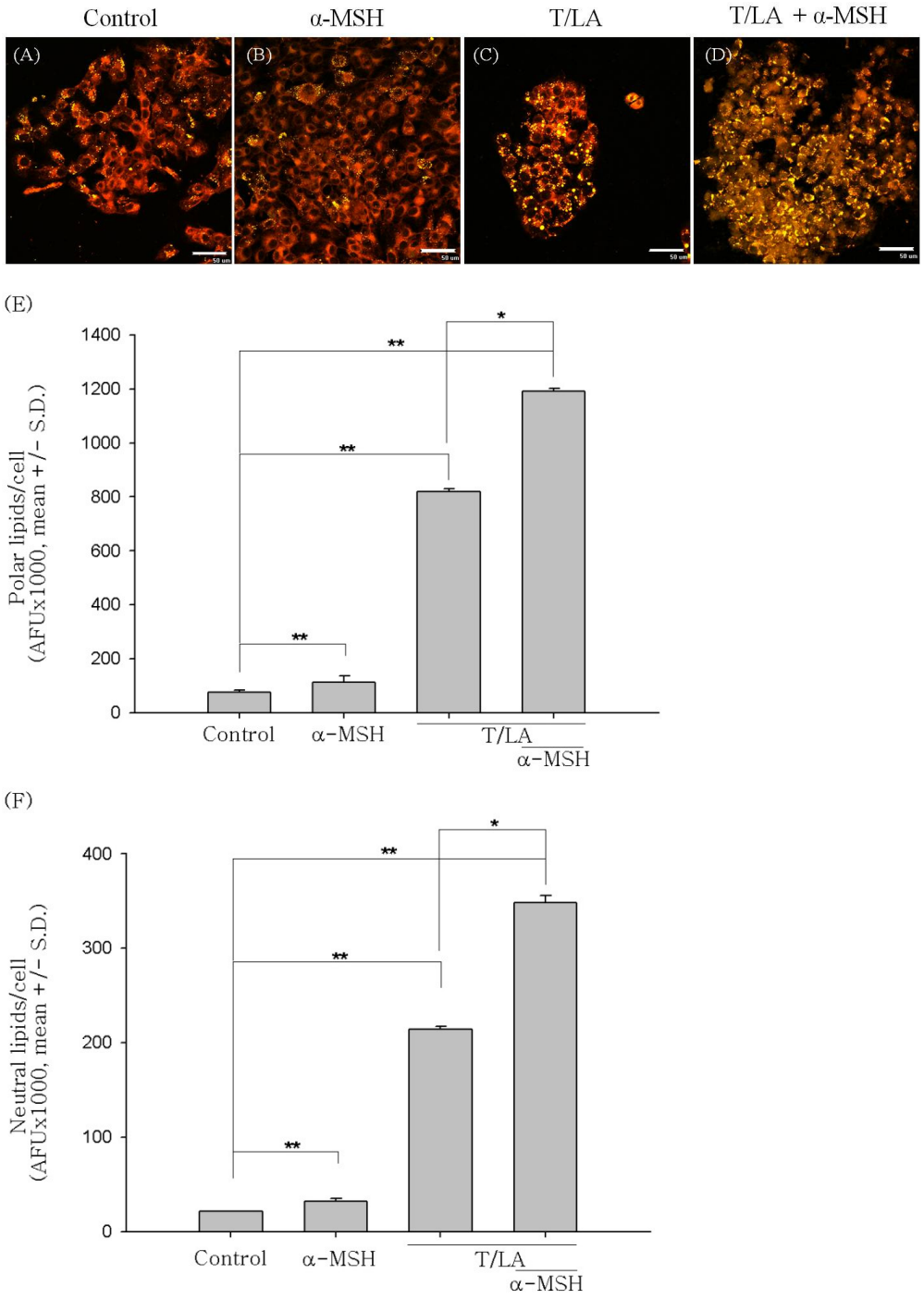


Figure 4. α-MSH induced lipid droplets in SZ95 sebocytes. The effect of α-MSH on the lipogenesis of sebocytes with or without pre-treatment with T/LA

Nile red staining showed a slight increase in cytosolic lipid accumulation in undifferentiated SZ95 sebocytes (A and B) by α-MSH treatment. T/LA treatment induced significant increase in lipogenesis. Moreover, α-MSH treatment further increased the lipid contents in SZ95 sebocytes which were pre-treated with T/LA (C and D). The production of polar (E) and neutral (F) are shown. Polar and neutral lipid was increased by α-MSH in SZ95 sebocytes treated with T/LA or in SZ95 sebocytes untreated with T/LA (E and F). T/LA exhibited a synergistic effect on polar and neutral lipids (E and F).
T/LA : testosterone and linoleic acid co-treatment, *: $p<0.05$, **: $P<0.01$

4. α-MSH increased SREBP-1c mRNA expression in SZ95 sebocytes but did not affect the mRNA expression of SREBP-1a.

To examine whether the increased lipid synthesis by α-MSH is related with the regulation of SREBP-1 gene expression, SREBP-1 gene expression was analyzed through real-time quantitative PCR. There were no significant changes in the SREBP-1a mRNA expression level in the sebocytes that were treated with α-MSH with or without pre-treatment with T/LA (Figure 5 A). On the other hand, α-MSH significantly increased the SREBP-1c mRNA level in both undifferentiated and differentiated SZ95 sebocytes (Figure 5 B). In addition, the SREBP-1c mRNA level in differentiated SZ95 sebocytes was more increased than in undifferentiated SZ95 sebocytes by α-MSH (Figure 5 B).

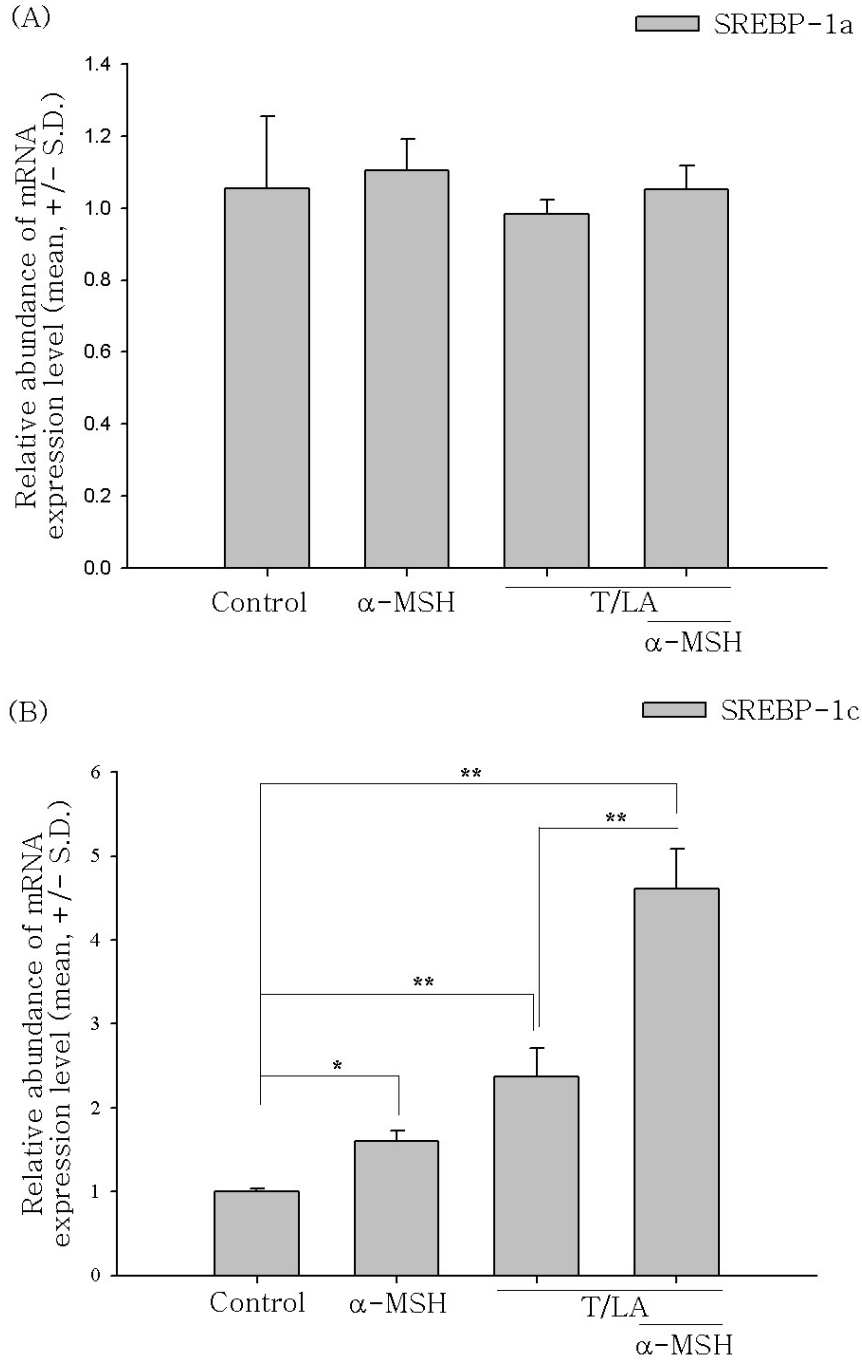


Figure 5. α-MSH induced SREBP-1c mRNA expression level in SZ95 sebocytes. α-MSH did not affect expression of SREBP-1a mRNA in T/LA-

untreated and –treated SZ95 sebocytes (A). However, SREBP-1c mRNA expression level was increased by α-MSH in T/LA-untreated SZ95 sebocytes (B). In T/LA-treated SZ95 sebocytes, the SREBP-1c mRNA expression level was more increased compared to the α-MSH that was treated in SZ95 sebocytes (B). Moreover, when 0.1 μM α-MSH was treated in T/LA-treated SZ95 sebocytes, the SREBP-1c mRNA expression level increased significantly (B).

T/LA : testosterone and linoleic acid co-treatment, *: $p<0.05$, **: $P<0.01$

5. IP_3 and PLC inhibitors inhibited the upregulation of SREBP-1c mRNA expression induced by α-MSH in both undifferentiated and differentiated SZ95 sebocytes.

Recently, it was demonstrated that α-MSH increased the intracellular calcium level of SZ95 sebocytes and that α-MSH-mediated calcium mobilization originated from calcium stores and was mediated by inositol triphosphate[19]. Moreover, α-MSH increased the MC1R immunoreactivity and lipid synthesis in SZ95 sebocytes in the presence of testosterone[19]. To examine whether α-MSH-mediated calcium mobilization affects lipid synthesis in SZ95 sebocytes, SREBP-1c mRNA expression in SZ95 sebocytes was detected using real-time quantitative PCR. 2-APB, an IP_3 receptor antagonist, inhibited α-MSH induced SREBP-1c mRNA increase in both undifferentiated and differentiated SZ95 sebocytes (Figure 6 A and B). Moreover, PLC inhibitor u-73122 prevented α-MSH induced SREBP-1c mRNA increase in both undifferentiated and differentiated SZ95 sebocytes (Figure 6 A and B).

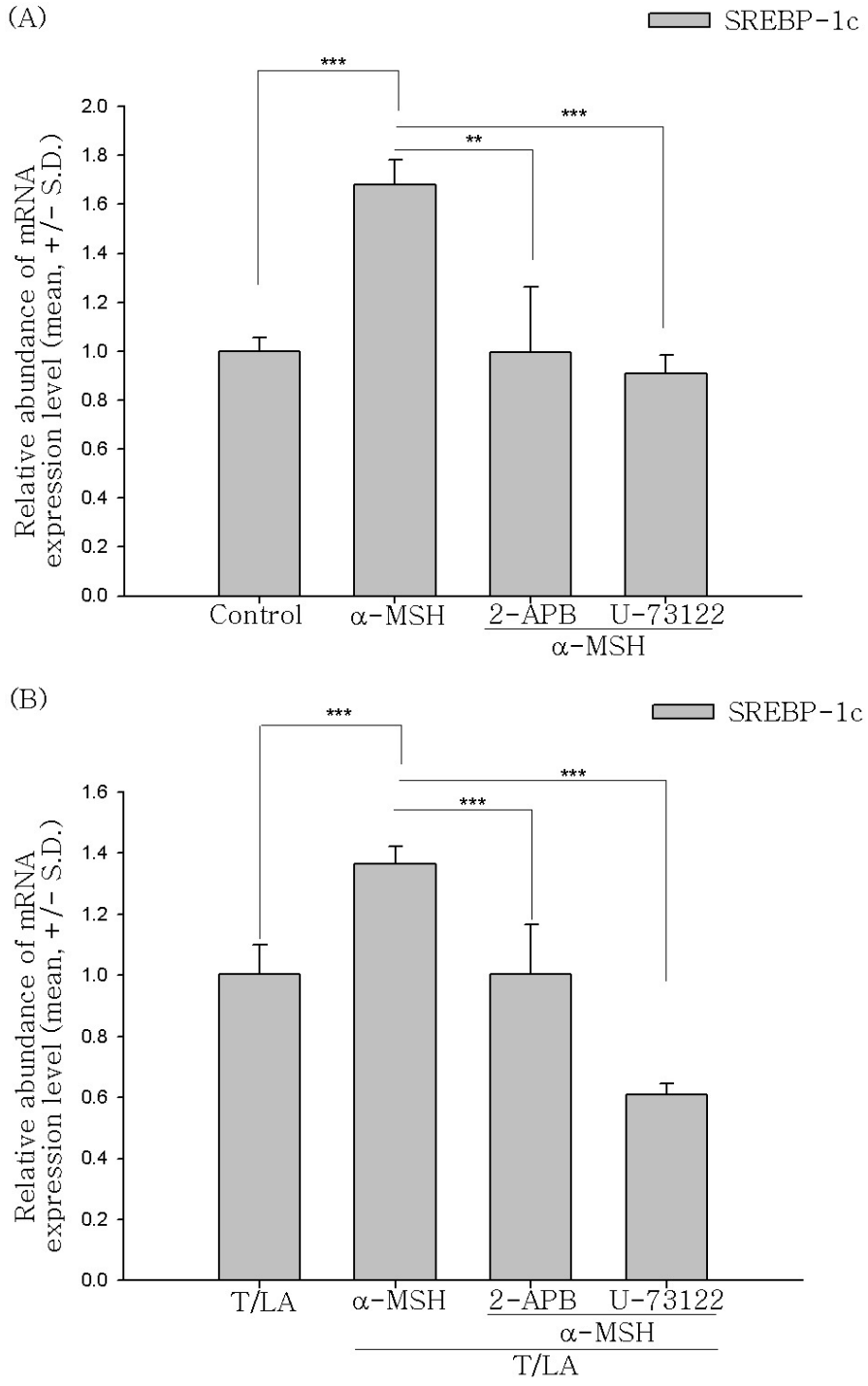


Figure 6. 2-APB and u-73122 inhibited SREBP-1c mRNA expression level

induced by α-MSH in both undifferentiated and differentiated SZ95 sebocytes. 75 μM 2-APB and 10 μM u-73122 inhibited the increment of SREBP-1c mRNA expression by α-MSH in undifferentiated SZ95 sebocytes (A). Similarly, 2-APB and u-73122 prevented α-MSH-induced SREBP-1c mRNA expression in differentiated SZ95 sebocytes (B).

T/LA : testosterone and linoleic acid co-treatment , **: $P<0.01$, *** : $P < 0.001$

6. HSD11β1 expression in sebaceous glands of acne vulgaris

The expression of HSD11β1 in the sebaceous glands of the lesional skin of patients with acne, sebaceous hyperplasia and folliculitis was observed through the immunohistochemical study. HSD11β1 expression was detected in the human sebaceous glands (Figure 7). The sebaceous glands in the lesional skin of acne showed increased immunoreactivity of HSD11β1 compared to those in the skin from sebaceous hyperplasia and folliculitis (Figure 7).

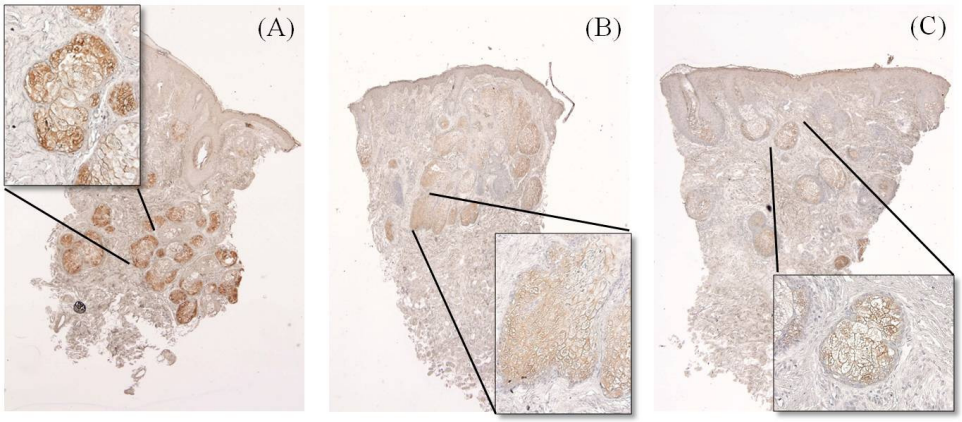


Figure 7. Expression of HSD11β1 in acne vulgaris was increased. HSD11β1 expression in sebaceous glands was confirmed by immunohistochemical staining. HSD11β1 was highly expressed in sebaceous gland in acne vulgaris. (A) Acne vulgaris (B) Sebaceous hyperplasia (C) Folliculitis (original magnification x40, inlet box: x400)

7. Dexamethasone increased HSD11β1 mRNA expression in SZ95 sebocytes.

To examine whether dexamethasone affects the expression of HSD11β1, real-time quantitative PCR was used. The HSD11β1 mRNA level was increased by dexamethasone in SZ95 sebocytes (Figure 8). The RU486 inhibited expression of HSD11β1 mRNA was also increased by dexamethasone (Figure 8).

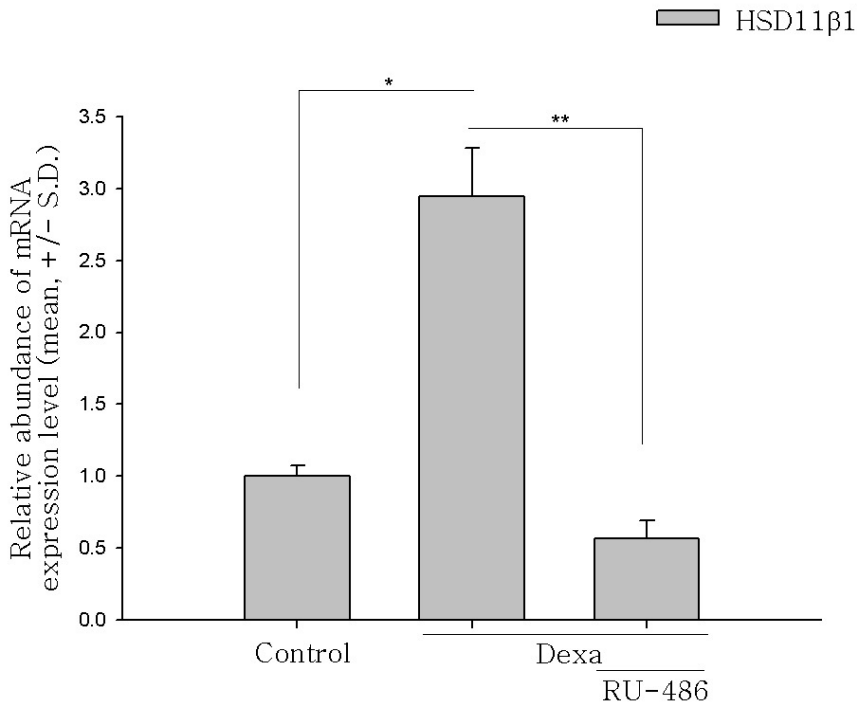


Figure 8. GR antagonist, RU-486, inhibited the upregulation of HSD11β1 mRNA expression by dexamethasone in SZ95 sebocytes. 1 μM dexamethasone increased HSD11β1 mRNA expression in SZ95 sebocytes. 1 μM RU-486 inhibited the increment of HSD11β1 mRNA expression by dexamethasone.

Dexa : dexamethasone, *: $P < 0.05$, **: $P < 0.01$.

8. Dexamethasone increased lipid droplets in cytoplasm of SZ95 sebocytes.

To examine the lipogenetic effects of dexamethasone on SZ95 sebocytes, Oil-red O staining was performed. When SZ95 sebocytes was treated with 1 μM dexamethasone, lipid droplets were increased in the cytoplasm of sebocytes than un-treated SZ95 sebocytes (control) (Figure 9 A and B). However, when SZ95 sebocytes were co-treated with GR antagonist RU-486 and dexamethasone, lipid droplets were not increased (Figure 9 B and C). This results accordance with previous report which has demonstrated that dexamethasone increased lipid droplets in SZ95 sebocytes via GR. Dexamethasone increased significantly polar and neutral lipids (Figure 9 E and F). The co-treatment of RU-486 and dexamethasone exhibited inhibition of polar lipids and neutral lipids (Figure 9 E and F).

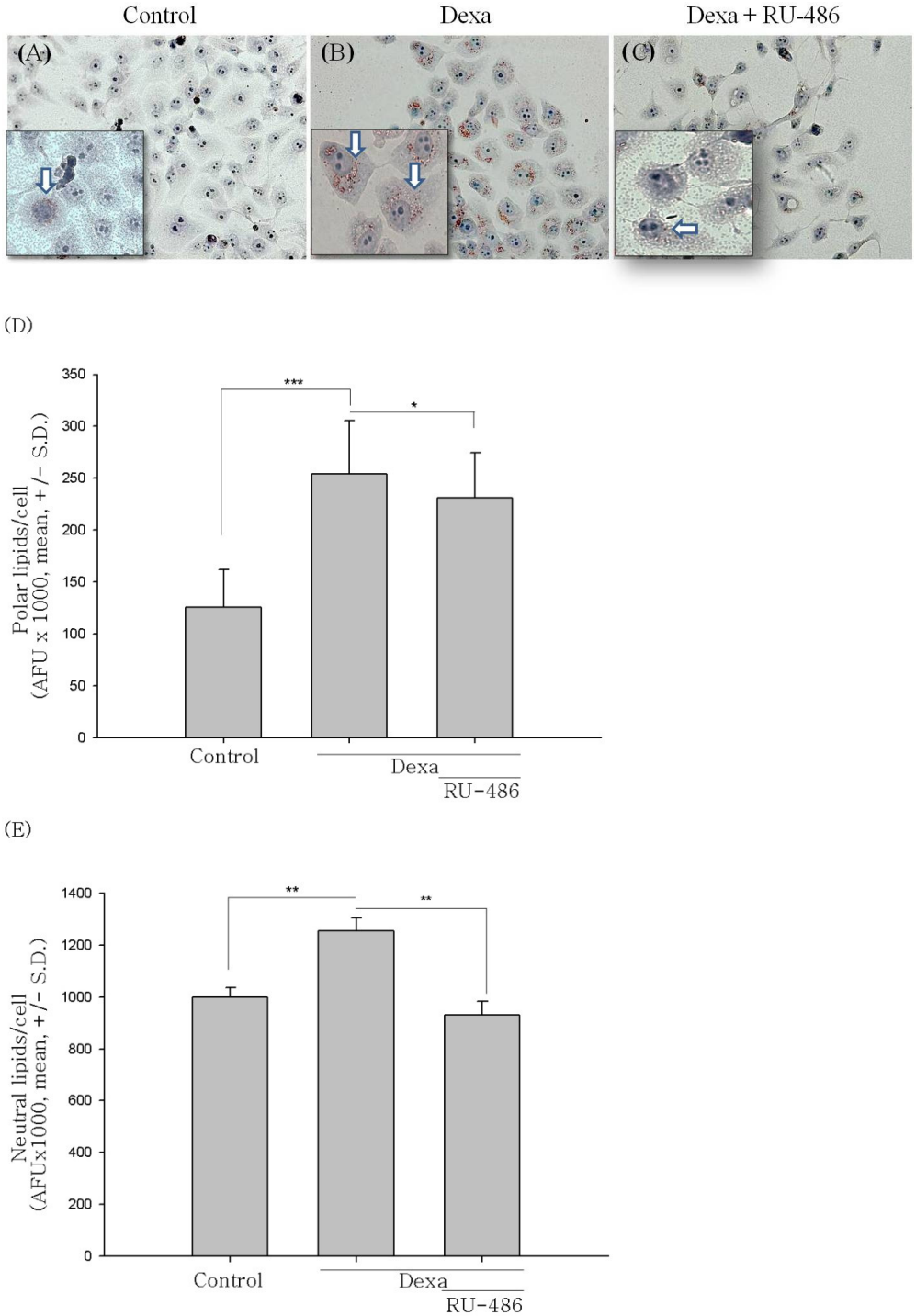


Figure 9. Dexamethasone increased lipid droplets in SZ95 sebocytes.

Dexamethasone increased lipid droplets in SZ95 sebocytes (B). RU-486 decreased lipid droplets that were increased by dexamethasone (C). The production of polar and neutral lipids was increased by dexamethasone (D and E). RU-486 inhibited the polar and neutral lipids that were increased by dexamethasone (D and E).

Dexa : dexamethasone, *: $P < 0.05$, **: $P < 0.01$.

9. Dexamethasone increased SREBP-1a and 1c mRNA expression in SZ95 sebocytes.

To examine whether the increase of lipids by dexamethasone is related with SREBP-1 gene expression, SREBP-1 gene expression was detected using real-time quantitative PCR. After treatment with dexamethasone, the SREBP-1a and SREBP-1c mRNA level in SZ95 sebocytes were increased (Figure 10 A and B). Moreover, the increment of SREBP-1a and 1c mRNA expression was inhibited by RU-486 (Figure 10 A and B).

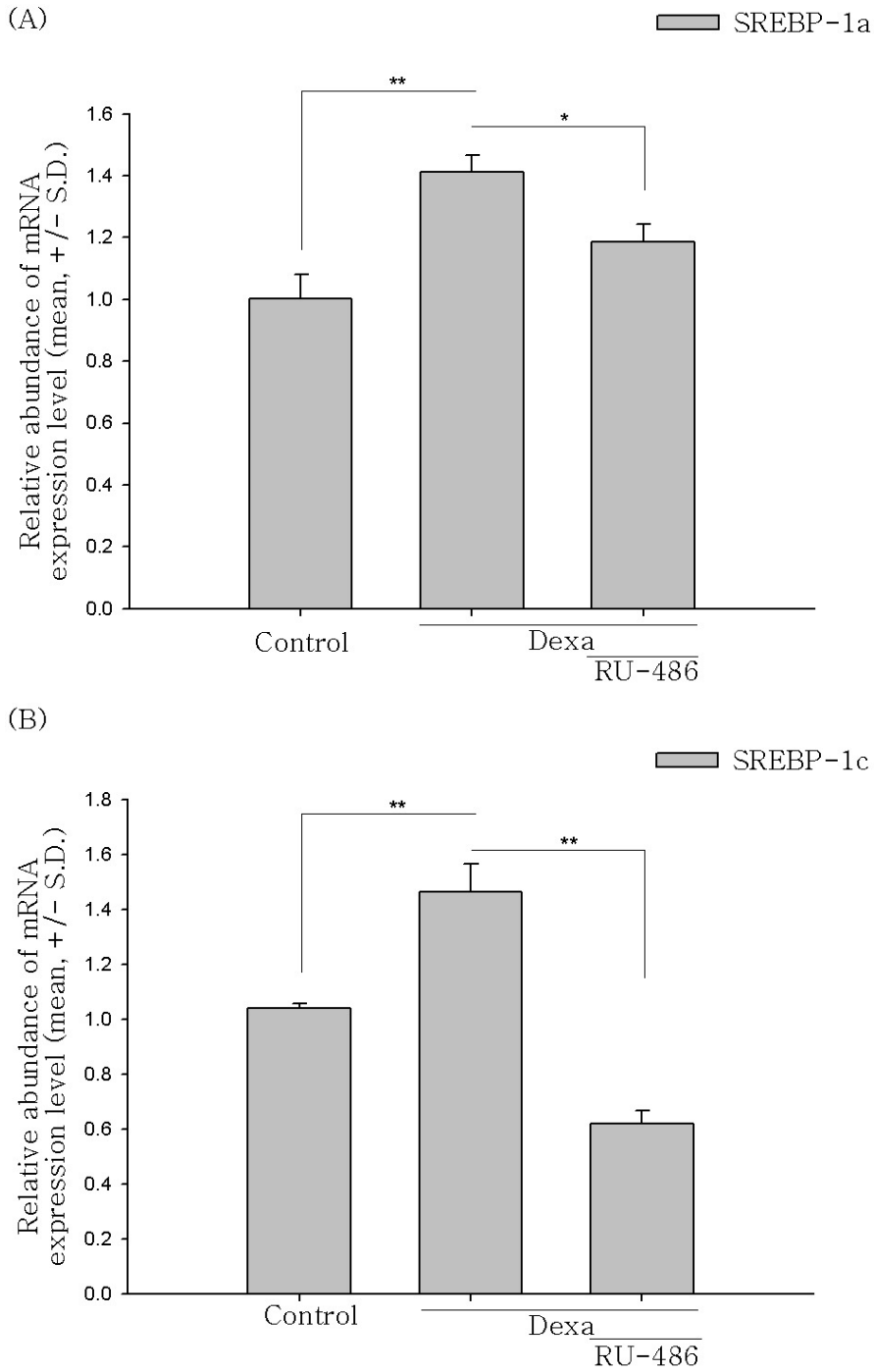


Figure 10. RU-486 inhibited the upregulation of SREBP-1a and 1c mRNA expression by dexamethasone in SZ95 sebocytes. 1 μM dexamethasone

increased SREBP-1a and -1c mRNA expression in SZ95 sebocytes (A and B). 1 μM RU-486 inhibited the increment of SREBP-1a and 1c mRNA expression by dexamethasone (A and B).

Dexa : dexamethasone, *: $P < 0.05$, **: $P < 0.01$

10. PPAR-γ agoinst and specific inhibitor of HSD11β1 inhibited the dexamethasone-induced HSD11β1 mRNA expression in SZ95 sebocytes.

It is well known that PPAR-γ agonists inhibit the expression of HSD11B1 in adipose tissues and adipocytes[43]. Moreover, it is known that PPAR-γ agonist pioglitazone does not affect lipid synthesis in human sebocytes[44]. To examine whether pioglitazone affects the mRNA expression of HSD11β1 in SZ95 sebocytes, HSD11β1 gene expression was detected using real-time quantitative PCR. Pioglitazone inhibited the expression of HSD11β1 mRNA, which was increased by dexamethasone (Figure 11). Similarly, PF-915275, a specific inhibitor of HSD11β1, inhibited the expression of HSD11β1 mRNA (Figure 11). However, pioglitazone and PF-915275 co-treatment did not show synergistic effects on HSD11β1 inhibition.

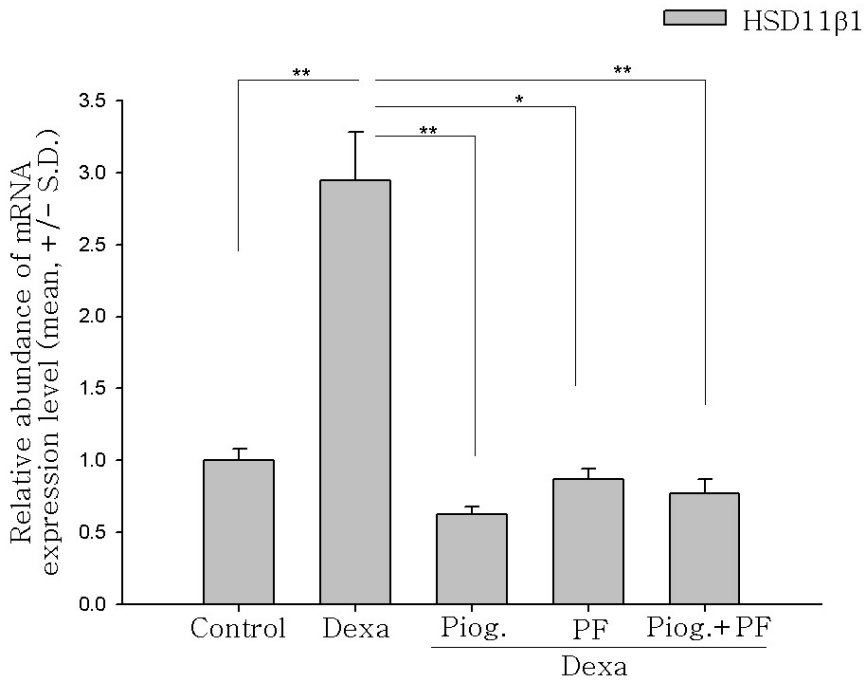


Figure 11. Pioglitazone and PF-915275 inhibited the upregulation of HSD11β1 mRNA expression by dexamethasone in SZ95 sebocytes. 1 μM

pioglitazone and 1 μM PF-915275 inhibited the increments of HSD11β1 mRNA expression, which was increased by dexamethasone. Co-treatment of pioglitazone and PF-915275 also prevented HSD11β1 mRNA to be induced by dexamethasone but did not show synergistic effects on HSD11β1 inhibition. Dexa : dexamethasone, Piog. : pioglitazone, PF : PF-915275, Piog.+PF : co-treatment of pioglitazone and PF-915275, *: $P < 0.05$, **: $P < 0.01$

11. Pioglitazone and PF-915275 inhibited the lipid synthesis of SZ95 sebocytes treated with dexamethasone.

To examine the lipogenetic effects of pioglitazone and PF-915275 on SZ95 sebocytes treated with dexamethasone, Oil-red O staining was performed. When 1 μM dexamethasone was treated to SZ95 sebocytes, lipid droplets were increased as compared to untreated SZ95 sebocytes (control) (Figure 12 A and B). However, when dexamethasone and pioglitazone, PPAR-γ agonist, or PF-915275, specific inhibitor of HSD11β1 were co-treated to SZ95 sebocytes, lipid droplets were not increased (Figure 12 B and C or D). Polar and neutral lipids increased by dexamethasone in SZ95 sebocytes (Figure 12 E and F). Co-treatment of pioglitazone and dexamethasone exhibited inhibition of polar lipids not neutral lipids (Figure 12 E and F). On the other hand, co-treatment of PF-915275 and dexamethasone exhibited inhibition of neutral lipids not polar lipids (Figure 12 E and F).

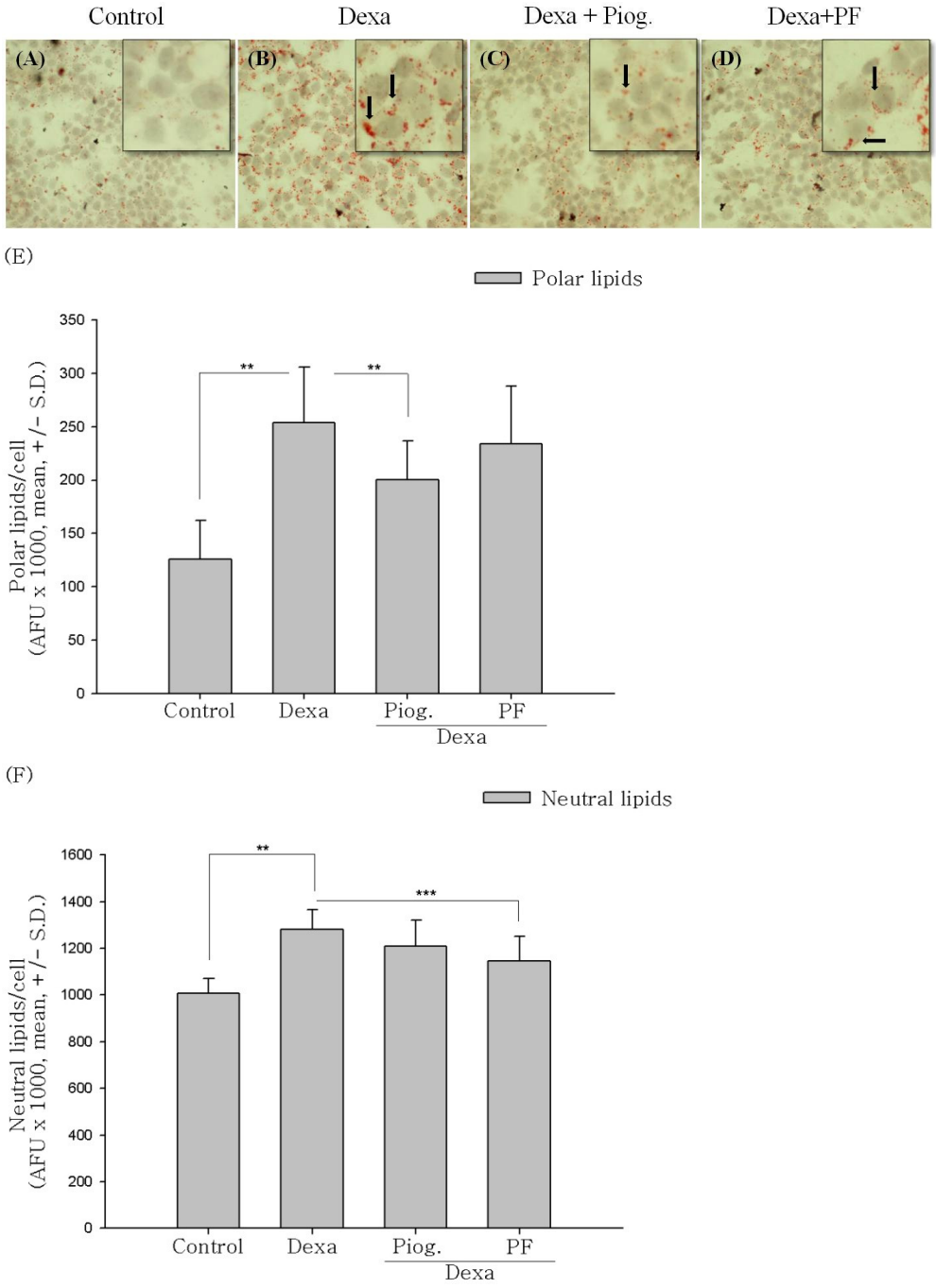


Figure 12. Pioglitazone and PF-915275 inhibited the upregulation of lipid droplets by dexamethasone in SZ95 sebocytes. Dexamethasone increased the lipid droplets in SZ95 sebocytes (A and B). Pioglitazone and PF-915275

reduced the lipid droplets induced by dexamethasone in SZ95 sebocytes (C and D). Arrows represent the lipid droplet stained by Oil red O. The production of polar and neutral lipids was increased by dexamethasone (E and F). Pioglitazone inhibited the polar lipids that were increased by dexamethasone (E). PF-915275 reduced the neutral lipids increased by dexamethasone (F).

Dexa: dexamethasone, Piog. : pioglitazone, PF : PF-915275, **: $P < 0.01$, ***: $P < 0.001$ (original magnification x200, inlet box: x400)

12. HSD11β1 inhibitors abolished the dexamethasone effect on the SREBP-1a and 1c expression in SZ95 sebocytes.

After treatment with dexamethasone, the SREBP-1a and SREBP-1c mRNA level were increased in SZ95 sebocytes. Pioglitazone inhibited the SREBP-1a mRNA but not the SREBP-1c mRNA, which were increased by dexamethasone (Figure 13 A and B). On the other hand, PF-915275 inhibited the SREBP-1a and 1c mRNA that were increased by dexamethasone (Figure 13 A and B).

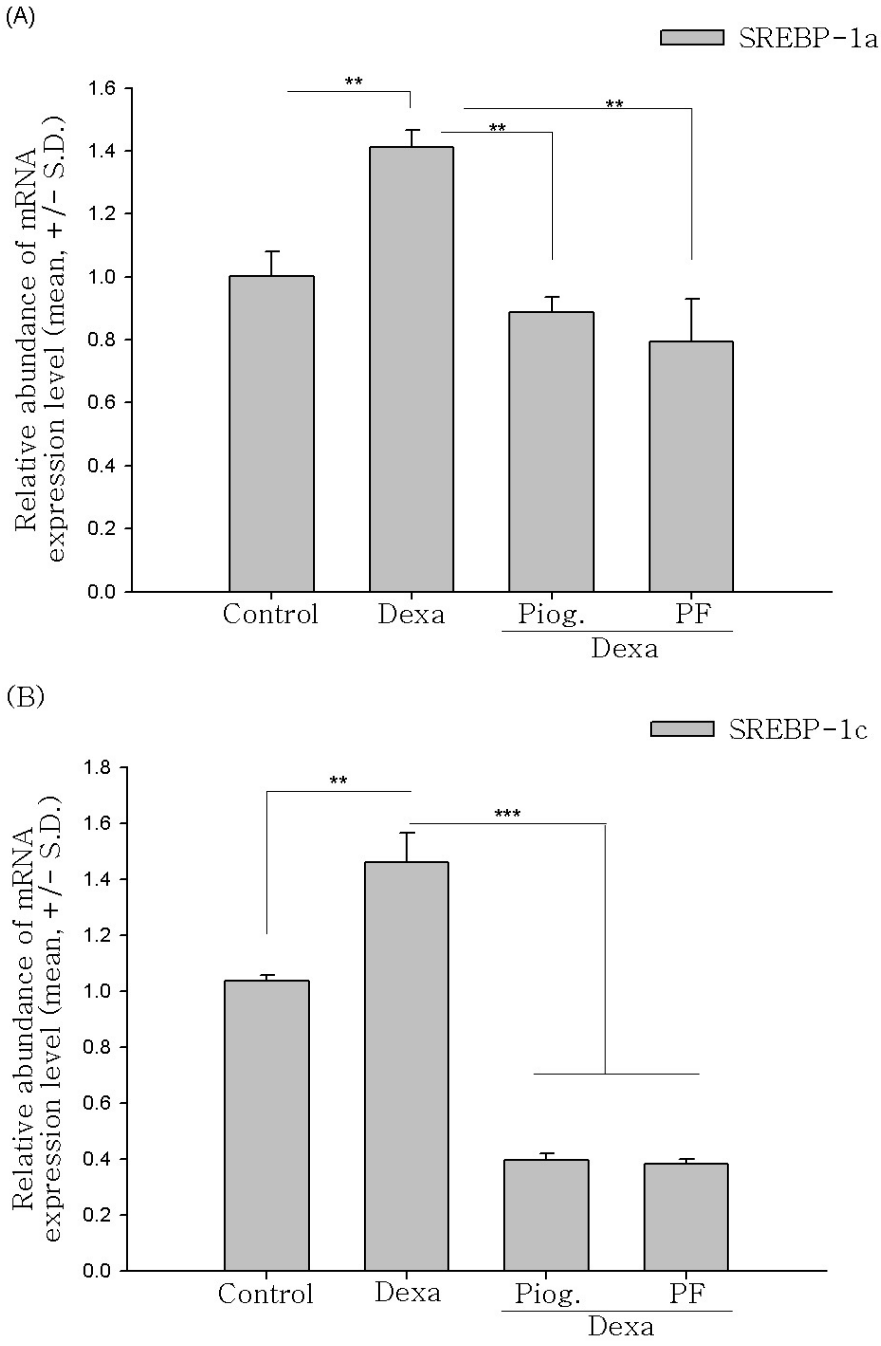


Figure 13. Pioglitazone and PF-915275 inhibited the upregulation of SREBP-1a and 1c mRNA expression by dexamethasone in SZ95 sebocytes. Pioglitazone inhibited the SREBP-1a mRNA expression induced by

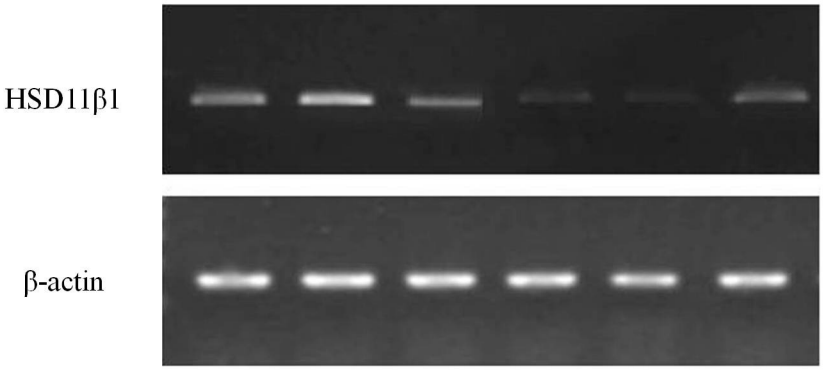
dexamethasone in SZ95 sebocytes (A). SREBP-1a and 1c mRNA expression that were increased by dexamethasone were prevented by PF-915275 in SZ95 sebocytes (B and C).

Dexa : dexamethasone, Piog. : pioglitazone, PF : PF-915275, **: $P < 0.01$, ***: $P < 0.001$

13. Dexamethasone did not affect SREBP-1a and 1c expression in SZ95 sebocytes transfected with the HSD11β1-direct siRNA.

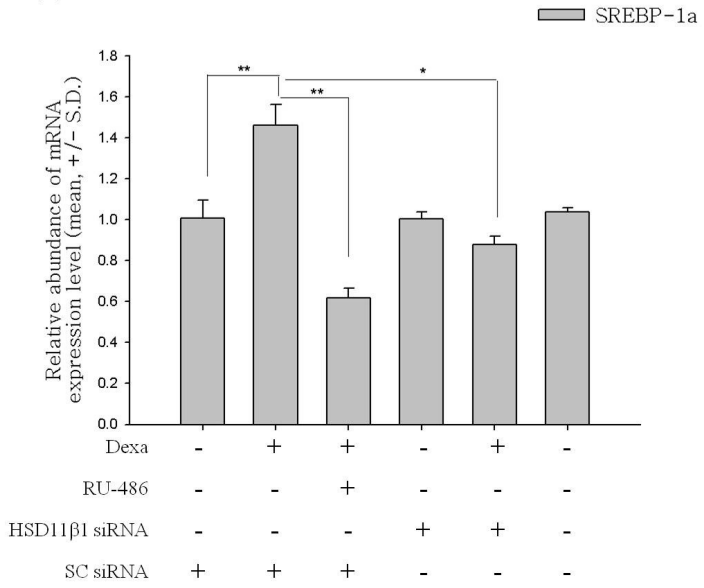
To confirm the effect of pioglitazone and PF-915275, the HSD11β1-directed siRNA pools and the negative control pool were transfected to SZ95 sebocytes. HSD11β1 mRNA expression was not detected in the SZ95 sebocytes that were transfected with HSD11β1-direct siRNA pools (Figure 14 A). Similarly, dexamethasone did not affect the HSD11β1 mRNA expression level in the SZ95 sebocytes transfected with HSD11β1-direct siRNA pools. The negative control pool did not affect the SZ95 sebocytes. Likewise, dexamethasone did not affect to the SREBP-1a and 1c mRNA expression in the SZ95 sebocytes that were transfected with HSD11β1-direct siRNA pools (Figure 14 B and C).

(A)



Dexa	-	+	+	-	+	-
RU-486	-	-	+	-	-	-
HSD11β1 siRNA	-	-	-	+	+	-
SC siRNA	+	+	+	-	-	-

(B)



Dexa	-	+	+	-	+	-
RU-486	-	-	+	-	-	-
HSD11β1 siRNA	-	-	-	+	+	-
SC siRNA	+	+	+	-	-	-

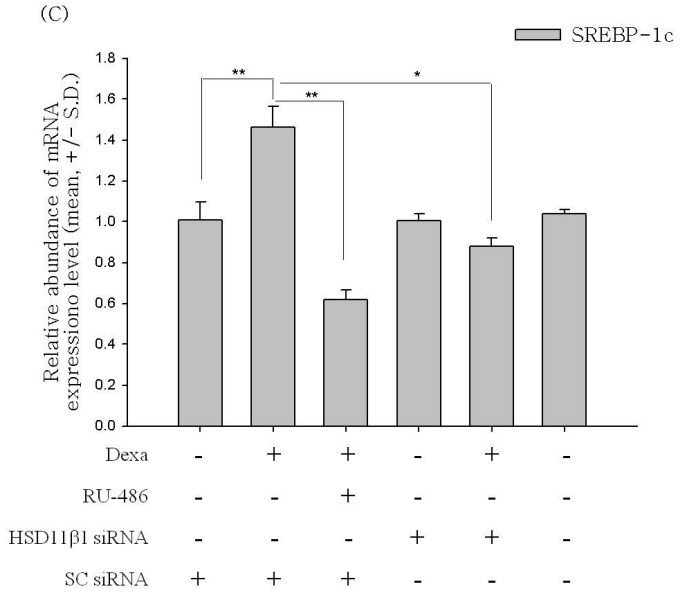
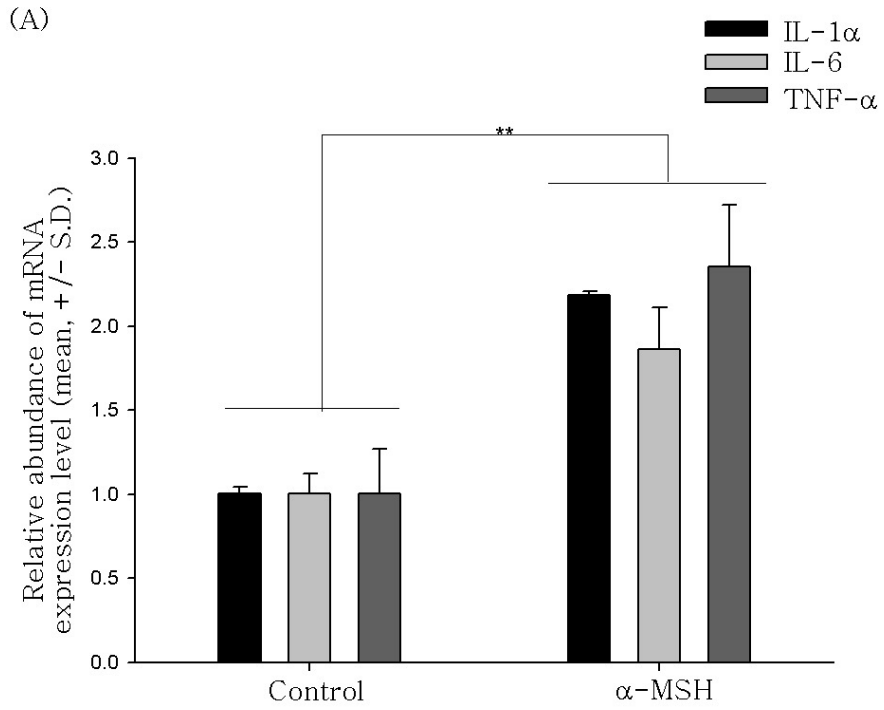


Figure 14. Dexamethasone did not affect SREBP-1a and 1c expression in SZ95 sebocytes transfected with the HSD11β1-direct siRNA. Expression analysis of the HSD11β1 gene in the SZ95 sebocytes was done by PCR. HSD11β1 expression in SZ95 sebocytes that were transfected SC siRNA pools was increased by dexamethasone (A). Co-treatment of RU-486 and dexamethasone inhibited HSD11β1 expression in SC siRNA pools transfected SZ95 sebocytes (A). On the other hand, dexamethasone did not affect the expression of HSD11β1 in SZ95 sebocytes that were transfected with HSD11β1-directed siRNA pools (A). Similarly, dexamethasone did not affect the expression of SREBP-1a and 1c mRNA in SZ95 sebocytes that were transfected with HSD11β1-directed siRNA pools (B and C).

Dexa : dexamethasone, SC siRNA : scramble siRNA, *: $P < 0.05$, **: $P < 0.01$

14. α-MSH induced mRNA expression of proinflammatory cytokines in SZ95 sebocytes.

To examine the expression of proinflammatory cytokines in SZ95 sebocytes that were treated with α-MSH, real-time quantitative PCR was performed. 0.1 μM α-MSH significantly increased the gene expression of proinflammatory cytokines including IL-1α, IL-6 and TNF-α in the SZ95 sebocytes (Figure 15 A). Similarly, 0.1 μM α-MSH also increased IL-1α, IL-6 and TNF-α gene expression in the differentiated SZ95 sebocytes (Figure 15 B).



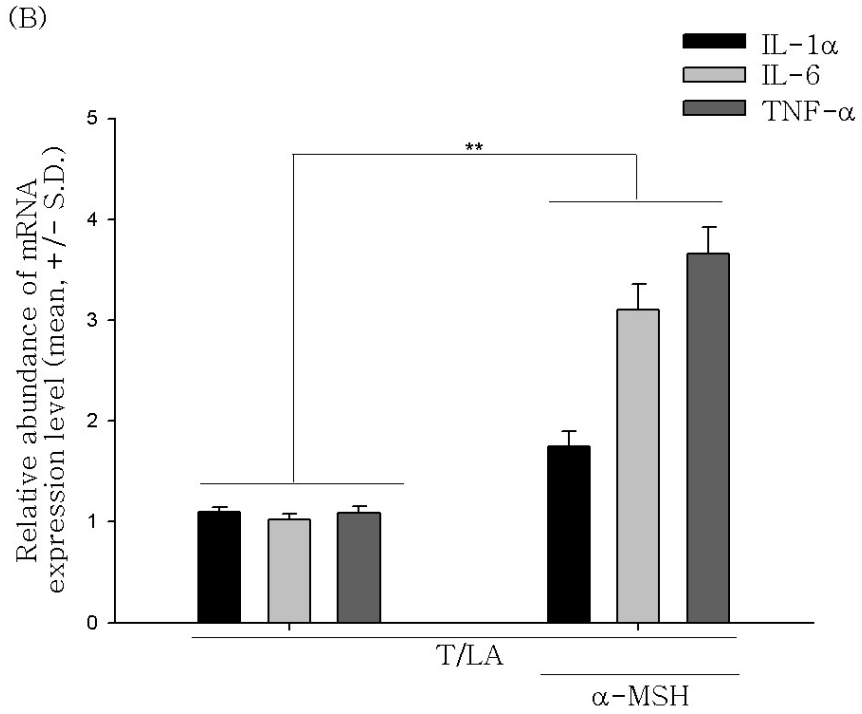


Figure 15. IL-1α, IL-6 and TNF-α mRNA expression were induced by α-MSH in undifferentiated and differentiated SZ95 sebocytes. IL-1α, IL-6 and TNF-α mRNA expression were determined in undifferentiated and differentiated SZ95 sebocytes after α-MSH treatment using real-time quantitative PCR (A and B). α-MSH induced mRNA expression level of IL-1α, IL-6 and TNF-α in undifferentiated and differentiated SZ95 sebocytes (A and B).

T/LA : testosterone and linoleic acid co-treatment, **: $P < 0.01$

15. α-MSH induced TLR-2 gene expression in the differentiated SZ95 sebocytes via MC5R.

To investigate the expression of toll-like receptor-2 (TLR-2) in SZ95 sebocytes treated with α-MSH, real-time quantitative PCR was performed. When 0.1 μM α-MSH treated to undifferentiated SZ95 sebocytes, gene expression of TLR-2 was decreased as compared to that of the untreated SZ95 sebocytes (Figure 16 A). On the other hand, 0.1 μM α-MSH increased TLR-2 gene expression in the differentiated SZ95 sebocytes (Figure 16 B).

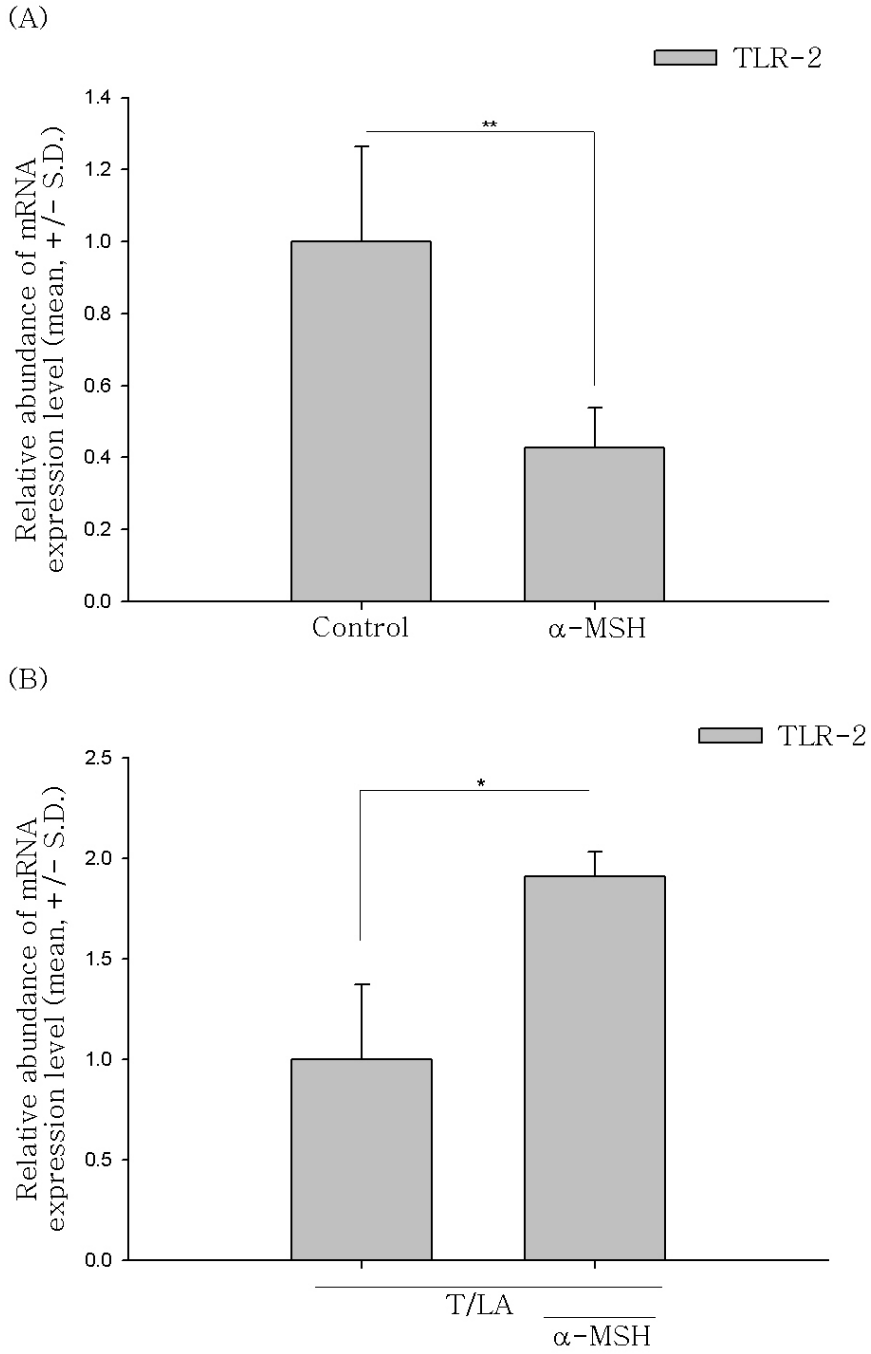


Figure 16. TLR-2 gene expression induced by α-MSH was only detected in differentiated SZ95 sebocytes. α-MSH decreased TLR-2 gene expression in undifferentiated SZ95 sebocytes (A). However, TLR-2 gene was increased by

α-MSH in differentiated SZ95 sebocytes (B).

T/LA : testosterone and linoleic acid co-treatment, * : $P < 0.05$

16. Proinflammatory cytokines increased HSD11β1 mRNA expression in SZ95 sebocytes.

After treatment with proinflammatory cytokines, the HSD11β1 mRNA level was increased by IL-1α, IL-6 and TNF-α in SZ95 sebocytes (Figure 17). However, IL-8 did not affect the expression of HSD11β1 mRNA expression in SZ95 sebocytes (Figure 17).

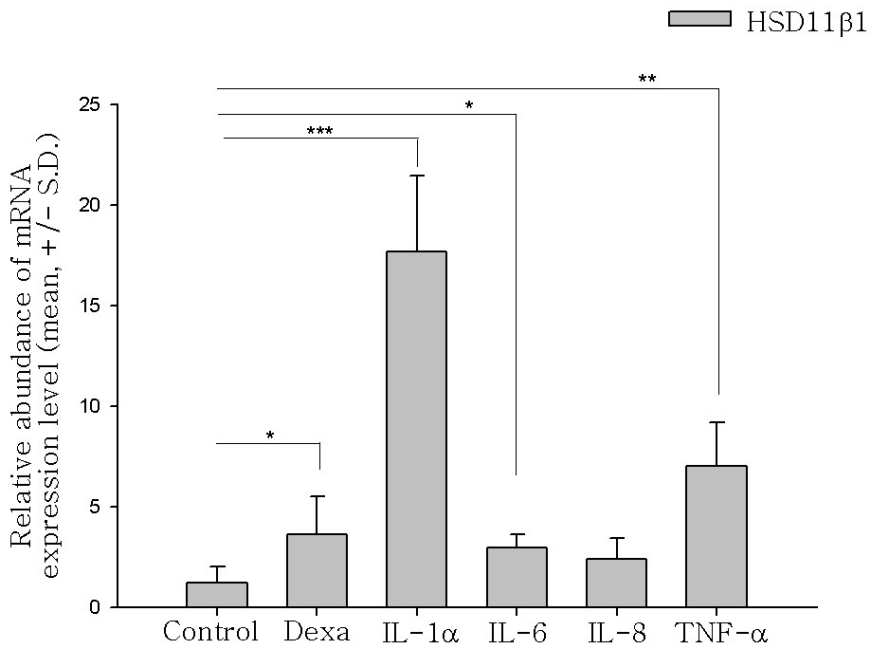


Figure 17. IL-1α, IL-6 and TNF-α but not IL-8 increased HSD11β1 mRNA expression level. Dexamethasone increased HSD11β1 mRNA expression in SZ95 sebocytes. Similarly, IL-1α, IL-6 and TNF-α induced HSD11β1 gene in SZ95 sebocytes but IL-6 did not induce HSD11β1 gene in SZ95 sebocytes. Dexa : dexamethasone, * : $P < 0.05$, ** : $P < 0.01$, *** : $P < 0.001$

17. TNF-α and dexamethasone increased TLR-2 gene expression in SZ95 sebocytes.

M Shibata (2009) found that two glucocorticoids, dexamethasone and cortisol, increased TLR-2 gene expression in cultured human keratinocytes without additional stimulation[45]. Moreover, glucocorticoids markedly enhanced TLR-2 gene expression in keratinocytes that had been stimulated with *P. acnes* or the inflammatory cytokines, TNF-α and IL-1α[45].

Therefore, to investigate the expression of TLR-2 in SZ95 sebocytes that were treated with dexamethasone, real-time quantitative PCR was performed. When 1 μM dexamethasone was applied as treatment to SZ95 sebocytes, the gene expression of TLR-2 was induced (Figure 18). In addition, RU-486, pioglitazone, and PF-915275 completely inhibited the induced TLR-2 gene expression by dexamethasone (Figure 18). Several inhibitors and activators of the mitogen-activated protein kinase (MAPK) were used to elucidate the mechanism underlying the enhancement of TLR-2 gene expression by dexamethasone in SZ95 sebocytes. Ro-31-8220, an inhibitor of MAPK phosphatase-1 (MAPK-1), inhibited the increment of TLR-2 gene expression by dexamethasone in SZ95 sebocytes (Figure 18). Anisomycin, an activator of p38 MAPK, also inhibited the increase in TLR-2 gene expression generated by the dexamethasone. On the other hand, the p38 MAPK inhibitor, SB202190, did not inhibit the increase in TLR-2 gene expression induced by dexamethasone (Figure 18).

Interestingly, TLR-2 gene expression was most increased by dexamethasone in SZ95 sebocytes that were stimulated with TNF-α (Figure 19).

The increment of TLR-2 gene expression in SZ95 sebocytes treated with dexamethasone and TNF-α was also inhibited by RO-31-8220 and anisomycin (Figure 19).

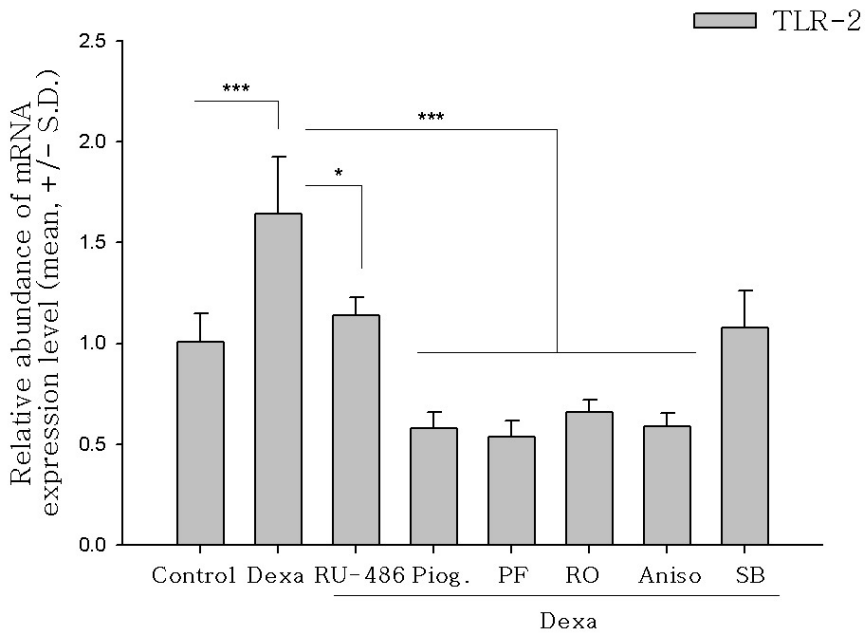


Figure 18. TLR-2 gene expression induced by dexamethasone was inhibited by RU-486, inhibitors of HSD11β1 and inhibitors of p38 MAPK. Dexamethasone increased TLR-2 gene expression in SZ95 sebocytes. RU-486 inhibited TLR-2 gene induction in dexamethasone treated SZ95 sebocytes. Also, TLR-2 gene induction was inhibited by pioglitazone, Ro31-8220 and anisomycin in dexamethasone treated SZ95 sebocytes. SB202190 did not inhibit the TLR-2 gene increased by dexamethasone in SZ95 sebocytes.

Dexa: dexamethasone, Pio: pioglitazone, PF: PF-915275, RO: Ro31-8220, SB: SB202190, Aniso: Anisomycin, *: $P < 0.05$, ***: $P < 0.001$

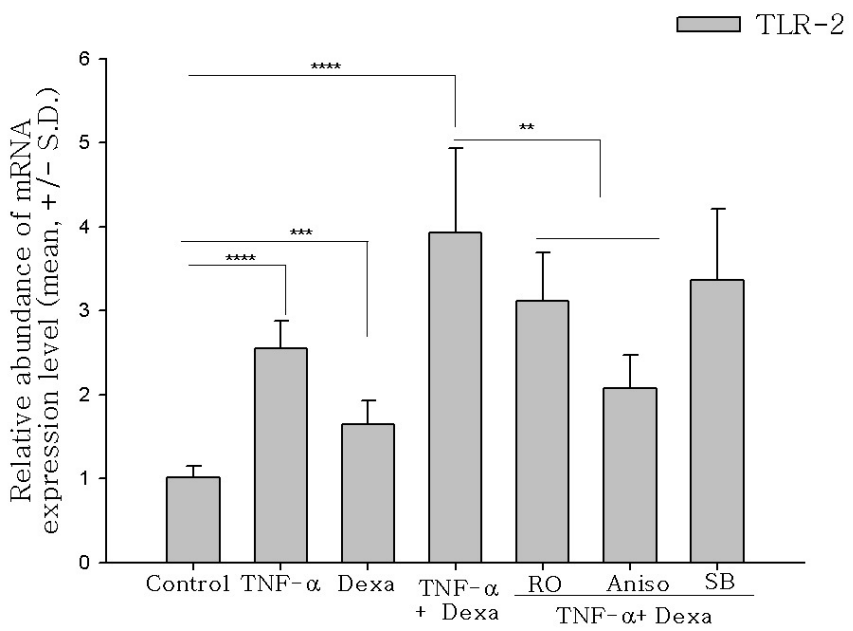


Figure 19. Dexamethasone induced TLR-2 gene expression in SZ95 sebocytes treated with TNF-α. TLR-2 gene expression was most increased by dexamethasone in SZ95 sebocytes that were stimulated with TNF-α. The induction of TLR-2 gene expression in SZ95 sebocytes treated with dexamethasone and TNF-α was also inhibited by RO-31-8220 and anisomycin. Dexa: dexamethasone, RO: Ro31-8220, SB: SB202190, Aniso: Anisomycin**,** **: $P < 0.01$, ***: $P < 0.001$

IV. DISCUSSION

In sebaceous glands and cultured human sebocytes, the expression of MC1R and MC5R was reported[16-17, 43]. Previously, it was reported that the expressions of these receptors were dependent on the differentiation status of sebocytes. While MC1R is constitutively expressed, MC5R expression is observed only in the differentiated sebocytes[43-44]. In line with previous reports, our result showed that MC5R was only detected on the differentiated SZ95 sebocytes induced by testosterone and linoleic acid. In this result, α-MSH increased only MC1R expression in undifferentiated SZ95 sebocytes and only MC5R expression in differentiated SZ95 sebocytes. These results suggest that in the undifferentiated SZ95 sebocytes, α-MSH mainly upregulates MC1R expression while in differentiated SZ95 sebocytes, α-MSH mainly upregulates MC5R expression. It was also observed that the effect of α-MSH on the lipogenesis of sebocytes was greater in the T/LA-induced differentiated sebocytes than in the undifferentiated cells. Based on these observations, these results suggest that the sebotrophic effect of α-MSH in SZ95 sebocytes via MC5R is superior to that of the sebotrophic effect happening via MC1R.

MCRs are seven-transmembrane G-protein coupled receptors that stimulate the cyclic adenosine monophosphate (cAMP) upon activation[47]. The MCRs-mediated intracellular signaling of α-MSH include the activation of adenylate cyclases that activates cAMP/PKA pathway, p38/MAP kinase pathway[48], activation of the signal transducer and activator of transcription (STAT)1 transcription factor[49], activation of PKC[50] and elevation of intracellular calcium[51]. The α-MSH-stimulated intracellular calcium ion concentration ($[Ca^{2+}]_i$) increase has been found in HEK 293 cells that were transfected with mouse MC5R[52-53]. It had been shown that MC peptides were able to modulate intracellular levels of calcium in normal human keratinocytes and HaCaT keratinocytes[54]. In a recent study, it was shown that α-MSH stimulation in SZ95 sebocytes via MC1R induces $[Ca^{2+}]_i$ elevation via the activation of PLC/IP_3/PKC pathways[19]. However, in that study, it was not investigated whether α-MSH/MC1R-mediated calcium signaling directly

regulates lipogenesis of SZ95 sebocytes.

SREBPs which belong to the family of transcription factors is known to regulate the transcriptional activation of various genes required for endogenous cholesterol, fatty acid (FA), triacylglycerol and phospholipid synthesis[55]. Currently, three SREBP isoforms—SREBP-1a, SREBP-1c, and SREBP2—have been identified and their different roles in lipid synthesis have been reported. SREBP-1c is involved in FA synthesis and insulin induced glucose metabolism. SREBP-2 is comparatively specific to cholesterol synthesis while SREBP-1a seems to be involved in both pathways[56]. In the presence of sterol, complexes of SREBP and SREBP-cleavage-activating protein (SCAP) reside in the endoplasmic reticulum (ER)[55]. However, in the absence of sterols, the SREBP-SCAP complex is free and travels to the Golgi apparatus to be processed[55]. Previously, it was reported that SREBP-1 regulated lipogenesis in the immortalized human sebocytes SEB-1, which is induced by insulin growth factor-1[57]. In this study, to investigate the effect of calcium signaling induced by α-MSH on lipogenesis of SZ95 sebocytes, SREBP-1 gene expression was observed. Based on the results, SREBP-1c was increased by α-MSH in undifferentiated and differentiated SZ95 sebocytes. Interestingly, in the differentiated SZ95 sebocytes, SREBP-1c mRNA expression was more increased than in undifferentiated SZ95 sebocytes. Moreover, SREBP-1c mRNA expression induced by α-MSH was inhibited by 2-APB and u-73122 in both SZ95 sebocytes. These results suggest that α-MSH induces lipid synthesis in SZ95 sebocytes by induction of SREBP-1c via the IP_3/PLC pathway.

Psychological stress can induce or exacerbate various skin disorders which are associated with abnormal permeability barrier function, including psoriasis and atopic dermatitis[58-60]. The secretion of glucocorticoids (GCs) is a classic endocrine response to stress. Excess GC from either an endogenous or exogenous source can result to abnormalities in epidermal structure and function[60]. It is well known that systemic administration of RU-486, GR antagonist, or antalarmin, inhibitor of CRH, can improve skin disorder induced by psychological stress[61-62]. In addition, since glucocorticoids antagonize the action of insulin and induce insulin resistance

when the amount is in excess, the pharmacological inhibition of HSD11β1 has been suggested to be a very attractive therapy for insulin resistance and other metabolic disorders such as type 2 diabetes, obesity and Cushing's syndrome[37-38, 63]. In this study, dexamethasone, a glucocorticoid, increased lipid droplets and HSD11β1 expression in SZ95 sebocytes. Moreover, RU-486 inhibited lipid synthesis and HSD11β1 expression induced by dexamethasone. These results indicate that the increase of HSD11β1 by dexamethasone via GR in SZ95 sebocytes enhances lipid synthesis in SZ95 sebocytes through augmentation of the GC reactivation. Furthermore, dexamethasone increased SREBP-1a and 1c gene expression in SZ95 sebocytes and the increment of SREBP-1a and 1c gene expression was inhibited RU-486. These results suggest that SREBP-1a and 1c involves lipid synthesis in SZ95 sebocytes by dexamethasone.

Peroxisome proliferator-related receptors (PPARs) are a subfamily of former 'orphan receptors' within the non-steroid receptor family of nuclear hormone receptors[64]. There are three subtypes (α,γ,δ); rat and human PPARδ are homologous to xPPARβ[65-67]. The finding, which states that PPARs have the potential to modulate lipogenesis, opens a new perspective on sebocyte pathophysiology [44, 68-70]. However, the distinct effect of PPAR isoforms on lipogenesis is not yet clear[44, 69-71]. The sebotrophic effect of PPARs is dependent on PPARs ligands[44]. M. Schuster, C.C *et al*., suggested that the sebostatic effect of PPARs not on the level of lipogenesis but by protecting cells from apoptosis and holocrine secretion[72]. It is well known that PPAR α and PPAR γ ligands reduce HSD11β1 activity and expression. Currently, PPARs are attractive therapeutic targets for antidiabetic therapy[73-75]. In this study, PPAR-γ agonist, pioglitazone, inhibited HSD11β1 expression and decreased the HSD11β1 expression increased by dexamethasone. Furthermore, pioglitazone alone did not induce lipid droplets in SZ95 sebocytes and inhibited increment of lipid droplets in the SZ95 sebocytes induced by dexamethasone. Based on results, PF-915275, a specific inhibitor of HSD11β inhibitor inhibited HSD11β1 gene expression, lipid droplets and SREBP-1 gene expression induced by dexamethasone. The present data suggest that

HSD11β1 involves lipid synthesis in SZ95 sebocytes; therefore, inhibitors of HSD11β1 including PPAR-γ agonist might have beneficial effects on sebum secretion by inhibiting the sebaceous lipogenesis.

In this study, α-MSH induced proinflammatory cytokines such as IL-1α, IL-6, and TNF-α, in undifferentiated and differentiated SZ95 sebocytes. Interestingly, in both SZ95 seobyctes, TNF-α mRNA expression was most increased by α-MSH. A number of proinflammatory cytokines, including IL-1α, IL-1β, IL-8, GM-CSF, and TNF-α, have been implicated in the inflammatory process of acne[76-78]. TNF-α, in particular, is regarded as a crucial cytokine in acne inflammation by involving a coordinated expression of adhesion molecules[79-81]. These results suggest that α-MSH contribute to the upregulation of IL-1α, IL-6, and TNF-α via MC1R and MC5R activation, thereby leading to the comedo formation, neutrophil infiltration, and sustained inflammation in the acne pathogenesis. Moreover, it is well known that inflammatory cytokines, such as IL-1β and TNF-α, induce HSD11β1 gene expression in adipocytes[22]. In present experiments, which use SZ95 sebocytes, it was found that the IL-1α, IL-6, and TNF-α increased HSD11β1 gene expression. These data suggest that proinflammatory cytokines contribute to the augmentation of GC reactivation.

It was also found through this study that α-MSH enhanced TLR-2 gene expression in differentiated SZ95 sebocytes. These data suggests that α-MSH may involve inflammatory and immune reactions in acne vulgaris lesions. Furthermore, dexamethasone remarkably enhanced TLR-2 gene expression in SZ95 sebocytes. The combination of dexamethasone and TNF-α synergistically increases TLR-2 gene expression. Recently, it was reported that synthetic corticosteroid budesonide enhances TLR-2 gene expression in both un-stimulated and lipopolysaccharide-treated keratinocytes[82].

This study tried to assess the intracellular signaling mechanism of glucocorticoid-enhanced TLR-2 gene expression by using several inhibitors and an activator. All of the data suggested that TLR-2 gene expression was negatively regulated by p38 mitogen-activated protein kinase (MAPK). These data suggest that p38 MAPK is

closely associated with dexamethasone-induced TLR-2 gene expression in SZ95 sebocytes.

Taken together, psychological stress induced α-MSH and glucocorticoids induce not only sebum secretion but also inflammation in acne vulgaris. The present findings suggest that receptors of α-MSH and HSD11β1 might be a future target for therapeutic intervention for the treatment of acne vulgaris.

V. CONCLUSION

The aim of this study is to investigate the sebogenesis-inducing effects of α-MSH, mediated by either MC1R or MC5R expression and differentiation status of sebocytes. This study also investigated the effects of HSD11β1 on sebogenesis in sebocytes. Lastly, this study investigated whether there is a possible cross-talk between α-MSH and HSD11β1 in acne vulgaris pathogenesis. The summary of the results are described below:

1. The effect of α-MSH on the lipogenesis of sebocytes was greater in the T/LA-induced differentiated sebocytes than in the undifferentiated cells. α-MSH induces lipid synthesis in SZ95 sebocytes by induction of SREBP-1c via the IP_3/PLC pathway.

2. SREBP-1a and 1c involves lipid synthesis in SZ95 sebocytes by dexamethasone. Specific inhibitors of HSD11β inhibited HSD11β1 gene expression, lipid droplets, and SREBP-1 gene expression that were induced by dexamethasone. The IL-1α, IL-6 and TNF-α increased HSD11β1 gene expression.

3. α-MSH enhanced TLR-2 gene expression in differentiated SZ95 sebocytes. Dexamethasone remarkably enhanced TLR-2 gene expression in SZ95 sebocytes. The combination of dexamethasone and TNF-α synergistically increased TLR-2 gene expression. TLR-2 gene expression was negatively regulated by p38 mitogen-activated protein kinase (MAPK).

In conclusion, psychological stress induced α-MSH and glucocorticoids induce not only sebum secretion but also inflammation in acne vulgaris.

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ABSTRACT (IN KOREAN)

α-멜라닌세포자극호르몬과 11β-하이드록시스테로이드 탈수산화효소가 SZ95 피지세포의 피지생성에 미치는 영향

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여드름은 모낭 피지기관의 만성 염증성 재발성 피부질환으로 임상적으로 면포, 농포, 결절, 낭종 등이 피지분비가 많은 얼굴, 목, 가슴 등에 나타나며 주로 사춘기에 발생하는 질환이다. 여드름의 병인은 모낭벽의 과각화, 피지분비의 증가, 여드름균의 증식 염증유발 등 크게 4가지로 언급되고 있다. 그러나 최근에는 이들 4가지 외에 정신적 스트레스에 의하여 여드름이 발생하는 기전을 추가적인 병인으로 제시하고 있다. 스트레스에 의해 시상하부－뇌하수체－부신축이 활성화 되면서 멜라노코르틴(melanocortin)과 당질코르티코이드 (glucocorticoids)와 같은 다양한 호르몬의 분비를 증가시킨다. 멜라노코르틴 중 하나인 α-멜라닌세포자극호르몬(α-MSH) 은 자신의 수용체인 MC1R 과 MC5R에 결합한다. α-MSH 은 인간 피지세포의 피지생성을 증가시키는 것으로 잘 알려져 있으며, 또한 최근 연구 결과에서 α-MSH이 피지세포의 MC1R을 통하여 세포 내 칼슘 이온 반응과 같은 중요 세포 신호 경로를 담당하는 것이 보고 되었다. 당질코르티코이드는 숙주의 중요한 생리조절활성기능을 조절한다. 비록 당질코르티코이드가 면역억제 및 항염증성 효능을 가지고 있지만, 임상적으로 전신 또는 국소 적인 당질코르티코이드 치료가 여드름 형성 반응을 불러일으키는 것 또한 잘 알려져 있다. 11β-하이드록시스테로이드 탈수산화효소 (HSD11β1)는 비활성 코르티손 (cortisone)을 활성화 된 코르티졸

(cortisol)로 전환하여 세포 내 당질코르티코이드 농도를 조절하는 역할을 한다.

인간의 피지세포가 α-MSH의 직접적인 세포 표적인 것은 자명해 보이나 아직까지 인간의 피지 샘에서 발현된 멜라노코르틴 수용체의 생물학적 영향에 대한 기전은 완전히 밝혀지지 않았다. 또한 당질코르티코이드가 피지생성에 미치는 영향 및 HSD11β1의 관여 가능성에 대한 연구는 아직까지 보고된 바 없다. 따라서 본 연구에서는 첫 번째로 피지세포의 분화 상태에 따라서 다르게 나타나는 MC1R 혹은 MC5R을 통한 α-MSH이 피지세포의 피지생성 및 염증성 사이토카인과 톨유사수용체-2 (toll-like receptor 2, TLR-2)의 발현에 미치는 영향에 대한 연구를 수행하였으며, 다음으로 피지세포에서의 HSD11β1의 발현과 사이토카인에 의한 발현 조절에 대한 연구 및 HSD11β1가 피지세포의 피지생성에 미치는 영향에 대한 연구를 수행하였다.

그 결과, MC5R의 발현은 오직 분화된 SZ95 피지세포에서만 확인 되었으며 분화된 세포 내에서 α-MSH에 의해 세포 내 피지 양의 증가가 분화되지 않은 세포에서 보다 더 높은 것을 확인하였다. 또한, SREBP-1a가 아닌 오직 SREBP-1c mRNA 의 발현만이 α-MSH에 의해 증가 되었으며, 이러한 증가는 IP_3 수용체의 길항제 및 PLC 저해제에 의해 억제되는 것을 미분화 세포 와 분화 세포에서 확인하였다. 다음으로 SZ95 피지세포 내에서 HSD11β1의 발현이 dexamethasone에 의해 증가하는 것을 확인하였으며 이는 당질코르티코이드 수용체의 길항제인 RU-486, 퍼옥시좀 증식체 활성화 수용체-γ (PPAR-γ) 활성제인 pioglitazone 및 HSD11β1 특정 억제제인 PF-915275에 의해 억제되었다. 이와 유사하게, dexamethasone에 의한 SZ95 피지세포의 피지생성 증가 또한 RU-486, PF-915275 및 pioglitazone에 의해 억제되었다. 뿐만 아니라 dexamethasone에 의한 SREBP-1a 과 1c의 mRNA의 발현 증가 역시 RU-486, PF-915275 및 pioglitazone에 의해 억제

되었다. 다음으로 α-MSH가 IL-1α, IL-6 그리고 TNF-α와 같은 염증성 사이토카인의 유전자 발현을 미분화 및 분화된 세포에서 모두 증가시키는 것을 확인하였다. 또한 이러한 IL-1α, IL-6 그리고 TNF-α를 SZ95 피지세포에 처리하였을 때 HSD11β1의 유전자 발현이 증가하는 것을 확인하였다. 마지막으로 α-MSH에 의한 TLR-2 유전자 발현의 증가는 오직 분화된 세포에서만 나타났다. dexamethasone 또한 SZ95 피지세포 내 TLR-2 유전자 발현을 증가 시키는 것을 확인하였으며 TNF-α와 함께 dexamethasone은 TLR-2 유전자 발현증가에 시너지 효과를 나타내었다.

이러한 모든 결과를 바탕으로 정신적인 스트레스에 의해 유도된 α-MSH와 당질코르티코이드는 여드름 에서의 피지 분비뿐만 아니라 염증반응에도 관여하는 것으로 보인다. 나아가서, 이러한 연구 결과를 바탕으로 α-MSH 수용체 와 HSD11β1는 여드름 치료를 위한 새로운 대상이 될 수 있다고 생각된다.

핵심되는 말 : 여드름, 피지세포, α-멜라닌세포자극 호르몬, 11β-하이드록시스테로이드 탈수산화효소, Toll-like receptor-2