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Original Article / 원저

Effects of *Raphani Semen* Ethanol Extracts on Skin Inflammation in HaCaT Keratinocytes

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萊菔子 에탄올 추출물이 HaCaT 피부각질형성세포에서 피부염증 감소에 미치는 영향

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Abstract

목적 : 본 연구는 인간피부각질형성세포(HaCaT keratinocytes) 모델을 TNF-α와 IFN-γ로 자극하여 萊菔子의 피부염증 감소 및 만성 염증성 질환에 미치는 영향을 알아보고자 하였다.

방법 : 萊菔子 에탄올 추출물(RSE)이 세포생존율에 미치는 영향을 확인하기 위하여 MTT assay를 시행하였다. 또한 RSE가 TNF-α와 IFN-γ로 자극한 HaCaT 세포에서 p-IκBα, p-ERK, p-JAK2, p-STAT1, p-STAT6의 발현과 periostin, TSLP 단백질 발현에 미치는 영향을 확인하였다.

결과 : RSE는 200㎍/㎖ 이하에서 세포 독성을 보이지 않았고, HaCaT keratinocytes에서 TNF-α와 IFN-γ자극에 의하여 증가된 IκBα, ERK의 인산화를 억제하였다. 또한 JAK2와 STAT1, STAT6의 인산화를 억제하였으며, periostin과 TSLP의 발현을 감소시켰다.

결론 : RSE는 HaCaT keratinocytes에서 pro-inflammatory cytokines 및 transcription factors의 발현을 감소시켜 피부염증 감소 효능을 보였고, 만성 염증성 질환에서 萊菔子의 사용 가능성을 확인하였다.

Key words : *Raphani semen*; HaCaT Keratinocyte; NF-κB/MAPK; JAK/STAT; TSLP/periostin

1. Introduction

Inflammation is an immune response in the skin against to the pathogenic microorganisms. However, impaired control of immune reaction can lead to chronic inflammation skin disease[1]. Skin consists of the epidermis, dermal layers, stratified keratinocytes, and dermis. As the outermost layer of the skin, stratum corneum consists of differentiated keratinocytes. The stratum corneum and skin surface microbial virulence factors including a wide range of molecules produced by pathogenic microorganisms play an important role in keeping the wholeness of the skin[2]. In this study, anti-inflammatory effects of Raphani semen ethanol extracts(RSE) were investigated through TNF-α and IFN-γ stimulated HaCaT keratinocytes.

There are two main theories regarding atopic dermatitis. Outside-in theory hypothesizes that the intrinsic damage to the skin barrier function caused by disturbed keratinocyte differentiation promotes the entry of allergens and activation of subsequent immune system. Meanwhile, the inside-out theory hypothesizes that the cascade of the immune response induced by Th2 activation in the skin results in the atopic dermatitis phenotype[3].

Leaves, seeds, and roots of *Raphanus sativus Linné* have been used for both edible and medical purposes. Above all, roots were mainly the subject of study, and the research on seeds was relatively small. Raphani semen, the dried ripe seed of *Raphanus sativus Linné* contains alkaloids, anthraquinon glycosides, terpenoids glycosides, steroids, tannins, carbohydrates, fats, oils, and flavonoids[4]. It has been reported that Raphani semen had anti-inflammatory and anti-asthmatic property and could be used for the treatment of intestinal inflammatory disorders[5,6]. Another study revealed that AITC, one of the ingredient of Raphani semen had inflammatory effects in human mast cell[7].

However, effects of Raphani semen in HaCaT keratinocytes were not investigated yet. So, this study aims to investigate the effects of RSE at various concentrations in an inflammatory environment induced by TNF-α and IFN-γ stimulated HaCaT keratinocytes through the expression of inflammatory cytokines and transcription factors.

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II. Materials and Methods

1. Reagents

Dulbecco's modified Eagle's medium(DMEM), fetal bovine serum(FBS), penicillin, and streptomycin were purchased from Life Technologies Inc.(Grand Island, NY, USA). 3-(4,5-Dimethylthiazol-2 -yl)-2, 5-diphenyltetrazolium bromide(MTT) reagent was imported from Sigma Chemical Co.(St. Louis, MO, USA). Primary antibodies against phospho-IκBα(cat. no. 2859), p-STAT1(cat. no. 9167), extracellular signal-regulated kinase(ERK)(cat. no. 9102), p-STAT6(cat. no. 56554), and STAT6(cat. no. 9362) were obtained from Cell Signaling Technology, Inc.(Danvers,

MA, USA). Primary antibodies against IκB-α(cat. no. sc-1643), p-ERK(cat. no. sc-7383), STAT1 (cat. no. sc-271661), periostin(cat. no. sc-398631), and β-actin(cat. no. sc-81178) as well as peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc.(Santa Cruz, CA, USA). Primary antibody against TSLP(cat no. ab188766) was purchased from Abcam, plc.(Cambridge, UK).

2. Preparation of RSE

The *Raphani semen* was purchased in Nanum herb(Yeongchen, Gyeongbuk, Korea). Dried and ground Raphani semen(200g) were extracted with 2ℓ 70% EtOH by maceration and the extracts were filtered. The filterate was evaporated in a rotary vacuum evaporator at 40℃, freeze-dried in vacuum using a freeze dryer. Finally, 15.25g of extracts(at a concentration of 50㎍/㎖) were obtained from 214.57g of *Raphani semen* and stored at -20℃ for bioassays.

3. Cell culture and sample treatment

The HaCaT keratinocytes were provided by Prof. Kyung-Tae Lee(Kyung Hee University, Seoul, Korea). The cells were cultured in DMEM supplemented with 10% FBS, penicillin(100U/㎖), and streptomycin(100㎍/㎖) in a 37℃ and 5% CO_2 incubator. Cells were treated with 50㎍/㎖, 100㎍/㎖, and 200㎍/㎖ for 1hr prior to stimulation with TNF-α and IFN-γ(10g/㎖) for the indicated time.

4. MTT assay

HaCaT keratinocytes were plated at a density of 5×10^4cells/well in 96 well plates. To determine the appropriate concentration of RSE which has no effect on cell viability, MTT assay was performed at 24hrs following treatment of RSE with various concentrations in HaCaT keratinocytes. Next day, the cells were treated with 50㎕ of MTT(5㎎/㎖) for 4hrs. The formazan precipitate was dissolved in DMSO, and absorbance was measured at 540㎚ using an Epoch microplate spectrometer(BioTek, Winooski, VT, USA).

5. Western blot analysis

Lysated cells were suspended in protein extraction solution(PRO-PREP™, Intron Biotechnology, Seongnam, Republic of Korea) and incubated for 20min at 4℃. Cell debris was eliminated by micro-centrifugation, followed by immediate freezing of the supernatant. The protein supernatant concentration was measured by using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Inc. CA, USA) according to the manufacturer's instructions. Each protein sample(30㎍) were electro-blotted transferred onto a polyvinylidene fluoride(PVDF) membrane followed by separation using 8-12% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Membranes were incubated for 30min with blocking solution(2.5 or 5% skim milk) at room temperature, followed by incubation overnight with a 1:1,000 dilution of primary antibody at 4℃. Membranes were washed three times with Tris-buffered saline/ Tween 20(TBS-T) and incubated with a 1:2,500 dilution of horseradish peroxidase-conjugated secondary antibody for 2hrs at room temperature. Membranes were again washed three times with TBS-T and then

developed by enhanced chemiluminescence(GE Healthcare Life Sciences, Chalfont, UK). The chemiluminescent blots were imaged on film. Densitometry analysis of the western blots was done by using ImageJ software(National Institutes of Health, Bethesda, MD). The intensity of the individual bands on western blot analysis was measured by gel analyzer tools of ImageJ and normalized either to β-actin to its total protein. The graphs represent normalized values as fold change over control condition.

6. Statistical analysis

The data reported have been expressed as the mean±standard deviation(SD). Data were analyzed using one-way analysis of variance (ANOVA) with Dunnett's test, and p-values<0.05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism(Ver. 5.00 for Windows, San Diego, CA, USA).

Ⅲ. Results

1. Effects of RSE on cell viability in HaCaT keratinocytes.

Cytotoxicity of RSE was tested in HaCaT cells to determine the appropriate concentrations. HaCaT cells were treated with different concentration of RSE ranging from 7.8125 to 500 for 24hrs. Since the result showed cytotoxicity at a concentration of 250㎍/㎖, the lower concentrations of 50㎍/㎖, 100㎍/㎖, and 200㎍/㎖ of RSE were set as the sample treatment concentration in this study(Fig. 1).

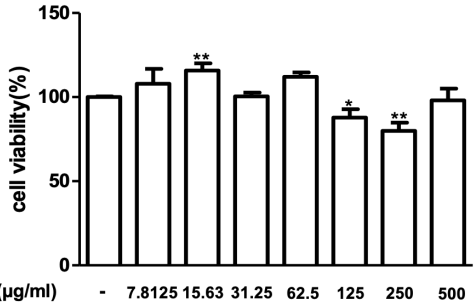

Fig. 1. Effects of RSE on Cell Viability in HaCaT Keratinocytes

HaCaT keratinocytes were incubated with the mentioned doses of RSE for 24hrs and cell viability was measured by MTT assay. The data shown represent mean±SD of three independent experiments. *p<0.05 and **p<0.01 vs the RSE non-treated group. RSE : Raphani semen ethanol extracts

2. Effects of RSE on p-IκBα expression in TNF-α and IFN-γ stimulated HaCaT keratinocytes.

Nuclear factor-κB(NF-κB), a transcription factor, exists in cytoplasm binding to inhibitor of kappa B alpha(IκBα). When IκBα is phosphorylated during an inflammatory reaction, NF-κB translocates to the nucleus, leading to transcription of iNOS, COX-2[8]. Therefore, Western blot assay was performed to confirm the expression of phosphorylation of IκBα(p-IκBα) protein inducing activation of NF-κB. In result, the RSE treatment of 100㎍/㎖ and 200㎍/㎖ significantly suppressed p-IκBα expression in TNF-α and IFN-γ stimulated HaCaT keratinocytes (Fig. 2).

3. Effects of RSE on p-ERK expression in TNF-α and IFN-γ stimulated HaCaT keratinocytes.

In addition to p-IκBα expression, the

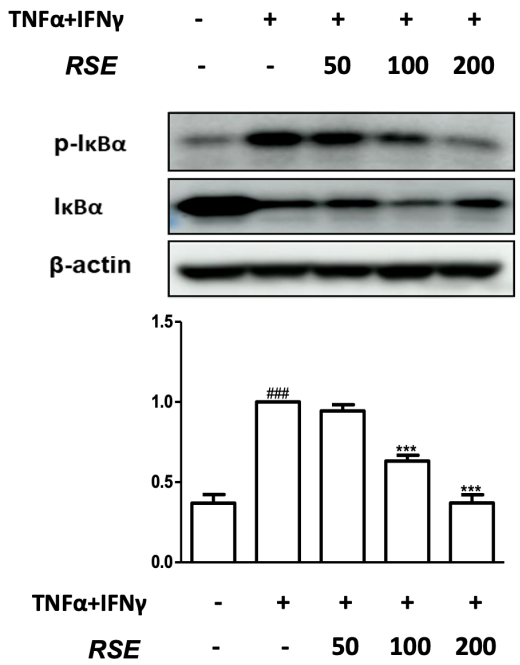

Fig. 2. Effects of RSE on p-IκBα Expression in TNF-α and IFN-γ Stimulated HaCaT Keratinocytes

HaCaT keratinocytes were pre-treated with Raphani semen for 1hr, and stimulated by TNF-α and IFN-γ (10ng/㎖), and the cells were incubated for 24hrs. Total proteins were prepared and western blotted for p-IκBα and IκBα using specific antibodies. β-actin is used as an internal control. Densitometry analysis of the blots was done using Image J software. The data shown represent mean±SD of three independent experiments. ###p<0.001 vs the control group, ***p<0.001 vs the TNF-α and IFN-γ treated group. RSE : Raphani semen ethanol extracts

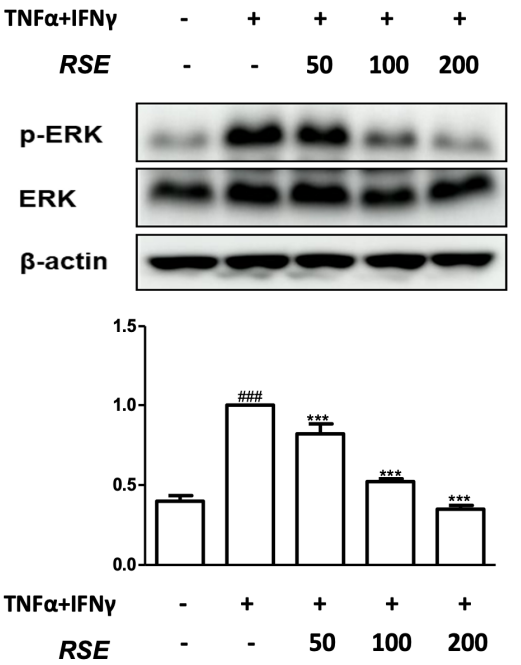

Fig. 3. Effects of RSE on p-ERK Expression in TNF-α and IFN-γ Stimulated HaCaT Keratinocytes

HaCaT keratinocytes were pre-treated with Raphani semen for 1hr, and stimulated by TNF-α and IFN-γ (10ng/㎖), and the cells were incubated for 24hrs. Total proteins were prepared and western blotted for p-ERK and ERK using specific antibodies. β-actin is used as an internal control. Densitometry analysis of the blots was done using Image J software. The data shown represent mean±SD of three independent experiments. ###p<0.001 vs the control group, ***p<0.001 vs the TNF-α and IFN-γ treated group. RSE : Raphani semen ethanol extracts

phosphorylation of mitogen-activated protein kinases(MAPK) which is involved in the activation of NF-κB was also confirmed. MAPKs consist of ERK, JNK, and p38. When MAPK pathway is activated, it increases cell activity and differentiation, playing an important role in the occurrence of inflammation. ERK activated by IL-1 and TNF-α is associated with signals that induce growth and differentiation of T cell and regulates the synthesis of IL-6, IL-12, IL-23, and TNF-α. Therefore, in order to determine whether the inhibitory mechanism of RSE passes through MAPK pathway, the phosphorylation of ERK was confirmed through western blot analysis[9-11].

RSE reduced p-ERK expression in dose dependent manner, indicating that RSE inactivated NF-κB signaling pathway through suppressing the phosphorylation of IκBα and ERK(Fig. 2, 3).

4. Effects of RSE on p-JAK2 expression in TNF-α and IFN-γ stimulated HaCaT keratinocytes.

JAK2/STAT1 pathway is one of the inflammatory s ignals activated by pro-inflammatory stimulation and plays an important role in iNOS expression with NF-κB signals[12]. To gain insight into the in hibitory mechanism of RSE on the Janus kinases (JAK)/signal transducer and activator of transcri ption proteins(STAT) signal cascade, we examine d the effects of RSE on p-JAK2 expression. The expression levels of p-JAK in the TNF-α and IF N-γ stimulated group significantly increased co mpared to the control group. In contrast, treatm ent with 50μg/㎖, 100μg/㎖, and 200μg/㎖ of RSE markedly reduced expression of p-JAK(Fig. 4).

5. Effects of RSE on p-STAT1 expression in TNF-α and IFN-γ stimulated HaCaT keratinocytes.

In response to cytokines like TNF-α and IFN-γ, STAT is activated by JAK kinases and MAP kinases[13]. So, we investigated the inhibitory effects of RSE on STAT1 phosphorylation using a western blot analysis. Results indicate that RSE significantly inhibited STAT1 phosphorylation in dose-dependent manner(Fig. 5).

6. Effects of RSE on p-STAT6 expression in TNF-α and IFN-γ stimulated HaCaT keratinocytes.

STAT6 activation is generally induced by stimulating IL-4 receptor. Activated STAT6 translocates from the cytoplasm to the nucleus, inducing target genes including IL-4, IL-5, and IL-13[14]. At the highest concentration of RSE(200μg/㎖) significantly suppressed expression of p-STAT6 (Fig. 6).

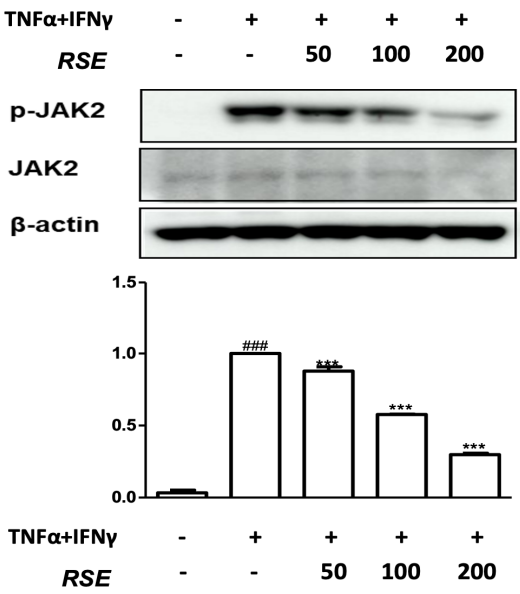

Fig. 4. Effects of RSE on p-JAK2 Expression in TNF-α and IFN-γ Stimulated HaCaT Keratinocytes

HaCaT keratinocytes were pre-treated with Raphani semen for 1hr, and stimulated by TNF-α and IFN-γ (10ng/㎖), and the cells were incubated for 24hrs. Total proteins were prepared and western blotted for p-JAK2 and JAK2 using specific antibodies. β-actin is used as an internal control. Densitometry analysis of the blots was done using Image J software. The data shown represent mean±SD of three independent experiments. ###p<0.001 vs the control group, ***p<0.001 vs the TNF-α and IFN-γ treated group. RSE : Raphani semen ethanol extracts

These results demonstrate that RSE inhibits the phosphorylation of STAT1 and STAT6 via JAK2 inactivation in TNF-α and IFN-γ induced inflammatory condition(Fig. 4-6).

7. Effects of RSE on periostin expression in TNF-α and IFN-γ stimulated HaCaT keratinocytes.

Periostin is well-known to exacerbate allergic

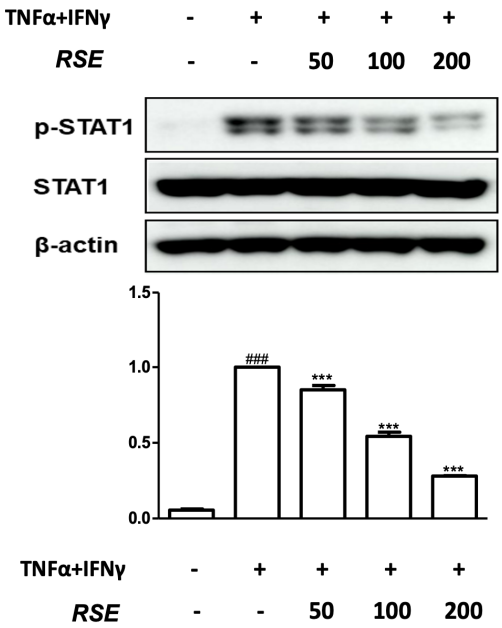

Fig. 5. Effects of RSE on p-STAT1 Expression in TNF-α and IFN-γ Stimulated HaCaT Keratinocytes

HaCaT keratinocytes were pre-treated with Raphani semen for 1hr, and stimulated by TNF-α and IFN-γ (10ng/㎖), and the cells were incubated for 24hrs. Total proteins were prepared and western blotted for p-STAT1 and STAT1 using specific antibodies. β-actin is used as an internal control. Densitometry analysis of the blots was done using Image J software. The data shown represent mean±SD of three independent experiments. ###p<0.001 vs the control group, ***p<0.001 vs the TNF-α and IFN-γ treated group. RSE : Raphani semen ethanol extracts

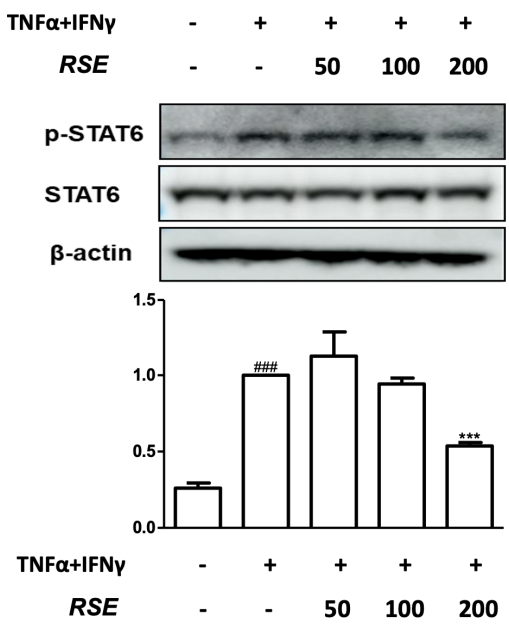

Fig. 6. Effects of RSE on p-STAT6 Expression in TNF-α and IFN-γ Stimulated HaCaT Keratinocytes

HaCaT keratinocytes were pre-treated with Raphani semen for 1hr, and stimulated by TNF-α and IFN-γ (10ng/㎖), and the cells were incubated for 24hrs. Total proteins were prepared and western blotted for p-STAT6 and STAT6 using specific antibodies. β-actin is used as an internal control. Densitometry analysis of the blots was done using Image J software. The data shown represent mean±SD of three independent experiments. ###p<0.001 vs the control group, ***p<0.001 vs the TNF-α and IFN-γ treated group. RSE : Raphani semen ethanol extracts

dermatitis by inducing the production of Thymic stromal lymphopoietin(TSLP) from keratinocytes, and serum periostin can reflect the severity of atopic dermatitis[15]. The expression of periostin requires the activation of STAT6[16].

The 200㎍/㎖ of RSE markedly suppressed expression of periostin(Fig. 8). RSE could decrease the expression of periostin via inhibition of p-STAT6 in HaCaT keratinocytes.

8. Effects of RSE on TSLP expression in TNF-α and IFN-γstimulated HaCaT keratinocytes.

TSLP promotes an inflammatory Th2 response by activation of dendritic cells, playing significant role in atopic dermatitis and other inflammatory disease. In epithelial cells, especially keratinocytes, TSLP was highly increased from atopic dermatitis subjects compared with healthy subjects[17].

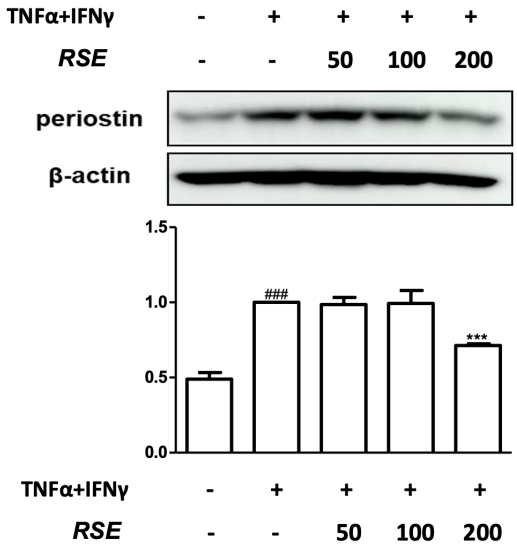

Fig. 7. Effects of RSE on periostin Expression in TNF-α and IFN-γ Stimulated HaCaT Keratinocytes

HaCaT keratinocytes were pre-treated with Raphani semen for 1hr, and stimulated by TNF-α and IFN-γ (10ng/㎖), and the cells were incubated for 24hrs. Total proteins were prepared and western blotted for periostin using specific antibodies. β-actin is used as an internal control. Densitometry analysis of the blots was done using Image J software. The data shown represent mean±SD of three independent experiments. ###p<0.001 vs the control group, ***p<0.001 vs the TNF-α and IFN-γ treated group. RSE : Raphani semen ethanol extracts

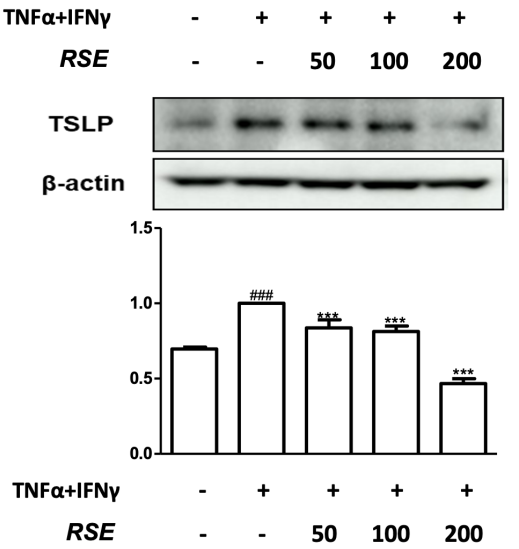

Fig. 8. Effects of RSE on TSLP Expression in TNF-α and IFN-γ Stimulated HaCaT Keratinocytes

HaCaT keratinocytes were pre-treated with Raphani semen for 1hr, and stimulated by TNF-α and IFN-γ (10ng/㎖), and the cells were incubated for 24hrs. Total proteins were prepared and western blotted for TSLP using specific antibodies. β-actin is used as an internal control. Densitometry analysis of the blots was done using Image J software. The data shown represent mean±SD of three independent experiments. ###p<0.001 vs the control group, ***p<0.001 vs the TNF-α and IFN-γ treated group. RSE : Raphani semen ethanol extracts, TSLP : Thymic stromal lymphopoietin

TNF-α and IFN-γ significantly increased the TSLP expression in HaCaT keratinocytes. However, RSE reduced the TSLP expression(Fig. 8). Production of TSLP is induced by periostin, and TSLP can induce the phosphorylation of STAT6[15,18]. These results suggest that RSE has anti-inflammatory effects by suppressing the production of TSLP via inhibiting the expression p-STAT6 and periostin in HaCaT keratinocytes.

Ⅳ. Discussion

The *Raphani semen* has been traditionally used as laxative, carminative, expectorant, and antitussive in Korea and China[19]. It has been demonstrated that Raphani semen has anti-bacterial, anti-inflammatory, antioxidant, anti-angiogenic, ACE inhibitory, anti-diabetic, expectorant, diuretic, and laxative effects[20-3]. The extraction of leaves has diuretic and laxative effects[24,25]. Also, the extraction of roots could be used for inflammatory disease, urinary problems, hemorrhoids, gastric illnesses, fungal disease, and D-galactosamine induced nephrotoxicity[24,26,27]. In previous studies,

anti-inflammatory functions of Rapahni semen were attributed to the inhibition of NF-κB, p-ERK, and IL-6 in HMC-1 cells and inactivating p38 MAPK and NF-κB pathways in experimental ulcerative colitis models[5-7]. Thus, in this study we suggest another important anti-inflammatory mechanism of RSE in HaCaT keratinocytes.

Keratinocytes, which account for 95% of epidermal cells, not only produce stratum corneums of the epidermis, but also produce several cytokines and play an important role in inflammatory diseases of the skin[28]. The human keratinocyte cell line(HaCaT) which is derived from human epidermal keratinocyte line and most similar to human keratinocytes has been used in the *in vitro*[29]. In this study, to make similar condition to atopic dermatitis skin we stimulated HaCaT cell with TNF-α and IFN-γ.

Atopic dermatitis inflammation is imbalance of Th1/Th2 immune reaction and IgE induced inflammation. In acute stage of atopic dermatitis Th2 signal dominates, meanwhile in a chronic state, Th2 switch to Th1 signal[30-2]. Th1 cells produce TNF-α and IFN-γ in chronic atopic dermatitis skin lesions. In HaCaT cells, stimulation of TNF-α and IFN-γ induces Th-2 related chemokines and cytokines like IL-1, IL-6, and IL-8[33-5].

In order to control the immune and inflammatory reactions, cytokine binds to a specific receptor and then transmits a signal to the target cell nucleus through a signal transmission pathway. Among the signaling pathways of cytokine, NF-κB and JAK/STAT are important transcription factors, and after being produced in ribosomes, they are introduced into the nucleus to promote gene expression, so inhibition of these actions can be crucial in treating atopic dermatitis[36].

NF-κB is present in cytoplasm by binding with IκBα in the form of heterodimerization[8]. However, stimuli induces cells to make IKK complexes composed of IKKα, IKKβ, and IKKγ, also called NF-κB essential modulator(NEMO) get activated[37]. The activated cells cause phosphorylation and 26 proteasome-mediated degradation of IκB which frees NF-κB. Free NF-κB is translocated into the nucleus and binds to DNA, which stimulates the expression of the target gene, causing inflammatory diseases[8].

NF-κB is activated by MAPKs. MAPKs activated by NO, cytokines, EGF, and pathogens induce the production of COX-2, cytokines, and control the cell activation and differentiation. MAPKs consist of three main groups: ERK, p38, and JNK, regulated with each other through associated signaling systems, and plays an important role in signaling systems of immune responses by regulating inflammatory responses in relation to activation of NF-κB[9,38]. ERK subfamily consists of ERK1, ERK2, and other atypical forms, such as ERK3, ERK4, ERK5, and ERK7. ERK1 and ERK2 are mainly found, so ERK 1/2 pathway has been involved in cell proliferation, growth, differentiation as well as cell migration, cell survival, and transcription[39]. In this study, RSE suppressed phosphorylation of IκBα and ERK, however phosphorylation of JNK and p38 did not show significant results. Suppressed expression of p-IκBα and p-ERK by RSE indicates that RSE has anti-inflammatory

effect through inactivating the NF-κB pathway via inhibition of MAPK pathway(Fig. 2, 3).

Another important transcription factor in signaling pathways of cytokines and growth factors is JAK/STAT pathway. JAK2/STAT1 pathway is one of the inflammatory signals activated by pro-inflammatory stimulation and plays an important role in iNOS expression with NF-κB signals[40]. JAK group including JAK1, JAK2, JAK3, and TYK2 is an pivotal proteins in signal transduction. STATs are activated by phosphorylation of JAK activation which requires the binding of ligands to cytokines and growth factors receptors[12]. Especially JAK2 is related with down stream proteins like STATs. So, JAK/STAT has been a major signaling pathway for crucial role in inflammation and cancer[41]. Induced by IFN-γ, JAK kinases directly and indirectly phosphorylate STAT1 at tyrosine residue at position 701(Tyr^{701}) and serine residue at position 727(Ser^{727}) respectively in a gPI3K-PKC dependent manner[42]. p-STAT1 converts into nucleus that activates transcription of pro-inflammatory media. A study on murine B cells indicated that the prolonged activation of the transcription factor STAT6 in B cells during chronic allergic inflammation resulted in IgE responses to viral and bacterial antigens resulting from the subsequent microbial infection that follows the onset of atopic dermatitis. These IgE responses stimulated further activation of mast cells resulting in inflammation[43]. It was observed that RSE inhibited STAT1 and STAT6 phospholyation via JAK2 inactivation in TNF-α and IFN-γ induced inflammatory condition(Fig. 4-6).

Through JAK/STAT pathway, keratinocytes secrete periostin in response to cytokine TSLP[44]. It was confirmed that atopic dermatitis did not occur when an antibody preventing the binding of periostin and receptor was administered to experimental mice and then house dust mite extract was applied to the mouse skin[16]. Also, according to the results of the recent study, serum periostin was significantly increased in children with exogenous atopic dermatitis, and also found to be correlated with SCORAD index and peripheral blood eosinophils. It means that periostin could be an indicator for understanding the pathological mechanism, degree of symptoms, and prognosis of atopic dermatitis[45]. TSLP acts as an initial stimulation factor for the skin immune response. When the skin is damaged and exposed to external antigens, TSLP is secreted from keratinocytes, inducing the secretion of inflammatory cytokines including IL-4, IL-13, IL-5, and TNF-α, but producing decreased levels of IL-10 and IFN-γ. IL-10 and IFN-γ down-regulate Th2 inflammation, especially IFN-γ distorts the Th1/Th2 balance in differentiating T cells[17,46].

Allergens such as house dust mite initiate inflammatory responses, then lymphocytes increase the expression of periostin in dermis. So periostin is acculmulated in dermis, resulting in up-regulating proliferation of TSLP-producing keratinocytes as well as promoting induction of TSLP expression which contributes to the accelerating Th2 inflammation. It means that periostin contributes to chronic inflammation and deteriorates allergic dermatitis by allowing immune cells to interact with non-immune

cells[15,16]. In the present study, RSE suppressed the expression of p-STAT6, periostin, and TSLP, suggesting that RSE could stop the loop involving the periostin(i.e., p-STAT6 → periostin → TSLP → p-STAT6 → periostin)(Fig. 6-8).

This study verified for the first time that RSE inhibited NF-κB and JAK/STAT pathway in inflammatory environment induced by TNF-α and IFN-γ stimulated HaCaT keratinocytes. Also, the possibility of RSE as an external preparation in inflammatory skin diseases such as atopic dermatitis was proved in this study.

Ⅴ. Conclusions

This study demonstrated mechanism of anti-inflammatory effects of RSE in TNF-α and IFN-γ stimulated HaCaT keratinocytes.

1. HaCaT cells were treated with RSE, and RSE did not show cytotoxicity below 250㎍/㎖, so concentrations of 50㎍/㎖, 100㎍/㎖, and 200㎍/㎖ of RSE were used in this study.
2. RSE suppressed the phosphorylation of IκBα and ERK in TNF-α and IFN-γ stimulated HaCaT keratinocytes.
3. RSE suppressed the phosphorylation of JAK2, STAT1, and STAT6 in TNF-α and IFN-γ stimulated HaCaT keratinocytes.
4. RSE inhibited the expression of periostin and TSLP in TNF-α and IFN-γ stimulated HaCaT keratinocytes.

These results showed that RSE could be used as a treatment for inflammatory disease through inhibiting NF-κB, MAPK and JAK/STAT pathways and suppressing the expression of periostin and TSLP.

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