Therapeutic effect of resveratrol on oxidative stress in Graves' ophthalmopathy orbital fibroblasts

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Directed by Professor Jin Sook Yoon

Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

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December 2014

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December 2014

ACKNOWLEDGEMENTS

I want to acknowledge the support of many people who have helped me to complete this work. First of all, I want to express my heartfelt gratitude to my preceptor, Prof. Jin Sook Yoon. She not only taught me, but also stimulated my interest. Her enthusiasm and constant exertions are great inspiration to me. I am also appreciative to Prof. Eun Jig Lee, Prof. Joon Haeng Lee, Prof. Suk-Woo Yang, and Prof. Kyungsoo Park for advising me to perform this study. This work would not have been possible without the help from Hyun Jung Lee and Min Kyung Chae. Many of teachers in medical school and residency made lasting impressions on me. To them I also offer my most sincere thanks.

Special thanks to my mentor, Prof. Sang Yeul Lee for directing and raising me. I am indebted to him and really respect him. He is the best thing that ever happened to me.

It is also appropriate to acknowledge my deep and sincere appreciation to my family and relatives, especially my elder brother and his wife who give a good model as a scholar to me, my lovely nieces, my mother-in-law who always provides confidence and support for me, and my reassuring brother-in-laws.

I am profoundly grateful to my parents for making me what I am. My glory lies in their love and sacrifices.

Above all things, I wish to thank my loving wife, Ji Yeun, and two children, Hyun Min and Hyun Yoo, the foundation of my life.

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<ABSTRACT>

Therapeutic effect of resveratrol on oxidative stress in Graves' ophthalmopathy orbital fibroblasts

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Graves' ophthalmopathy (GO) is a chronic inflammatory autoimmune disease that causes physical pain and considerable distress. However, it is difficult to manage patients with GO because multiple causative factors complicate its pathogenesis. Glucocorticoids, the most commonly used treatment for GO, have limited utility and many side effects. Recently, it has been suggested that oxidative stress contributes to the development and progression of GO. Therefore, we investigated the therapeutic effect of resveratrol, a potent antioxidant, on oxidative stress levels in GO orbital fibroblasts *in vitro*. Orbital fibroblasts were cultured from orbital connective tissues obtained from GO patients, and the production of reactive oxygen species (ROS) was induced with 2% cigarette smoke extract or 10 μM hydrogen peroxide. Treatment with 30 or 50 μM resveratrol resulted in reduced ROS production, and lower levels of oxidative stress-induced heme oxygenase-1. We also differentiated orbital

fibroblasts into adipocytes and found that resveratrol suppressed adipogenesis, reducing the number of adipocytes and suppressing the accumulation of lipid droplets. Treatment with 50 μM resveratrol also decreased ROS levels during adipogenesis. The extracellular signal-regulated kinase and c-Jun NH(2)-terminal kinase signaling pathways seemed to be associated with these results. This study supports the potential use of resveratrol in GO treatment.

Key words : adipogenesis, Graves' ophthalmopathy, orbital fibroblast, oxidative stress, reactive oxygen species, resveratrol

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I. INTRODUCTION

Graves' disease is a representative autoimmune disease affecting the thyroid gland. It is the most common cause of hyperthyroidism, and in 25% to 50% of cases the eye is involved as well.[1] Graves'ophthalmopathy (GO) manifests in various clinical signs and symptoms, ranging from mild ocular discomfort to severe pain and diplopia, eyelid retraction, chemosis, edema and erythema of periorbital tissues, proptosis, and even compressive optic neuropathy. The primary causes of GO pathogenesis are abnormal excessive hyaluronic acid accumulation and orbital adipogenesis resulting from autoimmune processes through thyrotropin receptors (TSHRs) and insulin-like growth factor 1 receptors.[1] Enlargement of the extraocular muscles and expansion of the orbital fatty connective tissues follow; orbital fibroblasts are known

to be the primary target cells of the disease process.[1-3]

GO causes patients physical pain and considerable distress. It is difficult to manage patients with GO because multiple causative factors complicate its pathogenesis. Glucocorticoids, the most common treatment for GO, are not very effective for the treatment of proptosis and the longstanding extraocular muscle involvement associated with fibrotic changes; they are also associated with many side effects.[4] Well-known and occasionally serious complications include cushingoid features, diabetes, hypertension, and osteoporosis. Thus, more effective, and safer long-term therapeutic agents for GO are required.

A large number of studies suggest that oxidative stress is associated with GO pathogenesis. The hypothesis that the formation of oxygen radicals is involved in the initiation of the immune response in Graves' disease was introduced in the 1980s.[5] Subsequently, a study in an experimental animal model showed that thyroid hormone enhances reactive oxygen species (ROS) generation and produces changes in tissue antioxidant systems.[6] Burch et al. have suggested that oxygen free radicals contribute to retroocular fibroblast proliferation in GO.[7] Bednarek et al. have also proposed that thyroid hormones accelerate basal metabolic rate and oxidative metabolism via the induction of mitochondrial enzymes and thyroid metabolic status influences changes in blood extracellular indices of ROS generation and free radical scavenging in Graves'disease.[8] Indeed, a number of recent studies have demonstrated that oxidative stress may play a role in the pathogenesis of GO.[9-11] Moreover, the hypothesis that GO orbital fibroblasts are hypersensitive to oxidative

stress has been proposed,[12] and several studies have demonstrated ROS in the retroocular fibroblasts and plasma of patients with GO.[13, 14] Finally Tsai et al. have detected oxidative deoxyribonucleic acid (DNA) damage in the urine of GO patients and demonstrated a positive correlation between this damage and clinical activity of GO.[9]

Given the focus on oxidative stress in GO pathogenesis, in this study we investigated the therapeutic effect of resveratrol (3,5,4'-trihydroxystilbene, Figure 1.), a potent antioxidant, in an *in vitro* model of GO.

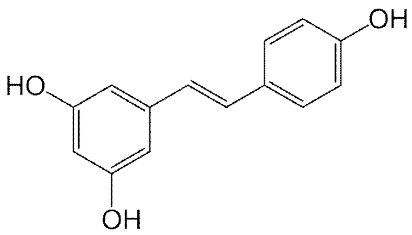


Figure 1. Chemical structure of resveratrol.

II. MATERIALS AND METHODS

1. Reagents

Resveratrol, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, the fluorescent probe propidium iodide (PI) and Oil Red O were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and gentamycin were purchased from Hyclone Laboratories,

Inc. (Logan, UT, USA). Fluorescent probes 2'7'-dichlorofluorescin (DCF) and dihydroethidine (DHE) were purchased from Molecular Probes, Invitrogen (Eugene, OR, USA). Insulin, carbaprostaglandin, and rosiglitazone were purchased from Boehringer-Mannheim (Manheim, Germany), Calbiochem (La Jolla, CA, USA), and Cayman (Ann Arbor, MI, USA), respectively. Anti- heme oxygenase (HO)-1 antibody, anti-extracellular signal-regulated kinase (ERK) antibody, anti-phospho-ERK antibody, anti-c-Jun NH(2)-terminal kinase (JNK) antibody, anti-phospho-JNK antibody, and anti-β-actin antibody were all obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Inhibitors of ERK (PD98059 and U0126), JNK (SP600125), protein kinase B (AKt/PKB; LY294002), and p38 (SB203580) were also purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2. Patients

Institutional Review Board (IRB)/Ethics Committee approval was obtained. This study adhered to the tenets of the Declaration of Helsinki, and written informed consent was obtained from all participants.

Orbital connective tissue was obtained from six GO patients who underwent orbital decompression surgery for severe proptosis. The patients did not have a history of radiotherapy and did not take steroid medication for at least 3 months prior to the surgery. They had euthyroid status and a clinical activity score < 4 at the time of surgery. Four control tissue samples were obtained from patients who underwent orbital wall reconstruction surgery for orbital wall fracture. Controls did not have

a history of GO or hyperthyroidism.

3. Orbital fibroblast culture

Orbital fibroblasts were cultured using published methods.[4] Tissue explants were minced and placed directly in culture dishes with DMEM containing 20% FBS, penicillin (100 U/mL), and gentamycin (20 μg/mL). Cells were incubated in a humidified 5% CO_2 incubator at 37°C and maintained in two 80-mm flasks with DMEM containing 10% FBS and antibiotics. Monolayers of proliferated preadipocytes were serially passaged by gently treating with trypsin/ethylenediaminetetraacetic acid (EDTA). Strains were stored in liquid N_2 and only used between the 3rd and 10th passages.

4. Cell viability (MTT) assay

Cell viability was assessed using the MTT assay, according to the manufacturer's (Sigma-Aldrich) protocol. Orbital fibroblasts from control and GO patients were seeded into 24-well culture plates ($1x10^5$ cells/well). Then, different concentrations of resveratrol (10, 30, 50, or 100 μM) were added for 24 hours. After treatment, cells were washed and treated with MTT (5 mg/ml) solution for 4 hours at 37°C. Then, the medium was removed and the converted dye was solubilized in ice-cold isopropanol. Absorbency was measured at 560 nm with background subtraction at 630 nm using a microplate reader (EL 340 Bio Kinetics Reader; Bio-Tek Instruments, Winooski, VT, USA).

5. Cigarette smoke extract (CSE) preparation and dose determination for CSE and H_2O_2

CSE was prepared from two commercially available filtered cigarettes containing 8.0 mg tar and 0.7 mg nicotine (Marlboro 20 class A, Philip Morris Korea, Inc., Seoul, Korea) using published methods.[4, 15] As previously reported, treatment with 1-5% CSE did not alter the viability of normal cells, while treatment with 2-5% CSE induced proliferation in GO cells. In addition, 10 μM H_2O_2 did not affect the viability of GO cells.[16] Therefore, 2% CSE and 10 μM H_2O_2 were used in our experiments.

6. Measurement of intracellular ROS in GO cells stimulated with CSE or H_2O_2

ROS levels were measured using 5-(and 6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA), an oxidant-sensitive fluorescent probe.[15] Cells were seeded at a density of $5x10^5$ cells per well in six-well plates to a total final volume of 2 ml and then treated with 2% CSE or 10 μM H_2O_2 for 30 minutes. To evaluate the effect of resveratrol on ROS levels, cells were pretreated with 30 μM or 50 μM resveratrol for 24 hours. After the medium was removed, cells were washed with PBS and incubated with 10 μM H_2DCFDA at 37°C for 30 minutes, and then 2% CSE or 10 μM H_2O_2 was added for 30 minutes. Subsequently, cells were trypsinized, washed, and resuspended in PBS. Thereafter, fluorescence intensity was measured with an IX71-F22PH inverted fluorescence microscope (Olympus Corp., Tokyo, Japan) and

flow-cytometric analysis was performed (ELITE flow cytometer, Coulter Cytometry, Inc., Hialeah, FL, USA). For each sample, more than 10,000 events were acquired. Cells were gated out and the analysis was performed using only live populations. Fluorescent cells were also examined microscopically (x100 magnification).

7. Western blot assay

Cells were washed with ice-cold PBS and lysed on ice for 30 minutes in cell lysis buffer consisting of 20 mM HEPES (pH 7.2), 10% glycerol (v/v), 10 mM Na_3VO_4, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM dithiothreitol, 1 μg/ml leupeptin, 1 μg/ml pepstatin, and 1% Triton X-100 (v/v). Lysates were centrifuged at 12,000 x *g* for 10 minutes, and cell homogenate fractions were stored at -70°C before use. The protein concentration of supernatant fractions was determined using the Bradford assay (BioRad, Hercules, CA, USA). Then, samples containing 50 μg protein were boiled in buffer and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis [SDS-PAGE, 10% (w/v)]. Proteins were transferred to polyvinylidene fluoride membranes (Immobilon; Millipore, Billerica, MA, USA), probed overnight with primary antibodies in Tris Buffer Saline Tween 20 (TBST), and washed three times with TBST. Immunoreactive bands were detected with horseradish peroxidase-conjugated secondary antibody, developed with an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA), and exposed to X-ray film (Amersham Pharmacia Biotech). The relative amount of each immunoreactive band was

quantified by densitometry and normalized to the level of β–actin in the sample.

8. Adipogenesis

Undifferentiated cells were forced to differentiate using a previously-published protocol.[4] Cells were grown to confluence in 6-well plates. Then, culture medium was changed to serum-free DMEM supplemented with 33 μM biotin, 17 μM pantothenic acid, 10 μg/ml transferrin, 0.2 nM T_3, 1 μM insulin, and 0.2 μM carbaprostaglandin. For the first 4 days, cells were incubated in 1 μM insulin, 1 μM dexamethasone, and 0.1 mM isobutylmethylxanthine. Differentiation was continued for 10 days, and media was replaced every 3 days. To further stimulate adipogenesis, 10 μM rosiglitazone, a peroxisome proliferator-activated receptor-gamma (PPARγ) agonist, was added on day 1. To evaluate the effect of resveratrol on adipocyte differentiation, cells were treated with resveratrol (10, 50, or 100 μM) for the entire 10-day differentiation period.

9. Oil red O staining

Cells were stained with Oil red O as described by Green and Kehinde.[17] A stock solution of Oil red O (0.5% of Oil Red O in isopropanol) was prepared. To make the working solution, 6 ml stock solution was mixed with 4 ml distilled water, left for 1 hour at room temperature, and filtered through a 0.2 μm filter. Cells were washed twice with PBS, fixed with 3.7% formalin in PBS for 1 hour at 4°C, and stained with

300 μl Oil red O working solution for 1 hour at room temperature. After dishes were washed with distilled water, they were inspected using an Axiovert light microscope (Carl Zeiss AG, Oberkochen, Germany) and photographed at x40 and x100 magnification using an Olympus BX60 light microscope (Olympus, Melville, NY, USA).

10. Statistical analysis

All experiments were performed at least three times using different strains, and samples were assayed in duplicate. For statistical analysis of ROS generation and western blots, means and standard deviations were calculated from normalized measurements taken from at least three samples harvested from different individuals. Multiple comparisons were performed using analysis of variance (ANOVA) with Tukey's multiple-comparison test, as a *post-hoc* test. The Wilcoxon signed-rank test was used to perform comparisons between cell groups or within cell groups treated with different concentrations of a compound or at different times. The test was two-sided with $\alpha = 0.05$ and was performed using IBM® SPSS® Statistics version 20 software.

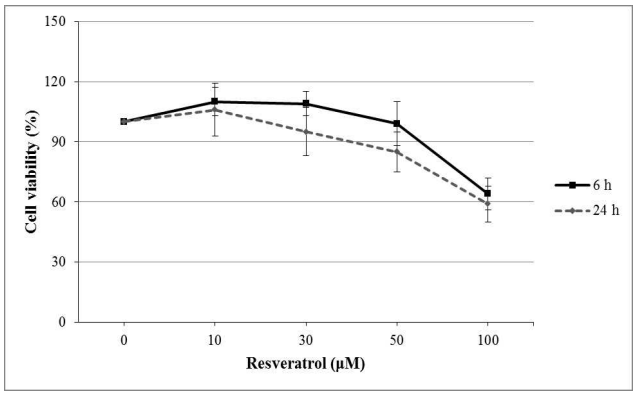
III. RESULTS

1. Effect of resveratrol on cell viability

The MTT assay was used to detect resveratrol cytotoxicity in GO and non-GO orbital fibroblasts. In GO and non-GO orbital fibroblasts treated with $\leq$ 50 μM of resveratrol for 6 hours, cell viability did not fall below

95%. However, a significant decrease in viability was observed after treatment with 100 μM resveratrol for 6 hours. When treated with ≤ 30 μM resveratrol for 24 hours, more than 90% of cells were viable (Figure 2.).

A.



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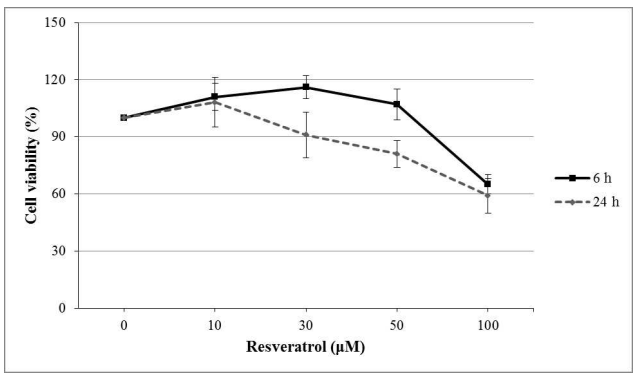


Figure 2. Effect of resveratrol on the viability of GO (A) and non-GO cells (B), measured using the MTT assay. Experiments were performed three times using different strains, and samples were assayed in duplicate. The results are presented as means ± SDs. GO, Graves' ophthalmopathy; MTT, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; h, hour(s).

2. Resveratrol decreased ROS levels induced by 2% CSE or 10 μM H_2O_2

Reduced levels of intracellular ROS were induced by treatment with of 2% CSE or 10 μM H_2O_2 when cells were pretreated with 30 or 50 μM resveratrol for 24 hours. Treatment with 50 μM resveratrol significantly decreased ROS levels (Figure 3.).

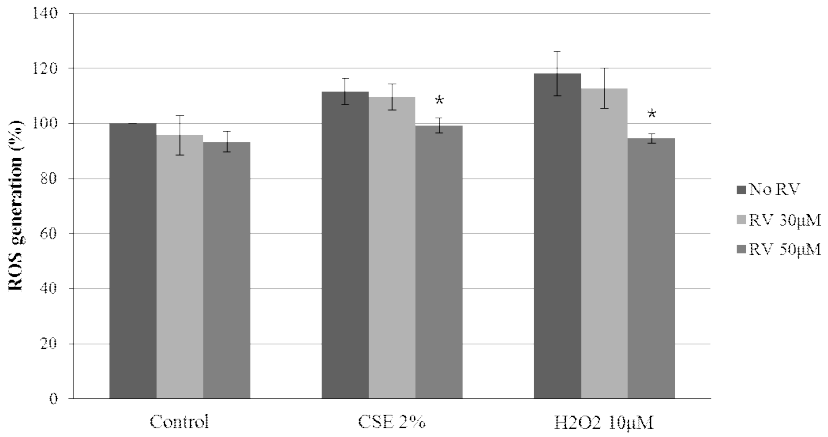


Figure 3. Effects of resveratrol on ROS generation. Experiments were performed three times using different strains, and samples were assayed in duplicate. Results are expressed as percentages of the control and presented as means ± SDs. $^{*}p < 0.05$ between RV treated and RV untreated cells. ROS, reactive oxygen species; CSE, cigarette smoke extract; RV, resveratrol.

3. HO-1 levels induced by 2% CSE or 10 μM H_2O_2 lowered after resveratrol treatment

Intracellular levels of HO-1 were higher in GO preadipocyte orbital fibroblasts treated with 2% CSE or 10 μM H_2O_2 than in controls. In cells pretreated with 30 or 50 μM resveratrol for 24 hours, there was a significant reduction in HO-1 levels (Figure 4.).

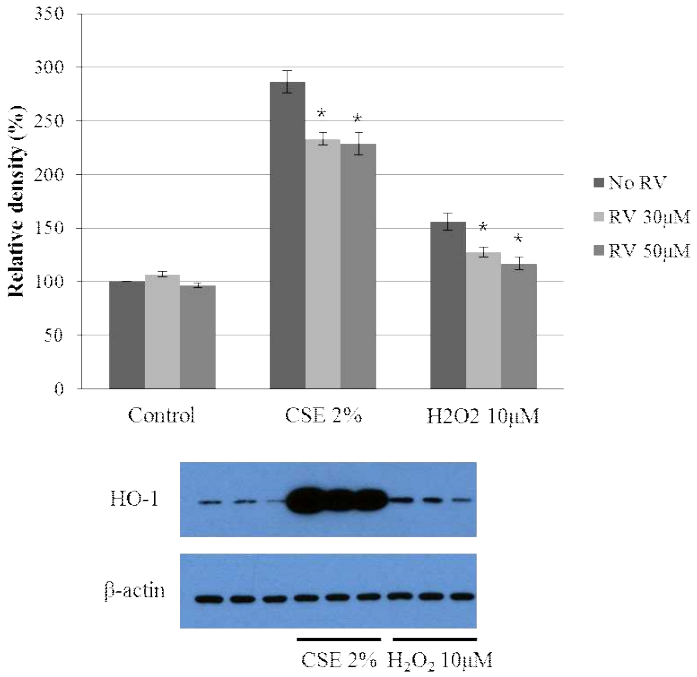


Figure 4. Effects of resveratrol on HO-1 levels in GO preadipocyte orbital fibroblasts. Experiments were performed three times using different strains, and samples were assayed in duplicate. Results are expressed as percentages of the control and presented as means ± SDs. $^{*}p < 0.05$ between RV treated and RV untreated cells. HO-1, heme oxygenase-1; CSE, cigarette smoke extract; RV, resveratrol.

4. Effects of resveratrol on adipogenesis of GO orbital fibroblasts

Undifferentiated orbital fibroblasts from GO patients were differentiated to adipocytes, stained with Oil red O, and examined with light microscopy. The number and size of differentiated cells increased over 10 days of differentiation. Treatment with 10 μM rosiglitazone significantly upregulated cellular differentiation. When resveratrol was added to the adipogenic medium for 3 days during the differentiation period, the number of adipocytes and the accumulation of lipid droplets induced by 10 μM rosiglitazone decreased in a dose-dependent fashion (Figure 5.).

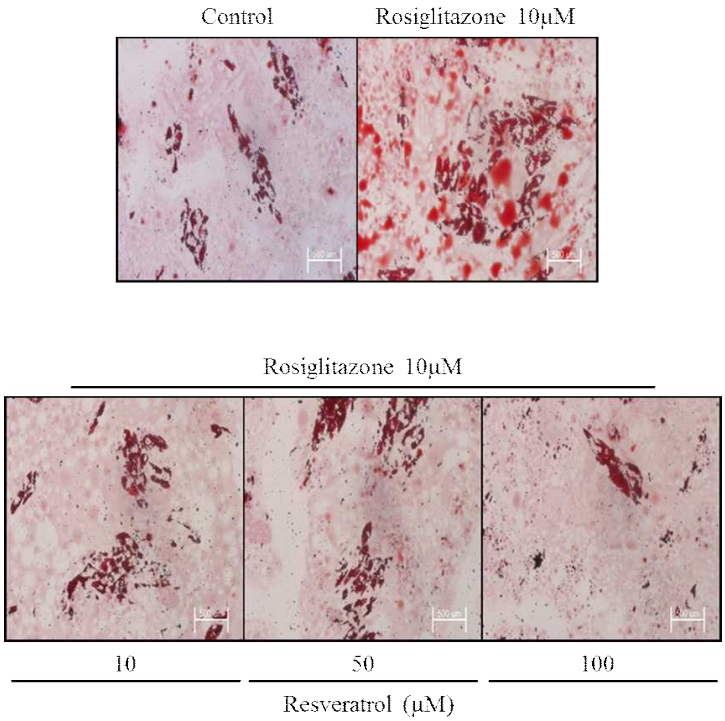


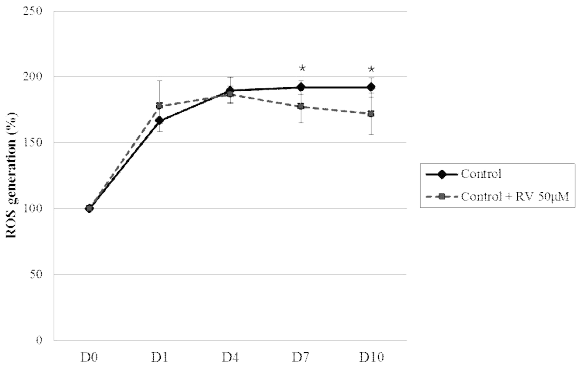
Figure 5. Effects of resveratrol on cellular differentiation to adipocytes. Experiments were performed three times using different strains. Cells were stained with Oil Red O and examined under light microscopy

(x100). Each bar indicates 500 μm.

5. Resveratrol decreased ROS generation during adipogenesis

Intracellular ROS generation was measured on days 0, 1, 4, 7, and 10 of adipocyte differentiation in GO cells. The increase in ROS levels was greatest on day 1, and subsequently ROS levels remained at roughly 200% of the control levels. Treatment with 50 μM resveratrol reduced ROS levels at day 7 and 10 compared with controls ($p < 0.05$, Figure 6A.). When exposed to 2% CSE or 10 μM H_2O_2, ROS levels in cells treated with 50 μM resveratrol were lower, but the difference was not statistically significant (cells treated with 2% CSE and untreated cells: $p = 0.068$ on day 7 and 0.144 on day 10, Figure 6B.; cells treated with 10 μM H_2O_2 and untreated cells: $p = 0.465$ on day 7 and 0.144 on day 10, Figure 6C.).

A.



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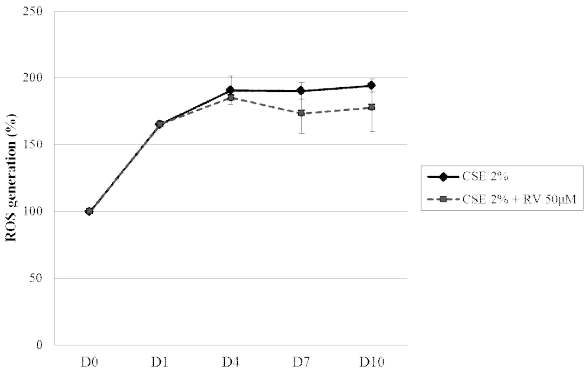


C.

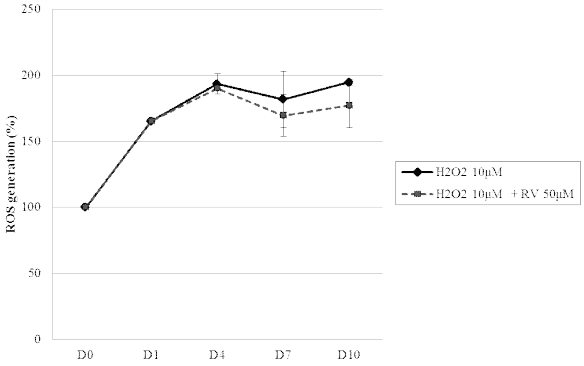


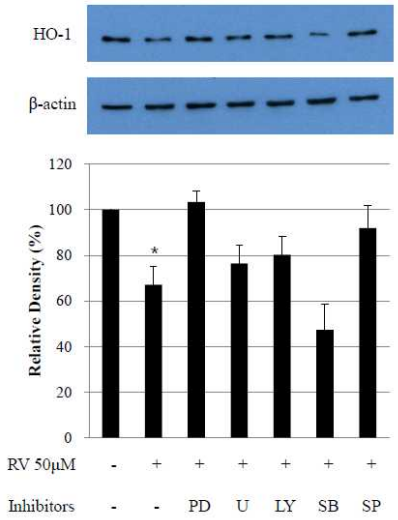
Figure 6. Effects of resveratrol on ROS generation during adipogenesis. Experiments were performed five times using different strains, and ROS levels after exposure to 2% CSE or 10 μM H_2O_2 were measured on day 1 in one strain. Results are expressed as percentages of the control and

presented with means ± SDs. $^{*}p < 0.05$ between RV-treated and RV-untreated cells on same day. ROS, reactive oxygen species; CSE, cigarette smoke extract; D, days after initiation of adipogenesis; RV, resveratrol**.**

6. Effects of resveratrol on expression of transcriptional regulators and HO-1

Treatment with 50 μM resveratrol for 24hours significantly attenuated expression of HO-1 in GO preadipocyte orbital fibroblasts. When cells were pretreated with PD (PD98059, inhibitor of ERK) and SP (SP600125, inhibitor of JNK) for 1 hour, reduced expression of HO-1 was observed (Figure 7A.). Expression of the transcriptional regulators phospho-ERK and phospho-JNK significantly increased after treatment with 50 μM resveratrol ($p < 0.05$), and decreased in response to inhibitors of each protein (PD and SP respectively, Figure 7B.).

A.



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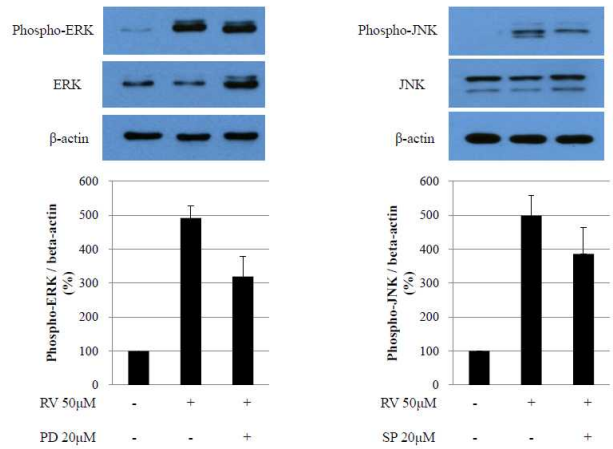

Figure 7. Effects of resveratrol on the expression of transcriptional regulators and HO-1 in GO preadipocyte orbital fibroblasts. Experiments were performed three times using different strains, and samples were assayed in duplicate. Results are expressed as percentages of the control and presented with means ± SDs. $^{*}p < 0.001$ between RV treated and RV untreated cells. HO-1, heme oxygenase-1; RV, resveratrol; PD

(PD98059), inhibitor of ERK; U (U0126), inhibitor of ERK; LY (LY294002), inhibitor of protein kinase B; SB (SB203580), inhibitor of p38; SP (SP600125), inhibitor of JNK.

IV. DISCUSSION

The autoimmune process induces an inflammatory reaction that produces ROS.[18] In turn, ROS result in oxidative damage to DNA in various autoimmune diseases.[19] To date, there have been many reports of the role of oxidative stress in the pathogenesis of Graves' disease.[20-22] ROS generation and increased oxidative stress has also been suggested as a mechanism for the development and progression of GO.[18]

Since ROS may contribute to the pathogenesis of GO, the possibility of treating GO with an antioxidant is attractive. Quercetin (3,3,4,5,7-penta-hydroxy flavonone) is a flavonoid phytoestrogen with potent antioxidant activity. Quercetin has been reported to inhibit inflammation, hyaluronan production, and adipogenesis in GO.[4, 16] Another study has demonstrated the protective effects of N-acetylcysteine and vitamin C in early stages of GO. Pretreatment with these antioxidants inhibits the stimulation of GO orbital fibroblast proliferation and the production of proinflammatory cytokines.[23] Finally, a recent randomized, double-blind, placebo-controlled clinical trial in 159 patients with mild GO demonstrated that selenium supplementation was associated with improved quality of life, less eye involvement, and slowed

progression of GO.[24] However, there is still controversy about the efficacy and safety of selenium supplementation.[25]

Resveratrol is a well-known polyphenolic flavonoid with potent antioxidant activity. It is derived from red grapes, berries, knotweed, peanuts and other plants. Resveratrol has a number of desirable biological properties; it is anti-carcinogenic, anti-inflammatory, free-radical scavenging, inhibits/induces apoptosis, and inhibits platelet aggregation.[26] Resveratrol has been shown to mediate death in a wide variety of cells and to have health benefits, especially in common age-related diseases such as cancer, type 2 diabetes, arthritis, and cardiovascular and neurologic diseases.[26, 27]

We showed that resveratrol treatment decreased ROS levels in an *in vitro* model of GO. ROS, induced by CSE or H_2O_2, decreased when primary cultured orbital fibroblasts from GO patients were treated with resveratrol. CSE and H_2O_2 are potent oxidants, and cigarette smoking is a well-known risk factor affecting GO incidence, severity, and response to treatment.[28] It has been suggested that interleukin (IL)-1 is associated with the detrimental effects of CSE in GO.[28] We also found resveratrol treatment significantly reduced ROS generation during adipogenesis. Although the decrease in ROS levels was not statistically significant in cells exposed to CSE or H_2O_2, differences in ROS production between resveratrol-treated and untreated cells reached marginal significance as time passed. This tendency was also demonstrated in a previous study of GO orbital fibroblasts, in which quercetin significantly reduced ROS levels during late-stage adipogenesis.[16]

We also found that resveratrol suppressed the adipogenesis of GO orbital fibroblasts. Our findings that resveratrol reduced the number of adipocytes and the accumulation of lipid droplets was similar to previously reported findings on the effect of quercetin in GO orbital fibroblasts.[16] Moreover, there is another report that resveratrol inhibits adipogenesis and induces apoptosis in 3T3-L1 mouse embryo fibroblasts.[29]

HO is a rate-limiting enzyme in the pathway by which heme is degraded into biliverdin/bilirubin.[30] There are two isoforms of HO, HO-1 and HO-2. HO-1 is induced by a variety of agents that cause oxidative stress.[31-33] HO-1 inhibits inducible nitric oxide synthase activity and protect against stress conditions.[34] It is upregulated in orbital fibroblasts by oxidative stress and adipogenic stimuli.[16] In our experiments, the levels of HO-1 induced by CSE or H_2O_2 in GO orbital fibroblasts were significantly lowered by treatment with resveratrol. Presumably, resveratrol reduces the need for HO-1 by decreasing ROS levels.

In TSHRs, which are associated with GO pathogenesis, signal transduction is mediated by a G protein cascade and a signaling pathway that includes ERK.[35] In addition to being influenced by downstream TSHR signaling processes, the effectors are influenced by various growth factors that work through mitogen-activated protein kinase (MAPK) cascades.[35] MAPK cascades are key signaling systems in the regulation of cell proliferation, survival, and differentiation.[36] It has been reported that resveratrol inhibits MAPK/extracellular signal-regulated kinase kinase (MEK) 1 > ERK 1/2 signaling.[37] In addition, other studies have demonstrated that resveratrol inhibits MAPK activity.[38, 39] Based on these

previous works, we investigated the effect of resveratrol on molecules related to the MAPK cascade, and our data show that ERK and JNK are associated with signal transduction of resveratrol to oxidative stress in GO.

This study has several limitations. Although cell viability was investigated in both GO and non-GO orbital fibroblasts, we did not measure resveratrol-induced changes in ROS or HO-1 levels in normal orbital fibroblasts. Our aim was to investigate the therapeutic effect of resveratrol in GO orbital fibroblasts, and this study demonstrated a significant effect of resveratrol on oxidative stress levels *in vitro*. In addition, an experiment under the activation of TSHRs using the anti-TSHR antibody could provide more information. Resveratrol has a large number of molecular targets, but we focused only on the MAPK cascade in our experiments. Resveratrol is a proven sirtuin-1 (SIRT1) activator.[26, 40] SIRT1, which belongs to the sirtuin family, is a key regulator of cellular defense and cell survival in response to stress.[41] Further studies are warranted to evaluate the therapeutic effects of resveratrol via a SIRT1 signal dependent pathway.

V. CONCLUSION

In this study, we found that resveratrol decreased levels of oxidative stress in primary cultured orbital fibroblasts from GO patients. It also decreased ROS levels during adipogenesis and suppressed adipogenesis. Thus, our results suggest the possibility of using resveratrol to treat GO.

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<ABSTRACT(IN KOREAN)>

갑상선안병증 환자의 안와섬유모세포에서 레스베라트롤이 산화성 스트레스에 미치는 치료적 효과

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갑상선안병증은 만성 염증 자가면역 질환으로 환자들의 정신적, 육체적 고통이 상당하다. 하지만, 갑상선안병증은 병리기전이 다양하고 복합적이기 때문에 치료에 어려움이 있고, 치료에 가장 널리 사용되는 스테로이드 제제는 제한된 효과와 많은 부작용으로 장기치료제로서 그 사용에 한계가 있다. 최근 갑상선안병증 발생 및 진행의 주요병리기전으로, 산화성 스트레스 기전이 보고되고 있다. 이에 강력한 항산화물질로 알려진 레스베라트롤을 이용하여 갑상선안병증 안와섬유모세포에서의 산화성 스트레스에의 효과를 생체외 실험 연구를 통해 살펴보았다. 갑상선안병증 환자로부터 얻어진 안와지방결체조직에서 안와섬유모세포를 분리 배양한 후 2%의 담배연기추출물와 10μM의 과산화수소를 이용하여 산화성 스트레스를 가해 활성산소를 증가시켰다. 증가된 활성산소가 30μM or 50μM의 레스베라트롤을 투여하였을 때 감소하였고, 산화성 스트레스에 의해 유도된 힘 옥시게나제-1 또한 30μM 혹은 50μM의 레스베라트롤 투여시 감소됨을 확인하였다. 안와

섬유모세포에서 지방세포로 분화를 유도하였는데, 레스베라트롤을 가한 경우 지방세포의 수와 지방방울의 감소를 보여 레스베라트롤이 지질생성을 억제함을 확인하였으며, 지질생성 과정에서 또한 50μM 레스베라트롤을 투여하였을 때 활성산소가 감소되었다. 이러한 반응에 ERK와 JNK가 관계된 세포내 신호전달경로가 관여하는 것으로 보였다.

본 연구결과는 갑상선안병증의 치료제로서 레스베라트롤의 가능성을 시사 하는 것이라 하겠다.

핵심되는 말 : 갑상선안병증, 레스베라트롤, 산화성 스트레스, 안와섬유모세포, 지질생성