

Original Article

2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-Glucoside modulated human umbilical vein endothelial cells injury under oxidative stress

Yan Guo^{1,2,#}, Wenxue Fan^{2,#}, Shuyu Cao², Yuefeng Xie², Jiancong Hong², Huifen Zhou², Haitong Wan², and Bo Jin^{2,*}

¹College of Basic Medicine & Public Health, ²College of Life Science, Zhejiang Chinese Medical University, Hangzhou, Zhejiang 310053, China

ARTICLE INFO

Received April 22, 2020

Revised July 24, 2020

Accepted July 27, 2020

*Correspondence

Bo Jin

E-mail: jinbo@zcmu.edu.cn

Key Words

Endoplasmic reticulum stress

Human umbilical vein endothelial cells

MicroRNAs

Oxidative damage

2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-Glucoside

ABSTRACT Endothelial cell injury is a major contributor to cardiovascular diseases. The 2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-Glucoside (TSG) contributes to alleviate human umbilical vein endothelial cells (HUVECs) injury through mechanisms still know a little. This study aims to clarify the TSG effects on gene expression (mRNA and microRNA) related to oxidative stress and endoplasmic reticulum stress induced by H₂O₂ in HUVECs. We found that TSG significantly reduced the death rate of cells and increased intracellular superoxide dismutase activity. At qRT-PCR, experimental data showed that TSG significantly counteracted the expressions of miR-9-5p, miR-16, miR-21, miR-29b, miR-145-5p, and miR-204-5p. Besides, TSG prevented the expression of ATF6 and CHOP increasing. In contrast, TSG promoted the expression of E2F1. In conclusion, our results point to the obvious protective effect of TSG on HUVECs injury induced by H₂O₂, and the mechanism may through miR16/ATF6/ E2F1 signaling pathway.

#These authors contributed equally to this work.

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of death worldwide [1]. Vascular endothelial dysfunction is a crucial factor in the occurrence of CVD. And oxidative stress is considered to be endothelial dysfunction major inducer. In particular, oxidative stress caused by ROS is a critical factor in the pathogenesis of vascular diseases. Thus, identifying more effective antioxidants is a promising strategy to prevent endothelial cell injury, which is of great significance for the prevention, clinical diagnosis, and monitoring of endothelial dysfunction and adverse events of CVD [2].

Antioxidant agents that attenuate the oxidative stress may have therapeutic applications in reducing endothelial cell damage. Many plant constituents exhibit antioxidant activity. For sample,

Wu *et al.* [3] showed that the *Polygonum orientale* flower extract can protect human umbilical vein endothelial cells (HUVECs) from hydrogen peroxide (H₂O₂)-triggered oxidative damage by enhancing the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase. 2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-Glucoside (TSG), the characteristic water-soluble component of *Polygonum multiflorum* Thunb, has shown various pharmacologic activities, including antioxidant, anti-inflammatory, anti-aging, and anti-atherosclerotic effects [4-6]. Neuroprotective effects of TSG against glutamate or H₂O₂-induced oxidative toxicity were confirmed between cell lines or rodent models [7,8]. Li *et al.* [9] confirmed that TSG decreased pulmonary aortic endothelial cell inflammatory injury induce by septic-serum via the ROS/MAPK/NF-κB signaling pathway. However, the protec-



This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. Copyright © Korean J Physiol Pharmacol, pISSN 1226-4512, eISSN 2093-3827

Author contributions: B.J. jointly conceived and designed the study with H.T.W.; Y.G., S.Y.C., and W.X.F. performed experiments; Y.G., Y.F.X., and J.C.H. analyzed the data; H.F.Z. and Y.G. drafted the manuscript; B.J. and H.T.W. revisited the manuscript critically for important intellectual content. All authors contributed equally to this work.

tive effect of TSG on H₂O₂-induced oxidative damage remains to be fully elucidated.

H₂O₂ acts as a signal molecule and second messenger involved in most of the redox metabolism reactions and processes of the cells, and is widely used to establish oxidative stress model [10]. Thus, stimulation with H₂O₂ is a good strategy for investigating vascular endothelial damage. In this study, we aimed to clarify the TSG effects on gene expression (mRNA and microRNA) related to oxidative stress induced by H₂O₂ in HUVECs.

METHODS

Materials

HUVECs cell line was purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside (TSG, CAS Number: 82373-94-2, purity: ≥ 98%) and TSG was dissolved in sterile water.

Cell cultures and cell treatment

The cells were cultured in RPMI 1640 with 10% fetal bovine serum. The medium was changed every 2 days and the cells were passaged with trypsin-EDTA. HUVECs were randomly divided into 4 groups: control group, H₂O₂ group, TSG (20 μg/ml) group and TSG (40 μg/ml) group. Cells in the control group were incubated with normal growth conditions. Those in the H₂O₂ group were incubated with H₂O₂ (200 μM) for 2 h and then recovered for 24 h. In the TSG (20 μg/ml, 40 μg/ml) groups, the cells were cultured with the medium containing different concentrations of TSG for 24 h before they were treated same as H₂O₂ group.

MTS assay

The MTS assay was used to assess cell viability. Before each experiment, HUVECs (5 × 10³ cells/well) were seeded in 96-well microtiter plates, and cells were treated with H₂O₂ or TSG according to the different experimental purposes. Subsequently, 20 μl MTS solution was added to each well, and the plates were incubated for 2 h. The absorbance was measured at 490 nm and used to calculate the relative ratio of cell viability.

In situ fluorescence detection of apoptosis

An annexin V-FITC apoptosis detection kit (MultiSciences; LiankeBio, Hangzhou, China) was used for the dual staining assay. Briefly, cells were harvested and washed with PBS. After adding the staining solution per the requirements of the kit, incubate with aluminum foil paper at room temperature for 15 min in the dark, and then place them in an ice bath in the dark. Then observed under a fluorescence microscope within one hour, Annexin V-FITC showed green fluorescence and propidium iodide (PI) showed red fluorescence.

Detection of SOD

We used Total Superoxide Dismutase Assay Kit with WST-8 (Beyotime Biotechnology, Shanghai, China) to detect the SOD activity in cell lysates. After treatments, cell lysates were prepared, and the protein concentration was measured using the BCA assay (Beyotime Biotechnology). The SOD activity levels were quantified according to the manufacturer's instructions.

RNA isolation and quantitative PCR (qRT-PCR)

Total RNA was extracted with TRIzol reagent. The complementary DNA was synthesized using Prime Script™ RT Master Mix (Perfect Real Time) (Takara, Dalian, China) for real-time

Table 1. The primer sequences

Name	Forward primer	Reverse primer
GAPDH	GGCATGGGTGAGAAGGATTCC	ATGTCACGCACGATTTCCTCCG
E2F1	GTTTCCAGAGATGCTCACCTTGTC	ACACCACACAGACTCCTTCCTT
ATF4	TTTGACAGCTAAAGTGAAGACTGA	GCTTCTTCTGGCGGTACCTA
ATF6	AATGCCAGTGTCCAGCAA	GCGCAGGCTGTATGCTGA
CHOP	ACTCTCCAGATTCCAGTCAGAG	GCCTCTACTTCCCTGGTCAG
GRP78	TGGGAGGAGTCATGACAAAA	GGGGTCGTTACCTTCATAG
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
miR-9	CGGAGATCTTTCTCTCTTC	CAAGAATTCGCCC GAACCAG
miR-16	TAGCAGCACGTAAATATTGGCC	CCTCTGACTGGAATTGTGAC
miR-21	ACACTCCAGCTGGGTAGCTTATCAGACTGATG	CTCAACTGGTGTGCTGGGA
miR-145	CGGAATTC AAGGTCAGTACAGCCTGGGAAC	CGCGGATCCTCAACCAGTGTCTTGAGAC
miR-204-5P	GCGAGCACAGAATTAATACGC	TCAGTGCACACTACAGAACTTTGT
miR-29b	TTCACAGTGGCTAAGTTCCGC	

polymerase chain reaction (RT-PCR) with conditions of 37°C for 15 min, 85°C for 5 sec, and storage at 4°C. The RT-PCR was performed in duplicate using SYBR[®] Prime Ex Taq TM II (Tli RNaseH Plus) (Takara) at 95°C for 10 sec, and then 95°C for 5 sec, 60°C for 20 sec for 40 cycles, and 95°C for 1 min, 55°C for 30 sec, 95°C for 30 sec. The primer sequences used in this study were as Table 1 showed.

TargetScanHuman database

TargetScanHuman database is a miRNA target gene prediction website for human. We inputted the effective miRNA based on the results of qRT-PCR to predict possible target genes.

Statistical analysis

GraphPad Prism 5 software was used for statistical analysis. Measurement data were compared using a one-way analysis of variance. $p < 0.05$ was considered statistically significant.

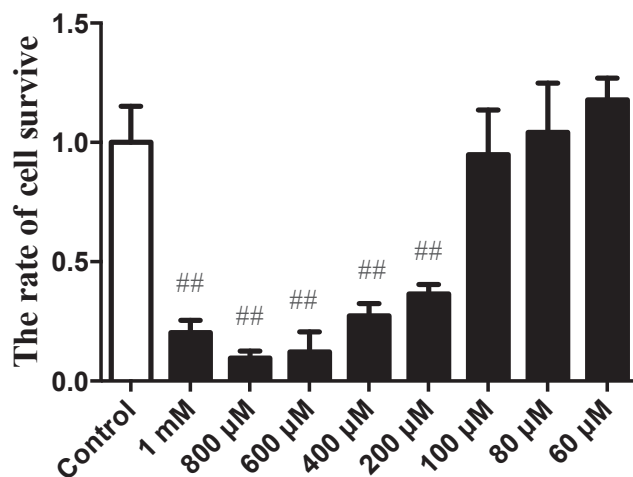


Fig. 1. H₂O₂ exposure induced the decrease of cell viability. HUVECs stimulated with or without on by H₂O₂. Values are expressed as mean \pm standard deviation (n = 3). HUVECs, human umbilical vein endothelial cells. ^{##} $p < 0.01$ vs. control.

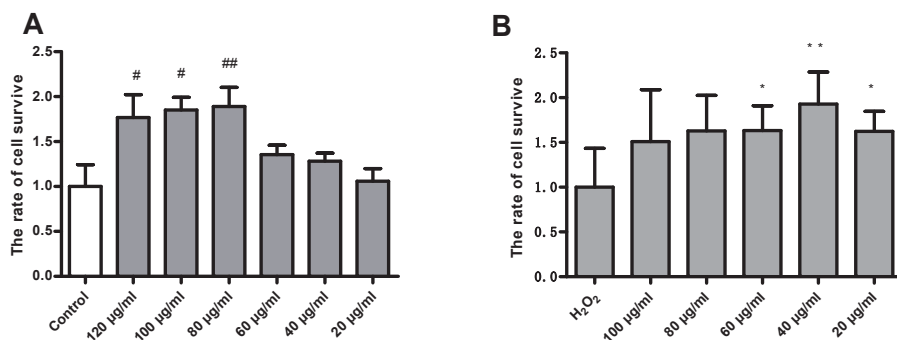


Fig. 2. TSG pre-treatment improved cell viability. TSG Treatment on HUVECs untreated (basal) (A) and H₂O₂-induced (B) for 24 h. Values are expressed as mean \pm standard deviation (n = 3). TSG, 2,3,5,4'-Tetrahydroxystilbene-2-O- β -D-Glucoside; HUVECs, human umbilical vein endothelial cells. [#] $p < 0.05$ and ^{##} $p < 0.05$ vs. control; ^{*} $p < 0.05$ and ^{**} $p < 0.05$ vs. H₂O₂ group.

RESULTS

H₂O₂ exposure induced the decrease of cell viability

To examine the cytotoxicity of H₂O₂ on HUVECs, cell viability was detected by the MTS assay. As shown in Fig. 1, treatment with H₂O₂ (60 μ M, 80 μ M, and 100 μ M) for 12 h had no effect on cell viability of HUVECs. However, cell viability of HUVECs decreased when the concentration of H₂O₂ increased to 200 μ M. Therefore, the concentrations of 200 μ M were chosen for H₂O₂, respectively, for the subsequent experiments.

TSG pre-treatment improved cell viability

TSG treatment at the concentrations of 120 μ g/ml, 100 μ g/ml, 80 μ g/ml, 60 μ g/ml, 40 μ g/ml, and 20 μ g/ml showed no cytotoxicity on HUVECs when compared with the control group (Fig. 2A). Furthermore, the decreased cell viability of HUVECs induced by H₂O₂ was improved with TSG treatment (40 μ g/ml and 20 μ g/ml), suggesting that TSG rescued H₂O₂-induced cell injury in HUVECs (Fig. 2B).

TSG pre-treatment suppressed apoptotic cell death

Cell death (annexin-V/PI) was explored in HUVECs treated 24 h with TSG in the presence or absence of H₂O₂ (200 μ M). H₂O₂ can induce various cell death, including apoptosis and necrosis. We measured the Annexin V positive and PI positive HUVECs of each group to determine the state of the cells. As shown in Fig. 3, the treatment of HUVECs with 200 μ M H₂O₂ significantly increased the apoptotic cell death. And pre-incubation with TSG significantly attenuated H₂O₂-induced apoptotic cell death.

TSG pre-treatment ameliorated H₂O₂ induced loss of SOD in HUVECs

SOD is an important metal enzyme that can specifically remove superoxide anions and protect the human body from

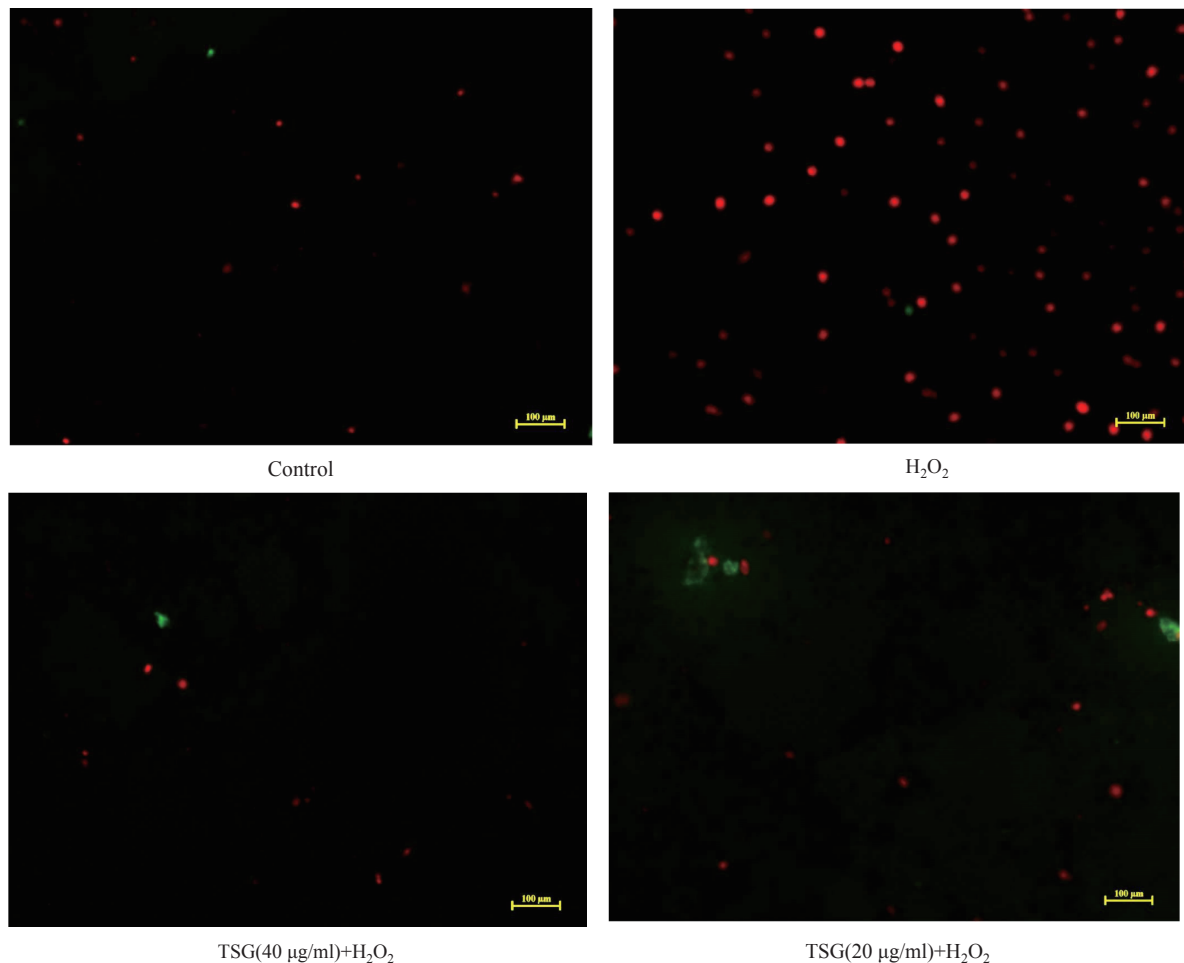


Fig. 3. TSG pre-treatment suppressed apoptotic cell death. Representative images of staining with Annexin V-FITC (green) and PI (red) acquired using a laser scanning microscope (scale $\times 100 \mu\text{m}$). The images were captured within the same field and then merged. Values are expressed as mean \pm standard deviation ($n = 3$). TSG, 2,3,5,4'-tetrahydroxystilbene-2-O- β -D-glucoside; PI, propidium iodide.

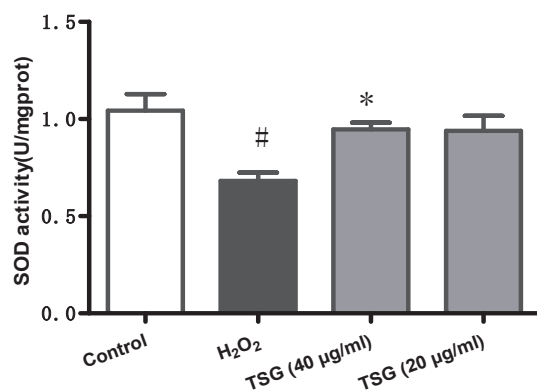


Fig. 4. TSG pre-treatment ameliorated H₂O₂ induced loss of SOD in HUVECs. SOD activity was been detected. Values are expressed as mean \pm standard deviation ($n = 3$). TSG, 2,3,5,4'-tetrahydroxystilbene-2-O- β -D-glucoside; SOD, superoxide dismutase; HUVECs, human umbilical vein endothelial cells. [#] $p < 0.05$ vs. control; ^{*} $p < 0.05$ vs. H₂O₂ group.

oxidative damage. Moreover, SOD is a key mitochondrial antioxidant. We measured the intracellular SOD activity of each group to determine the extent of their oxidative damage. The result of the antioxidant system of SOD was shown in Fig. 4. The activity of SOD significantly decreased in H₂O₂-treated group compared with that in the control group, and was significantly activated by TSG pre-treatment with increasing concentrations.

Effect of TSG pre-treatment in miRNA expression in H₂O₂ induced HUVECs

Studies have shown that miRNA can regulate the expression of most genes in cells. In the H₂O₂-treated HUVECs, the expression of miR-9-5p, miR-16, miR-21, miR-29b, miR-145-5p, and miR-204-5p were significantly upregulated. TSG antagonized H₂O₂-induced miR-9-5p, miR-16, miR-21, miR-29b, miR-145-5p, and miR-204-5p synthesis (Fig. 5A–F).

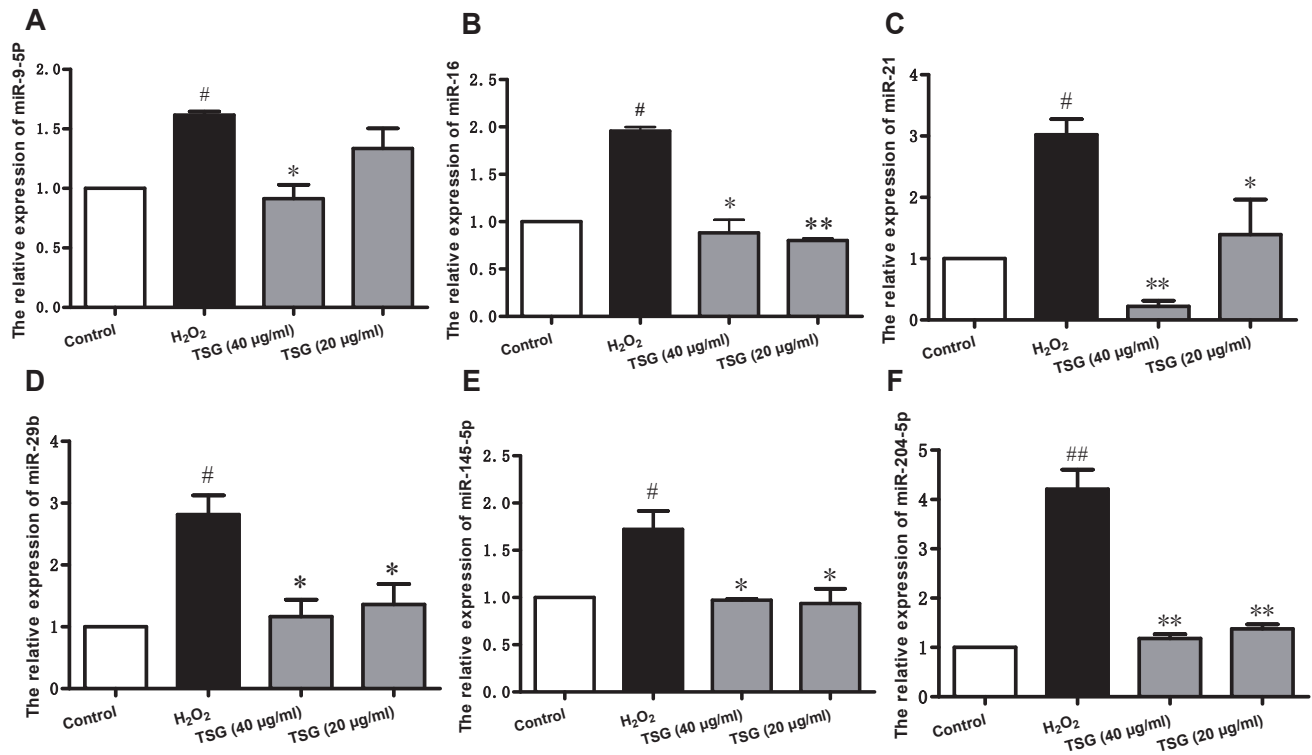


Fig. 5. Effect of TSG pre-treatment in miRNA expression in H₂O₂ induced HUVECs. qRT-PCR was used to detect the (A) miR-9-5p, (B) miR-16, (C) miR-21, (D) miR-29b, (E) miR-145-5p, and (F) miR-204-5p synthesis. Values are expressed as mean ± standard deviation (n = 3). TSG, 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside; HUVECs, human umbilical vein endothelial cells. #p < 0.05 and ##p < 0.01 vs. control; *p < 0.05 and **p < 0.01 vs. H₂O₂ group.

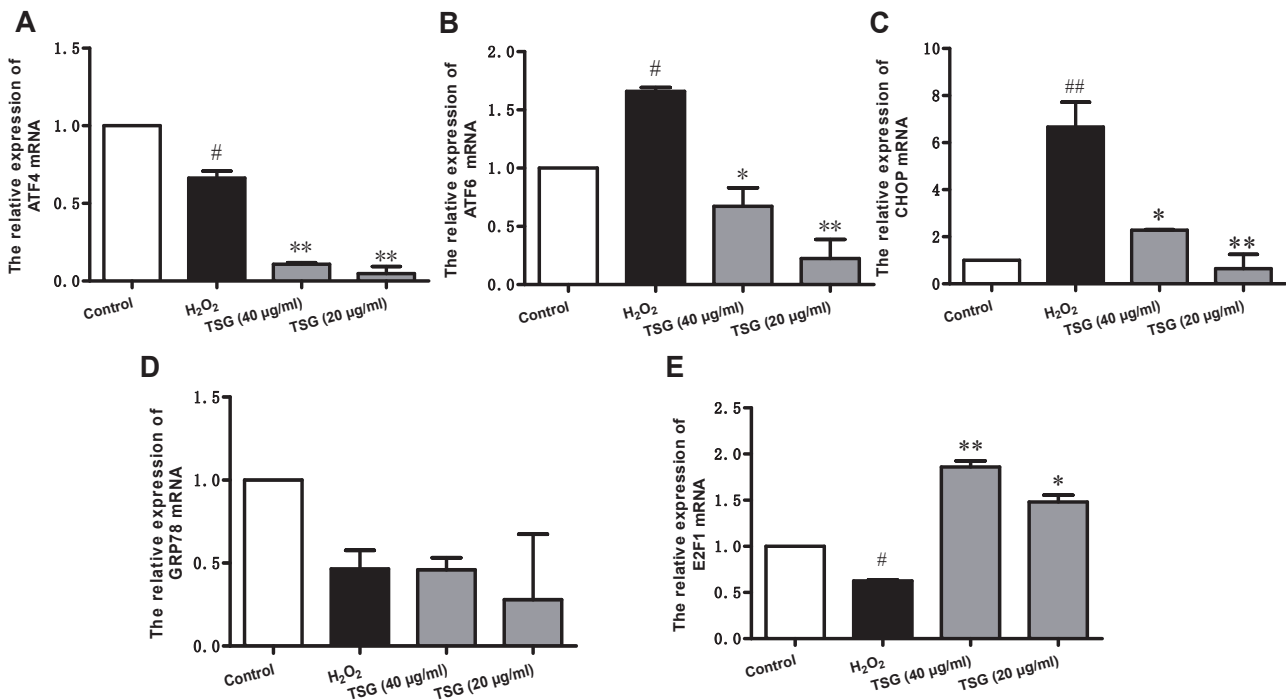


Fig. 6. Effect of TSG on ER stress in HUVECs. qRT-PCR was used to detect the (A) ATF4, (B) ATF6, (C) CHOP, (D) GRP78, and (E) E2F1 mRNA synthesis. Values are expressed as mean ± standard deviation (n = 3). TSG, 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside; ER, endoplasmic reticulum; HUVECs, human umbilical vein endothelial cells. #p < 0.05 and ##p < 0.01 vs. control; *p < 0.05 and **p < 0.01 vs. H₂O₂ group.

Effect of TSG on endoplasmic reticulum (ER) stress in HUVECs

The ER has recently emerged as an alternative target to induce cell death, because prolonged ER stress results in the induction of apoptosis. The levels of ER stress markers (ATF4, ATF6, CHOP, GRP78) and E2F1 mRNA were examined with qRT-PCR. In the H₂O₂-treated HUVECs, the expression of ATF6 and CHOP mRNA was significantly upregulated while the ATF4, GRP78 and E2F1 mRNA were significantly downregulated. TSG antagonized H₂O₂-induced ATF6, CHOP and E2F1 mRNA synthesis. However, TSG has no effects on reversing the expression of ATF4 and GRP78 mRNA (Fig. 6A–E).

DISCUSSION

CVD are major contributor to death associated with endothelial dysfunction. Effective drugs to prevent endothelial dysfunction are important. In the present research, our results showed that TSG could mitigate H₂O₂-induced decrease of cell vitalities and antioxidative enzyme activities and decrease the level of oxidative stress and ER stress in HUVECs. And the safety of TSG has been well proved. Our result showed that TSG at the concentration had no effect on the cell viabilities of HUVECs. Notably, the concentration of 40 µg/ml and 20 µg/ml showed a property to inhibit H₂O₂-induced oxidative stress and ER stress.

SOD is an important metal enzyme that can specifically remove superoxide anions and protect the human body from oxidative damage. It is closely related to the occurrence and development of many diseases. After we measured the total SOD activity in cells, we found that TSG can reduce the oxidative damage of H₂O₂-induced HUVECs by increasing the SOD activity in cells. In addition, the results of in situ fluorescence detection of apoptosis also visually show that TSG significantly inhibits H₂O₂-induced HUVECs apoptotic cell death.

miRNAs are small endogenous non-coding RNAs of 18–24 nucleotides in length, which are widely present in plants and animals and are involved in various biochemical regulations of the body, including cell proliferation, senescence, death, cell cycle regulation and apoptosis [11–13]. For example, studies have investigated the role of miR-9-5p in angiogenesis and apoptosis in HUVECs injury, and dual luciferase reporter gene assay verified that miR-9-5p targeted CXCR4 [14], while miR-16, miR-29b exerts a specific tumor suppressive effect by targeting the oncogene Bcl-2 [15,16]. As a key regulator of apoptosis in many types of

human tumors, Bcl-2 is also positively regulated by miR-21 [17]. In addition, miR-145 has a negative regulatory effect on caspase-3 and inhibits the apoptosis process of cells [18]. Moreover, studies have shown that HUVECs were suppressed by conditioned media from lung cancer cells with miR-204 overexpression [19]. Based on the above research, we explored whether TSG has effects on the expressions of miRNAs in H₂O₂-induced HUVECs. And the results showed that TSG can play a protective role against HUVECs oxidative damage via reducing the expression of miR-9-5p, miR-16, miR-21, miR-29b, miR-145-5p and miR-204-5p. These may also prompt us to further study the antioxidant mechanism of TSG in the future consider miR-9-5p, miR-16, miR-21, miR-29b, miR-145-5p, and miR-204-5p.

ER stress occurs after oxidative stress. ER represents a compartment able to sense several cellular stresses and to trigger, as last resort, cell death [20]. ER stress markers included the ATF4, ATF6, CHOP, and GRP78. ATF4/ATF6 [21,22] which activates transcription factor (ATF/CREB) family, molecular chaperone GRP78 in the endoplasmic reticulum, and CHOP, a transcription factor that mediates endoplasmic reticulum stress-induced apoptosis [23], P53 [5], etc. have an important relationship with oxidative stress and apoptosis. Our results showed that TSG may exert its protective effect against HUVECs oxidative damage via decreasing the expressions of ATF6 and CHOP. TSG had little effects on the expression of ATF4 and GRP78. Meanwhile, we made use of the TargetScanHuman database to find that ATF6 may be the target gene of miR-16 (Fig. 7).

E2F1 is known to exert different effects on cell growth and apoptosis depending on the cell context. ER stress-mediated E2F1 down-regulation may contribute to the life/death cell decision under prolonged ER stress. Our results indicated that the low expression of E2F1 will promote HUVECs oxidative damage, while TSG can play its protective role against HUVECs oxidative damage via increasing the expression of E2F1. Pagliarini *et al.* [24] demonstrated that ATF6 controls the expression of E2F1 during the UPR program execution through direct binding to an ERSE site within the E2F1 gene promoter. Therefore, we could hypothesize that TSG regulate the expression of E2F1 through ATF6.

In summary, TSG can effectively protect HUVECs from oxidative damage induced by H₂O₂. Its main regulatory mechanism may be related to miR16/ATF6/E2F1 signaling pathway. And the molecular mechanism of its specific role remains to be further explored.

ACKNOWLEDGEMENTS

This work was supported by the Natural Science Foundation of Zhejiang Province, China (LY17H280007).

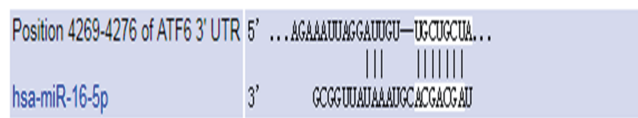


Fig. 7. The predicted result of TargetScanHuman database.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

- Ueda P, Woodward M, Lu Y, Hajifathalian K, Al-Wotayan R, Aguilar-Salinas CA, Ahmadvand A, Azizi F, Bentham J, Cifkova R, Di Cesare M, Eriksen L, Farzadfar F, Ferguson TS, Ikeda N, Khalili D, Khang YH, Lanska V, León-Muñoz L, Magliano DJ, et al. Laboratory-based and office-based risk scores and charts to predict 10-year risk of cardiovascular disease in 182 countries: a pooled analysis of prospective cohorts and health surveys. *Lancet Diabetes Endocrinol.* 2017;5:196-213.
- Scioli MG, Storti G, D'Amico F, Rodríguez Guzmán R, Centofanti F, Doldo E, Céspedes Miranda EM, Orlandi A. Oxidative stress and new pathogenetic mechanisms in endothelial dysfunction: potential diagnostic biomarkers and therapeutic targets. *J Clin Med.* 2020;9:1995.
- Wu Q, Liu XX, Lu DY, Li YT, Sun J, Lan YY, Liu T. [Protective effect of Polygonum orientale flower extract on H₂O₂-induced oxidative damage of HUVEC cells]. *Zhongguo Zhong Yao Za Zhi.* 2018;43:1008-1013. Chinese.
- Büchter C, Zhao L, Havermann S, Honnen S, Fritz G, Proksch P, Wätjen W. TSG (2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside) from the Chinese herb Polygonum multiflorum increases life span and stress resistance of *Caenorhabditis elegans*. *Oxid Med Cell Longev.* 2015;2015:124357.
- Zhao J, Liang Y, Song F, Xu S, Nian L, Zhou X, Wang S. TSG attenuates LPC-induced endothelial cells inflammatory damage through notch signaling inhibition. *IUBMB Life.* 2016;68:37-50.
- Zhao J, Xu S, Song F, Nian L, Zhou X, Wang S. 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside protects human umbilical vein endothelial cells against lysophosphatidylcholine-induced apoptosis by upregulating superoxide dismutase and glutathione peroxidase. *IUBMB Life.* 2014;66:711-722.
- Lee SY, Ahn SM, Wang Z, Choi YW, Shin HK, Choi BT. Neuroprotective effects of 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside from Polygonum multiflorum against glutamate-induced oxidative toxicity in HT22 cells. *J Ethnopharmacol.* 2017;195:64-70.
- Xie M, Zhang G, Yin W, Hei XX, Liu T. Cognitive enhancing and antioxidant effects of tetrahydroxystilbene glucoside in Aβ1-42-induced neurodegeneration in mice. *J Integr Neurosci.* 2018;17:355-365.
- Li W, Sun R, Zhou S, Ma J, Xie Y, Xu B, Long H, Luo K, Fang K. 2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside inhibits septic serum-induced inflammatory injury via interfering with the ROS-MAPK-NF-κB signaling pathway in pulmonary aortic endothelial cells. *Int J Mol Med.* 2018;41:1643-1650.
- Di Marzo N, Chisci E, Giovannoni R. The role of hydrogen peroxide in redox-dependent signaling: homeostatic and pathological responses in mammalian cells. *Cells.* 2018;7:156.
- Kuosmanen SM, Kansanen E, Sihvola V, Levonen AL. MicroRNA profiling reveals distinct profiles for tissue-derived and cultured endothelial cells. *Sci Rep.* 2017;7:10943.
- Incalza MA, D'Oria R, Natalicchio A, Perrini S, Laviola L, Giorgino F. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascul Pharmacol.* 2018;100:1-19.
- Nemecz M, Alexandru N, Tanko G, Georgescu A. Role of microRNA in endothelial dysfunction and hypertension. *Curr Hypertens Rep.* 2016;18:87.
- Yi J, Gao ZF. MicroRNA-9-5p promotes angiogenesis but inhibits apoptosis and inflammation of high glucose-induced injury in human umbilical vascular endothelial cells by targeting CXCR4. *Int J Biol Macromol.* 2019;130:1-9.
- Sun CY, She XM, Qin Y, Chu ZB, Chen L, Ai LS, Zhang L, Hu Y. miR-15a and miR-16 affect the angiogenesis of multiple myeloma by targeting VEGF. *Carcinogenesis.* 2013;34:426-435.
- Chung HJ, Choi YE, Kim ES, Han YH, Park MJ, Bae IH. miR-29b attenuates tumorigenicity and stemness maintenance in human glioblastoma multiforme by directly targeting BCL2L2. *Oncotarget.* 2015;6:18429-18444.
- Chao J, Guo Y, Li P, Chao L. Role of kallistatin treatment in aging and cancer by modulating miR-34a and miR-21 expression. *Oxid Med Cell Longev.* 2017;2017:5025610.
- Pan Y, Ye C, Tian Q, Yan S, Zeng X, Xiao C, Wang L, Wang H. miR-145 suppresses the proliferation, invasion and migration of NSCLC cells by regulating the BAX/BCL-2 ratio and the caspase-3 cascade. *Oncol Lett.* 2018;15:4337-4343.
- Liu X, Gao X, Zhang W, Zhu T, Bi W, Zhang Y. MicroRNA-204 deregulation in lung adenocarcinoma controls the biological behaviors of endothelial cells potentially by modulating Janus kinase 2-signal transducer and activator of transcription 3 pathway. *IUBMB Life.* 2018;70:81-91.
- Rutkowski DT, Kaufman RJ. A trip to the ER: coping with stress. *Trends Cell Biol.* 2004;14:20-28.
- Zhang JJ, Zhang YZ, Peng JJ, Li NS, Xiong XM, Ma QL, Luo XJ, Liu B, Peng J. Atorvastatin exerts inhibitory effect on endothelial senescence in hyperlipidemic rats through a mechanism involving downregulation of miR-21-5p/203a-3p. *Mech Ageing Dev.* 2018;169:10-18.
- Zhang M, Zhou SH, Li XP, Shen XQ, Fang ZF, Liu QM, Qiu SF, Zhao SP. Atorvastatin downregulates BMP-2 expression induced by oxidized low-density lipoprotein in human umbilical vein endothelial cells. *Circ J.* 2008;72:807-812.
- Yi TN, Zhao HY, Zhang JS, Shan HY, Meng X, Zhang J. Effect of aspirin on high glucose-induced senescence of endothelial cells. *Chin Med J (Engl).* 2009;122:3055-3061.
- Pagliarini V, Giglio P, Bernardoni P, De Zio D, Fimia GM, Piacentini M, Corazzari M. Downregulation of E2F1 during ER stress is required to induce apoptosis. *J Cell Sci.* 2015;128:1166-1179.