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Journal of Nutritional Biochemistry 23 (2012) 1410–1416

Journal of Nutritional Biochemistry

Resveratrol reduces vascular cell senescence through attenuation of oxidative stress by SIRT1/NADPH oxidase-dependent mechanisms☆

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Received 22 March 2011; received in revised form 23 August 2011; accepted 30 August 2011

Abstract

Objective: Senescence of vascular cells contributes to the development of cardiovascular diseases and the overall aging. This study was undertaken to investigate the effects of resveratrol (Res) on amelioration of vascular cell aging and the role of SIRT1/nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathway.
Methods and Results: Adult male Wistar rats were treated with a high-fat/sucrose diet (HFS) in the presence or absence of Res for 3 months. HFS and *in vitro* treatment with high glucose increased the senescence cells and reactive oxygen species production in rat aorta and cultured bovine aortic endothelial cells (BAECs), respectively, which was attenuated by Res treatment. Res protected against HFS- or high-glucose-induced increase in NADPH oxidase p47phox expression and decrease in SIRT1 level. Apocynin, a NADPH oxidase inhibitor, down-regulated p47phox protein expression, but had no influence on SIRT1 protein; sirtinol, a SIRT1 inhibitor, aggravated the decrease in SIRT1 protein level and the increase in p47phox protein expression induced by high glucose.
Conclusion: Our studies suggested that Res was able to reverse the senescence process in aorta induced by HFS in rats or induced by the exposure to high glucose in cultured BAECs. The underlying mechanism is at least SIRT1/NADPH oxidase pathway dependent.
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Keywords: Resveratrol; SIRT1; NADPH oxidase; Vascular cell senescence

1. Introduction

It is well established that age-related diseases are closely associated with the senescence of vasculature [1–3]. Vascular senescence plays a crucial role in the development of atherosclerosis in people with or without diabetes [1]. Obviously, senescence of vascular cells will lead to decreased functions of organs/tissues and increased risk level of various cardiovascular diseases and accelerated aging. Epidemiological data [4,5] has indicated that the young people with signs of early vasculature senescence have an increased risk level of developing cardiovascular diseases, which suggested that prevention of aging should start with prophylaxis of vascular aging [6]. Therefore, slowing of vasculature senescence will likely reduce the risk level of developing cardiovascular disorders and slow the overall aging process [7].

There is no doubt that reactive oxygen species (ROS) plays a necessary role in the aging process including vascular cell senescence [8–10]. So, the ability to resist or prevent oxidative stress is a key determinant of longevity, and it follows that therapies aimed at reducing the oxidative burden would have significant antiaging effects. A substantial evidence demonstrated that the main source of ROS production in the vasculature is reduced nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidase [11,12].

Resveratrol (*trans*-3,5,4′-trihydroxystilbene, Res), a polyphenol phytoalexin, has a variety of diverse biochemical and physiological functions, including antiaging effects, and has attracted extensive attention [13–16]. The mechanism of Res in antisenescence is extremely complicated and has not been clarified. The antioxidant properties and the ability of activating silencing information regulator (SIRT1) may mediate the antisenescence effect of Res. For example, Res has direct free-radicals-scavenging capacity due to its structure of hydroxyl groups. Besides, Res can decrease lipid peroxidation by

☆ Grants, sponsors and funding sources: the National High Technology Research and Development Program of China (2010AA023003).

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doi:10.1016/j.jnutbio.2011.08.008

chelating metals [17,18]. Nevertheless, the free-radical-scavenging ability is very limited relative to the strong free-radical-producing ability of the human body itself [19].

There are few studies on the effect of Res on the senescence of vascular cells, and results are conflicting. Some studies have shown that Res can reduce senescence of vascular cell [20,21], while others failed to show the same effect [22,23]. It has been shown that Res can reduce oxidative stress in vascular cells by decreasing the expression or the activity of NADPH oxidase [24,25]. A substantial number of studies in rodents reported that NADPH oxidase-mediated O_2^- production in arteries [26,27] is higher in aged rodents in comparison to that of younger animals, indicating that the expressions of NADPH oxidase tend to increase in aged blood vessels [28]. Given the aforementioned results, we suppose that Res can attenuate vascular cell aging through decreasing the NADPH oxidase expression. Additionally, studies have shown that SIRT1, a NAD^+-dependent protein deacetylases, also plays a critical role of the antisenescence effect of Res [14,29–32]. Overexpression or activation of SIRT1 in human umbilical vein endothelial cells (HUVECs) has been shown to slow the senescence process [20,21]. Inhibition of SIRT1 led to accelerate the senescence program in vessels of rats and HUVECs [33,34]. A study by Schilder et al. implied that SIRT1 may be an upstream regulator of NADPH oxidase in HUVECs [23]. However, the effect of Res on the vasculature senescence and its association with p47phox and SIRT1 have not been well established in animals. Therefore, in this study, we investigated the effect of Res on the senescence process in aorta of rats induced by high-fat/sucrose diet and in cultured bovine aortic endothelial cells (BAECs) induced by the exposure to high glucose (Glu). We also studied the effects of Res on NADPH oxidase and SIRT1, and further explored the relationships between NADPH oxidase and SIRT1 in cell experiment.

2. Materials and methods

2.1. Chemicals or reagents and antibodies

Res was from Biological Co., Ltd. NanJing, Zelang (JiangSu, China). Senescence-associated β-galactosidase (SA-β-gal) staining kit and dichlorofluorescein diacetate (DCFH-DA) were purchased from Beyotime Institute of Biotechnology (JiangSu, China); dihydroethidium (DHE) was from Molecular Probes (Eugene, OR, USA). Goat polyclonal anti-NADPH oxidase p47phox was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-SIRT1 and anti-Nucleolin were from Abcam Limited (Cambridge, UK); mouse anti-β-actin antibody, sirtinol and apocynin were from Sigma (St. Louis, MO, USA). Other reagents used were of the highest grade commercially available.

2.2. Animals and diets

Animals were cared for according to the *Guiding Principles in the Care and Use of Laboratory Animals* published by the US National Institutes of Health. Experiments described in this study were approved by the Tongji Medical College Council on Animal Care Committee. Animals were kept on a regular 12-h:12-h light/dark cycle at a controlled temperature (25°C±2°C) and relative humidity (65%–75%).Thirty-six Wistar male rats (197±10 g), from Shanghai Sippr-VK lab animal Co. Ltd., were divided into four groups on average and maintained on a normal diet (CON) containing 12.96 kJ/g (10.19% fat, 26.48% protein and 63.33% carbohydrate), high-fat/sucrose diet (HFS) containing 17.09 kJ/g [54.22% fat, 18.33% protein and 27.45% carbohydrate (19.65% starch, 7.8% sucrose)] and HFS plus Res [50, 100 mg/kg body weight (bw)] for 14 weeks. At the end of the experiment, three animals in each group were pressure-perused for SA-β-gal staining as markers of vascular cell senescence, and other animals were sacrificed after an overnight fasting. Blood samples were collected by decapitation. Fresh thoracic aorta about 1 cm in length for ROS determination and the remaining ones were used for reverse transcriptase polymerase chain reaction (RT-PCR) and Western blot analysis.

2.3. Analysis of blood samples

Levels of fasting plasma Glu were assayed with glucose oxidase-peroxidase assays (BIOSINO Biotechnology and Science Inc., Beijing, China). Levels of total cholesterol (TC) and triglycerides (TG) were measured by using enzymatic colorimetric assays (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) following the manufacturer's guidelines.

2.4. Cell culture

BAECs (no. C-003-5C) were obtained from Health Science Research Resources Bank (Osaka, Japan) and maintained at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum as described previously [35]. Confluent cultures were detached with trypsin/EDTA and plated on 96-well culture plates for evaluating cell viability and cellular ROS level, in 24-well culture plates for measurement of SA-β-gal activity or in 100-mm-diameter dishes for analysis of protein level of NADPH oxidase p47phox and SIRT1 expression.

2.5. Measurement of the viability of BAECs

Cells were seeded at a concentration of 5×10^3 cells 200 μl/well and then treated with Res and high Glu (concentration 33 mM) for 24 h, followed by measurements of cell viability as previously described [36]. Briefly, the cultured wells were treated for 4 h with solution (10 μl/well) provided in the Cell Counting Kit-8 (Dojindo, Japan). The level of cellular viability was measured at 450 nm by using a microplate reader.

2.6. SA-β-gal staining in aorta and BAECs

Rats were anesthetized with sodium pentobarbital (30 mg/kg). The central portion of thoracic aorta was excised after perfusion fixation for SA-β-gal activity measurement according to previously described instructions [37]. Levels of senescence in cultured BAECs were evaluated by quantifying the activity of SA-β-gal with the staining solution provided in the SA-β-gal assay kit as previously described [38].

2.7. Measurement of ROS levels in aorta of rats and BAECs

DHE was used for *in situ* detection of ROS [39,40] in the aorta of rats. Fresh cross sections (5 μm) of unfixed but frozen aorta were immediately incubated with 5 M DHE at 37 C for 15 min in a humidified chamber. Fluorescence level was then visualized with a fluorescence microscope. After incubation in high Glu with or without Res (0.01, 0.1, 1.0 μM) for 24 h, ROS production in cultured BAECs was detected by adding DCFH-DA (10 μM) to the culture media for 20 min, followed by the quantifications with spectrometry (488 nm as the excitation length and 525 nm as the emission length).

2.8. Real-time RT-PCR analysis

Total RNA was extracted from thoracic aorta using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Target mRNAs were quantified by using the SYBR green-based qRT-PCR kit (TaKaRa BIO Inc., Dalian) and specific oligo primers in a real-time PCR machine (7900HT, Applied Biosystems, Foster City, CA, USA). The efficiency of the PCRs was determined using a series of dilutions of a standard vascular sample. The specificity of the product was assessed by melting curve analysis. Gene expression was determined by using the $2^{-\Delta\Delta Ct}$ method. Gene expression of Sirt1 (NM_001107627.1) and NADPH oxidase p47phox (AF260779) is presented as fold change relative to control. The mRNA level of glyceraldehyde 3-phosphate dehydrogenase (NM_017008) was quantified as an endogenous control. The forward and reverse primers for Sirt1 were TTC AGA ACC ACC AAA GCG and CAG CAA GGC GAG CAT AAA, respectively. The primers for NDAPH oxidase p47phox were AGG TGG TAT GAT GGG CAG CGT G and CCT CGG TTT GGC TTC ATC TGG C. The primers for GADPH were GCA AGT TCA ACG GCA CAG and GCC AGT AGA CTC CAC GAC AT.

2.9. Western blot analysis

Thoracic aorta tissues were homogenated and lysed in radioimmunoprecipitation assay lysis buffer (1% Triton X-100, 1% deoxycholate, 0.1% sodium dodecyl sulfate). The BAECs treated with high Glu and various concentrations of Res for 72 h were lysed in cell plasma and nuclear protein extracts kit (Boster, China) according to the manufacturer's instructions. Tissue and cell lysates with equal amount of proteins were subjected to Western blotting. Levels of target proteins were probed with the specific primary antibodies against the target protein and the species-specific second antibodies conjugated to horseradish peroxidase. Immunoreactive bands were detected by means of an ECL plus Western Blotting Detection System (Amersham Biosciences, Little Chalford, UK) according to the manufacturer's instructions. Quantitative analysis of the relative density of the bands in Western blots was performed by Gel Pro 3.0 software (Biometra, Goettingen, Germany). Data were corrected for background, standardized to β-actin or nucleolin as optical density (OD/mm^2).

2.10. Statistical analysis

All data were inputted in Excel and are expressed as means±S.E. Statistical analyses of data were performed using one-way analysis of variance with SPSS 12.0 software (SN: 59245 46841 40655 89389 09859 21671 21957 29589 12). A difference between groups of $P<.05$ was considered significant.

3. Results

3.1. Treatment with Res prevented the HFS-induced changes in body weight gain and serum levels of Glu and lipids

By the end of the treatment periods, rats fed with HFS gained more weight than others, while Res decreased the weight of rats. After exposure to different doses of Res, levels of serum Glu and lipids (TC and TG) initially increased by HFS were decreased (Table 1).

3.2. Treatment with Res decreased the marker of senescence in aorta induced by HFS in rats or by high Glu in cultured BAECs

The histological appearances of representative vessels in different groups were shown in Fig. 1A. In contrast to vessels of controls, the high-fat/sucrose group showed an accumulation of SA-β-gal-positive cells in arteries. However, Res can apparently reduce the blue cells in vessels.

To investigate the concentration range of Res on protection of BAECs against senescence, we detected the effect of Res (0.01, 0.1, 1.0, 10 μM) on the viability of BAECs incubated in high Glu for 24 h with CCK-8 assay. To determine whether or not the high Glu-induced change in viability of BAECs was due to the change in osmolarity, some BAECs were treated with a high concentration of mannitol. The high level of mannitol did not decrease viability, suggesting that the high Glu-reduced vability of BAECs was caused by increased osmolarity. Res (0.01, 0.1, 1.0 μM) protected against high-Glu-induced decrease in the viability of cells in a dose-dependent manner (Fig. 1D). To further study the effect of Res on senescence of vascular cells, senescence was induced in BAECs by high Glu in the presence of 0–1 μM of Res. As shown in Fig. 1B–C, the number of SA-β-gal-positive cells was significantly increased by the exposure to high Glu. However, the increase was prevented by various amounts of Res. The results of high-Glu-induced endothelial cells senescence were consistent with other groups' studies [41].

3.3. Res prevented ROS production induced by HFS in aorta or by high Glu in BAECs

Recently acquired evidence has pointed out that ROS, which exerts damaging effects through oxidative stress, is a primary contributor to the cell senescence process [42]. ROS production was measured *in vitro* and *in vivo* in our studies. As shown in Fig. 2A–B, ROS level in aorta was significantly elevated by HFS. Application of Res prevented the HFS-induced increase in ROS production in a dose-dependent manner. To further investigate the effect of Res in preventing ROS production in vasculatures, ROS production was induced by high Glu in the presence of different amounts of Res. As shown in Fig. 2C, the production of ROS was significantly increased with high Glu incubation and decreased with Res treatment dose dependently. Together, these results suggested that ROS in part can be prevented and/or delayed by Res.

Table 1
Effects of Res on weight, serum Glu, TC, TG concentrations in rats fed HFS diet

	CON	HFD	HR1	HR2
Weight (g)	375.78±26.25	440.89±37.13 *	418.44±27.33	400.67±33.07 #
FPG (mmol/L)	5.86±0.63	7.33±1.76 *	6.20±0.89	5.95±0.63 #
TG (mmol/L)	0.93±0.24	1.50±0.23 *	1.01±0.25 #	1.20±0.59
TC (mmol/L)	1.06±0.26	1.55±0.29 *	1.29±0.24	1.15±0.28 #

Thirty-six rats were randomly divided into four groups on average, namely, CON, HFS, HR1 and HR2. Data were shown as mean±S.D., *n* (weight)=9, *n* (others)=6. FPG: fasting plasma glucose, CON: normal diet-fed rats, HFS: high-fat/sucrose diet-fed rats, HR1: 50 mg/kg·bw Res+HFS, HR2:100 mg/kg·bw Res+HFS.

* P<.05 vs. CON.

\# P<.05 vs. HFS.

3.4. Application of Res reversed the changes in expressions of NADPH oxidase p47phox and SIRT1 in aorta of animals induced by HFS and in cultured BAECs induced by high Glu

To investigate the mechanism by which Res prevents the HFS- and high-Glu-induced ROS production, expressions of p47phox and SIRT1 were studied because P47phox, a cytosolic membrane in NADPH oxidase, plays a crucial role in the production of superoxide anions [43–46] and SIRT1 is a known antioxidation agent. We observed that HFS diet apparently increased mRNA and protein expression of p47phox in aorta of rats, while treatment with increasing concentrations of Res attenuated HFS-induced up-regulation of p47phox. We also found that HFS decreased mRNA and protein expression of SIRT1 in aorta. Res enhanced HFS-induced down-regulation of SIRT1 in both concentrations; the higher dose was more effective than the lower one (Fig. 3A, B, D and E). Fig. 3G and H depicted the dose–response effects of Res on protein levels of p47phox and SIRT1 in BAECs. The protein level of p47phox was elevated by the treatment with a high level of Glu, but the increase was reversed by an increasing amount of Res. The protein level of SIRT1 was suppressed by a high level of Glu, and the suppression was reversed by Res completely. Levels of p47phox and SIRT1 were then scatter-plotted; as shown in Fig. 3C, F and I, expressions of p47phox and SIRT1 were negatively correlated in both aorta and culture BAECs. Together, these results demonstrate that Res can reduce oxidative stress by blocking the superoxide-dependent ROS production and promoting the SIRT1-mediated antioxidation system.

3.5. SIRT1/NADPH oxidase pathway may play an important role in Res reducing BAECs senescence

To further examine the relationship between SIRT1 and NADPH oxidase, BAECs were preincubated with sirtinol as inhibitor of SIRT1 or apocynin as inhibitor of NADPH oxidase before incubation with high Glu. Results (Fig. 4) demonstrated that apocynin also showed the same beneficial effect as Res decreasing the protein of NADPH oxidase expression, and sirtinol up-regulated NADPH oxidase expression and presented the reverse effect of Res, suggesting that SIRT1 plays an inhibitory role in expression of p47phox in BAECs. We also found that sirtinol down-regulated protein of SIRT1 levels, but apocynin did not, suggesting that expression of SIRT1 is not controlled by p47phox. Together, these results indicate that SIRT1 plays its function through inhibiting expression of p47phox in BAECs.

4. Discussion

It is well established that Res is an antiaging reagent, but much of the associated mechanisms remain unknown. In this study, we investigated the effect of Res in vascular senescence induced either by HFS in aorta of rats or by exposure to high Glu in cultured BAECs. There are several novel observations in this study: (a) To our knowledge, we demonstrated that Res attenuated oxidative-stress-induced vascular cell senescence in animals for the first time. (b) Res can inhibit ROS production by down-regulating the NADPH oxidase expression, accompanied by up-regulation of SIRT1. (c) Our observations provided the first evidence that SIRT1 functions as a novel upstream regulator for NADPH oxidase signaling involved in the onset and process of vascular cell aging.

The Res-mediated prevention of vasculature senescence induced in aorta by the HFS diet or by exposure to a high level of Glu in vascular endothelial cells is tightly linked to the Res-mediated reduction in ROS generation. This vasoprotective effect of Res is especially important because the HFS diet used in this animal study is very similar to modern human diets in industrialized societies and increased exposure to a high level of Glu occurs in people with diabetes,

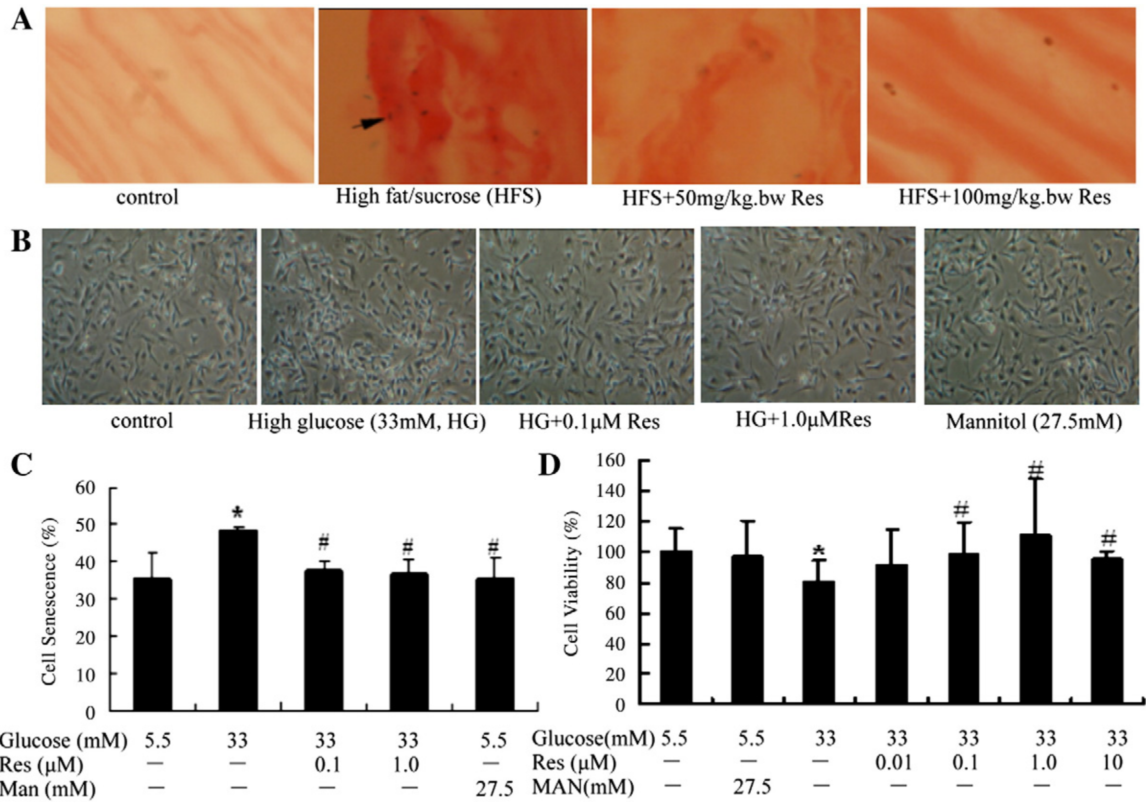

Fig. 1. Res prevents the senescence induced in aorta by HFS diet and in cultured BAECs by the exposure to high Glu. (A) Wistar rats were fed either normal diet or HFS in the absence or presence of different amounts of Res for 14 weeks. A portion of the thoracic aorta was collected and stained for the presence of SA-β-gal. The SA-β-gal-staining cells were visualized by microscope (×1000). (B–C) BAECs were incubated for 7 days with different concentrations of Glu (5.5 mM for control and 33 mM for other groups) in the presence of Res (0.1 or 1 μM) as noted. Some cells were treated with mannitol (27.5 mM) as an osmotic control for the same length of time. SA-β-gal activity was stained and visualized by using fluorescence microscope (×100), quantified and presented as mean±S.D. (D) Effect of Res on the viability of BAECs. *P<.05 vs. control; #P<.05 vs. high Glu.

which is becoming pandemic now. There is accumulating evidence suggesting that many of the changes in senescent vascular cells are in accordance with those in human atherosclerosis [1]. For example, the interaction between monocytes and vascular endothelial cell has been strengthened in the senescent endothelial cell, which contributes to the promotion of atherosclerosis [47]. Additionally, an increased rate of senescence of vascular cells was observed in the progression of diabetes [48–51], which can lead to various diabetic

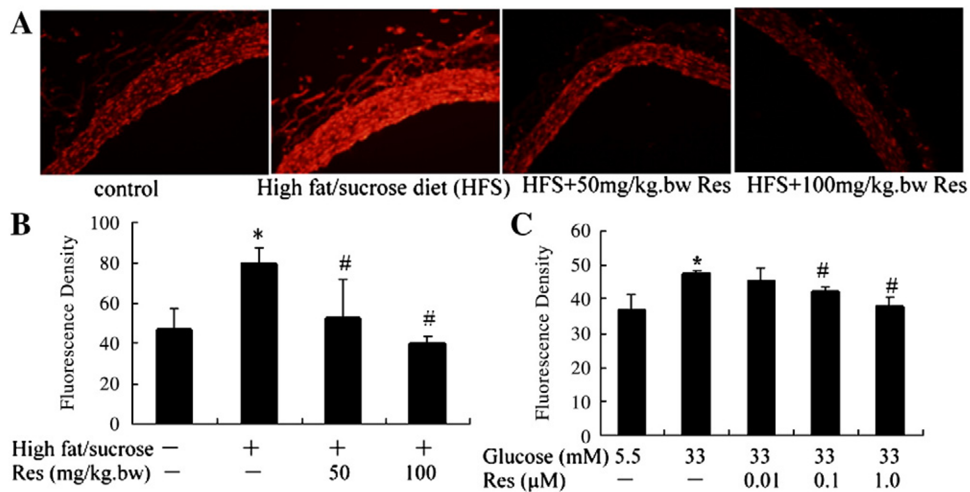

Fig. 2. Res prevented increase in ROS production in rat aortas fed with HFS and BAECs incubated with high Glu. (A–B) ROS in the aortas of the rats was detected by using dihydroethidium (DHE) which reacts with ROS and forms ethidium bromide (ETH) that binds to DNA and produces red fluorescence signal, visualized with fluorescence microscope (×200) and quantified. Fluorescence intensities in randomly selected areas of the images were quantified by using the IPP image analysis software. Results were presented as mean ±S.D. (n=3 for HFS and HFS+50 mg/kg Res, n=4 for control and HFS+100 mg/kg Res). *P<.05 vs. control; #P<.05 vs. HFS. (C) BAECs were incubated with media containing 33 mM Glu in the presence of various amounts of Res as noted. ROS in the cells was detected by using DCFH-DA and quantified with a microplate reader at 480/525 nm. Results represent mean ±S.D. of five independent experiments. *P<.05 vs. control; #P<.05 vs. high Glu.

Fig. 3. Treatment with Res reverses expressions of SIRT1 and P47 that are altered by HFS or high Glu in aorta or cultured BAECs. The mRNAs and proteins of p47phox (A, D) and SIRT1 (B, E) in the thoracic aortas of the rats were quantified with real-time RT-PCR or detected with Western blotting. The quantitative results represent mean±S.D. of three independent tests. (G, H) BAECs were incubated with media containing either 5.5 mM or 33 mM of Glu in the presence of Res as noted for 72 h. Proteins of p47phox and SIRT1 were detected by using Western blotting and quantified. Results represent mean±S.D. of three independent experiments. Results in A, B, D, E, G and H were scatter-plotted and presented in C, F and I. *P<.05 vs. control; #P<.05 vs. HFS or high Glu.

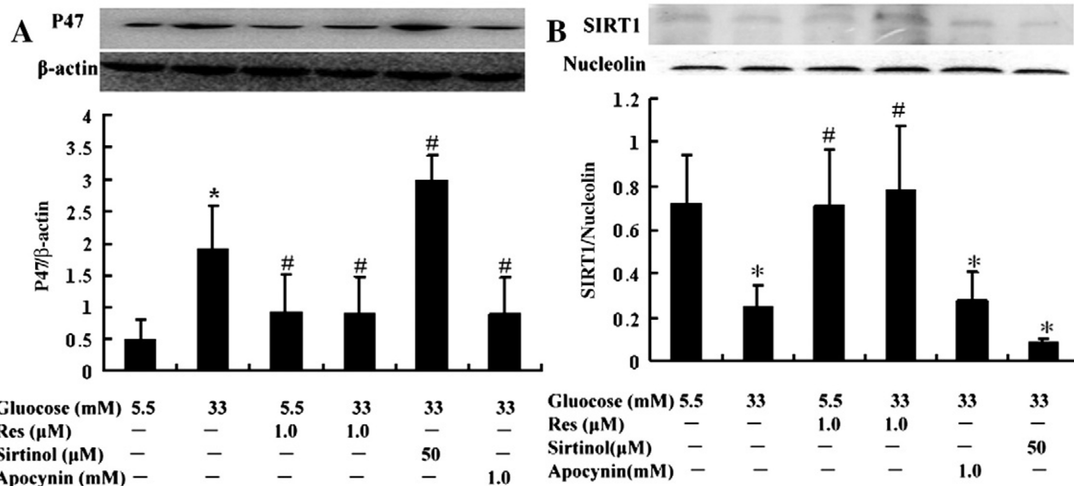

Fig. 4. Influence of apocynin and sirtinol on protein expression of P47 (A) and SIRT1 (B) in BAECs. After pretreatment with sirtinol (50 μM) and apocynin (1 mM) for 1 h, BAECs were incubated with media containing 5.5 mM or 33 mM Glu in the absence or presence of Res as noted for 72 h. Proteins were extracted by cell nucleus/plasma extraction kit, and proteins of p47phox and SIRT1 were detected with Western blotting, quantified and normalized to β-actin or nucleolin as noted. Results represent mean±S.D. of three independent experiments. *P<.05 vs. CON; #P<.05 vs. high Glu.

complications such as atherosclerotic cardiovascular disorders. Therefore, it is likely that the antiaging action of Res will contribute to its cardiovascular protective effects *in vivo*.

Furthermore, *in vitro*, we found that high-Glu-induced cell senescence was reversed by Res at a certain range of concentrations. Concentrations of Res in micromolar levels are sufficient to prevent endothelial cell from senescence, which is consistent with studies by other laboratories [6,52,53]. The levels of Res used in our studies are generally consistent with dietary intake concentration estimated by Zamora-Ros et al., who estimated that the amount of dietary intake for men and women is on average 1629 μg/day and 235 μg/day, respectively; these amounts would result in peak serum concentrations of approximately 2.06 μM and 0.29 μM free Res in a 70-kg person [52,54–56]. In the effort to use Res as a caloric restriction mimic, it is important that the concentrations of Res in the blood have to be in the range of effective concentrations without adverse effects. Otherwise, the antiaging effect of Res may always be achievable [23].

It is known that increased ROS production in aging vessels is mainly caused by the enhanced activity and/or expression of NADPH oxidase [26,57–59]. NADPH oxidase, composed of a membrane-associated unit and a cytosolic component, is a membrane-associated enzyme catalyzing the one-electron reduction of oxygen with NADH or NADPH as the electron donor [60]. The cytosolic unit contains p47phox, p40phox, p67phox and Rac, which all bind to the membrane-associated component upon activation [61]. We determined the expression of the cytosolic NADPH oxidase subunit p47phox, which plays an essential and crucial role in activating NADPH oxidase activity and subsequently in the production of superoxide anions *in vitro* and *in vivo* [43,46], as a main regulator of NADPH oxidase activation by organizing the complex [61]. Barry-Lane et al. have previously shown that p47phox is required for atherosclerosis lesion progression in ApoE−/− mice [62]. Mukai et al. have reported that the level of p47phox mRNA in aorta in rats increased significantly with spontaneous hypertension compared with Wistar-Kyoto rats, while p22phox mRNA level was not altered [63]. Donato et al. demonstrated that older men exhibited elevated venous endothelial cell p47phox compared to young subjects [64]. Here, we show for the first time that Res can reverse the expression of p47phox stimulated by exposure to high Glu or by HFS diet. These observations suggest that it is possible to prevent or reduce the NADPH oxidase-dependent oxidative stress, vascular senescence, and atherosclerosis by targeting specific subunits of the NADPH oxidase such as p47phox.

Our aforementioned results suggested that Res protects vascular cell senescence in part by reducing the production of ROS through decreasing NADPH oxidase expression. Meanwhile, SIRT1 plays an important role in extending life span in many organisms. Overexpression of SIRT1 can prevent the oxidative-stress-induced premature senescence [65], and inhibition of SIRT1 by using either siRNA or chemicals abolishes the antioxidation and antiaging effect of Res in endothelial cells [20,23]. The relationship between SIRT1 and p47phox is unclear. Here, we show that treatment with Res alters expressions of both SIRT1 and p47phox genes. Expression of p47phox is increased when SIRT1 is inhibited. In contrast, inhibition of p47phox does not affect the expression of SIRT1. Thus, it appears that SIRT1 is an upstream inhibitor of p47phox. These results provide new understanding about the mechanism by which Res slows vasculature senescence and the overall aging process.

Acknowledgments

This work was supported by the National High Technology Research and Development Program of China (2010AA023003). In particular, we appreciate the valuable suggestions and guidance of Prof. Wenhong Cao (Department of Nutrition, University of North Carolina at Chapel Hill 27759, NC, USA) with polishing language.

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