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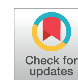
The protective effect of resveratrol on vascular aging by modulation of the renin–angiotensin system



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Background and aims: This study evaluated the effects of resveratrol on arterial aging and the renin–angiotensin system (RAS) in mice and vascular smooth muscle cells (VSMCs).

Methods: Aging mice were divided into control and resveratrol groups. Histological changes, inflammation, oxidative stress, RAS components, and the expression of AMP-activated protein kinase (AMPK), silent information regulator T1 (SIRT1), peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α), and anti-oxidative enzymes was measured in thoracic aortas of 24-month-old mice. The effect of resveratrol on fibrosis, cell senescence, and RAS components was also investigated in VSMCs stimulated by angiotensin (Ang) II.

Results: Aorta media thickness, inflammation, fibrosis, and oxidative stress were significantly lower in the resveratrol group than in the control group. Resveratrol treatment decreased serum Ang II level and the aortic expression of prorenin receptor (PRR) and angiotensin converting enzyme (ACE), and increased serum Ang-(1–7) level and the expression of ACE2, Ang II type 2 receptor (AT2R), and Mas receptor (MasR). Resveratrol increased the expression of phosphorylated AMPK, SIRT1, PGC-1α, phosphorylated endothelial nitric oxide synthase and superoxide dismutase 1 and 2, and decreased that of NADPH oxidase 2 and 4. In Ang II-stimulated VSMCs, resveratrol treatment markedly decreased the number of senescence associated β-galactosidase stained cells and pro-fibrotic protein expression and increased the expression of AT2R and MasR.

Conclusions: Resveratrol protects against arterial aging and this effect is associated with reduced activity of the PRR–ACE–Ang II axis and stimulation of the ACE2–Ang-(1–7)–ATR2–MasR axis.

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1. Introduction

Aging is accompanied by vascular structural and functional changes, which are implicated in the increased cardiovascular risk of the elderly population [1]. Arterial aging is characterized by increased luminal diameter, intimal and medial thickening, reorganization of the extracellular matrix, vascular stiffening, and endothelial dysfunction [2]. Theories to explain the mechanism of arterial aging include mitochondrial dysfunction, oxidative stress, altered calcium regulation, increased DNA protein and lipid oxidation, inflammation, and the activation of the renin–angiotensin-system (RAS) [3]. Chronic activation of the RAS promotes end-stage organ injury, which is related to aging through the increases in tissue and mitochondrial oxidative stress [4]; the activity or responsiveness of the RAS also changes with aging [5].

Angiotensin (Ang) II is the main effector molecule implicated in the RAS-associated vascular aging [4]. Ang II acts through two

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distinct receptor subtypes: Ang II type 1 receptor (AT1R) and Ang II type 2 receptor (AT2R) [6]. Activation of the AT1R promotes vasoconstriction, reactive oxygen species (ROS) production, extracellular matrix remodelling, and inflammation [7]. Activation of the AT2R inhibits cell growth, inflammation, and fibrosis [8,9]. Therefore the effects of AT2R seem to counterbalance the detrimental effects mediated by Ang II and the AT1R and to protect against the progression of end-organ damage [6]. Angiotensin–converting enzyme (ACE) converts Ang I to Ang II, and angiotensin-converting enzyme 2 (ACE2) metabolizes Ang II and generates Ang-(1–7) [10]. Ang-(1–7) acts at the Mas receptor (MasR) [11], and the ACE2-Ang-(1–7)-MasR axis exerts vasodilator, anti-proliferative, and anti-fibrotic actions, which oppose those of the ACE–Ang II–AT1R axis [12]. The prorenin receptor (PRR) binds renin and its precursor, prorenin [13], and is known to play an integral role in tissue-specific RAS, such as in the brain [13]. The binding of prorenin or renin to the PRR activates the intracellular signalling pathway and allows for the conversion of angiotensinogen to Ang I and Ang I to Ang II by the prorenin–PRR complex [13–15].

Resveratrol is a natural plant polyphenol and an activator of AMP-activated protein kinase (AMPK), silent information regulator T1 (SIRT1), known as sirtuin 1, and the anti-oxidative enzyme superoxide dismutase (SOD) [16,17]. AMPK directly phosphorylates serine 1177 and activates endothelial nitric oxide synthase (eNOS) to increase nitric oxide production [18]. Resveratrol has been shown to increase eNOS phosphorylation through AMPK activation in hypertensive rats and mice [19]. SIRT1 activates several transcriptional factors, such as peroxisome proliferator activated receptor-γ co-activator 1α (PGC-1α) and class O forkhead box, and attenuates oxidative stress in metabolic and aging-related diseases [20]. PPARα regulates the cellular state, energy metabolism, development, and lipid metabolism, and PPARα activators have anti-inflammatory, anti-angiogenic, anti-apoptotic, and anti-oxidative effects. PPARα activation in turn activates AMPK, which results in the phosphorylation of protein kinase B (Akt/PKB) and eNOS activation [21–23]. Resveratrol has been shown to attenuate inflammation, oxidative stress, and endothelial dysfunction by modulating the PPARα signalling pathway in diabetic nephropathy and renal lipotoxicity [24,25].

Accumulating evidence suggests that Ang II signalling affects sirtuin signalling and causes mitochondrial dysfunction [26,27]. Overexpression of SIRT1 in vascular smooth muscle cells (VSMCs) and resveratrol-induced activation of SIRT1 in mice downregulate AT1R expression [28]. Resveratrol increases SOD and SIRT1 levels and suppresses cardiomyocyte death induced by Ang II [29]. These findings suggest that resveratrol may protect mitochondria by blocking the RAS. We have previously reported that the aorta of aging mice exhibits altered expression of the PRR–ACE–Ang II–AT1R axis and the ACE2–MasR axis [30]. In this study, we hypothesized that resveratrol would attenuate the aging process in the aorta in mice and Ang II-induced VSMC injury by modulating RAS components and by activating AMPK–SIRT1–PGC-1α signalling and PPARα signalling.

2. Materials and methods

2.1. In vivo experiments

The Animal Care Committee of the Catholic University approved the experimental protocol. Eighteen-month-old male C57BL/6 mice were purchased from the Korea Research Institute of Bioscience and Biotechnology (Chungcheongbuk-do, Republic of Korea). Mice were housed in a temperature- and light-controlled environment with a 12:12-h light–dark cycle and had free access to water. Mice were divided into two groups. The control group (Cont, $n = 7$) received normal mouse chow (PicoLab Rodent Diet 20 5053, LabDiet, St. Louis, MO, USA), and the resveratrol-treated group (Resv, $n = 7$) received a mixture of resveratrol (40 mg/kg; Sigma-Aldrich, St Louis, MO, USA) and normal chow for 6 months. The mice were sacrificed at the age of 24 months. Each mouse was placed in an individual mouse metabolic cage (Tecniplast Gazzada, Italy) and was allowed access to water and food for 24 h, and were maintained in fasting state for 8 h before sacrifice.

2.2. Cell culture and in vitro experiments

VSMCs were grown in smooth muscle cell medium (ScienCell, Carlsbad, CA, USA) supplemented with 5% fetal bovine serum (FBS) (WISENT INC, Principale, St-Jean-Baptiste, Qc, Canada) in a humidified atmosphere of 95% air, 5% CO_2 at 37 °C. VSMCs were used between passages 10 and 13. VSMCs were plated at a density of 3×10^5 cells per well in 6-well plates for 3days and then were changed to smooth muscle cell medium with 1% FBS including AngII (Sigma Life Science) 100 nM with or without resveratrol 25 μM for 24 h. Cells were harvested at the end of the treatment for further analysis. The dosage of Ang II [31,32] and resveratrol [33] was chosen based on previous reports.

2.3. Histological and microscopic analyses

Thoracic aorta tissues were embedded in low-temperature melting paraffin, and 4-μm-thick sections were processed and stained with haematoxylin–eosin (HE). HE-stained sections were analysed with a colour image analyser (TDI Scope Eye, Version 3.5 for Windows, Olympus, Tokyo, Japan). The thickness of the media was assessed by measuring the cross-sectional length of the media at 10 different positions in six different sections per animal.

2.4. Immunohistochemistry

Deparaffinized tissue sections were processed for immunohistochemistry as described elsewhere [34], using primary antibodies to AT1R (Santa Cruz Biotechnology, TX, USA), AT2R (Novus Biologicals, Littleton, CO, USA), MasR (Novus Biologicals), 8-hydroxy-2′-deoxyguanosine (8-OHdG, Japan Institute for the Control of Aging, Shizuoka, Japan), 3-nitrotyrosine (Santa Cruz Biotechnology), and transforming growth factor-β (TGF-β, R&D Systems, MN, USA). All sections were assessed using a colour image analyser (TDI Scope Eye, Version 3.5 for Windows) and quantified using ImageJ software.

2.5. Immunofluorescence analysis

We used immunofluorescence analysis to analyse the expression of collagen IV (Abcam, Cambridge, UK) and fibronectin (Proteintech Group Inc, IL, USA), using a tyramide signal amplification fluorescence system (PerkinElmer, Waltham, MA, USA) as described elsewhere [24].

2.6. Western blot analysis

Total protein was extracted from the thoracic aorta tissues using Pro-Prep Protein Extraction Solution (Intron Biotechnology, Gyeonggi-Do, Republic of Korea) according to the manufacturer's instructions. Western blot analysis was performed using the following antibodies: Tumor necrosis factor-α (TNF-α, Abcam), ACE (Santa Cruz Biotechnology), ACE2 (R&D Systems), PRR (Sigma Life Science, MO, USA), AT1R (Santa Cruz Biotechnology), AT2R (Novus Biologicals), MasR (Novus Biologicals), endothelial nitric oxide

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