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EDITORIAL

Resveratrol: A Multifunctional Compound Improving Endothelial Function

Editorial to: "Resveratrol Supplementation Gender Independently Improves Endothelial Reactivity and Suppresses Superoxide Production in Healthy Rats" by S. Soylemez et al.

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Abstract The red wine polyphenol resveratrol boosts endothelium-dependent and -independent vasorelaxations. The improvement of endothelial function by resveratrol is largely attributable to nitric oxide (NO) derived from endothelial NO synthase (eNOS). By stimulating eNOS expression, eNOS phosphorylation and eNOS deacetylation, resveratrol enhances endothelial NO production. By upregulating antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and suppressing the expression and activity of NADPH oxidases, resveratrol inhibits superoxide-mediated NO inactivation. Some resveratrol effects are mediated by sirtuin 1 (SIRT1) or estrogen receptors, respectively.

Introduction

Resveratrol is a polyphenol phytoalexin present in a variety of plant species, including white hellebore (*Veratrum grandiflorum O. Loes*), *Polygonum cuspidatum*, grapes, peanuts and mulberries [1–3]. Resveratrol attracted little interest until 1992 when it was postulated to explain some of the cardioprotective effects of red wine. Since then, accumulating reports have shown that resveratrol can prevent or slow the progression of a wide variety of diseases, including cancer, cardiovascular disease, ischemic injuries and Alzheimer's disease. The compound has also been shown to extend the lifespan of various organisms from yeast to vertebrates [1, 2, 4].

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Resveratrol induces vasorelaxation in vitro

In organ chambers in vitro, resveratrol inhibits the contractile response to noradrenaline [5] and causes relaxation of the phenylephrine-precontracted rat aorta [5]. The vasorelaxant activity of resveratrol has also been observed in the mesenteric and uterine arteries of guinea pigs [6], and in porcine coronary arteries [7]. Both endothelium-dependent and endothelium-independent mechanisms are involved in resveratrol-induced vascular relaxation [2]. The endothelium-dependent vasorelaxation is largely attributable to NO, whereas the endothelium-independent relaxation is likely to be mediated by some ion channels including voltage-gated K^+ channels, big Ca^{2+}-activated K^+ channels or voltage-gated Ca^{2+} channels [8, 9].

Resveratrol improves endothelial function in vivo

Endothelial dysfunction (characterized as an impairment of endothelium-dependent relaxation) is an early event in the development of atherosclerosis and is present even before structural changes occur in the vasculature. All major risk factors for atherosclerosis such as hyperlipidemia, diabetes, hypertension and smoking are associated with endothelial dysfunction [10].

Oral treatment with resveratrol results in the enhancement of agonist-stimulated, endothelium-dependent relaxations, demonstrating its therapeutic potential. Such an improvement in endothelial function has been shown in hypertensive rats [11–13], diabetic rats and mice [14, 15] and hypercholesterolemic rabbits [16].

In the current issue of *Cardiovascular Drugs and Therapy*, Soylemez et al. provide evidence that in vivo

resveratrol supplementation also improves endothelial responsiveness in healthy rats [17]. This indicates the potential of resveratrol for health promotion and disease prevention.

Resveratrol enhances NO synthesis by modulating eNOS expression and activity

The resveratrol-induced improvement of endothelial function is largely attributable to nitric oxide (NO) derived from endothelial NO synthase (eNOS). Resveratrol enhances NO synthesis and decreases NO inactivation (Fig. 1).

Resveratrol enhances eNOS expression We have shown that resveratrol [18] and red wine rich in resveratrol [19] enhance the expression of eNOS in human endothelial cells. Resveratrol is known to be an activator of the protein deacetylase Sirtuin 1 (SIRT1) [20]. The effect of resveratrol on eNOS expression is likely to be mediated by SIRT1. Calorie restriction leads to the enhanced expression of SIRT1 and eNOS upregulation [21]; the endothelium-specific overexpression of SIRT1 results in an increase in eNOS expression in mice [22]. In human coronary arterial endothelial cells, resveratrol-induced eNOS expression can be prevented by the knockdown of SIRT1 [23].

Resveratrol enhances eNOS phosphorylation The treatment of endothelial cells with nanomolar concentrations of resveratrol leads to the rapid phosphorylation of eNOS at serine 1177 and an increase in eNOS enzymatic activity [24, 25]. In human endothelial cells, this effect seems to be mediated by the estrogen receptor ERα that is localized in a "signalsome complex" within caveolae [25]. Resveratrol rapidly activates ERα in caveolae, leading to eNOS activation via the activation of Gα, Cav-1, Src and MAPK (ERK1/2) in a manner similar to that elicited by estradiol [25]. In addition, a recent study demonstrated that resveratrol prevented hyperglycemia-induced endothelial dysfunction via the activation of adenosine monophosphate-activated protein kinase (AMPK) [26].

Resveratrol decreases eNOS acetylation SIRT1 deacetylates ε-acetyllysine residues in histones and many non-histone proteins including eNOS. SIRT1 deacetylates eNOS at lysine residues in the calmodulin-binding domain and thereby stimulates eNOS activity. The short-term treatment

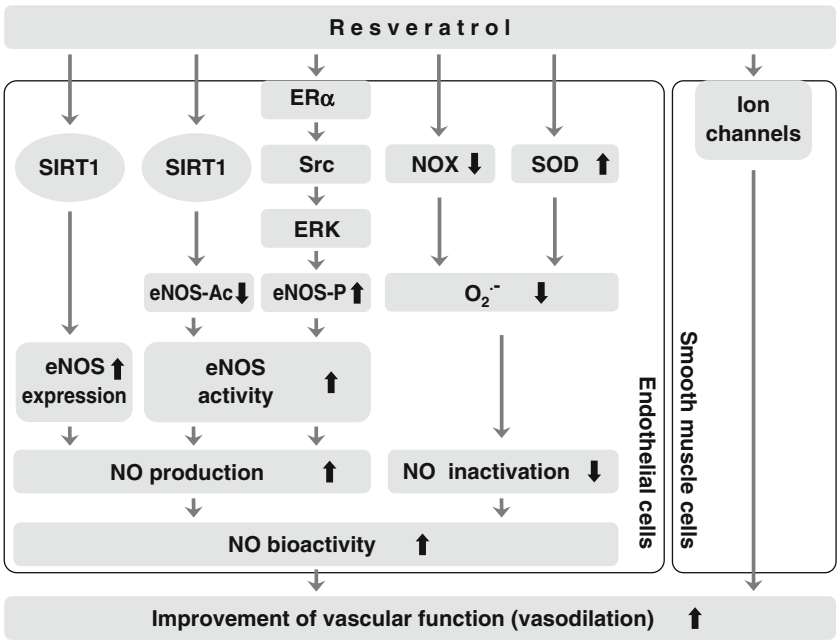

Fig. 1 Mechanisms of resveratrol-induced improvement of vascular function. Resveratrol induces vasorelaxation through both endothelium-dependent and -independent mechanisms. Resveratrol increases endothelial NO production by SIRT1-dependent eNOS upregulation, SIRT1-dependent eNOS deacetylation and estrogen receptor ERα-dependent, ERK1/2-mediated eNOS phosphorylation. By decreasing the expression and activity of vascular NADPH oxidases (NOX) and enhancing the expression of superoxide dismutases (SOD), catalase and glutathione peroxidases, resveratrol decreases superoxide-mediated NO inactivation. The resulting elevation in NO bioactivity is likely to mediate the endothelium-dependent relaxation. Ion channels seem to be involved in the endothelium-independent effects of resveratrol

of endothelial cells with resveratrol leads to SIRT1 activation and eNOS deacetylation. Resveratrol-induced endothelial NO production can be significantly reduced by siRNA-mediated SIRT1 knockdown [27].

Resveratrol reduces superoxide-mediated NO breakdown

NO can be rapidly inactivated by superoxide. There is evidence that resveratrol can reduce vascular superoxide levels through multiple mechanisms (see below). This enhances NO bioavailability and is likely to contribute to the improvement of endothelial function by resveratrol.

The direct antioxidant activity of resveratrol is poor As a polyphenolic compound, resveratrol has been shown to be a scavenger of hydroxyl, superoxide and metal-induced radicals [2]. However, the direct antioxidant effects of resveratrol are rather poor; resveratrol is less potent than other well-established antioxidants such as ascorbate and cysteine [2]. In addition, resveratrol is ineffective at scavenging superoxide anions generated enzymatically by a hypoxanthine/xanthine oxidase (HX/XO) system and/or inhibiting XO [28, 29]. Therefore, the direct antioxidant effects of resveratrol are likely to play only a secondary role in vasoprotection, and the protective properties of resveratrol are more likely to be attributable to effects on pro- and antioxidatives gene products.

Resveratrol inhibits NADPH oxidase activity and expression Reactive oxygen species (ROS) can be produced by several enzyme systems in the vascular wall with NADPH oxidases being the predominant source of ROS [30]. In the current issue of *Cardiovascular Drugs and Therapy*, Soylemez et al. demonstrate that resveratrol supplementation attenuates angiotensin II- or NADPH-induced superoxide production [17], which is compatible with previous studies [29, 31]. This might result from the reduced expression and/or activity of vascular NADPH oxidases. In endothelial cells, resveratrol attenuates oxidized low-density lipoprotein-stimulated NADPH oxidase activity by reducing the membrane translocation of Rac1, which is required for the assembly of the active NADPH oxidase complex [32]. Similarly, resveratrol restores endothelial function in type II diabetes by inhibiting the TNFα-induced activation of NADPH oxidase [15]. Resveratrol treatment also decreases the expression of NADPH oxidase (gp91phox, i.e. Nox2) [15]. Recent data from our laboratory demonstrate that resveratrol also decreases the expression of NADPH oxidase (Nox4) in endothelial cells [33].

Resveratrol enhances the expression of antioxidant enzymes Living organisms have developed a number of antioxidant enzyme systems to maintain their survival against oxidative stress. Major cardiovascular enzymatic antioxidants include superoxide dismutases (SOD) and catalase and glutathione peroxidases (GPx) [34]. Resveratrol treatment upregulates the expression of catalase and GPx1 in cultured arteries [35]. We have recently shown that resveratrol enhances the expression of SOD1 and GPx1 in endothelial cells, and this mechanism contributes to the reduction of endothelial oxidative stress by resveratrol [33].

Involvement of estrogen receptors and the question of gender differences

Resveratrol activates nuclear and extranuclear estrogen receptors (ER) [24, 36, 37]. Both ERα and ERβ are expressed in vascular smooth muscle and endothelial cells [38]. The rapid enzymatic activation of eNOS by resveratrol is mediated by plasma membrane-associated ER. In bovine aortic endothelial cells, ERα and ERβ are both involved in resveratrol-induced eNOS phosphorylation [24]. In human umbilical vein endothelial cells (HUVEC), however, the effect of resveratrol on eNOS phosphorylation is mediated by ERα, but not ERβ [25].

In addition to the rapid non-genomic effect on eNOS activity, resveratrol also enhances eNOS expression. Although estrogen itself increases eNOS expression in an ER-dependent manner [25, 39], the effect of resveratrol on eNOS expression seems to be ER-independent [18], but rather SIRT1-mediated [23].

Because vascular relaxation to estrogen shows gender-dependent differences [31, 40], vascular effects of resveratrol could be different between males and females. However, that does not seem to be the case. In the study by Soylemez et al., resveratrol decreased superoxide production, increased plasma nitrite/nitrate levels and enhanced acetylcholine-induced relaxation in endothelium-denuded arteries from both male and female rats [17]. No major gender differences were found. This is compatible with a previous study demonstrating that resveratrol has similar protective effects on oxidative DNA damage in male and female stroke-prone spontaneously hypertensive rats [41].

Taken together, a wide variety of signaling mechanisms including transcriptional and post-translational effects contribute to the improvement of endothelial function and enhanced vasorelaxation produced by resveratrol.

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Disclosures None

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