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Molecular mechanisms underlying pharmacological stimulation of eNOS expression and eNOS activity

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Endothelium-derived nitric oxide (NO) generated by endothelial NO synthase (eNOS) is a key regulator of endothelial function. Vascular NO induces vasodilation, inhibits platelet aggregation and adhesion, prevents oxidative modification of LDL cholesterol, limits vascular smooth-muscle-cell proliferation, and decreases the expression of proinflammatory genes that promote the development of atherosclerosis. In the presence of cardiac risk factors or cardiovascular disease, endothelial function is impaired. A growing amount of evidence indicates that oxidative stress – an imbalance between endogenous reactive oxygen species (ROS) and antioxidants in favor of the former – contributes markedly to endothelial dysfunction. A crucial aspect of endothelial dysfunction is the reduced bioactivity of NO. A dominant mechanism reducing bioavailability of vascular NO relates to its rapid oxidative inactivation by the ROS superoxide anion. In addition, evidence indicates that persisting oxidative stress renders eNOS dysfunctional, such that it ceases to produce NO and produces superoxide instead. In recent years, we have identified a number of compounds that can revert these events and improve the bioavailability of NO [1-3]. Below we describe the effects of trans-resveratrol as an example.

Trans-resveratrol, a naturally occurring phytoalexin found in grapes and wine, is likely to contribute to the potential of red wine in preventing human cardiovascular disease. We have published previously, that resveratrol upregulates eNOS expression and enhances eNOS activity [5,4]). In addition to its (moderate) direct antioxidant effect, we have found that resveratrol regulates gene expression of pro- and antioxidative enzymes in the cardiovascular system.

Resveratrol is an activator of sirtuin 1 (SIRT1) [6], a NAD^+-dependent histone deacetylase. SIRT1 facilitates the formation of heterochromatin, the more tightly packed form of chromatin associated with histone hypoacetylation and gene repression. Multiple non-histone targets have also been described for SIRT1. These include some transcription factors or cofactors such as the tumor suppressor p53, the forkhead box class O (FOXO) transcription factors, nuclear factor κB (NF-κB), and peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) [7]. Recent studies point to SIRT1 as a key regulator of angiogenesis, vascular tone and endothelial function.

Incubation of human umbilical vein endothelial cells (HUVEC) and HUVEC-derived EA.hy 926 cells with resveratrol resulted in a concentration- and time-dependent upregulation of superoxide dismutases (SOD)1, 2 and 3. SIRT1 inhibition with sirtinol or knockdown of SIRT1 by sRNAi markedly reduced the upregulation of SOD1 and 2 with little effect on SOD3. Also glutathione peroxidase 1 (GPx1) and catalase were upregulated by resveratrol. Sirti-

nol and SIRT1 knockdown prevented the effect on GPx1, but had no significant effect on the enhancement of catalase. The same resveratrol regimen downregulated the expression of NADPH oxidase Nox4, which occurred independently of SIRT1. (6*R*)-5,6,7,8-tetrahydro-L-biopterin (BH_4) is an essential cofactor of eNOS. BH_4 is sensitive to oxidation and diminished in oxidative stress. BH_4 is synthesized from guanosine 5'-triphosphate (GTP) via a de novo pathway by the rate-limiting enzyme GTP cyclohydrolase I (GCH1). Treatment of EA.hy 926 cells with resveratrol enhanced the expression of GCH1, which was reversed by sirtinol or knockdown of SIRT1 by siRNA.

Atherosclerotic apolipoprotein E knockout (ApoE-KO) mice were treated with resveratrol (30 or 100 mg/kg/day for 7 days via gavage). Resveratrol treatment significantly reduced the levels of superoxide in the heart, which was associated with an upregulation of SOD1-3, GPx1, and catalase. In parallel, mRNA expression of NADPH oxidase subunits Nox2 and Nox4 were reduced. Resveratrol also inhibited the translocation of p47phox and Rac1 and decreased the activity of the NADPH oxidase complex in heart membrane fractions. The cardiac content of malondialdehyde, an indicator of lipid peroxidation and a biomarker for oxidative stress, was reduced by resveratrol treatment. Resveratrol also reduced 3-nitrotyrosine levels in the heart. ApoE-KO mice are an animal model of oxidative stress, and eNOS was found in an uncoupled state, producing superoxide instead of NO. This was probably due to a relative deficiency of BH_4. Treatment of ApoE-KO mice with resveratrol enhanced the expression of GCH1 and significantly increased BH_4 levels in the heart. This was associated with reduced superoxide production by eNOS.

These data demonstrate that resveratrol protects against vascular oxidative stress in vitro and in vivo, by changing the expression pattern of pro- and antioxidative genes in an advantageous manner. The data also indicate that many of these effects are mediated by SIRT1, thereby identifying SIRT1 as a key regulator of cardiovascular oxidative stress.

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