Upregulation and Activation of eNOS by Resveratrol

To the Editor:
Wallerath et al\(^1\) reported that the stilbene derivative trans-resveratrol, an ingredient of red wine, stimulates acute nitric oxide (NO) release from vascular endothelial cells and induces upregulation of endothelial nitric oxide synthase (eNOS) gene expression after 24 to 72 hours incubation. Resveratrol-enhanced eNOS expression and activity were considered to contribute to the vasoprotective activity of red wine. However, this conclusion may be premature, because contradicting findings and possible adverse consequences of eNOS overexpression have to be considered.

(1) Conducting tone measurements on endothelium-preserved arteries, we and others detected no resveratrol-induced vasodilatations.\(^2,3\) Consistently, real-time measurements of resveratrol-mediated NO release from porcine coronary arteries revealed only nonsignificant elevations of 15±6% over basal NO levels.\(^3\) In contrast, Wallerath et al\(^1\) determined acute NO effects of resveratrol in a cell culture system of EA.hy 926 and calculated eNOS activity indirectly by assessment of cGMP increase in reporter cells which may not reflect the situation in preserved native endothelium.

(2) Wallerath et al\(^1\) suggested resveratrol is a major antioxidant constituent of red wine. However, many studies showed that other phenols (eg, gallic acid, flavonoids, and tannins) are present in red wines in far higher concentrations and exhibit greater antioxidant activity than resveratrol. Moreover, the free trans-resveratrol content of the wines tested by Wallerath et al\(^1\) was higher (29.8 and 73.6 mol/l, respectively) than in most red wines (with concentrations <10 μmol/l). Additionally, we found superoxide scavenging capacity and inhibition of superoxide-generating enzymes by resveratrol to be several orders of magnitude lower compared with other wine phenols (Taubert et al, unpublished data, 2002), making resveratrol intake with red wine unlikely to delay superoxide-induced NO degradation.

(3) Recent evidence indicates that resveratrol is nearly completely metabolized to the 4′-O-glucuronide before entering systemic circulation,\(^4\) so that in vivo vascular effects may be limited to this conjugate, whereas biological effects in the present study were only assessed employing unconjugated resveratrol.

(4) It has not been unequivocally proven that eNOS upregulation exerts only beneficial vascular effects. Conversely, long-term overexpression of eNOS has been recently found to accelerate atherogenesis in apoE-deficient mice.\(^5\)

In conclusion, we believe that summarized evidence to date is not sufficient to propose a vasoprotective activity of resveratrol in vivo. Hence, it appears speculative to postulate resveratrol to be implicated in the cardioprotection of red wine.

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**Response**

Taubert and Berkels raise four points: (1) the acute effect of resveratrol on eNOS activity; (2) the relative antioxidant capacity of resveratrol; (3) the effect of resveratrol in vivo; and (4) the physiological consequences of an enhanced eNOS expression.

(1) The major finding of our paper is the upregulation of eNOS gene expression by resveratrol. In addition, we showed an activation of eNOS after short-term exposure of endothelial cells to resveratrol. This is consistent with the findings of others, who demonstrated endothelium-dependent relaxations in rat aorta (see Introduction of our paper). Also, in porcine coronary arteries (the model cited by Taubert and Berkels), Jager and Nguyen-Duong\(^1\) found acute relaxant effects of resveratrol.

(2) Other polyphenols (such as gallic acid, caffeic acid, flavonoids, and tannins) are present in red wine in higher concentrations than resveratrol. We have tested many of these for their effect on eNOS expression and eNOS-derived NO production. For gallic acid, caffeic acid, and a number of flavonoids, we found no effect on eNOS expression; quercetin even down-regulated the expression of eNOS (Wallerath et al, unpublished data, 2002). Goldberg et al\(^2\) found resveratrol concentrations of 20 to 60 μmol/L in various red wines; French wines were in the high range. This is similar to the concentrations reported in the present paper.

(3) Bertelli et al showed that after oral administration of resveratrol in doses corresponding to reasonable wine intake, pharmacologically relevant concentrations of resveratrol appear in plasma.\(^3\) They also demonstrated that resveratrol accumulated in organs such as heart, liver, and kidney.\(^3\) Furthermore, after oral administration of resveratrol to rats, we found an enhanced eNOS mRNA expression in several tissues (Wallerath et al, unpublished data, 2002).

(4) It is widely accepted that a moderate upregulation of eNOS is associated with beneficial cardiovascular effects.\(^4\) However, in several types of pathophysiology, the upregulation of eNOS goes along with “uncoupling” and dysfunction of the enzyme.\(^4\) Under these conditions, superoxide is generated from the oxygenase domain instead of NO. In most cases, addition of (6R)-5,6,7,8-tetrahydrobipterin (and L-arginine) can restore NO production by eNOS. The apoE-deficient mice in the study by Ozaki et al\(^5\) showed a 10-fold overexpression of eNOS compared with wild-type mice. The authors themselves presented evidence for eNOS “uncoupling” and superoxide production, which can be corrected with (6R)-5,6,7,8-tetrahydrobipterin.

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