Endothelial GTPCH in eNOS Uncoupling and Atherosclerosis

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Nitric oxide (NO) produced from endothelial NO synthase (eNOS) has antiatherosclerotic properties, including inhibition of cell growth, leukocyte adhesion, and platelet adherence and aggregation.1 NO bioavailability is decreased in various cardiovascular diseases such as hypercholesterolemia and atherosclerosis attributable to reduced NO synthesis and increased NO consumption by reactive oxygen species (ROS).2 A critical determinant of eNOS activity is its cofactor tetrahydrobiopterin (BH4),3,4 which facilitates electron transfer from the eNOS reductase domain and maintains the heme prosthetic group in its redox active form.5 Moreover, BH4 promotes and stabilizes eNOS protein monomers into the active homodimeric form of the enzyme.6 BH4 bioavailability in the vasculature appears to be regulated by the following (Figure): (1) a de novo pathway using the rate-limiting enzyme GTP-cyclohydrolase I (GTPCH); (2) a salvage pathway from the synthetic pterin, sepiapterin, which is metabolized to BH4 by sepiapterin reductase (SR) and endothelial dihydrofolate reductase (DHFR); and (3) oxidative degradation of BH4 to dihydrobiopterin (BH2) that is inactive for eNOS cofactor function.7,8 When BH4 levels are inadequate, the enzymatic reduction of molecular oxygen by eNOS is no longer coupled to L-arginine oxidation, resulting in generation of superoxide rather than NO. This phenomenon is referred as “eNOS uncoupling” and contributes to vascular oxidative stress and endothelial dysfunction.5

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Clinical and animal studies demonstrated that “uncoupled eNOS” plays an important role in endothelial dysfunction. Acute administration of BH4 improves impairment of endothelial dependent relaxation (EDR) associated with hypercholesterolemia, atherosclerosis, hypertension, and cigarette smoking.8 Laursen et al demonstrated that incubation of isolated vessels from ApoE knockout (ApoE-KO) mice with sepiapterin, a precursor to BH4, improves EDR by reducing superoxide production derived from eNOS.9 Of note, overexpression of eNOS in the endothelium in ApoE-KO mice fed with high-fat diet (ApoE-KO/eNOS-Tg) increases uncoupled eNOS, thereby accelerating atherosclerosis.10 In these ApoE-KO/eNOS-Tg mice, BH4 levels in aorta are decreased compared with those in wild-type controls, and dietary BH4 supplementation restores NO synthesis from eNOS.10 Mice made hypertensive by DOCA-salt (deoxycorticosterone acetate salt) feeding show that superoxide derived from NADPH oxidase reacts with NO to form peroxynitrite, thereby inducing oxidative degradation of BH4, resulting in eNOS uncoupling and impairment of EDR, which is rescued by BH4 treatment.11 These findings suggest that increased eNOS protein alone is insufficient to maintain NO synthesis in hypercholesterolemia and that adequate BH4 levels are essential to prevent enzymatic uncoupling. However, all the previous reports used high pharmacological doses of sepiapterin or BH4 (often >100-fold in excess of physiological concentrations). Thus, it remains unclear whether their beneficial effect is attributable to nonspecific antioxidant effect of BH4 or specific effect on eNOS coupling. Moreover, it remains unclear whether adequate eNOS cofactor function is related to absolute BH4 levels in the endothelium, or whether the relative balance between reduced BH4 and oxidized BH2 is more important.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Takaya et al provide the evidence that endothelial BH4 levels play a critical role in atherosclerosis accelerated by uncoupled eNOS. Based on the fact that the rate-limiting BH4 synthesis enzyme is GTPCH, they crossed ApoE-KO/eNOS-Tg mice with transgenic mice with endothelial-targeted expression of GTPCH (ApoE-KO/eNOS-Tg/GTPCH-Tg). A previous study reported that transgenic mice expressing endothelial GTPCH (GTPCH-Tg) increase endothelial BH4 levels by 3- to 4-fold without elevation of plasma BH4 levels.12 In the current study, authors obtained several important findings. First, ApoE-KO/eNOS Tg mice fed with a standard chow (total cholesterol: ≈12 mmol/L) caused greater atherosclerotic lesion formation, which was associated with an increase in uncoupled eNOS-derived superoxide production in a similar fashion to a high fat–fed ApoE-KO/eNOS Tg mice (total cholesterol: ≈50 mmol/L).10 Of note, eNOS-Tg mice which do not increase BH4 levels have been shown to induce eNOS uncoupling.13 These data indicate that eNOS coupling is directly related to eNOS-BH4 stoichiometry. Second, GTPCH overexpression in ApoE-KO/eNOS-Tg mice increased vascular BH4 levels and decreased superoxide production from uncoupled eNOS, which was associated with reduced plaque area. Consistent with this, Alp et al demonstrated that GTPCH overexpression in “high fat–fed” ApoE-KO mice (total cholesterol: 30 to 40 mmol/L) show an increase in aortic BH4 levels and improvement of EDR and atherosclerotic lesion formation, which is associated with reduction of endothelial superoxide production and eNOS
uncoupling as compared with ApoE-KO mice. It has been speculated that the extent of hypercholesterolemia may affect BH4 metabolism. However, effects of GTPCH on eNOS uncoupling and atherosclerotic lesion were similar between the study by Alp et al and the current study. Thus, these findings further support the concept that BH4 availability is a critical determinant of eNOS coupling in atherosclerosis, thereby regulating NO-mediated endothelial function and atherosclerotic lesion formation.

Previous reports show that supplementation of the general antioxidant vitamin C improves endothelial dysfunction in smokers, in subjects with hypercholesterolemia, and in patients with diabetes mellitus or coronary artery disease. Underlying mechanism includes the effects of vitamin C on BH4 levels by preventing oxidation of BH4 to BH2 rather than by increasing its synthesis. It is postulated that vitamin C may stabilize BH4 by its reductase capacity; however, Kuzkaya et al recently showed that vitamin C can reduce the BH3 radical to regenerate BH4. Effects of long-term vitamin C treatment on atherosclerotic lesion in mice have been variable. Large scale clinical trials failed to demonstrate a beneficial effect of antioxidant supplements on cardiovascular disease morbidity and mortality. d’Uscoio et al reported that vitamin C, but not vitamin E, increases BH4/BH2 ratio and eNOS activity in ApoE−/− mice fed a high-fat Western diet. In the current study, Takaya et al showed that both vitamin C treatment and endothelial GTPCH overexpression decreased vascular superoxide production to the same extent, whereas only endothelial GTPCH overexpression reduced the accelerated atherosclerosis in ApoE-KO/eNOS-Tg mice with a standard chow. The reason for this discrepancy of vitamin C effect between their studies is unclear, but it could be because of the difference in the dose of vitamin C or the extent of oxidative stress (high-fat versus non–high-fat diet). Importantly, vitamin C-induced decrease in superoxide in the current study was mediated through its antioxidative effect rather than its effect on eNOS uncoupling. This is because L-NAME treatment increased superoxide production from the endothelium only in aortas from GTPCH overexpressed ApoE-KO/eNOS-Tg mice, but not in those from vitamin C–treated animals. Consistent with this, GTPCH overexpression partially restored the eNOS dimer to monomer ratio, suggesting an increase in “coupled” eNOS, whereas vitamin C treatment had no effect. These findings suggest that a general reduction of vascular superoxide alone by vitamin C treatment is insufficient to retard atherosclerosis. Rather, restoration of eNOS/BH4 stoichiometry and eNOS coupling by augmented endothelial BH4 biosynthesis is important to restore disease progression.

The information presented by Takaya et al strongly supports a critical role of endothelial GTPCH, as a regulator of BH4 synthesis, in atherosclerosis induced by eNOS uncoupling (Figure). There are many unanswered questions. How is vascular BH4 metabolism regulated in physiological (exercise training) and pathophysiological states (atherosclerosis, hypertension, diabetes, etc) in terms of BH4 biosynthesis, oxidative degradation, and regeneration? What are molecular mechanisms by which uncoupled eNOS accelerates atherosclerotic lesion formation? Oxidized bipterin BH2, which has no eNOS cofactor activity, can compete with BH4 for eNOS binding. Does BH4/BH2 ratio play a role in controlling generation of NO and superoxide from eNOS, eNOS activity, and endothelial function in vivo? BH4 is also a critical cofactor for iNOS isoforms which are involved in atherosclerosis. What is the role of “uncoupled” iNOS in atherosclerosis? Addressing these questions will be essential to our understanding the mechanism of atherosclerosis in which eNOS uncoupling plays an essential role. Increasing BH4 availability by modulating GTPCH is a new strategy to restore NO-mediated endothelial function and reduce pathophysiology of atherosclerosis.

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References
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