

## Tetrahydrobiopterin: Mediator of Endothelial Protection

Zvonimir S. Katusic, Livius V. d'Uscio

During the past twenty years, the essential role of endothelial cells in preservation of vascular homeostasis has been well established.<sup>1,2</sup> Protection of the vascular endothelium against harmful influences of circulating substances including excessive levels of lipids has been a major therapeutic approach in prevention of atherosclerosis. While endothelial dysfunction, defined as a loss of biologically active nitric oxide (NO) produced in the endothelium, has been recognized as a prime target for prevention and reversal of atherosclerotic process, identification of exact molecular mechanisms responsible for the loss of endothelial NO have been more difficult to determine. In the current issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Alp et al<sup>3</sup> report a series of elegant studies on genetically modified mice supporting the concept that NO synthase cofactor, tetrahydrobiopterin (Figure 1), is an important vascular protective molecule. The beneficial effect of tetrahydrobiopterin in apolipoprotein E (ApoE)-deficient mice appears to be mediated by preservation of endothelial nitric oxide synthase (eNOS) activity and production of nitric oxide in endothelial cells.

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In the early 1990s it was recognized that in the presence of suboptimal concentrations of tetrahydrobiopterin, activation of purified neuronal nitric oxide synthase (nNOS) causes "uncoupling of NOS" with subsequent increase in production of superoxide anions.<sup>4,5</sup> These findings have been confirmed and extended to eNOS.<sup>6-8</sup> This concept captured the imagination of vascular biologists for several reasons: (1) impaired biosynthesis of tetrahydrobiopterin can help to explain reduced production of NO and endothelial dysfunction in a variety of vascular diseases; (2) dysfunctional eNOS could become a major source of superoxide anions and contribute to oxidative stress in vascular disease; (3) tetrahydrobiopterin itself is a potent reducing molecule and could be attractive molecular target for oxidation; and (4) therapeutic interventions could be designed to increase endothelial concentration of tetrahydrobiopterin and "recouple eNOS". Initial studies in isolated coronary arteries supported the notion that inhibition of tetrahydrobiopterin biosynthesis may alter endothelial function by increasing production of oxygen-derived free radicals.<sup>9</sup> This was followed by measurements of BH4 levels in intact arteries demonstrating that single layer of endothelial

cells account for more than 60% of total tetrahydrobiopterin present in the vascular wall.<sup>10,11</sup> Consistent with these findings, it was demonstrated that GTP cyclohydrolase I, a rate-limiting enzyme in biosynthesis of BH4, is expressed and active in vascular endothelium.<sup>12-14</sup> These early reports were followed by a number of studies on experimental animals and humans demonstrating the beneficial effect of tetrahydrobiopterin supplementation on endothelial dysfunction induced by hypercholesterolemia, diabetes, hypertension, and smoking (see recent review by Werner et al<sup>15</sup>). Oxidative stress is the common mechanism in pathogenesis of vascular disease, and accelerated oxidative degradation of tetrahydrobiopterin may provide an explanation for the consistent beneficial effect observed in a variety of animal models and patients with endothelial dysfunction. This hypothesis is strongly supported by the reported ability of an antioxidant, vitamin C, to stimulate eNOS enzymatic activity by increasing intracellular concentration of BH4.<sup>16-19</sup> The effect of vitamin C appears to be mediated by chemical stabilization of tetrahydrobiopterin.

The beneficial effect of tetrahydrobiopterin supplementation is based on the assumption that an insufficient amount of tetrahydrobiopterin is present in diseased arteries. However, measurements of tetrahydrobiopterin levels in aortas of atherosclerotic animals reported controversial results. Studies by Ozaki et al<sup>20</sup> and Vasquez-Vivar et al<sup>21</sup> reported reduced tetrahydrobiopterin in aortas of hypercholesterolemic mice and rabbits, respectively. In contrast, d'Uscio and colleagues<sup>19</sup> reported increased tetrahydrobiopterin levels in aortas of ApoE-deficient mice. In the present study by Alp et al<sup>3</sup> levels of tetrahydrobiopterin were not measured in control wild-type animals; however, presented findings are consistent with the reported increase of tetrahydrobiopterin in ApoE-deficient mice.<sup>19</sup> The most likely explanation for the controversial findings is that studies by Ozaki et al<sup>20</sup> and Vasquez-Vivar et al<sup>21</sup> used atherosclerotic animals that had about 30 times higher circulating levels of cholesterol as compared with control animals. On the other hand, in studies by d'Uscio et al<sup>19</sup> and Alp et al,<sup>3</sup> total cholesterol levels were increased only about 3 times. Thus, it appears that hypercholesterolemia tends to increase tetrahydrobiopterin levels, whereas extremely high cholesterol levels can reduce availability of tetrahydrobiopterin. This finding is consistent with existing literature demonstrating stimulatory effect of proinflammatory cytokines on biosynthesis of tetrahydrobiopterin.<sup>13,14,22</sup> These findings are also in line with reported increased plasma levels of neopterin, a byproduct of tetrahydrobiopterin biosynthesis, in patients with atherosclerosis and coronary syndrome.<sup>23,24</sup> Interestingly, in the present study by Alp et al,<sup>3</sup> additional increase in tetrahydrobiopterin due to over-expression of GTP cyclohydrolase I in endothelium of ApoE-deficient mice provided protection against atherosclerosis. This observation suggests that despite an apparent adaptive increase of tetrahydrobiopterin levels in aorta of ApoE-deficient mice, there appears to be a relative shortage of the NOS cofactor. Genetic

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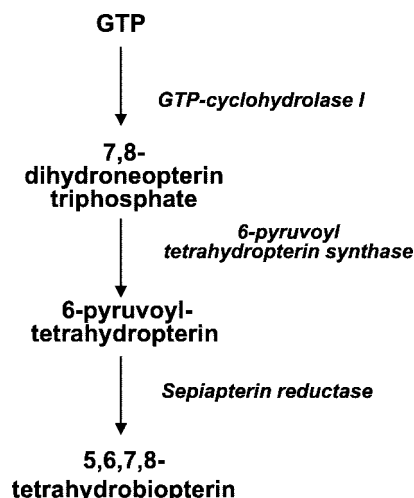
From the Department of Anesthesiology and Molecular Pharmacology, Mayo Clinic, Rochester, MN.

Correspondence to Zvonimir S. Katusic, Department of Anesthesiology and Molecular Pharmacology, Mayo Clinic, 200 First Street Southwest, Rochester, MN 55905. Email katusic.zvonimir@mayo.edu

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The major steps of tetrahydrobiopterin biosynthesis. GTP=guanosine 5'-triphosphate.

supplementation of tetrahydrobiopterin preserved production of NO, reduced endothelial formation of superoxide anions, and protected vascular wall against atherosclerosis. Thus, tetrahydrobiopterin could be added to the list of endogenously produced antiatherosclerotic molecules, whereas endothelial GTP cyclohydrolase I is emerging as a potential new therapeutic target.

Results of the study by Alp and colleagues<sup>3</sup> are interpreted as additional evidence to support "eNOS uncoupling hypothesis" as an important mechanism of endothelial dysfunction. Studies by several different groups suggest that "eNOS uncoupling" could be prevented by tetrahydrobiopterin supplementation.<sup>9,11,25</sup> However, as correctly pointed out by Alp et al,<sup>3</sup> direct in vivo evidence of eNOS uncoupling is missing because of limitations of methodologies available to detect vascular superoxide anions.<sup>26</sup> Furthermore, it remains puzzling how hypercholesterolemia-induced increase in tetrahydrobiopterin concentration in vascular wall may lead to uncoupling of eNOS in ApoE-deficient mice. Further studies of mechanisms involved in control of vascular GTP cyclohydrolase I expression and activity, as well as metabolism of tetrahydrobiopterin in endothelial cells in vivo, may ultimately provide explanation for the role of tetrahydrobiopterin in eNOS uncoupling. Irrespective of the exact mechanism underlying vascular protective effect of tetrahydrobiopterin, the study by Alp and colleagues<sup>3</sup> provides strong evidence that tetrahydrobiopterin is an important endogenous antiatherosclerotic molecule.

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