

Sepiapterin Treatment in Atherosclerosis

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Tetrahydrobiopterin (BH₄) plays an important role in functional and metabolic cellular homeostasis, with additional effects on proliferation,^{1,2} immune responsiveness,^{3,4} and neuronal activity.⁵⁻⁷ Mutations in either de novo biosynthetic or regeneration (salvage) pathways result in BH₄ deficiency associated with diminished levels of serotonin and dopamine with progressive neurologic symptoms.^{8,9} The phenotypic presentation of these synthetic mutations can be predicted in large part by the role of BH₄ as an obligatory cofactor in phenylalanine, tryptophan, and tyrosine hydroxylases (the rate-limiting enzymes for catecholamine and serotonin synthesis). The function of BH₄ in these aromatic amino acid hydroxylases involves redox-active donation of electrons and reductive enzyme activation and is associated with a tightly coupled system for regeneration of BH₄ from the oxidized dihydrobiopterin.^{8,10}

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In addition to its role in the biosynthesis of monoamine neurotransmitters, BH₄ serves as an essential cofactor in all isoforms of nitric oxide synthases (NOS), with the Km for BH₄ for NOS several orders of magnitude lower than for the aromatic amino acid hydroxylases: NOS, ≈0.3 μmol/L vs ≈3 μmol/L for phenylalanine hydroxylase, ≈30 μmol/L for tyrosine hydroxylase and tryptophan hydroxylase, suggesting tight coupling of the cofactor with enzyme.⁸ However, the precise function(s) of BH₄ in NOS enzymatic activity is not as well defined as in the aromatic amino acid hydroxylase enzymes and may vary according to enzyme isoform. For endothelial NOS (eNOS), BH₄ has been reported to modulate the heme iron environment and stabilize and increase L-arginine binding, thus resulting in allosteric modulation of enzyme activity.^{11,12} The importance of BH₄ in regulating protein dimerization, while critical in iNOS, is diminished for eNOS.¹³ Importantly, eNOS has been demonstrated to generate superoxide in a calcium/calmodulin-dependent fashion that is influenced by BH₄ levels, as well as the availability of L-arginine substrate.^{12,14} This NADPH-dependent formation of superoxide anion in the absence of NO production has been referred to as uncoupling of NOS activity.¹⁵ Superoxide formation from eNOS is critically controlled by BH₄, with increasing production of superoxide occurring at low levels of reduced pterin, even in the presence of L-arginine.^{12,14}

More recently, partially oxidized analogues of BH₄ have been shown to also enhance rates of superoxide formation from purified eNOS in the presence of saturating L-arginine concentrations, suggesting that the ratio of reduced and oxidized biopterin may be physiologically important in determining rates of NO production versus uncoupled superoxide formation from eNOS.¹⁶

The increase in oxidant formation that appears to accompany most, if not all, disease processes associated with endothelial-dependent vascular dysfunction may potentially be a result of inadequate BH₄ concentrations within the vasculature, with ensuing uncoupling of eNOS, increased superoxide formation and diminished NO formation. Thus, there is intense interest in the potential for BH₄ to modulate eNOS activity. Numerous groups have recently reported beneficial effects of acute (≤60 minutes) administration of BH₄ or analogs such as sepiapterin on endothelial function in both clinical and experimental models of hypercholesterolemia, atherosclerosis, hypertension, and cigarette smoking.¹⁷⁻²¹ Additionally, the beneficial effects of vitamin C administration on endothelial function are in part due to stabilization of intracellular BH₄ with resultant enhanced NO bioactivity.^{22,23} A similar mechanism of BH₄ stabilization may underlie the influence of folate supplementation on eNOS activity.²⁴

Alterations in BH₄-dependent eNOS activity have also been implicated in diabetic vascular dysfunction where sepiapterin has been reported to diminish eNOS-derived superoxide in human vascular segments.²⁵ Acute administration of a BH₄ analog augmented endothelial-dependent relaxation in a streptozotocin model of diabetes.²⁶ Spontaneously diabetic BB rats have diminished GTP-cyclohydrolase 1 activity and decreased BH₄ levels, while sepiapterin treatment for 48 hours normalized BH₄ levels and increased NO production from endothelial cells isolated from diabetic BB rats.²⁷ In an insulin-resistant rat model, chronic oral administration of BH₄ also increased vascular BH₄ content and improved endothelial-dependent vascular function.²⁸ Given these positive outcomes after administration of BH₄, either longer term in vitro exposures or chronic administration studies are warranted in models of atherosclerosis.

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Vasquez-Vivar et al²⁹ report their interesting findings on the influence of 6 hours sepiapterin incubation on vascular BH₄ levels and endothelial function in vessels isolated from rabbits fed a high-cholesterol diet. They demonstrate a marked reduction in vascular BH₄ content in hyperlipidemic vessels compared with controls, which could be restored by incubation with sepiapterin. These findings are in agreement with the relatively few other studies reporting on BH₄ content in diseases associated with vascular dysfunction. While vascular BH₄ levels have been reported to be

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normal in spontaneously hypertensive rats (SHR) before the development of hypertension, BH₄ levels are decreased in SHR with established hypertension.^{30,31} Several models of diabetes have also reported decreased vascular BH₄ content.^{27,28}

In contrast to the improvement of endothelial function seen in acute studies, however, long-term exposure to sepiapterin resulted in a further derangement in response to either acetylcholine or the calcium ionophore A23187, while endothelial-independent function was not altered. These data are similar to those reported by Tsutsui et al³² in which canine middle cerebral arteries exhibited diminished relaxation to A23187 after 24-hour incubation with 100 μmol/L sepiapterin. In that study, the authors concluded that the inhibition of endothelial-dependent relaxation was due to enhanced generation of superoxide anion, as exogenous superoxide dismutase (SOD) ameliorated the effects of sepiapterin. It is well-recognized that BH₄ itself can redox cycle and thus generate superoxide.³² This effect is likely to be enhanced in the setting of vascular relaxation studies, in which the O₂ concentration is typically 95%. However, in the present report, only the hyperlipidemic vessels demonstrated a sepiapterin-dependent inhibition of endothelial-dependent relaxation. It should be noted that the normal vessels did not demonstrate an increase in BH₄ content after incubation with sepiapterin, while the rise in BH₄ content with sepiapterin supplementation did not exceed levels seen in control vessels. If the effects on vessel relaxation are secondary to BH₄ autoxidation, this would suggest that there is also at least a relative deficiency of SOD in the hyperlipidemic vessels. Alternatively, in the face of a functionally abnormal eNOS, could sepiapterin supplementation result in sepiapterin-dependent superoxide generation from eNOS itself, as has been described for the purified enzyme?¹⁶ The effect of either exogenous SOD or a cell-permeant SOD mimetic would be informative.³³ On the other hand, BH₄ can serve to scavenge superoxide with a rate constant of 10⁵ M⁻¹ · s⁻¹ and reacts with peroxynitrite.^{12,19,34} Thus, the effects of sepiapterin are the result of extent of salvage pathway activity and conversion to BH₄, balanced with rates of BH₄ autoxidation and consumption by peroxynitrite, as well as sepiapterin binding to eNOS in competition with BH₄.

The inhibitory effect of 6 hours of sepiapterin treatment also contrasts with 2 studies of 8-week in vivo administration of BH₄ demonstrating improved endothelial function in models of SHR and diabetes.^{28,31} Are these differences due to unrecognized secondary reactions of sepiapterin as opposed to BH₄ itself? The report by Vasquez-Vivar et al²⁹ opens new avenues of investigation with respect to the role of biopterins in vascular dysfunction.

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