Published in final edited form as:
Curr Vasc Pharmacol. 2012 November ; 10(6): 705–708.

Tetrahydrobiopterin: A Vascular Redox Target to Improve Endothelial Function

Keith M. Channon*
Department of Cardiovascular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, UK

Abstract

Loss of normal endothelial function and bioactivity of nitric oxide (NO), associated with increased production of reactive oxygen species (ROS), are characteristics of cardiovascular disease states. There is good experimental evidence that these abnormalities are causally related to cardiovascular disease pathogeneses, and are amenable to therapeutic intervention. However, simple attempts to increase NO levels or reduce “oxidative stress”, for example using non-selective anti-oxidant drugs, have shown no benefit as treatments of cardiovascular disease. Increasing evidence highlights the need to better understand NO and ROS mediated signaling mechanisms in endothelial function, in order to identify more rational and selective therapeutic targets. The NO synthase co-factor, tetrahydrobiopterin (BH4) is a redox active molecule which regulates NO and ROS production by NO synthase and provides an exemplar of redox dependent signaling in the endothelium, with relevance to cardiovascular disease. Loss of endothelial cell BH4 is observed in cardiovascular disease states and results in loss of NO, but increased ROS production by endothelial NO synthase. Genetic mouse models of augmented endothelial cell BH4 synthesis have shown proof of concept that endothelial cell BH4 can alter cardiovascular disease pathogenesis, but clinical trials of BH4 therapy in vascular disease have been limited by systemic oxidation and limited endothelial cell uptake of BH4. In contrast, some existing therapies such as statins appear to exert favourable effects on endothelial cell BH4 and endothelial NO synthase function. Identifying specific redox mechanisms and targets in the endothelium will provide new potential targets for future drug treatments.

Keywords

Endothelium; nitric oxide; tetrahydrobiopterin; reactive oxygen species

Endothelial Function and Inflammation in Cardiovascular Disease

Cardiovascular disease states such as diabetes, atherosclerosis and hypertension, are all characterised by abnormalities in endothelial function and by inflammation, both locally and systemically. One critical aspect of normal endothelial function is production of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS). eNOS modulates blood flow and pressure

*Address correspondence to this author at the Department of Cardiovascular Medicine, John Radcliffe Hospital, OX3 9DU, Oxford, UK; Tel +44 1865 572783; Fax +44 1865 572784; keith.channon@cardiov.ox.ac.uk.

Conflict of Interest
The authors confirm that this article content has no conflicts of interest.

Europe PMC Funders Author Manuscripts

and has a number of important anti-atherogenic effects. In humans, endothelial function is deficient in subjects with vascular disease states and correlates with risk factor profile. More importantly, several prospective studies have now identified deficient NO mediated endothelial function as a quantitative, independent predictor of adverse cardiac events. Numerous animal model studies have contributed further strong evidence that NO, generated by eNOS, has key roles in vascular disease pathogenesis.

Despite the pathogenetic importance of eNOS, therapeutic strategies targeting NO production in disease states have been largely disappointing. Short-term overexpression of eNOS can improve NO-mediated endothelial function and reduce inflammatory cell recruitment. However, many studies have shown that eNOS protein levels are *increased* rather than decreased in disease states such as diabetes [1]. Furthermore, long term transgenic overexpression of eNOS in the ApoE-/- mouse model of atherosclerosis paradoxically accelerates plaque formation, due to increased production of reactive oxygen species (ROS) [2]. Clinical drug therapies that reduce long term cardiovascular risk through targeting known risk factors also improve NO-mediated endothelial function. However, there is no evidence that primarily targeting NO-mediated endothelial function can alone reduce vascular disease progression or cardiovascular risk in diabetes. Indeed, simple strategies aimed at pharmacological NO generation, such as the use of nitrate drugs, have shown no prognostic clinical benefits and are paradoxically associated with adverse consequences such as impaired vasomotor responses and increased vascular oxidative stress [3].

Reactive Oxygen Species and Endothelial Dysfunction in Cardiovascular Disease

Europe PMC Funders Author Manuscripts

The loss of normal NO mediated endothelial function in cardiovascular disease states is both associated with and causally linked to an increase in the production of reactive oxygen species (ROS) such as superoxide, hydroxyl and hydrogen peroxide. These ROS can react rapidly with NO, for example forming the reactive nitrogen species (RNS) peroxynitrite, which both diminishes the bioavailability of nitric oxide and results in nitration of protein, lipid and DNA targets. In diabetes and other cardiovascular disease states, increased ROS production correlates inversely with NO mediated endothelial function, and is associated with increasing cardiovascular risk factor profile. Increased ROS/RNS production in cardiovascular disease states led to the concept of “oxidative stress” with the presumption that these ROS and RNS are uniformly damaging and contribute to disease pathogenesis. The oxidative stress concept led rapidly to the notion that simple chemical strategies to reduce ROS/RNS action through “anti-oxidant” drug treatments should lead to modification of disease pathogenesis and would yield clinical benefit. However, large-scale clinical trials of drugs such as vitamin C, vitamin E and other “anti-oxidant” combinations has shown no benefit in patients with cardiovascular disease states. It is now clear that the “oxidative stress” hypothesis was neither rational nor adequately addressed by the clinical trials, since many of the available “anti-oxidants” tested in these studies have largely uncharacterised and/or unpredictable effects on ROS/RNS biology *in vivo*, and because the notion of “oxidative stress” is a gross simplification and misunderstanding of the role of ROS/RNS in cardiovascular disease pathogenesis.

In contrast to the idea that all ROS/RNS production exerts “oxidative stress”, ROS and RNS are highly regulated, specific effectors of cellular signalling, through interactions leading to post translational modification of proteins and other biomolecules. These biological effects are wide ranging and as important as other signalling modalities, for example redox modification of protein SH groups being analogous with protein phosphorylation at OH groups. Recent evidence reveals that, through these signalling roles, ROS and RNS control processes such as cell growth and apoptosis, inflammation, mitochondrial function and intermediary metabolism, cell migration and contraction. More importantly, proof of principal experiments in knockout mouse models demonstrate that specific modification of ROS signalling, targeting particular enzymatic sources of ROS, in specific cell types, has important effects of cardiovascular pathophysiology and vascular disease. Thus, ROS/RNS signalling is a rational and valid concept for identifying specific therapeutic targets to intervene in vascular disease pathogenesis, but requires a more informed approach to target specific tractable mechanisms, rather than the irrational and simplistic concept of targeting “oxidative stress”.

Tetrahydrobiopterin – A Redox Sensor and Effector

The NOS cofactor, tetrahydrobiopterin (BH4), regulates NOS enzymatic activity and NO-ROS signalling, and provides an exemplar of how understanding a single redox mechanism, in the endothelial cell, can have important implications for strategies aiming to improve endothelial function in diabetes and other vascular disease states [4]. BH4 is required for enzymatic activity of all mammalian NOS enzymes, through mechanisms that include direct participation in L-arginine oxidation by molecular oxygen, to yield NO, and contribution to structural integrity of the NOS homodimer through shared interactions with both monomers. Functionally, BH4-dependent NOS regulation is important in cardiovascular disease because BH4-deficient NOS is not inactive, but becomes ‘uncoupled’: electron transfer from NADPH *via* flavins in the reductase domain, continues to form the haem-oxygen intermediate at the active site, but without BH4 the reduction of the haem-oxygen intermediate is not coupled to L-arginine oxidation, so that superoxide anion rather than NO is produced [4]. The alteration in NO *vs.* ROS production by BH4-depedent regulation of eNOS may alter the relative formation of alternative species such as peroxynitrite, nitrate/nitrite and, in particular, nitroxyl anion (HNO), that may have important roles in vascular physiology. Thus, regulation of NOS coupling *vs.* uncoupling expands the biological repertoire of NOS signalling to include not just NO, but also redox signalling through ROS production.

BH4 is synthesised within cells from GTP *via* a three step pathway; the first and rate limiting step is catalysed by GTP cyclohydrolase 1 (GTPCH: EC 3.5.4.16). Regulation of eNOS by BH4 can exert biologically important effects in vascular pathophysiology and in the pathogenesis of atherosclerosis. In mouse models with either systemic BH4 deficiency (the *hph-1* mouse [5, 6]), or with endothelial overexpression of either GTPCH [7, 8] or eNOS [9], relative BH4 deficiency is sufficient to cause eNOS uncoupling [9], and directly contributes to disease pathogenesis. Conversely, restoration or augmentation of endothelial BH4, by transgenic over-expression of GTPCH, is sufficient to both normalise endothelial

Europe PMC Funders Author Manuscripts

function and ROS production and to reduce or functional and structural abnormalities in diabetes [8] and atherosclerosis [7].

The level and activity of GTPCH is the key determinant of endothelial cell BH4 levels [10, 11] that are modulated by BH4 oxidation to the inactive form, dihydrobiopterin (BH2), such that BH4 acts a redox 'sensor', whereby increased ROS/RNS production leads to loss of BH4. Oxidation of BH4 can be regenerated by 'recycling', catalysed by dihydrofolate reductase (DHFR) [12] which appears to be important in regulating BH4 availability, particularly in conditions where BH4 biosynthesis is reduced [13].

Can Endothelial Redox Signalling by BH4 Provide Therapeutic Targets?

The failure of non-specific 'antioxidant' drugs to confer any benefit in patients with cardiovascular disease emphasizes the need to identify and validate specific redox targets that are amenable to therapeutic intervention. As a biologically validated target, BH4 may be such a potential opportuntity. Several clinical studies have used pharmacological BH4 supplementation to target endothelial function. However, the majority of these studies involved acute intra-arterial administration of high doses of BH4 into either the brachial or coronary arteries, reporting an improvement of NO-mediated endothelial function but with the limitation that the high dose of BH4 is confounded by non-specific 'anti-oxidant' effects rather than effects mediated through eNOS. These studies have demonstrated elevations of plasma BH4 concentrations to between 50-100 uM [14, 15], whereas plasma BH4 levels in healthy subjects and patients with atherosclerosis are typically 10-50 nM [16]. Some investigators have attempted to account for these effects by comparing the effects of BH4 to other known antioxidants. For example, BH4 restored endothelial function in chronic smokers, whereas tetrahydroneopterin (NH4, which has equipotent antioxidant capability *in vitro*) had no effect, suggesting restored eNOS coupling as the underlying mechanism [17]. Similarly, the 6S-stereoisomer of BH4 showed no effect on endothelial function following a glucose challenge [18]. In a randomised, placebo-controlled clinical trial of chronic oral BH4 supplementation in otherwise healthy subjects with elevated cholesterol [19], NO-mediated endothelial function was improved after four weeks of treatment, although it is not possible to be sure whether this effect is due to alteration in eNOS coupling or an alternative mechanism; a reduction in plasma F_2-isoprostanes, a marker of oxidative stress, implies a favourable effect on systemic antioxidant status. One further study showed an improvement in blood pressure with oral BH4 in hypertensive patients, however this study was neither randomised nor placebocontrolled [20]. In contrast, a randomised controlled trail of oral BH4 in patients with established severe coronary artery disease showed no benefit, due to significant oxidation of BH4, forming the inactive form, BH2, and limited uptake of BH4 in to the vascular wall from the plasma [21]. Thus, like the non-specific 'antioxidants' of studies 20 years ago, even a more rationally-targeted redox mechanism is subject to major limitations as a therapeutic target. Nevertheless, probing the mechanisms of existing drugs reveals important redox mechanisms that likely contribute to the therapeutic effects.

Statins inhibit HMG CoA reductase, and in doing so impact on isoprenylation of proteins, such as the small G-protein, Rac-1. In human blood vessels, statins lead to a rapid increase in vascular tissue BH4 and an improvement in endothelial function, mediated by

upregulation of GTPCH, that is observed *ex vivo*, isolated from effects on LDL cholesterol, and much more rapidly *in vivo* than any observed change in circulating LDL [22]. Furthermore, the improvement in endothelial function and increase in vascular BH4 levels are accompanied by a reduction in plasma BH4 levels, that reflect the inhibitory effects of statins on systemic inflammation [16]. Thus, pharmacological actions on vascular redox signalling through BH4 are a component of the rapid, 'pleiotropic' effects of statins, and illustrate the validity of vascular redox signalling as a therapeutic target.

Future Opportunties

Identifying tractable mechanisms that will provide rational approaches to target BH4 as a therapy in cardiovascular disease will require a systematic programme to determine how altered BH4 synthesis and/or BH4 levels are either increased or decreased in different cell types in cardiovascular states, and to develop new drugs and delivery approaches that can modulate BH4 dependent effects in a cell specific and disease specific manner. For example, targeting BH4 endothelial cells might require increased BH4 synthesis and/or recycling, but given the limitation of systemic BH4 supplementation new small molecules will be required. Cell- and disease- specific high throughput screens must be based on integrated biological readouts incorporating effects on GTPCH expression and activity, BH4 oxidation and recycling, and downstream signaling effectors. Redox mechanisms in the vascular wall represent a rich source of new therapeutic approaches and targets, but taking advantage of this potential will require rational understanding of mechanisms and effects.

Acknowledgements

British Heart Foundation, NIHR Oxford Biomedical Research Centre.

References

[1]. Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation. 2006; 113:1708–14. [PubMed: 16585403]

[2]. Ozaki M, Kawashima S, Yamashita T, et al. Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. J Clin Invest. 2002; 110:331–40. [PubMed: 12163452]

[3]. Schulz E, Tsilimingas N, Rinze R, et al. Functional and biochemical analysis of endothelial (dys)function and NO/cGMP signaling in human blood vessels with and without nitroglycerin pretreatment. Circulation. 2002; 105:1170–5. [PubMed: 11889009]

[4]. Alp NJ, Channon KM. Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. Arterioscler Thromb Vasc Biol. 2004; 24:413–20. [PubMed: 14656731]

[5]. Khoo JP, Nicoli T, Alp NJ, Fullerton J, Flint J, Channon KM. Congenic mapping and genotyping of the tetrahydrobiopterin-deficient hph-1 mouse. Mol Genet Metab. 2004; 82:251–4. [PubMed: 15234340]

[6]. Khoo JP, Zhao L, Alp NJ, et al. A pivotal role for tetra-hydrobiopterin in pulmonary hypertension. Circulation. 2005; 111:2126–33. [PubMed: 15824200]

[7]. Alp NJ, McAteer MA, Khoo J, Choudhury RP, Channon KM. Increased endothelial tetrahydrobiopterin synthesis by targeted transgenic GTP-cyclohydrolase I overexpression reduces endothelial dysfunction and atherosclerosis in ApoE-knockout mice. Arterioscler Thromb Vasc Biol. 2004; 24:445–50. [PubMed: 14707037]

[8]. Alp NJ, Mussa S, Khoo J, et al. Tetrahydrobiopterin-dependent preservation of nitric oxide-mediated endothelial function in diabetes by targeted transgenic GTP-cyclohydrolase I over expression. J Clin Invest. 2003; 112:725–35. [PubMed: 12952921]

[9]. Bendall JK, Alp NJ, Warrick N, et al. Stoichiometric relationships between endothelial tetrahydrobiopterin, eNOS activity and eNOS coupling *in vivo*: Insights from transgenic mice with endothelial-targeted GTPCH and eNOS over-expression. Circ Res. 2005; 97:864–71. [PubMed: 16179591]

[10]. Crabtree MJ, Tatham AL, Al-Wakeel Y, et al. Quantitative regulation of intracellular endothelial nitric oxide synthase (eNOS) coupling by both tetrahydrobiopterin-eNOS stoichiometry and biopterin redox status: Insights from cells with tet-regulated GTP cyclohydrolase I expression. J Biol Chem. 2009; 284:1136–44. [PubMed: 19011239]

[11]. Tatham AL, Crabtree MJ, Warrick N, Cai S, Alp NJ, Channon KM. GTP cyclohydrolase I expression, protein, and activity determine intracellular tetrahydrobiopterin levels, independent of GTP cyclohydrolase feedback regulatory protein expression. J Biol Chem. 2009; 284:13660–8. [PubMed: 19286659]

[12]. Crabtree MJ, Tatham AL, Hale AB, Alp NJ, Channon KM. Critical role for tetrahydrobiopterin recycling by dihydrofolate reductase in regulation of endothelial nitric-oxide synthase coupling: relative importance of the *de novo* biopterin synthesis *vs*. salvage pathways. J Biol Chem. 2009; 284:28128–36. [PubMed: 19666465]

[13]. Crabtree MJ, Hale AB, Channon KM. Dihydrofolate reductase protects endothelial nitric oxide synthase from uncoupling in tetrahydrobiopterin deficiency. Free Radic Biol Med. 2011; 50:1639–46. [PubMed: 21402147]

[14]. Setoguchi S, Hirooka Y, Eshima K, Shimokawa H, Takeshita A. Tetrahydrobiopterin improves impaired endothelium-dependent forearm vasodilation in patients with heart failure. J Cardiovasc Pharmacol. 2002; 39:363–8. [PubMed: 11862115]

[15]. Mayahi L, Heales S, Owen D, et al. (6R)-5,6,7,8-tetrahydro-L-biopterin and its stereoisomer prevent ischemia reperfusion injury in human forearm. Arterioscler Thromb Vasc Biol. 2007; 27:1334–9. [PubMed: 17413035]

[16]. Antoniades C, Shirodaria C, Crabtree M, et al. Altered plasma *vs*. vascular biopterins in human atherosclerosis reveal relationships between endothelial nitric oxide synthase coupling, endothelial function, and inflammation. Circulation. 2007; 116:2851–9. [PubMed: 18040031]

[17]. Heitzer T, Brockhoff C, Mayer B, et al. Tetrahydrobiopterin improves endothelium-dependent vasodilation in chronic smokers : evidence for a dysfunctional nitric oxide synthase. Circ Res. 2000; 86:E36–E41. [PubMed: 10666424]

[18]. Ihlemann N, Rask-Madsen C, Perner A, et al. Tetrahydrobiopterin restores endothelial dysfunction induced by an oral glucose challenge in healthy subjects. Am J Physiol Heart Circ Physiol. 2003; 285:H875–882. [PubMed: 12730050]

[19]. Cosentino F, Hurlimann D, Delli Gatti C, et al. Chronic treatment with tetrahydrobiopterin reverses endothelial dysfunction and oxidative stress in hypercholesterolaemia. Heart. 2008; 94:487–92. [PubMed: 17916662]

[20]. Porkert M, Sher S, Reddy U, et al. Tetrahydrobiopterin: a novel antihypertensive therapy. J Hum Hypertens. 2008; 22:401–7. [PubMed: 18322548]

[21]. Cunnington C, Antoniades C, Shirodaria C, et al. Systemic and vascular oxidation limits efficacy of oral tetrahydrobiopterin treatment in patients with coronary artery disease. Circulation. 2012; 125:1356–66. [PubMed: 22315282]

[22]. Antoniades C, Bakogiannis C, Leeson P, et al. Rapid, direct effects of statin treatment on arterial redox state and nitric oxide bioavailability in human atherosclerosis *via* tetrahydrobiopterin-mediated endothelial nitric oxide synthase coupling. Circulation. 2011; 124:335–45. [PubMed: 21730307]