Ascorbic acid and tetrahydrobiopterin: looking beyond nitric oxide bioavailability

Judy M. Muller-Delp*

Department of Physiology and Functional Genomics, University of Florida, PO Box 100274, 1600 SW Archer Road, Gainesville, FL 32610, USA

Online publish-ahead-of-print 10 September 2009

This editorial refers to ‘Ascorbic acid and tetrahydrobiopterin potentiate the EDHF phenomenon by generating hydrogen peroxide’ by A. Garry et al., pp. 218–226, this issue.

Endothelial dysfunction, manifested as reduced vasodilatory capacity, occurs in arteries exposed chronically to cardiovascular risk factors. Hyperglycaemia, hypercholesterolaemia, hypertension, ageing, and smoking have all been associated with endothelial dysfunction. Although the molecular basis of endothelial dysfunction remains incompletely understood, shared mechanisms appear to involve NO loss.

Both tetrahydrobiopterin (BH4) and ascorbic acid have been shown to enhance endothelial function in humans and in animal models. As a result, investigations of the cardiovascular implications of treatment with exogenous BH4 and ascorbic acid have focused on the endothelial effects of these compounds and their contribution to chemical stabilization of the enzyme endothelial nitric oxide synthase (eNOS) and protection of NO from scavenging by free radicals, respectively. Both BH4 and ascorbic acid may contribute to promoting NO-dependent vasodilation; however, both compounds possess redox capabilities that may alter vascular reactivity independent of their effects on the bioavailability of NO.

In a study in this issue of Cardiovascular Research, Garry et al. investigated the possibility that pro-oxidant effects of BH4 and ascorbic acid potentiate NO-independent vasodilatory responses of arterial rings to both the G-protein-coupled agonist, acetylcholine (ACh), and the endoplasmic reticulum Ca2⁺-ATPase (SERCA) inhibitor, cyclopiazonic acid (CPA). These authors reported that in the presence of molecular oxygen, both BH4 and ascorbic acid increased extracellular hydrogen peroxide (H2O2), thereby potentiating endothelium-dependent dilation. Vasodilatory responses to ACh and CPA were assessed in the presence of NOS and cyclooxygenase inhibitors to eliminate NO and vasodilatory prostanoids. Scavenging of BH4- and ascorbic acid-generated H2O2 with catalase inhibited the potentiation of the vasodilatory responses to CPA and ACh, and treatment with exogenous H2O2 mimicked the potentiation of vasodilation that occurred in response to treatment with BH4 and ascorbic acid. These authors have previously reported that H2O2 enhances vasodilatory responses to CPA through a mechanism that involves an increase in Ca2⁺ store depletion, with a subsequent increase in Ca2⁺ mobilization promoting the opening of hyperpolarizing calcium-activated potassium channels (Kca) (see Figure 1). In this most recent manuscript, Garry et al. describe a novel mechanism whereby H2O2 generated upon oxidation of supplemental BH4 or ascorbic acid enhances endothelium-dependent vasodilation through a mechanism that is NO-independent and which appears to involve hyperpolarization.

In models of endothelial dysfunction, endothelium-derived hyperpolarizing factor (EDHF)-induced relaxations increase in a compensatory manner in arteries in which NO-dependent relaxations are impaired. The results of Garry et al. suggest that supplemental BH4 and/or ascorbic acid could reverse endothelial dysfunction through potentiation of dilation occurring in response to EDHF as well as through stabilization of eNOS function and preservation of NO.

This report from Garry et al. also suggests that ascorbic acid and BH4, through generation of H2O2 in the interstitial space, could modulate vascular smooth muscle tone independent of plasma changes in BH4 and ascorbic acid. These findings suggest that although supplementation has been primarily attributed to endothelial effects of these compounds, attention should be given to the interstitial levels of these compounds that occur with supplementation, and of which little is currently known. Although these investigators did discern the effects of luminal vs. abluminal application of H2O2, they did not directly assess the possible pro-oxidant effects of H2O2 on relaxation to CPA and ACh in arteries denuded of endothelium. Thus, the direct effects of BH4 or ascorbic acid-induced increases in interstitial H2O2 on vascular smooth muscle tone were not determined in this study, and the possibility remains that pro-oxidant effects of BH4 and ascorbic acid occur through direct effects on vascular smooth muscle tone.

The endothelium and vascular smooth muscle are electrotonically coupled via gap junctions, and Garry et al. found that blockage of gap junctions with connexin-mimetic...
peptides superseded the potentiation of vasodilation to ACh and CPA by $H_2O_2$. In their report, these authors propose that the pro-oxidant effects of ascorbic acid and BH4 enhance EDHF-type vasodilatory responses by generating $H_2O_2$. In prior work, these authors have demonstrated that $H_2O_2$ can enhance EDHF-type relaxations by potentiating $Ca^{2+}$ release from endothelial stores (Figure 1). In addition, these investigators have previously shown that BH4 modulates arterial function through effects on gap junctions and electrotonic signalling; however, the direct effects of BH4- or ascorbic acid-generated $H_2O_2$ on membrane potential of either the endothelium or vascular smooth muscle of intact rings remains to be determined. The current study cannot eliminate the possibility that the pro-oxidant effects of these compounds occur through direct alteration of ion channels or gap junctions, thereby altering membrane potential. Future studies should focus on elucidating the cellular targets of BH4- and ascorbic acid-generated $H_2O_2$ and explaining the causative relationship between $H_2O_2$, increasing intracellular $Ca^{2+}$, and modulation of membrane potential by $K_{Ca}$ channel activity.

The data presented in the study by Garry et al. raises the possibility that $H_2O_2$ generated from ascorbic acid and BH4 within a setting of endothelial dysfunction may amplify the EDHF phenomenon. Thus, the beneficial effects of supplementation with these compounds may extend beyond their role in sustaining NO bioavailability. It remains to be determined whether these pro-oxidant effects of BH4 and ascorbic acid described by Garry et al. improve endothelial function in vivo; however, this potential mechanism for reversal of endothelial dysfunction clearly warrants further investigation.

Conflict of interest: none declared.

References