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Computational analysis of interactions of oxidative stress and tetrahydrobiopterin reveals instability in eNOS coupling

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ABSTRACT

In cardiovascular and neurovascular diseases, an increase in oxidative stress and endothelial dysfunction has been reported. There is a reduction in tetrahydrobiopterin (BH_4), which is a cofactor for the endothelial nitric oxide synthase (eNOS), resulting in eNOS uncoupling. Studies of the enhancement of BH_4 availability have reported mixed results for improvement in endothelial dysfunction. Our understanding of the complex interactions of eNOS uncoupling, oxidative stress and BH_4 availability is not complete and a quantitative understanding of these interactions is required. In the present study, we developed a computational model for eNOS uncoupling that considers the temporal changes in biopterin ratio in the oxidative stress conditions. Using the model, we studied the effects of cellular oxidative stress ($Q_{supcell}$) representing the non-eNOS based oxidative stress sources and BH_4 synthesis (Q_{BH4}) on eNOS NO production and biopterin ratio (BH_4/total biopterins (TBP)). Model results showed that oxidative stress levels from 0.01 to 1 $nM \cdot s^{-1}$ did not affect eNOS NO production and eNOS remained in coupled state. When the $Q_{supcell}$ increased above 1 $nM \cdot s^{-1}$, the eNOS coupling and NO production transitioned to an oscillatory state. Oxidative stress levels dynamically changed the biopterin ratio. When $Q_{supcell}$ increased from 1 to 100 $nM \cdot s^{-1}$, the endothelial cell NO production, TBP levels and biopterin ratio reduced significantly from 26.5 to 2 $nM \cdot s^{-1}$, 3.75 to 0.002 μM and 0.99 to 0.25, respectively. For an increase in BH_4 synthesis, the improvement in NO production rate and BH_4 levels were dependent on the extent of cellular oxidative stress. However, a 10-fold increase in Q_{BH4} at higher oxidative stresses did not restore the NO-production rate and the biopterin ratio. Our mechanistic analysis reveals that a combination of enhancing tetrahydrobiopterin level with a reduction in cellular oxidative stress may result in significant improvement in endothelial dysfunction.

1. Introduction

An increase in oxidative stress causes endothelial dysfunction in cardiovascular and neurovascular diseases (Fatehi-Hassanabad et al., 2010; Di Marco et al., 2015; Koizumi et al., 2016; Santilli et al., 2015). Vascular diseases risk factors including hyperglycemia and hypertension increase the production of reactive oxygen species (ROS) along the vascular wall (Fatehi-Hassanabad et al., 2010; Forstermann & Munzel, 2006). Endothelial dysfunction is characterized by a reduction in nitric oxide (NO) bioavailability and a concurrent increase of ROS production (Forstermann & Munzel, 2006; Headley et al., 2016). In endothelial cells, oxidative stress can cause uncoupling of the endothelial nitric oxide synthase (eNOS). The uncoupling of eNOS shifts the eNOS production of NO to superoxide ($O_2^{\bullet -}$) (Forstermann & Munzel, 2006; Alderton et al., 2001). The extent of eNOS uncoupling is predominantly determined by the availability of tetrahydrobiopterin (BH_4), an essential cofactor of eNOS that plays an important role in maintaining normal endothelial function (Bendall et al., 2014; Chen et al., 2014). The enhancement of BH_4 bioavailability holds therapeutic potential for improvement of endothelial dysfunction (Starr et al., 2013; Wang et al., 2014; Cai et al., 2005), whereas other studies have shown limited (Cunnington et al., 2012) or no improvement (Nystrom et al., 2004; Worthley et al., 2007) in endothelial function. Our understanding of the complex interactions of eNOS uncoupling, oxidative stress and BH_4 availability are not complete and a quantitative understanding of these interactions is required.

In normal physiologic conditions, biopterin is primarily present in reduced form (BH_4) because of low levels of oxidative stress (Nurkiewicz et al., 2010). In oxidative stress conditions, ROS can react with NO to form reactive nitrogen species (RNS) (Forstermann & Munzel, 2006; Potdar & Kavdia, 2009). An increase in the production

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of ROS (including $O_2^{\bullet-}$, ·OH and $CO_3^{\bullet-}$) and RNS (including peroxynitrite ($ONOO^-$) and $\cdot NO_2$) leads to oxidation of BH_4 to dihydrobiopterin (BH_2) (Kar & Kavdia, 2011; Katusic et al., 2009). Both BH_2 and BH_4 can compete with similar affinity for binding to eNOS (Crabtree et al., 2008). The binding of BH_2 to eNOS leads to the uncoupling of eNOS resulting in $O_2^{\bullet-}$ production (Kar & Kavdia, 2011; Katusic et al., 2009; Presta et al., 1998; Marchal et al., 2004). Studies have reported that the uncoupling of eNOS is correlated to the biopterin ratio (ratio of BH_4 to oxidized biopterins ($BH_3 + BH_2$)) (Vasquez-Vivar et al., 2002) and/or the total biopterin (TBP) levels (Alp et al., 2004; Alp et al., 2003). Recent experimental (Crabtree et al., 2008; Noguchi et al., 2011; Takeda et al., 2009) studies have reported that the extent of eNOS uncoupling is better associated to the biopterin ratio. Also the results from the previous endothelial cell based computational model, developed to investigate eNOS uncoupling related NO and $O_2^{\bullet-}$ production, showed that the ratio of reduced biopterin to total biopterin ([BH_4]/[TBP]) is the key parameter that determines the extent of eNOS uncoupling (Kar & Kavdia, 2011; Kar et al., 2012). However, the model was limited by its inability to predict the temporal changes in BH_4 concentration and biopterin ratio.

A change in biopterin ratio can dynamically affect endothelial cell oxidative stress levels (Vasquez-Vivar et al., 2002). In addition, oxidative stress can alter the activity of guanosine triphosphate cyclohydrolase I (GTPCH). GTPCH is a key enzyme in *de novo* synthesis pathway for the endogenous production of BH_4 (Katusic et al., 2009; Crabtree et al., 2009). The activity of GTPCH is reported to increase (Shimizu et al., 2003; Shimizu et al., 2005) or reduce (Meininger et al., 2000) in oxidative stress conditions. Shimizu et al. (Shimizu et al., 2003; Shimizu et al., 2005) reported that the long exposures of endothelial cells to H_2O_2, ·OH and $ONOO^-$ induced GTPCH mRNA expression and resulted in an increase of only BH_4 levels and not the oxidized forms of biopterin. The GTPCH inhibitor reduced the BH_4 levels in ROS and RNS exposed vascular endothelial cells. Meininger et al. (Meininger et al., 2000) reported a decrease in GTPCH activity with a proportional decrease in the BH_4 levels in diabetic rats. The endothelial cell NO synthesis from the diabetic rats was only 18% compared to that of normal animals.

Several experimental studies have examined the interactions of oxidative stress and BH_4 enhancement on the biopterin ratio and endothelial dysfunction (Crabtree et al., 2008; Alp et al., 2003; Sasaki et al., 2008). Crabtree et al. (Crabtree et al., 2008) reported a decrease in the biopterin ratio from 1:1 (BH_4:BH_2) in non-supplemented cells to 1:6 in 10 μM BH_4 supplemented hyperglycemic endothelial cells. They also reported a 40% decrease in NO production for BH_4 supplemented hyperglycemic endothelial cells. Alp et al. (Alp et al., 2003) reported that BH_4 comprised only 10% of the total biopterin content in diabetic-GTPCH overexpressing transgenic mice as compared to 80% in control GTPCH overexpressing transgenic mice. This decrease in the biopterin ratio was attributed to a 2–3 fold increase in $O_2^{\bullet-}$ production in diabetic-GTPCH overexpressing transgenic mice. Sasaki et al. (Sasaki et al., 2008) reported that $O_2^{\bullet-}$ production increased 1.6 fold in diabetic mice as compared to non-diabetic control mice and BH_4 supplementation suppressed $O_2^{\bullet-}$ production in diabetic mice.

The results from these studies demonstrate that there are complex biochemical interactions between BH_4, oxidized biopterins, ROS and RNS that modulates eNOS uncoupling. These interactions may also dynamically change the biopterin ratio through BH_4 oxidation and GTPCH activity or expression regulation. There is a lack of quantitative understanding of the interaction of oxidative stress and BH_4 on biopterin ratio and eNOS uncoupling. In the present study, we developed a computational model for eNOS uncoupling that takes into account the temporal changes in biopterin ratio in the cellular oxidative stress conditions. Using the model, we analyzed the effect of cellular oxidative stress and BH_4 on the eNOS based NO production rate and biopterin ratio. The model results will be helpful in guiding the experimentation in this high priority area of cardiovascular research.

2. Materials and methods

2.1. Model description

We developed an endothelial cell computational model that included the eNOS biochemical pathway, cellular oxidative stress, BH_4 synthesis and downstream reactions involving NO, ROS, RNS and biopterins (BH_4, BH_3 and BH_2). Fig. 1 shows the interaction of oxidative stress and BH_4 synthesis with the eNOS biochemical pathways and the downstream reactions. The eNOS biochemical pathway related NO and $O_2^{\bullet-}$ production depends on the relative availability of BH_4 and BH_2, respectively, which is a function of the biopterin ratio. In this study, the biopterin ratio is defined as the ratio of reduced biopterin to total biopterin ([BH_4]/[TBP]). The concentration of BH_4 and BH_2 depends on the rate of synthesis and oxidation of BH_4 (Vasquez-Vivar, 2009). The sources of oxidative stress in endothelial cells include NADPH oxidase, xanthine oxidase (XOD) and mitochondrial electron transport chain (Dikalov, 2011; Brown & Griendling, 2009). Each of these distinctive oxidative stress sources produces $O_2^{\bullet-}$ at different rates (Quijano et al., 2007; Quinlan et al., 2012a; Spiekermann et al., 2003) and results in enhanced ROS and RNS production in endothelial cells (Dikalov, 2011; Dikalova et al., 2010).

The eNOS biochemical pathway and downstream reactions with the respective rate constants modeled in this study are presented in Tables 1 and 2. The biochemical pathway for eNOS based NO and $O_2^{\bullet-}$ production are detailed in our previous work (Kar & Kavdia, 2011; Kar et al., 2012). In brief, the key reactions from the eNOS biochemical pathway for NO production include; (i) the binding of the co-factor BH_4 and substrates (L-arginine and O_2) to eNOS, (ii) the oxidation of L-arginine to *N*-hydroxyl-L-arginine (NHA) through enzyme substrate complexes (from eNOS-[Fe^{III}-O_2^-]-BH_4-Arg, E1 to eNOS-(Fe^{III})-BH_4-NHA, E2) and (iii) the oxidation of NHA to form NO and citrulline through enzyme substrate complexes (from eNOS-(Fe^{III})-BH_4-NHA, E2 to eNOS-(Fe^{III})-NO-BH_4, E4) (Kar & Kavdia, 2011; Chen & Popel, 2006). The eNOS biochemical pathway for $O_2^{\bullet-}$ production involves the binding of co-factor BH_2 and substrates (L-arginine and O_2) to eNOS (Klatt et al., 1994; Berka & Tsai, 2000; Berka et al., 2004; Berka et al., 1996). However, the inability of BH_2 to transfer electron to the eNOS heme results in the dissociation of the eNOS-substrate complex eNOS-[Fe^{III}-O_2^-]-BH_2-Arg, E5 to form $O_2^{\bullet-}$ (Marchal et al., 2004; Berka et al., 2004).

The oxidative stress and BH_4 synthesis are represented by production rate terms for $O_2^{\bullet-}$ ($Q_{supcell}$) and BH_4 (Q_{BH4}). $Q_{supcell}$ ($M \cdot s^{-1}$) represents the sum of $O_2^{\bullet-}$ production rate from non-eNOS based sources including NADPH and xanthine oxidase, and mitochondria. Q_{BH4} ($M \cdot s^{-1}$) represents the rate of BH_4 synthesis by GTPCH. We have accounted for the extracellular diffusion of BH_2 (k_{38}) since BH_4 is reported to have very low permeability across the endothelial cell membrane (Schmidt & Alp, 2007) and BH_3 has an extremely short half-life (Patel et al., 2002).

The main downstream reactions include (i) the reaction between NO and $O_2^{\bullet-}$ to form the RNS peroxynitrite ($ONOO^-$), which is in an acid-base equilibrium with peroxynitrous acid (ONOOH), (ii) the formation of RNS ($\cdot NO_2$) and ROS (·OH and $CO_3^{\bullet-}$) from the interaction of $ONOO^-$ with CO_2 and NO, respectively and by dissociation of ONOOH, (iii) the self and SOD-catalyzed dismutation of $O_2^{\bullet-}$ to form H_2O_2, (iv) the oxidation of BH_4 to biopterin radical (BH_3) by ROS ($O_2^{\bullet-}$, ·OH and $CO_3^{\bullet-}$) and RNS ($ONOO^-$ and $\cdot NO_2$), and (v) the oxidation of BH_4 and BH_3 to BH_2 by O_2.

2.2. Model assumptions

1) The current model is developed for dimeric eNOS since $O_2^{\bullet-}$ generated from monomeric eNOS is negligible compared to dimeric eNOS (Forstermann & Munzel, 2006; Benson et al., 2013).