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Contribution of membrane permeability and unstirred layer diffusion to nitric oxide–red blood cell interaction

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HIGHLIGHTS

- We developed a model of nitric oxide (NO) transport to a red blood cell (RBC).
- Effect of diffusion transport resistances on NO–RBC interaction was analysed.
- Intracellular diffusion of NO does not affect NO–RBC interaction.
- Membrane transport resistance is dominant if membrane permeability (P_m) < 0.21 cm s^{-1}.
- Extracellular diffusion transport resistance is dominant if $P_m > 0.44$ cm s^{-1}.

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ABSTRACT

Nitric oxide (NO) consumption by red blood cell (RBC) hemoglobin (Hb) in vasculature is critical in regulating the vascular tone. The paradox of NO production at endothelium in close proximity of an effective NO scavenger Hb in RBCs is mitigated by lower NO consumption by RBCs compared to that of free Hb due to transport resistances including membrane resistance, extra- and intra-cellular resistances for NO biotransport to the RBC. Relative contribution of each transport resistance on NO–RBC interactions is still not clear. We developed a mathematical model of NO transport to a single RBC to quantify the contributions from individual transport barriers by analyzing the effect of RBC membrane permeability (P_m), hematocrit (Hct) and NO–Hb reaction rate constants on NO–RBC interactions. Our results indicated that intracellular diffusion of NO was not a rate limiting step for NO–RBC interactions. The extracellular diffusion contributed 70–90% of total transport resistance for $P_m > 1$ cm s^{-1} whereas membrane resistance accounts for 50–75% of total transport resistance for $P_m < 0.1$ cm s^{-1}. We propose a narrow P_m range of 0.21–0.44 cm s^{-1} for 10–45% Hct, respectively, below which membrane resistance is more significant and above which extracellular diffusion is a dominating transport resistance for NO–RBC interactions.

1. Introduction

Nitric oxide (NO) acts as a potent vasodilator in vasculature (Furchgott and Zawadzki, 1980; Ignarro et al., 1987) and is a key player in physiological and pathophysiological processes (Moncada et al., 1991). In vasculature, NO homeostasis is maintained with its production by endothelial cells and consumption by smooth muscle cells and red blood cell (RBC) hemoglobin (Hb). It is established that Hb is an effective scavenger of NO ($k_{Hb-NO} \sim 10^7$ M^{-1} s^{-1}) (Cassoly and Gibson, 1975; Doyle and Hoekstra, 1981; Eich et al., 1996; Moncada et al., 1991). This has raised an important question about how NO is able to escape its potent scavenger Hb to maintain NO bioavailability in the vasculature. The paradox of vasoactive nitric oxide (NO) production in the close proximity of an effective NO scavenger Hb encapsulated in red blood cells has resulted in multitude of theoretical and experimental studies to examine NO interaction with RBCs (Deonikar and Kavdia, 2010a; Han and Liao, 2005; Huang et al., 2007; Liao et al., 1999; Vaughn et al., 2000).

The NO consumption by RBCs is much lower than that of NO consumption by equivalent amount of free Hb (Carlsen and Comroe, 1958; Deonikar and Kavdia, 2010b; Vaughn et al., 2001). A series of transport resistances are reported to slow the NO–RBC interactions. In blood vessels, a red blood cell-free layer near the vessel wall provides diffusional resistance for NO transport (Liao et al., 1999). Furthermore, an unstirred layer surrounding an RBC can provide additional diffusional resistance (Liu et al., 2002, 1998b). The membrane of the RBC can also act as a resistance for

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NO transport into the RBC. Sakai et al. (2008) recently reported that a diffusional transport resistance is also present inside the RBC. Although it is established that RBCs consume NO at a lower rate than free Hb, the mechanisms that lead to the lower consumption are still under intense investigation.

Several experimental studies have reported that RBC membrane permeability plays a critical role in the NO–RBC interactions. In the literature, RBC membrane permeability for NO varies four orders of magnitude from 0.0415 to 40 cm s^{-1} (Liu et al., 2007; Tsoukias and Popel, 2002; Vaughn et al., 2001). Han and Liao (2005) observed an increase in NO diffusion into the RBCs when they reduced the submembrane cytoskeleton compared to that with an intact submembrane cytoskeleton. Additionally, a study by Huang et al. (2001) reported that the NO–RBC interaction increased and decreased when RBC membrane was chemically modified with azide and bis(sulfosuccinimidul)-suberate, respectively. Recently, we reported that a RBC membrane permeability of 0.0415–0.4 cm s^{-1} is required to maintain sufficient NO bioactivity (Deonikar and Kavdia, 2010a). In a computational analysis of an earlier experimental study by Azarov et al. (2005), Huang et al. (2007) suggested that the binding of deoxygenated hemoglobin at the band-3 protein in submembrane cytoskeleton increased the membrane permeability NO by approximately ~10 fold than that under oxygenated conditions, thereby allowing more NO under deoxygenated conditions and less NO under oxygenated conditions through the RBC membrane for NO–RBC interactions.

Experimental and theoretical analyses have proposed that the extracellular diffusional resistance plays a major role in slowing NO–RBCs interactions (Liu et al., 2002; Liu et al., 1998b). Given that the extracellular diffusional resistance lowers the oxygen uptake by RBCs (Huxley and Kutchai, 1981), it is extrapolated that the transport of similar sized gaseous molecule NO is also limited to the RBCs by the extracellular diffusional resistance (Huxley and Kutchai, 1981; Liu et al., 2002). By comparing the measured rate of NO disappearance in presence of RBCs and oxyHb, Liu et al. (2002) concluded that the lower consumption of NO by RBCs is due to extracellular diffusional resistance.

The understanding of NO–RBC interactions is also critical in developing and optimizing the Hb based oxygen carriers (HBOC) because the NO bioavailability at smooth muscle cell layer for vasodilatation is dependent on NO consumption in the vascular lumen by the HBOC. Diffusional resistance and permeability are important design parameters for HBOC. Sakai et al. (2008) reported that the internal diffusional resistance and not the lipid membrane barrier is responsible for lowering the consumption of NO by the Hb vesicles designed to carry oxygen, and they extrapolated this to NO–RBC interactions. A recent study by Azarov et al. (2011) concluded that extracellular diffusion was the rate limiting step for NO–RBC and NO–Hb vesicle interactions while NO membrane permeability was the rate limiting step for NO–RBC microparticle interactions.

The understanding of NO–RBC interactions will also provide insight into the mechanisms of NO export out of the RBCs. RBCs have been reported to preserve NO bioactivity in the form of *S*-nitrosohemoglobin (SNOHb) (Jia et al., 1996; Stamler et al., 1997). In addition, deoxyHb can act as a nitrite reductase to form NO under deoxygenated conditions (Crawford et al., 2006; Gladwin et al., 2006). Export of NO from either SNOHb or nitrite reductase pathway would strongly depend on the transport resistances in the RBC and vascular lumen.

Thus, the quantification of individual transport resistance can provide useful insights in NO–RBC interactions. To understand the individual contributions of transport resistances including membrane permeability, extra-, and intra- cellular resistances on NO–RBC interactions, we developed a computational model of NO transport to a single RBC. We predicted the NO concentration profile across the unstirred plasma layer, the RBC membrane and the RBC core for different values of NO concentration outside the unstirred plasma layer, Hct and RBC membrane permeability. We also estimated the NO concentrations inside and outside the RBC membrane as a function of extracellular diffusional resistance and RBC membrane permeability.

2. Mathematical model

2.1. Model geometry

The NO biotransport to a single RBC at steady state was modeled in spherical geometry as shown in Fig. 1. The spherical model consisted of three concentric spheres representing the RBC core, the RBC membrane, and an unstirred plasma layer (PL). The inner sphere represents the RBC-hemoglobin and the outer sphere represents the unstirred plasma layer. The RBC membrane separates the inner sphere to the outer sphere and has a thickness of δ. NO diffuses from the unstirred plasma layer into the RBC membrane and reaches the RBC core to react rapidly with Hb. We simulated how NO–RBC interactions are affected from various hemodynamic, biophysical and biochemical parameters.

2.2. Model equation

The steady state NO biotransport in the spherical geometry is represented as follows:

$$D^i_{NO}\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C_{NO}}{\partial r}\right)-R_i=0 \quad (1)$$

where, C_{NO} represents the NO concentration, D^i_{NO} represents the diffusivity of NO in the respective region. R_i represents the net reaction rate to describe the NO consumption in each region. In the RBC core NO reacts with Hb. The reaction rate is given as $R_{cyt}=k_{NO-Hb}C_{NO}C_{Hb}$. k_{NO-Hb} is the reaction rate constant for NO–Hb reaction in the RBC core and C_{Hb} represents the hemoglobin concentration inside the RBC. No reaction is considered in the RBC membrane, hence $R_m=0$. In the unstirred plasma layer surrounding the RBC, NO autooxidation reaction is considered and the reaction rate is given as $R_{pl}=k_{NO-O_2}C_{NO}{}^2C_{O_2}$, where k_{NO-O_2} represents the reaction rate constant for auto-oxidation reaction of NO in the unstirred plasma layer and C_{O_2} represents the oxygen concentration in the unstirred plasma layer.

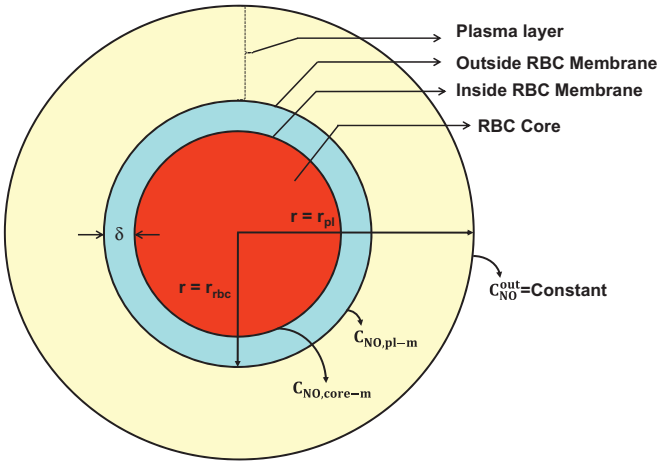

Fig. 1. Model geometry. NO biotransport to a single RBC is modeled in spherical geometry. The model consists of three concentric spheres. The inner sphere represents the RBC enclosed by an outer sphere representing unstirred plasma layer surrounding the RBC. NO concentration outside the unstirred plasma layer is considered constant.