Microscale Analysis for quantifying Nitric Oxide induced Hypoxemia in Methemoglobinemia

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Abstract

Methemoglobinemia is a disorder of the blood caused by abnormally high levels of methemoglobin (MetHb) in the red blood cell (RBC) resulting from simultaneous uptake of oxygen (O_2) and nitric oxide (NO) by the human lungs. MetHb is produced in the RBC by irreversible oxidation of the oxygen carrying ferrous ion (Fe^{2+}) of the heme group of the hemoglobin (Hb) molecule to its non-oxygen binding ferric state (Fe^{3+}). The oxidation is induced by NO, which is directly inhaled by the patient or synthesized in excess by L-Arginine present in endothelial cells, or is produced from oxidizing nitrates ingested through drinking water or pharmaceutical agents.

In this work, we study the role of NO in the pathophysiology of methemoglobinemia and perform quantitative analysis of the relation between levels of NO inhaled by the patient and the severity of the disease. Reactions of NO occurring in the RBC with both Hb and oxyhemoglobin are considered in conjunction with the reaction between oxygen and Hb to form oxyhemoglobin and that for the reduction of MetHb to Hb.

Our dynamic simulations of NO and O_2 uptake in the RBC under continuous exposure to both gases reveal that at the end of the pulmonary transit time of 1 s, the oxygen saturation in the RBC equilibrate at 98% while breathing NO-free room air but decreases monotonically to 93% as the concentration of NO increases to 5 ppm and further down to 50% when the NO level in the blood increases to 80ppm. We show that an NO level of 10 ppm or higher while breathing in room air may be considered to be the critical NO concentration for Methemoglobinemia since it causes severe hypoxemia in patients by decreasing the oxygen saturation level to below its critical value of 91%.

We simulate the effects of oxygen therapy on MetHb and oxygen saturation levels in the blood and quantify the severity of hypoxemia by stratifying Methemoglobinemia patients who are oxygen responsive from those who fail to respond to pure oxygen. Our dynamic simulations reveal that ventilating patients with pure oxygen serves as an effective therapeutic strategy for NO levels of less than 10ppm in the RBC. Otherwise, Methemoglobinemia may be treated with methylene blue 1% solution (10mg/ml, 1-2 mg/kg) administered intravenously slowly over five minutes. This treatment is affected through the enzyme-inducing effect of methylene blue on levels of diaphorase II (NADPH methemoglobin reductase) which helps in the reduction of MetHb (Fe^{3+}) back to hemoglobin (Fe^{2+}) by providing an electron. Our kinetic modeling of this metabolic pathway reveals that methylene blue restores the iron in Hb to its oxygen-carrying state in less than a second of reaching the RBC.

Introduction

Methemoglobinemia is a disorder characterized by the presence of a higher than normal level of methemoglobin (MetHb) in the blood. It is a compound formed from hemoglobin (Hb) by oxidation of

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iron atom from ferrous to ferric state. MetHb lacks the electron that is needed to form a bond with oxygen and hence, is incapable of oxygen transport. Generally, red blood cells (RBCs) are continuously exposed to various oxidant stresses and so MetHb is continually produced in humans. Typically, less than 1 percent of the total circulating hemoglobin in a healthy adult is present in the form of MetHb. However, injury or toxic agents convert a larger proportion of hemoglobin into MetHb. In healthy children, the ferric iron in MetHb is readily reduced to the ferrous state through the function of enzyme called as cytochrome b5 oxidase (also referred to as methemoglobin reductase), which is present in erythrocytes and other cells.

In this paper, we have concentrated in microscale modeling i.e., reactive uptake of NO in the RBC for the quantification of Methemoglobinemia. When a person is exposed continuously to NO, the reactions will attain equilibrium and the whole transport of NO through the lung will be diffusion controlled. Here spatial averaging is performed over the volume of the RBC. This model is used to obtain the increased levels of Methemoglobin inside RBC that is responsible for causing Methemoglobinemia and also estimate the levels of inhaled NO that could be fatal.

Reaction Chemistry

Nitric Oxide as previously discussed has rich reaction chemistry in Human Blood. The reactions that take place inside the RBC in presence of NO and O_2 are given as:

$$Hb + n.O_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Hb(O_2) \quad (1)$$

$$NO + HbO_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} MetHb + NO_3^{-1} \quad (2)$$

$$NO + Hb \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} HbNO \quad (3)$$

$$MetHb + b5_{Red} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} Hb + b5_{ox} \quad (4)$$

Reaction (1) shows that Oxygen-Hb equilibrium is assumed to follow Hill Kinetics with Hill constant, *'n'* equal to 2.34. The equilibrium rate constant of Hill kinetics is calculated using

$K_1 = \frac{H^n}{P_{50}^{\ n}}$, where H= 7.4x10^5 Torr and P_{50}= 26 Torr. (1)

Absolute value of the reaction rate constant for NO induced oxidation of oxyhemoglobin to MetHb (2) was calculated by Eich and co-workers, (1996). Sheele et al., (1999) reported the reaction rate constant of the reaction between NO and deoxyhemoglobin as 2x10^7 $M^{-1}s^{-1}$. They measured the dissociation rates of Nitrosylhemoglobin (3). Also, Dissociation rate constant of MetHb is obtained to be 2.47 x10^{-3} s^{-1} from Power et al., (2007). Reaction (4) shows the enzymatic MetHb reduction by reduced cytochrome *b*5 back to normal hemoglobin. In normal RBCs, MetHb is maintained at a level of less than 1% of total hemoglobin through two metHb-reducing pathways. One of these systems is the redox cycle consisting of cytochrome b5 (cytb5) and cytochrome b5-metHb reductase (b5R), which uses NADH as an electron transfer to cytb5 ("cytb5-NADH system"). The other pathway uses flavin as an electron carrier for the reduction of MetHb coupled with NADPH oxidation, catalyzed by NADPH-dependent

flavin reductase (FR) ("flavin-NADPH system"). Here we have considered first reduction pathway, because cytb5-NADH system is estimated to be responsible for more than 95% of metHb-reducing capacity under experimental conditions. Value of k_4 as 6.2×10^3 $M^{-1}s^{-1}$; and k_{-4} as 0.583 $M^{-1}s^{-1}$ are given by Kuma , (1981) and Abe et al., (1979).

Mathematical Model

The diffusion-reaction equations in Lagrangian coordinates for a single RBC of any arbitrary geometry and volume Ω, and external surface area $\partial\Omega$ are given by,

$$\alpha_{O_2}\frac{\partial P_{O_2,rbc}}{\partial t} = D_{O_2}\alpha_{O_2}\nabla^2 P_{O_2,RBC} - R_1 \quad (6)$$

$$[Hb]_T\frac{\partial S_{HbO_2}}{\partial t} = D_{Hb}[Hb]_T\nabla^2 S_{HbO_2} + R_1 - R_2 \quad (7)$$

$$\alpha_{NO}\frac{\partial P_{NO,rbc}}{\partial t} = D_{NO}\alpha_{NO}\nabla^2 P_{NO,RBC} - (R_2 + R_3) \quad (8)$$

$$[Hb]_T\frac{\partial S_{MethHb}}{\partial t} = D_{Hb}[Hb]_T\nabla^2 S_{MethHb} + R_2 - R_4 \quad (9)$$

$$[Hb]_T\frac{\partial S_{HbNO}}{\partial t} = D_{Hb}[Hb]_T\nabla^2 S_{HbNO} + R_3 \quad (10)$$

where $P_{i,rbc}$ is the partial pressure of physically dissolved gas *'i'* (NO or O_2) in the RBC, S_j is the fractional hemoglobin *'j'* saturation, ∇^2 is the three dimensional Laplacian in the local coordinate in the RBC, D_i and D_{Hb} are the diffusion coefficients of gas *'i'* and hemoglobin inside the RBC, R_j is the net rate of conversion for corresponding reaction (j), $[Hb]_T$ is the total intra-erythrocytic hemoglobin (free+bound), and α_i is the solubility of gas *'i'* in the RBC. Since [b5R] is given to be 0.07 μM in Higasa et al. (1998), the scale of R_4 is very low as compared to R_2. Hence it can be ignored in Equation (9). Since the details of the NO concentration in the vasculature is not of interest to us, we assume that the total endothelial generated NO is consumed inside the RBC, i.e. it is not available for s-GMP activation. The uptake rate of oxygen depends on the alveolar partial pressure of oxygen, P_{AO2}, which is around 100 Torr.

Results and Discussion

We have taken initially deoxygenated blood i.e. arterial P_{O2} to be 40 torr corresponding to 75% S_{HbO2} for carrying out the simulation. This is because blood returning to the heart from the tissues has a low P_{O_2} (40 mmHg) and travels to the lungs via pulmonary arteries. The pulmonary arteries form pulmonary capillaries, which surround alveoli.

After oxygenation blood moves into the pulmonary veins which return to the left side of the heart to be pumped to the systemic tissues. Fig. 1(a) shows the dynamic simulation for S_{HbO2}. It can be noticed from the figure that when alveolar NO is nearly zero, oxygen saturation attains equilibrium at the value of 0.97 and the oxygen tension increases to 100 torr. Oxygen diffuses (moves through the membrane separating the air and the blood) from the high pressure in the alveoli (100 mmHg) to the area of lower pressure of the blood in the pulmonary capillaries (40 mmHg). It has been found that under this case oxygen saturation reaches a steady state within 0.8-1 sec of exposure of oxygen. Normally 1 sec is the

transit time for oxygen consumption in red blood cell. Fig.1(b) shows the dynamic profile for S_{MetHb} in red blood cell. As discussed earlier 1% is known to be the level of MetHb in normal hemoglobin. But we can see that as the concentration of NO increases, MetHb saturation increases so much that it leads to the onset of methemoglobinemia. We have obtained the plot for 2 second but chopped it off in 1 second as this is the transit time of gas inside RBC. Moreover we have assumed initial condition that of deoxygenated blood inside RBC that is partial pressure of O_2 inside RBC to be of 40 torr.

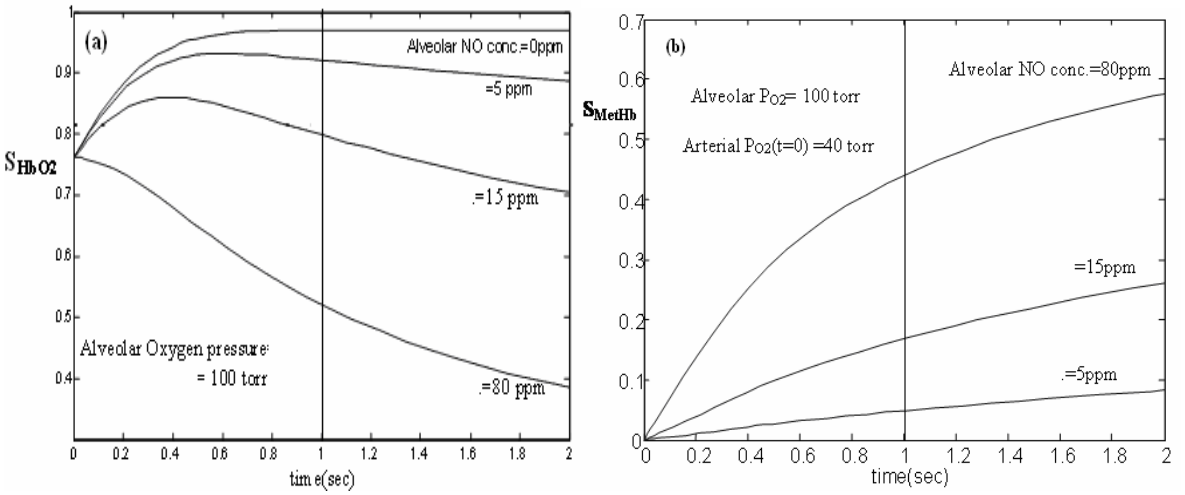

Fig.1. Dynamic Profile of (a) S_{HbO2} and (b) S_{MetHb} inside RBC for different values of alveolar NO concentration**.**

Therapeutic Strategy for Methemoglobinemia

Methemoglobinemia results from the oxidation of the heme moiety in the hemoglobin molecule from the ferrous to the ferric state. Auto-oxidation of Hb to MetHb occurs spontaneously at a slow rate in normal individuals, converting 0.5 to 3 percent of the available Hb to MetHb per day. The only physiologically important pathway for reducing MetHb back to Hb is the NADH-dependent reaction catalyzed by cytochrome b5 reductase (b5R). An alternative pathway is an enzyme utilizing NADPH generated by glucose-6-phosphate dehydrogenase (G6PD) in the hexose monophosphate shunt as a source of electrons. However, there is normally no electron carrier present in red blood cells to interact with NADPH methemoglobin reductase. . As a result, electron acceptors or redox dyes, such as methylene blue (MB), and flavin are required for this pathway to work. A congenital deficiency in the NADH-dependent cytochrome b5 methemoglobin reductase can be inherited in an autosomal recessive pattern. Patients who are homozygous for this enzymatic deficiency have congenital methemoglobinemia and may exhibit lifelong cyanosis. In order to treat this cyanosis, we decided to supply patient with oxygen therapy.

Fig. 2 shows the dynamic simulation of oxygen saturation with increasing oxygen supply for NO concentration of 5ppm. It shows that as oxygen supply increases above 250 torr, critical saturation increases and moves towards left till 0.2 sec and after that all follow the same trend with minute difference in values. From various plots for different NO concentration, we concluded that pretreatment with oxygen is useful for lower concentration of NO i.e. below 10ppm because supplying excess oxygen brings oxy-saturation to near about 91% which is the safe limit for normal human being. However when NO concentration is more than 10 ppm, excess oxygen supply does not play significant role. In such cases, we need to see other options available to us. Most significant and popular option for treatment of

methemoglobinemia is Methylene Blue. Methylene blue is the first-line antidotal therapy. Methylene blue works as a cofactor for the enzyme NADPH-methemoglobin reductase. Methylene blue is oxidized into leukomethylene blue by accepting an electron from NADPH in the presence of NADPH-methemoglobin reductase. Leukomethylene blue then donates this electron to MetHb resulting in its conversion back to Hb. The initial dose is 1-2 mg/kg over 5 min. Its effects should be seen in approximately 20 min to 1 h. Fig.3 shows that MetHb saturation reaches to its minimum value within 0.1 second of methylene blue reaching RBC irrespective of alveolar NO concentration. The prophylactic preoperative methylene blue administration in a patient with congenital methemoglobinemia significantly decreased the MetHb level and increased the fractional oxygen saturation with a consequent increase of the safety margin against perioperative hypoxemia.

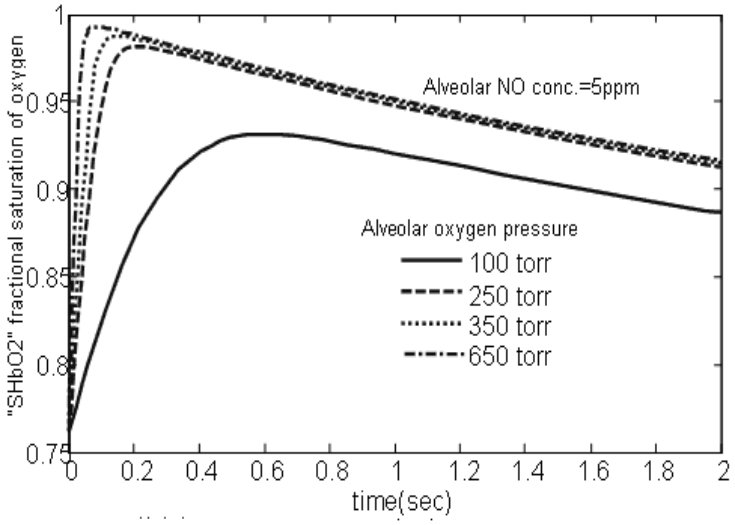

Fig. 2. Dynamic profile of oxygen saturation during oxygen therapy (alveolar NO conc. = 5 ppm).

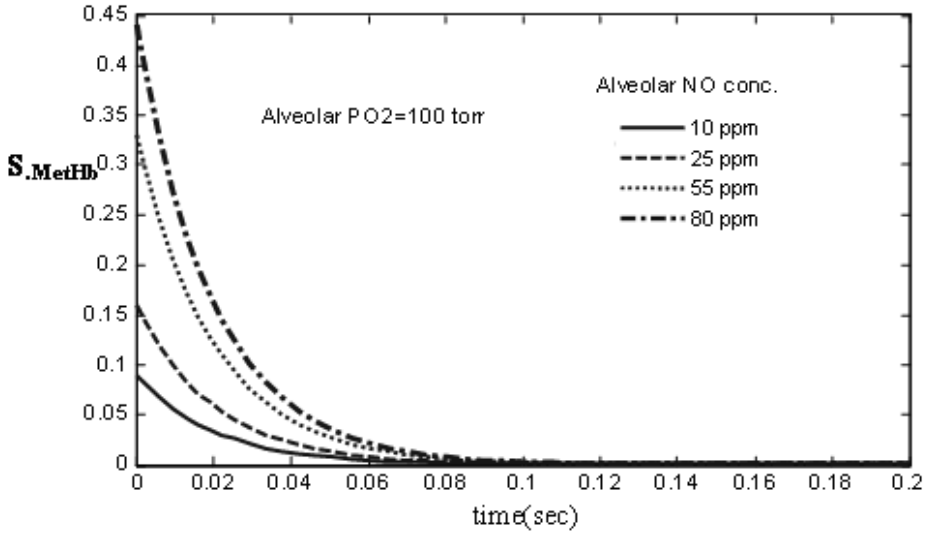

Fig. 3. Dynamic profile of S_{MetHb} where NADPH-methemoglobin reductase is activated by Methylene Blue for MetHb reduction to Reduced Hb for different NO concentration.

Conclusions

In this work, we have modeled the simultaneous uptake of Nitric Oxide and Oxygen by Red Blood Cell in order to quantify methemoglobinemia. The rate equations for reactions of NO with oxy- and deoxy-hemoglobin are developed and these equations are used to develop analytical expressions for O_2 and NO diffusing capacities of RBC for continuous exposure of NO. We have used LS based spatial averaging approach to solve these expressions. During continuous exposure to NO, we have assumed that the reactions achieve equilibrium and we have used it to obtain fractional saturation of various compounds. Dynamic simulation of our mathematical model shows that for higher NO concentration i.e. 10 ppm, oxy-saturation falls more rapidly going below the limit of 91% which is the safe limit for normal human being as discussed earlier. Also MetHb saturation can go upto 40% for 80 ppm of NO leading to fatal conditions of extreme methemoglobinemia. In order to cure this disease, we dealt with two kinds of treatment. First one is excess supply of oxygen which helps to cure cyanosis which occur upto 10 ppm of NO inside RBC. For NO concentration higher than this, first treatment does not work and so treatment with methylene blue is given more emphasis. Methylene blue works as a cofactor for the enzyme NADPH-methemoglobin reductase which helps in rapid conversion of MetHb back to Hb. It was observed from the plots that conversion process is activated within 0.1 second of methylene blue reaching inside RBC reducing MetHb to almost zero.

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