ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 11 & 12, 2005

Mini Review

Redox Biology of Blood Revisited: The Role of Red Blood Cells in Maintaining Circulatory Reductive Capacity

PAUL W. BUEHLER and ABDU I. ALAYASH

ABSTRACT

There is an increasing recognition of the role of red blood cells (RBCs) in cell signaling above and beyond its oxygen (O_2)-carrying function. A recent forum published in the December 2004 issue of *Antioxidants & Redox Signaling* focused on redox biology of blood and the intricate signaling pathways of RBCs or its free components, *i.e.,* hemoglobin, with the vasculature. The forum provided an up-to-date source of information on this emerging and exciting area of blood biology and the underlying redox chemistry. In the current short review, we have revisited the topic of redox biology of blood and focused on yet another emerging area of research, which deals with the reductive power of blood and the physiological implications. *Antioxid. Redox Signal.* 7, 1755–1760.

INTRODUCTION

THE ARTICLES featured within the forum on redox biology (*Antioxidants & Redox Signaling*, Vol. 6, December, 2004) highlighted several critical emerging areas of research. Two of these articles dealt with both *in vitro* and *in vivo* redox chemistry of heme proteins and possible contributions of these reactions to pathology (25) and nonenzymatic degradation of heme originating from the dominant red blood cell (RBC) protein, hemoglobin (Hb) and subsequent reactive oxygen species (ROS) generation (20). Both articles provided insights into the abnormalities of hemoprotein structure and function that can be induced under conditions of oxidative stress. The role of nitric oxide (NO) interactions with Hb and subsequent influence on oxidative modification to Hb's normal allosteric function was reviewed in depth. Additionally, NO interaction at the level of heme iron and the effect of Hb/NO interaction at important amino acids (βCys_{93}) were detailed (5). RBC and vascular function (8), as well as signaling mechanisms between RBC components, particularly Hb, and the vasculature (6), provided insight into Hb-mediated vascular control of oxygen (O_2) sensors such as hypoxia inducible factor (HIF) through ATP, NO, and nitrite (NO_2^-) under normal and pathological conditions. O_2 distribution and microcirculatory regulation (29) were reviewed in light of O_2 delivery and the resulting effect on blood flow within the microcirculation. Additionally, current concepts in Hb based O_2 carrier (HBOC) redox chemistry and ROS actions on microcirculatory permeability (3), as well as HBOC redox influences on HIF and heme oxygenase (HO-1), were demonstrated in systems of *in vitro* oxidative stress (31).

The field of redox biology of blood is rapidly advancing and encompasses several important areas as emphasized in the December issue's forum. The present update will focus on the intricate role of RBCs in maintaining circulatory protective antioxidant levels. In the current review, we will further expand on these concepts, taking into consideration the role of RBCs and their role in antioxidant redox cycling of endogenous ascorbic acid (AA).

Laboratory of Biochemistry and Vascular Biology, Division of Hematology, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD.

Disclaimer: The opinions and assertions contained herein are the scientific views of the authors and are not to be construed as policy of the United States Food and Drug Administration.

ASCORBIC ACID AND THE RED BLOOD CELL

The evolution of certain vertebrates, particularly humans, is accompanied by a loss of L-gulonolactone oxidase, the final enzyme required for the endogenous synthesis of AA (7). This evolutionary characteristic is shared by other species, such as most nonhuman primates, fruit bats, and guinea pigs; thus, dietary intake of AA is essential for maintaining plasma and tissue reductive capacity in these species. AA primarily functions to remove superoxide anion ($O_2^{\cdot -}$) and singlet oxygen (22) and is itself oxidized to one and two electron-oxidized forms, these products being the AA radical (AAR) and dehydroascorbic acid (DHA), respectively (27, 30). Interestingly, AA also functions to remove other ROS generated by protein-bound redox metals (18) and xanthine oxidase (19). Nandi *et al.* (21) have demonstrated that vertebrates incapable of producing endogenous AA due to a lack of L-gulonolactone oxidase possess greater tissue levels of superoxide dismutase (SOD); however, despite increased levels of SOD and other ROS-detoxifying agents [*e.g.,* glutathione (GSH), catalase, and α-tocopherol], oxidative damage persists in the absence of AA intake.

Given the critical nature of AA as a plasma and tissue reducing agent, even in the presence of other up-regulated means of reductive capacity, consideration of its role in preventing ROS generation in the presence pro-oxidative agents in blood has recently been recognized. May *et al.* (16), using ferricyanide as an extracellular oxidant, suggested a two-tiered system of redox recycling of low concentrations of the AAR by human RBCs under minimal oxidative stress (tier one) followed by a backup system of RBC recycling of large quantities of DHA under conditions of increased oxidative stress (tier two). This reaction was initially introduced, also by May *et al.* (15), to occur in vascular endothelial cells and found to be dependent on cellular membrane-bound NADH and NADPH oxidoreductases when oxidative stress is less severe and via an intracellular GSH-dependent reduction of DHA when oxidative stress is more severe. RBC accumulation of DHA as a result of pro-oxidative conditions originates in part outside of the RBC during the two-electron oxidation of AA, which is subsequently transported reversibly in competition with glucose by the type 1 glucose transporters (GLUT1) spanning the RBC membrane (12, 30). Alternatively, DHA may be lost altogether by irreversible ring opening and further degradation, removing a pool of potentially reversible oxidized AA. RBC concentrations representative of a 45% hematocrit can regenerate the AA present in whole blood every 3 min. Experimental evidence suggests that recycling of AA by the RBC seems to contribute toward an additional antioxidant reserve of blood when compared with experiments evaluating plasma AA in the absence of RBCs (14). Therefore, it appears clear that an organism's own red cells may contribute to important redox defense mechanisms.

HBOC'S OXIDATIVE NATURE

Oxidative chemistry

HBOCs represent a class of therapeutic agents that demonstrate varying degrees of oxidative potential originating at their heme iron centers in terms of autoxidation (Reaction 1), oxo-ferryl Hb formation (Reaction 2), oxo-ferryl Hb radical formation (Reaction 3), rhombic heme formation and degradation (Reaction 4), and NO dioxygenation (Reaction 5) (1):

$$HbFe^{2+}O_2 \rightarrow HbFe^{3+} + O_2^{\cdot -} \quad \text{(Reaction 1)}$$

$$HbFe^{2+}O_2 + H_2O_2 \rightarrow HbFe^{4+}{=}O + H_2O + O_2 \quad \text{(Reaction 2)}$$

$$HbFe^{3+} + H_2O_2 \rightarrow {}^{\cdot}HbFe^{4+}{=}O + H_2O \quad \text{(Reaction 3)}$$

$$HbFe^{4+}{=}O + H_2O_2 \rightarrow HbFe^{3+} + O_2^{\cdot -} + H_2O \rightarrow \text{Heme degradation}^{*} \quad \text{(Reaction 4)}$$

$$HbFe^{2+}O_2 + NO \rightarrow HbFe^{3+} + NO_3^{-} \quad \text{(Reaction 5)}$$

[$HbFe^{2+}O_2$ is oxyhemoglobin, $HbFe^{2+}$ is ferrous Hb, and $HbFe^{3+}$ is ferric Hb or methemoglobin.]

Certain HBOCs contribute significantly to a prooxidative environment in the microcirculation, leading to vascular endothelial injury and permeability (2, 4). This effect may be due to differing rates of Reactions 1–4 among HBOCs. Additionally, the nature of HBOCs' volume of distribution generally limits their pharmacokinetic exposure to the plasma volume (23) and may lead to derangements in normal oxidized AA and RBC recycling. Conditions of significant blood loss (traumatic hemorrhage) or reduced hematocrit (hemolytic anemia) result in a simultaneous loss of RBC volume/plasma volume/AA concentration and RBC volume, respectively. Each of these conditions may alter the balance of plasma AA reducing capability in the presence of an HBOC either by the combined loss of RBC volume/AA concentration or by the loss of RBC volume alone. Thus, cell-free HBOCs represent an interesting contribution to model systems, which test RBC reductive capacity.

Model of HBOCs and RBC/AA recycling

Figure 1 (bottom) is a modified representation of the two-tiered model of RBC and oxidized AA recycling proposed by May *et al.* (16) using a generic HBOC as the extracellular prooxidant in place of ferricyanide. As shown in Fig. 1 (bottom left), oxidized heme iron in its non–O_2-carrying $HbFe^{3+}$ form may be reduced back to its O_2-carrying form $HbFe^{2+}$ directly by transmembrane-bound NADH oxidoreductase coupled to intracellular AA oxidation. Figure 1 (bottom right) represents the extracellular reduction of $HbFe^{3+}$ and oxidation of AA to $HbFe^{2+}$ and DHA, respectively. When $HbFe^{3+}$ is abundant in the plasma due to autoxidation or NO dioxygenation, excess DHA is transported into the RBC by GLUT1, reduced by GSH, and transported out of the RBC as AA. DHA is also capable of being reduced to AA at the RBC surface by the NADH oxidoreductase system (bottom left). Alternatively, DHA may be lost to hydrolysis by ring opening and formation of 2,3-diketo-L-gulonic acid when RBC volume is insufficient, intra- or extracellular AA concentration is low, or extracellular prooxidant levels become overwhelming. The upper portion of Fig. 1 represents a hypothetical interplay between AA and its role in vascular O_2 sensing and oxidative insult. This concept will be expanded on in a brief discussion of the complex relationship between AA/RBCs and HBOC efficacy/toxicity.

The role of AA/RBCs in the control of oxidized HBOCs in vitro

The first studies evaluating the role of RBCs and AA in limiting persistence of oxidized extracellular Hb were per-

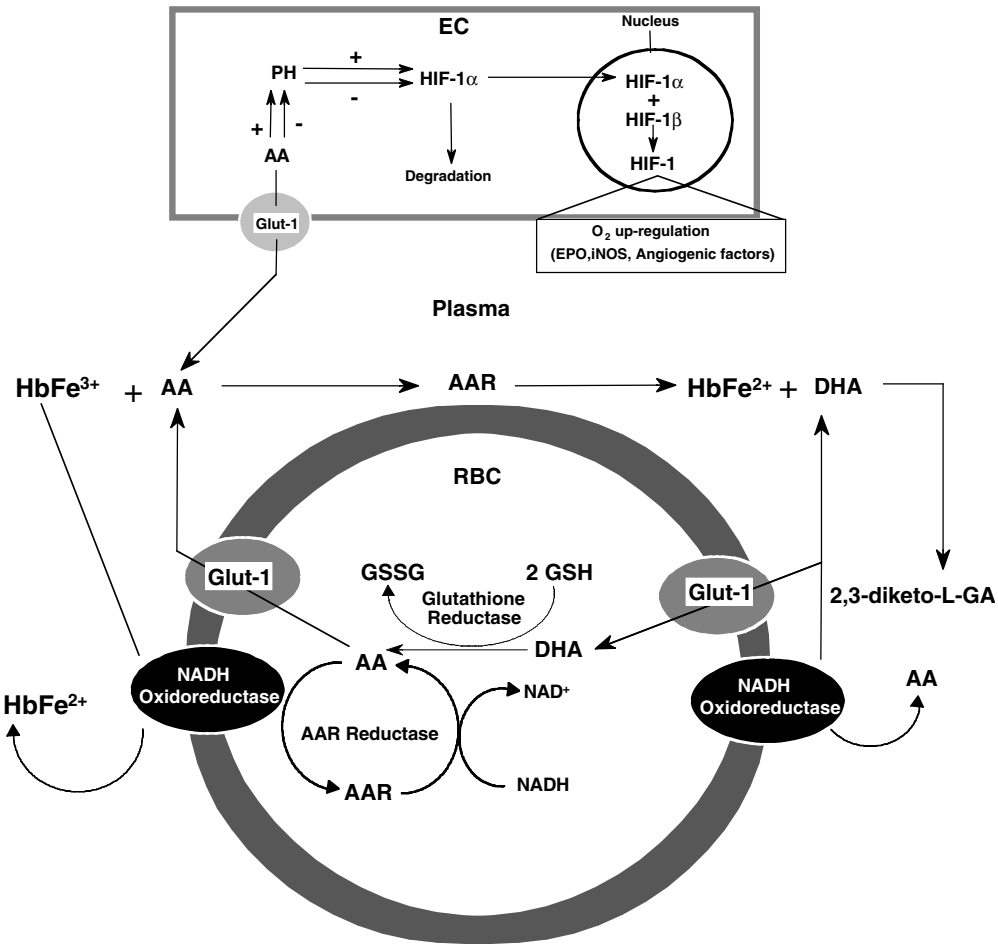

FIG. 1. A hypothetical interaction between endothelial cells (EC), plasma, RBCs, and AA in the presence of a prooxidant (cell-free $HbFe^{3+}$). (**Top**) Within the EC, AA acts as a cofactor for prolyl hydroxylase (PH). The presence of normal cellular AA concentrations (+) allows for PH activation (+) in the degradation process of HIF-1α. When O_2 and potentially AA are low (−), PH is inactive (−) and HIF-1α is translocated into the nucleus after combining with HIF-1β to form HIF-1. As a result, up-regulation of factors influencing increased tissue oxygenation occurs. In the presence of increased plasma oxidants such as $HbFe^{3+}$, intracellular AA may be depleted by either GLUT1 transport (14) or simple diffusion (26). Epo, erythropoietin; iNOS, inducible NO synthase. (**Bottom**) Reductive processes in the plasma and RBC are primarily dependent on AA (left), which reduces plasma oxidants such as $HbFe^{3+}$ (left) to $HbFe^{2+}$ (right) and DHA (left) through formation of the AA radical intermediate (AAR). Alternative, $HbFe^{3+}$ may be reduced by RBC membrane-bound oxidoreductases (left). DHA represents a renewable source of AA, as it is either reduced in the RBC following glucose type 1 transport (GLUT1) or by membrane-bound oxidoreducase activity (right). Cellular AA may be moved out of the RBC or function within the RBC in coordination with membrane-bound oxidoreductase and AAR reductase. Alternatively, DHA can be lost to irreversible ring opening, forming 2,3-diketo-L-gulonic acid (2,3-diketo-L-GA) and degradation products.

formed by McGown *et al.* (17). This work demonstrated that addition of oxidized HBOC ($HbFe^{3+}$) to human whole blood and washed human RBCs in the presence of physiologic concentrations of AA (~0.1 m*M*) reduced $HbFe^{3+}$ to $HbFe^{2+}$ by 40–50%. Moreover, an important physiologic mechanism for controlling oxidation of HBOCs was revealed. Since the initial work of McGown, a limited number of studies have focused on extracellular Hb as affected by plasma and RBC reducing components. Reduction of $HbFe^{3+}$ to $HbFe^{2+}$ in the presence of plasma reducing agents such as AA, GSH, and β-NADH was studied for several naturally occurring Hbs [HbA_0, Hb S (sickle Hb), and *Lumbricus* Hb] and a number of HBOCs (10). Dorman *et al.* (10) suggest that the redox potential of naturally occurring and chemically modified Hbs plays an important role in the resistance to oxidation and susceptibility to reduction of certain Hbs by plasma reducing agents. Den Boer *et al.* (9) evaluated the percent reduction resulting from incubation of 91% oxidized Hb (total Hb concentration = 3%) in the presence of human RBCs and found a 27% reduction in oxidized Hb. The addition of 0.2 m*M* AA to the RBC/Hb cocktail further reduced the oxidized Hb by another 42%. Taken together, these studies clearly indicate a synergistic effect occurring between RBCs and AA in reductive processes of oxidized extracellular Hb.

We conducted similar *in vitro* experiments to evaluate the role of differing simulated hematocrit levels over a range of 0–40% on HBOC ($HbFe^{3+}$) (300 μ*M*) reduction in the presence of physiologic AA concentration (0.1 m*M*) following 5 h

of incubation at 37°C. Our unpublished data indicate that incubating a simulated hematocrit of 1% (0.1 ml of washed RBCs/10 ml phosphate-buffered), in the presence of 0.1 m*M* AA exhibits a nearly identical rate of reduction to 10% (1 ml of washed RBCs/10 ml phosphate-buffered), 20% (2 ml of washed RBCs/10 ml phosphate-buffered) and 40% (4 ml of washed RBCs/10 ml phosphate-buffered) simulated hematocrits in the presence of 0.1 m*M* AA. Incubation with 300 μ*M* $HbFe^{3+}$, 0.1 m*M* AA, and 0% hematocrit had essentially no effect on $HbFe^{3+}$ reduction. Human packed RBCs average 8.0×10^6 cells/μl; thus, a 1% simulated hematocrit in this experiment represents ~8.0×10^8 RBCs/ml of solution, whereas a simulated hematocrit of 40% represents ~3.2×10^{10} RBCs/ml of solution. Given a physiological AA concentration (0.1 m*M*) in this experiment, there appears to be a sufficient RBC reducing capacity in spite of a 40-fold decrease in RBC volume. Thus, one potential means of limiting HBOC-induced oxidative injury and maintenance of the functional O_2 carrying form ($HbFe^{2+}$) may be accomplished by coadministration of AA, even when massive red cell loss occurs.

The role of AA/RBCs in HBOC reduction in vivo

The principles of AA/RBC redox cycling originally suggested by McGown as a physiologic means of limiting exposure to oxidized HBOCs have since been tested *in vivo*. Faivre *et al.* (11) found that HBOC oxidized by 30–40% in plasma sampled over 36 h following a 50% exchange transfusion in guinea pigs. The majority of HBOC ($HbFe^{3+}$) formation occurred within the first 12–24 h post exchange. Similarly, following a 50% exchange transfusion of HBOC ($HbFe^{3+}$), ~40% was reduced back to HBOC ($HbFe^{2+}$) over the initial 12–24 h post exchange. A single infusion of 2 m*M* AA immediately after HBOC exchange transfusion limited oxidation to 20% over 12–24 h. With the exception of the Faivre study, most small animal models of blood loss with subsequent HBOC treatment utilize various strains of rat, all of which produce endogenous AA. The rat is reported to produce 38 ± 4 μg of AA/mg of liver microsmes/h, whereas the guinea pig, nonhuman primates, and humans must rely on dietary intake of the vitamin (7).

We have obtained data (unpublished) to demonstrate the novel multifunctional aspects of endogenous AA in preventing HBOC redox toxicity. Using electron paramagnetic resonance spectrometry, we have found that AA reduces $HbFe^{3+}$ to $HbFe^{2+}$ in the presence of RBCs and reduces both ferryl hemoglobin ($HbFe^{4+}$) and associated globin chain-bound free radicals following a 20% exchange transfusion in rabbits.

A reliable model representative of HBOC efficacy and toxicity may be most appropriately demonstrated in species with similar circulatory AA reductive capacity to humans. In a follow-up study and given the guinea pig's inability to synthesize AA, we compared it with the rat in an effort to evaluate HBOC oxidative changes. Figure 2A and B show the visible spectra (soret region) of polymerized bovine Hb (PolyHbBv) in plasma sampled over 48 hours following a 50% blood/PolyHbBv exchange transfusion in the guinea pig and rat, respectively. The pharmacokinetic data (not shown) demonstrate greater HBOC ($HbFe^{3+}$) exposure [area under the plasma HBOC ($HbFe^{3+}$) concentration–time curve] following transfusion in the guinea pig versus the rat. Although we have not yet directly assessed the levels of ascorbate (before and after exchange transfusion) in rats versus guinea pigs, in view of the fact that ascorbate synthesis in guinea pig is lacking, these data may indirectly suggest a role for endogenous ascorbate in controlling Hb oxidative chemistry in rats as opposed to the guinea pigs.

Efficacy of HBOC solutions may be determined, in part, by their influence on vascular O_2 sensing and activation of downstream mediators, which maintain normal oxygenation of tissues. Both microcirculatory O_2 content (pO_2) (24) and cellular AA concentrations (28) are demonstrated to have significant influences on HIF expression, a global O_2 sensor in vascular tissue and regulator of downstream mediators of O_2 adaptive responses (*e.g.,* erythropoietin, inducible NO synthase, and vascular endothelial growth factor). When pO_2 and/or AA levels are low within vascular tissue, stabilization of cytosolic HIF-1α leads to transport of the protein into the nucleus after complexation with HIF-1β to form HIF-1, and subsequent up-regulation of numerous genes to meet global and local O_2 demand. Activated prolyl hydroxylases (PH) within the cytosol function to destabilize HIF-1α and prevent its nuclear transport (13). O_2, 2-oxoglutarate (cosubstrate), and AA (cofactor) all function in the activation of PH; thus, independent depletion of cellular O_2 (via increased mitochondrial respiration) or AA leads to increased HIF-1 expression. Alternatively, depletion of both O_2 and AA may result in quantitatively dissimilar HIF-1 expression as would be observed

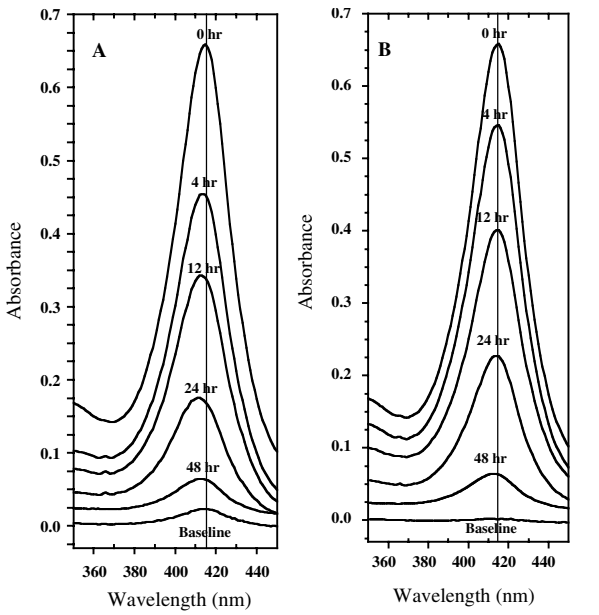

FIG. 2. The plasma clearance of glutaraldehyde PolyHbBv in the guinea pig and the rat following a 50% PolyHbBv for blood exchange transfusion (ET). The UV-visible spectral changes in the soret region of PolyHbBv within plasma from the end of ET (0 h) until 48 h post end of ET for the guinea pig (**A**) and rat (**B**) are shown. Each species demonstrates differences in plasma clearance patterns within this region of the spectrum; most noticeable, however, is the gradual shift in the individual spectra of PolyHbBv in guinea pig plasma from $HbFe^{2+}O_2$ (λ_{max} = 415 nm) toward that of $HbFe^{3+}$ (λ_{max} = 405 nm).

under circumstances of either independent loss of cellular O_2 or AA. The complex influences of both O_2 and AA on HBOC efficacy/potential toxicity as determined by expression of HIF-1 and $HbFe^{3+}$ persistence, respectively, represent interesting challenges in elucidating the nature of HBOC-induced HIF-1 expression, as being mediated by either suboptimal O_2 delivery and/or AA depletion resulting from HBOC oxidation.

CONCLUSION

The December 2004 issue of *Antioxidants & Redox Signaling* provided a highly informative and thought-provoking group of articles on the redox biology of blood. The role of RBCs in O_2 transport and its physiology is well established. We are hopeful that these articles will continue to generate interest in this and other equally important, but not yet fully exploited, roles of RBCs in areas such as O_2 sensing, transport of NO or its metabolites, and the redox cycling of AA.

ABREVIATIONS

AA, ascorbic acid; AAR, ascorbic acid radical; DHA, dehydroascorbic acid; GLUT1, type 1 glucose transporter; GSH, glutathione; Hb, hemoglobin; $HbFe^{2+}$, ferrous hemoglobin; $HbFe^{3+}$, ferric hemoglobin or methemoglobin; $HbFe^{2+}O_2$, oxyhemoglobin; HBOC, hemoglobin-based oxygen carrier; HIF, hypoxia-inducible factor; NO, nitric oxide; O_2, oxygen; $O_2^{\cdot -}$, superoxide anion; PH, prolyl hydroxylase; pO_2, oxygen content; PolyHbBv, polymerized bovine hemoglobin; RBC, red blood cell; ROS, reactive oxygen species; SOD, superoxide dismutase.

REFERENCES

1. Alayash AI. Oxygen therapeutics: can we tame haemoglobin? *Nat Rev Drug Discov* 3: 152–159, 2004.
2. Baldwin AL. Modified hemoglobins produce venular interendothelial gaps and albumin leakage in the rat mesentery. *Am J Physiol* 277(2 Pt 2): H650–H659, 1999.
3. Baldwin AL. Blood substitutes and redox responses in the microcirculation. *Antioxid Redox Signal* 6: 1019–1030, 2004.
4. Baldwin AL, Wiley EB, and Alayash AI. Comparison of effects of two hemoglobin-based O_2 carriers on intestinal integrity and microvascular leakage. *Am J Physiol Heart Circ Physiol* 283: H1292–H1301, 2002.
5. Bonaventura C, Fago A, Henkens R, and Crumbliss AL. Critical redox and allosteric aspects of nitric oxide interactions with hemoglobin. *Antioxid Redox Signal* 6: 979–991, 2004.
6. Buehler PW and Alayash AI. Oxygen sensing in the circulation: "cross talk" between red blood cells and the vasculature. *Antioxid Redox Signal* 6: 1000–1010, 2004.
7. Chatterjee IB. Evolution and the biosynthesis of ascorbic acid. *Science* 182: 1271–1272, 1973.
8. Crawford JH, Chacko BK, Kevil CG, and Patel RP. The red blood cell and vascular function in health and disease. *Antioxid Redox Signal* 6: 992–999, 2004.
9. den Boer PJ, Bleeker WK, Rigter G, Agterberg J, Stekkinger P, Kannegieter LM, de Nijs IM, and Bakker JC. Intravascular reduction of methemoglobin in plasma of the rat in vivo. *Biomater Artif Cells Immobilization Biotechnol* 20: 647–650, 1992.
10. Dorman SC, Kenny CF, Miller L, Hirsch RE, and Harrington JP. Role of redox potential of hemoglobin-based oxygen carriers on methemoglobin reduction by plasma components. *Artif Cells Blood Substit Immobil Biotechnol* 30: 39–51, 2002.
11. Faivre B, Menu P, Labrude P, Grandgeorge M, and Vigneron C. Methemoglobin formation after administration of hemoglobin conjugated to carboxylate dextran in guinea pigs. Attempts to prevent the oxidation of hemoglobin. *Artif Cells Blood Substit Immobil Biotechnol* 22: 551–558, 1994.
12. Himmelreich U, Drew KN, Serianni AS, and Kuchel PW. ^{13}C NMR studies of vitamin C transport and its redox cycling in human erythrocytes. *Biochemistry* 37: 7578–7588, 1998.
13. Kivirikko KI, Myllyla R, and Pihlajaniemi T. Protein hydroxylation: prolyl 4-hydroxylase, an enzyme with four cosubstrates and a multifunctional subunit. *FASEB J* 3: 1609–1617, 1989.
14. May JM, Qu ZC, and Whitesell RR. Ascorbic acid recycling enhances the antioxidant reserve of human erythrocytes. *Biochemistry* 34: 12721–12728, 1995.
15. May JM, Qu ZC, Neel DR, and Li X. Recycling of vitamin C from its oxidized forms by human endothelial cells. *Biochim Biophys Acta* 1640: 153–161, 2003.
16. May JM, Qu ZC, and Cobb CE. Human erythrocyte recycling of ascorbic acid: relative contributions from the ascorbate free radical and dehydroascorbic acid. *J Biol Chem* 279: 14975–14982, 2004.
17. McGown EL, Lyons MF, Marini MA, and Zegna A. Reduction of extracellular methemoglobin by erythrocytes. *Biochim Biophys Acta* 1036: 202–206, 1990.
18. Mukhopadhyay CK and Chatterjee IB. Free metal ion-independent oxidative damage of collagen. Protection by ascorbic acid. *J Biol Chem* 269: 30200–30205, 1994.
19. Mukhopadhyay CK and Chatterjee IB. NADPH-initiated cytochrome P450-mediated free metal ion-independent oxidative damage of microsomal proteins. Exclusive prevention by ascorbic acid. *J Biol Chem* 269: 13390–13397, 1994.
20. Nagababu E and Rifkind JM. Heme degradation by reactive oxygen species. *Antioxid Redox Signal* 6: 967–978, 2004.
21. Nandi A, Mukhopadhyay CK, Ghosh MK, Chattopadhyay DJ, and Chatterjee IB. Evolutionary significance of vitamin C biosynthesis in terrestrial vertebrates. *Free Radic Biol Med* 22: 1047–1054, 1997.
22. Nishikimi M. Oxidation of ascorbic acid with superoxide anion generated by the xanthine–xanthine oxidase system. *Biochem Biophys Res Commun* 63: 463–468, 1975.
23. Palaparthy R, Kastrissios H, and Gulati A. Pharmacokinetics of diaspirin cross-linked haemoglobin in a rat model of hepatic cirrhosis. *J Pharm Pharmacol* 53: 179–185, 2001.
24. Ratcliffe PJ, O'Rourke JF, Maxwell PH, and Pugh CW. Oxygen sensing, hypoxia-inducible factor-1 and the regulation

of mammalian gene expression. *J Exp Biol* 201(Pt 8): 1153–1162, 1998.
25. Reeder BJ, Svistunenko DA, Cooper CE, and Wilson MT. The radical and redox chemistry of myoglobin and hemoglobin: from in vitro studies to human pathology. *Antioxid Redox Signal* 6: 954–966, 2004.
26. Rose RC. Transport of ascorbic acid and other water-soluble vitamins. *Biochim Biophys Acta* 947: 335–366, 1988.
27. Rose RC and Bode AM. Biology of free radical scavengers: an evaluation of ascorbate. *FASEB J* 7: 1135–1142, 1993.
28. Salnikow K, Donald SP, Bruick RK, Zhitkovich A, Phang JM, and Kasprzak KS. Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress. *J Biol Chem* 279: 40337–40344, 2004.
29. Tsai AG, Cabrales P, Hangai-Hoger N, and Intaglietta M. Oxygen distribution and respiration by the microcirculation. *Antioxid Redox Signal* 6: 1011–1018, 2004.
30. Wilson JX. The physiological role of dehydroascorbic acid. *FEBS Lett* 527: 5–9, 2002.
31. Yeh LH and Alayash AI. Effects of cell-free hemoglobin on hypoxia-inducible factor (HIF-1alpha) and heme oxygenase (HO-1) expressions in endothelial cells subjected to hypoxia. *Antioxid Redox Signal* 6: 944–953, 2004.

Address reprint requests to:
Abdu I. Alayash, Ph.D.
FDA/CBER
National Institutes of Health
Building 29, Room 112
8800 Rockville Pike
Bethesda, MD 20892

E-mail: alayash@cber.fda.gov

Received after revision April 29, 2005; accepted June 20, 2005.

This article has been cited by:

1. C.E. Angulo-Valadez, M.C. Reyes-Becerril, G.M.J. Romero, R. Cepeda-Palacios, D.R. López-Aguilar, T. Zenteno, F. Ascencio. 2011. Antioxidant enzymes in erythrocytes from goats seropositive to the sheep nose bot fly (Oestrus ovis L., Diptera: Oestridae) infection. *Veterinary Parasitology* . [CrossRef]
2. Manuela Blasa, Donato Angelino, Lorenzo Gennari, Paolino Ninfali. 2011. The cellular antioxidant activity in red blood cells (CAA-RBC): A new approach to bioavailability and synergy of phytochemicals and botanical extracts. *Food Chemistry* **125**:2, 685-691. [CrossRef]
3. Oliver Neubauer, Stefanie Reichhold, Lukas Nics, Christine Hoelzl, Judit Valentini, Barbara Stadlmayr, Siegfried Knasmüller, Karl-Heinz Wagner. 2010. Antioxidant responses to an acute ultra-endurance exercise: impact on DNA stability and indications for an increased need for nutritive antioxidants in the early recovery phase. *British Journal of Nutrition* **104**:08, 1129-1138. [CrossRef]
4. Michalis G. Nikolaidis, Athanasios Z. Jamurtas. 2009. Blood as a reactive species generator and redox status regulator during exercise. *Archives of Biochemistry and Biophysics* **490**:2, 77-84. [CrossRef]
5. Alcir Luiz Dafre, Tiago A.S. Brandão, Evaldo Reischl. 2007. Involvement of vertebrate hemoglobin in antioxidant protection: chicken blood as a model. *Canadian Journal of Zoology* **85**:3, 404-412. [CrossRef]