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FORUM EDITORIAL

Clearance and Control Mechanisms of Hemoglobin from Cradle to Grave

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Abstract

Hemoglobin is a highly reactive molecule, and besides its oxygen-carrying capacity, it has multiple enzymatic and ligand-binding activities that have only recently been explored as fundamental pathophysiologic mechanisms. Nitric oxide neutralization, generation of potentially toxic radical species, and heme-mediated inflammation are among the most extensively studied mechanisms of Hb-mediated pathology. Extracellular Hb has an established role in sickle cell disease and other hemolytic disorders. However, extracellular Hb seems also to have relevant disease-modifying activities in many other important pathologic conditions, such as malaria and atherosclerosis. In this Forum, we summarize the current knowledge of mechanisms of Hb toxicity. Special emphasis is given to the highly efficient endogenous scavenger and detoxification pathways, such as α-hemoglobin stabilizing protein (AHSP), haptoglobin, hemopexin, CD163, and heme oxygenase. Systemic and local activity of these pathways finally determines the impact of extracellular Hb on physiology and tissue homeostasis. *Antioxid. Redox Signal.* 12, 181–184.

Multiple physiologic and pathologic conditions are associated with red blood cell (RBC) lysis and release of hemoglobin (Hb) into extracellular compartments. Hemoglobin is a highly reactive molecule, and besides its oxygen-carrying capacity, it has multiple enzymatic and ligand-binding activities that have only recently been explored as fundamental pathophysiologic mechanisms (1). The most paradigmatic condition associated with intravascular free Hb exposure is hemolysis in sickle cell disease, and therefore, many disease processes such as Hb-associated systemic and pulmonary hypertension, vasculopathy, and Hb/heme-triggered inflammation have initially been explored in the context of this genetic disorder (8). However, several other diseases and conditions have been described to be Hb-related pathologies. The most severe form of malaria, cerebral malaria, has very distinct links to cell-free Hb and heme reactivities (7). Atherosclerosis with intraplaque hemorrhage serves as another potentially important example of how extracellular Hb could worsen disease processes within oxidative and inflammatory susceptible tissues (3, 13). Finally, experiences with hemoglobin-based oxygen carriers (HBOCs) are potentially the most extensive source of preclinical and clinical information on the extracellular effects of Hb within the vascular and tissue compartments (6).

Although the understanding of the precise mechanisms of Hb toxicity has considerably expanded, other research has explored effective physiologic systems that control the adverse activities of Hb from early erythropoiesis to heme degradation in macrophages. It is an intriguing and conceivable idea that these Hb "detoxification systems" have allowed the evolutionary success of heme-globin–based oxygen transport (Fig. 1).

A precise understanding of the molecular processes underlying Hb-mediated pathology will be instrumental in finally defining valid strategies to control Hb-mediated diseases. The unmet need for effective therapeutic strategies in hemoglobin-related pathology was just emphasized by the disappointing news from a failed clinical trial that investigated a nitric oxide (NO)-modulating strategy in sickle cell disease (see http://public.nhlbi.nih.gov/newsroom/home/GetPressRelease.aspx?id=2650). In this Forum, we summarize the current knowledge about Hb toxicity, together with an in-depth overview of the most recent understanding of Hb scavenging and detoxification pathways.

In the first review, Buehler and D'Agnillo give a biochemical and physiological perspective on the known consequences of extracellular Hb on eukaryotic physiology. A decisive idea raised in this article is that Hb toxicity is a multifactorial process that cannot simply be traced back to nitric oxide (NO) inactivation (that is the most extensively studied mechanism) or any other single reaction. Rather, NO neutralization, heme release, and heme-driven oxidative processes interact with

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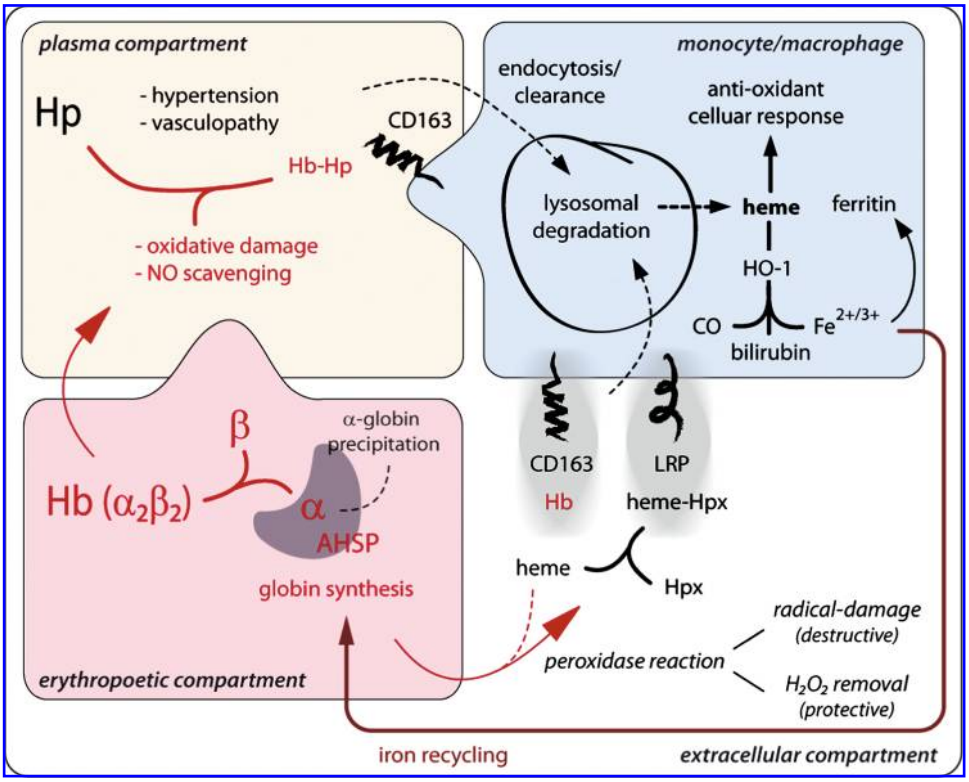

FIG. 1. Schematic representation of compartmentalized hemoglobin toxicity, scavenger proteins, and detoxification pathways.

other factors to yield the full physiological mosaic of Hb-associated adverse effects. Maladaptive (damaging) and adaptive (protective) responses to extracellular Hb and heme, respectively, can now be impressively reproduced in novel animal models of human disease, such as the transgenic sickle cell mice. In their article, Belcher *et al*. discuss the complex sequence of extracellular Hb/heme accumulation, oxidant exposure, inflammation, and vascular damage in the context of sickle cell disease vascular pathology.

Oxidative control of Hb or its subunits is essential not only after erythrocyte destruction and extracellular Hb accumulation. It is equally important to control redox activity and the structural integrity of single α-globin chains before they assemble to form tetrameric ($\alpha_2\beta_2$) hemoglobin during erythropoesis. Mollan *et al*. explain the essential role of the evolutionarily conserved α-hemoglobin-stabilizing protein (AHSP) in fine tuning RBC precursor maturation. As a chaperone, AHSP rapidly captures newly synthesized Hb α-globin chains within a low-affinity α-globin: AHSP complex. AHSP supports α-globin folding, controls oxidative chemistry at its heme group, and prevents α-subunit precipitation. Subsequently, AHSP allows tetrameric Hb formation by slowly releasing bound subunits into the higher-affinity $\alpha\beta$–globin interaction.

A series of reviews summarizes the major physiologic scavenger pathways that exist to detoxify and clear Hb when it is released from bursting RBCs. Haptoglobin (Hp) is a plasma glycoprotein with a genetically determined dimeric or multimeric structural assembly ($\alpha_2\beta_2 \ldots \alpha_x\beta_x$). Each Hp β-subunit can bind one Hb dimer with an extremely high affinity to form an irreversible and high-molecular-weight Hb:Hp protein complex. Although Hp was identified as the primary plasmatic Hb scavenger in the early days of physiological biochemistry, some essential functions of this molecule have been explored only recently. However, it is now recognized that Hp has the potential to attenuate some of the major adverse effects of Hb, such as vasoactivity, oxidative tissue damage, and peroxidative self-destruction of the Hb heme and globin moieties (2, 4). Although Hp drastically changes the physiologic effects of Hb *in vivo*, the ligand-binding and enzymatic properties at its heme sites remain preserved (2). Therefore, the unaltered kinetics of heme-based reactions within the complex suggest distinct functions of Hb:Hp during hemoylsis, inflammation, or wound healing. Wicher *et al*. review the evolutionary framework of Hb and heme scavengers. Haptoglobin first appeared in bony fish. There, the protein gained its high-affinity Hb-binding function from an ancient innate immunity protease. Later, other Hb-binding proteins, such as the SRCR proteins CD163 and pit54, appeared; these originated from a completely different cysteine-rich protein family. The result of these evolutionary events is a redundant and highly regulated system of soluble capture proteins and cellular receptors that effectively shield mammalian species from Hb-related damage (10).

Although Hp is generally considered to have protective activities, the polymorphic Hp phenotype (Hp 2-2) is linked to a higher incidence of oxidative stress–associated diseases, such as micro- and macrovascular complications in diabetes patients. In the future, this genotype–disease relation might offer the possibility to identify a high-risk (Hp 2-2) population of patients deserving personalized prevention or treatment

strategies or both. Distinct differences between the dimeric Hp 1-1 and multimeric Hp 2-2 in respect to their capability to control Hb-mediated peroxidative processes could be the basis for these clinical observations and may form the rationale for targeted treatment interventions. These basic and clinical aspects of Hp and its unique polymorphism are reviewed in the article by Levy *et al*.

After depletion of Hp and with oxidation of the heme iron, heme eventually dissociates from globin. Hempoexin (Hpx) is the primary scavenger for free heme that backs up the plasma Hb-binding capacity provided by haptoglobin. The physiologic role of hemopexin was highlighted in Hp/Hpx double-knockout mice that are prone to severe liver inflammation, splenomegaly, and oxidative endothelial damage (12). Like cell-free Hb, heme sequestered within hemopexin is cleared from the circulation and from heme-exposed tissues by a dedicated receptor-clearance pathway (CD91/LRP). In addition to heme-binding, other biologic functions of hemopexin involve regulatory functions in cell signaling and cell survival. The diverse facets of this multifunctional plasma protein are summarized in the article by Tolosano *et al*. The Hb:Hp and heme:Hpx complexes are finally cleared by their respective endocytosis receptors CD163 and CD91/LRP before they are degraded by lysosomal proteases. Nielsen *et al*. review our current knowledge about the molecular function of these carrier protein–dependent receptor systems and how activation of downstream metabolic pathways can link toxic heme/Hb exposure to antioxidant and antiinflammatory cellular responses (9).

Three original contributions close this Forum. Butt *et al*. show that significant species differences exist in the redox status of extracellular Hb that is secreted through the kidney. The significant interspecies differences that exist in important antioxidant systems (*i.e*., extensive ascorbate synthesis in rats but not in guinea pigs) are a potential explanation for these findings and may also in part explain the role of ascorbate in variable adaptive ferritin synthesis. These data support the concept of appropriate animal species and models in the investigation of Hb-related pathology. Asleh *et al*. investigated the intriguingly opposing effects of the two antioxidants, ascorbic acid and α-tocopherol on Hb-driven HDL peroxidation. The results presented in their article can help us to understand the pharmacogenomic interactions of Hb, Hp phenotypes, and different antioxidant strategies in atherosclerosis-prone individuals. In the final article of this Forum, Widmer *et al*. add a provocative novel facet to the complex biology of extracellular Hb. Although the H_2O_2-degrading (peroxidase) activity of Hb has been known for decades, this activity was largely considered destructive because the radicals produced in the peroxidase reaction of Hb–H_2O_2 could damage circulating biomolecules and tissues. With a complementary repertoire of cell-biology assays, comprehensive gene-expression analysis, and biochemical methods, Widmer *et al*. showed that Hb has the potential to absorb a significant amount of oxidative impact within its peptide structure. This self-destructive process involves amino acid oxidation, globin chain cross-linking, and finally precipitation of the altered protein (5, 11). Thereby, extracellular Hb acts as a protective peroxidase that can attenuate H_2O_2-mediated oxidative stress and preserve cellular viability during H_2O_2 exposure. These observations strongly imply that extracellular Hb could be a double-edged sword with both damaging and protective effects on tissue homeostasis, similar to those of H_2O_2. The net physiologic impact is likely dependent on specific environmental factors, such as the presence of Hp or the level of macrophage CD163 expression.

We hope that the series of reviews and original contributions collected in this Forum will provide a comprehensive source of knowledge and stimuli for the growing community of researchers and clinicians involved in the fascinating field of hemoglobin-related pathology.

Author Disclosure Statement

No conflict of interest exists. The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any agency determination or policy.

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