Toxicity of Acellular and Cellular Hemoglobins

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This presentation reflects the views of the speaker and should not be construed to represent FDA's views or policies
Oxidative Pathways of Acellular and Cellular Hemoglobins

- Hemoglobin-based oxygen carriers (HBOCs) (free Hb)
- Hemolytic anemias (free Hb/microparticles)
- Stored blood “storage lesions” (free Hb/microparticles)
Oxygen Therapeutics “Blood Substitutes”

- RBC
- PFC Emulsion
- Tetramer
- Conjugated Tetramer
- Polymer
- Encapsulated Hb
HBOCs: Associated Adverse Events

- Transient hypertension
- Gastrointestinal symptoms
- Pancreatic and liver enzyme elevation
- Myocardial infarction; cardiac arrhythmias
- Renal Injury
- Mortality

Mechanisms of Hemoglobin Oxidation and Control

- **Autoxidation** (Erythroid cells and mature RBCs)
  - **CAT/GSSG**
  - $\text{H}_2\text{O} + \text{O}_2$
  - $\text{H}_2\text{O}_2$
  - $\text{O}_2^-$
  - $\text{O}_2 + \text{H}_2\text{O}_2$
  - **SOD**

- **Redox cycles and oxidative modifications** (Circulation)
  - **Damage Associated Molecular Pattern (DAMP)**
    - **Molecule**
    - **TLR-4** (inflammation)
    - **HO-1** induction
    - Altered cell metabolism

- **Degradation**
  - **AHSP**
  - **βTyr145**
  - **HP**
  - **HPX**
  - **AIM**

- **FerrylHb/Ferryl radical** (protein-bound heme adduct)

- **Ferrous** $\alpha_2\beta_2$

- **Ferric** $\alpha_2\beta_2$

- **Heme release and degradation**
Transition of Hemoglobin Into Higher Redox States (Pseudoperoxidase) and Subsequent Changes in β Subunit’s “Oxidative Hotspot”

Dorsal Skin Fold Chamber Model Measures Vaso-occlusion in Transgenic Sickle Mice after Hemoglobin Infusion

Effects of Haptoglobin/Hemopexin on Stasis in Sickle Cell Mice

Heme Triggers Vaso-occlusion Through Activation of TLR4 Signaling on Endothelial Cells

(Damage Associated Pattern Molecule (DAMP))

Pathophysiologic Consequences of RBC Storage

- Decreased deformability
- Impaired flow in vivo
- Impaired O₂ delivery
- Hemolysis
- Disordered NO homeostasis

Proposed Oxidative Pathways within Red Blood Cells Leading to Generation of Microparticles

Young RBC

Membrane

Cytosol

HbFe^{II}-O_{2} (oxy/deoxy)

O_{2}-(SOD)
autoxidation
H_{2}O_{2} (CAT)

MetHb reductase
NADPH/NADH

HbFe^{III} (MetHb)

Old/Sickle RBC

HbFe^{II}^{IV}/
HbFe^{IV}

H_{2}O_{2}

Band 3 clustering

O_{2}-(SOD)
autoxidation
H_{2}O_{2} (CAT)

MetHb reductase
NADPH/NADH

HbFe^{III} (MetHb)

Free Hb

Microparticles

Buehler et al., Antioxid Redox Signal 14:1713, 2011

Camus et al., Blood 125:3805, 2015
Autoxidation of Hemoglobin-Townes Mice Microparticles
(Time = 0 hr.)

(A) Wavelength (nm)

(B) Absorbance at 280 nm

Absorbance

Wavelength (nm)

Absorbance at 577 nm

HbA control
mMp_AA
mMp_AS
mMp_SS

mMp_SS
mMp_AS
mMp_AA

heme

protein

Time (min)
Time-dependent Changes in the Redox States of Hemoglobin in Microparticles from Townes Mice

![Graph A: MethHb % vs Time (hrs)](image1)

![Graph B: OxyHb % vs Time (hrs)](image2)
Quantitative Proteomic Analysis of AA, AS and SS Mice Samples

Gene Ontological Functional Classes

- antioxidant activity (GO:0016209)
- binding (GO:0005488)
- catalytic activity (GO:0003824)
- enzyme regulator activity (GO:0030234)
- nucleic acid binding transcription factor activity (GO:0001071)
- receptor activity (GO:0004872)
- structural molecule activity (GO:0005198)
- translation regulator activity (GO:0045182)
- transporter activity (GO:0005215)

Abundance of antioxidant enzymes in microparticles from AA, AS and SS samples (t=0)

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Circulating SCD-MPs Mitochondrial Bioenergetic Impairment and Oxidative Stress in Vascular Endothelial Cells (HUVEC)

Heme oxygenase-1 expression in HUVEC

Mitochondrial superoxide generation in HUVEC by MitoSOX fluorescence (red)

Circulating Sickle MPs exhibit band 3 phosphorylation at Tyr-21
Proposed Mechanism of Hemoglobin-induced Toxicity (mitochondrial dysfunction)

HbFe$^{2+}$/HBOC

Oxidative stress

HbFe$^{3+}$

Hp

Heme (DAMP)

HPx

TLR4

Hb

BV

Fe$^{2+}$

CO

Ferritin

HO-1

NF-κB

Mitochondria

Summary and Conclusions

Free hemoglobin:

- Oxidative pathways, subsequent radical migration to $\beta$ subunits ($\beta\text{Cys93}$) and loss of heme, a DAMP molecule play a central role in oxidative toxicity of hemoglobin.
- Oxidation products of Hb (ferric and a persistent ferryl Hb) induce mitochondrial dysfunction and translocation of heme oxygenase to mitochondria.

Microparticles:

- Oxidative pathways within human and mouse derived microparticles follow similar trends which may be influenced by changing reductive capacity of microparticles.
- Microparticles promote impairment of cellular bioenergetics which are directly impacted by the redox state of the heme iron.
In Conclusion:

“The storage lesion” is a misnomer.

In fact, relevant biochemical, structural and functional changes are multifactorial and complex.

Impact of storage on hemoglobin oxygenation and NO homeostasis is likely to be transient and of uncertain significance.

In contrast, perturbations of RBC membrane are likely to adversely affect blood flow in the microcirculation.

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