Nitrite bioactivation by Globin X in zebrafish blood and effects on heart regeneration

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Beneficial Effects of Nitrite

Role of circulating nitrite and S-nitrosohemoglobin in the regulation of regional blood flow in humans

Mark T. Gladwin1, James H. Shelhammer2, Alan N. Schechter2, Margaret E. Pease-Fye4, Myron A. Waclawiw4, Julio A. Panza5, Frederick P. Ozonime6, and Richard O. Cannon III7

11482-11487 | PIAS | October 10, 2000 | vol. 97 | no. 21

Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation

Kenyatta Cosby1, Kristine S Partovi2, Jack H Crawford1, Rakesh P Patel1, Christopher D Reiter1, Sabrina Marty2, Benjamin K Yang3, Myron A Waclawiw4, Gloria Zaloe1, Xiuli Xu6, Kris T Huang2, Howard Shields6, Daniel B Kim-Shapiro5,7, Alan N Schechter2, Richard O Cannon III7,8, and Mark T Gladwin2,4,4

VOLUME 9 | NUMBER 12 | DECEMBER 2003 NATURE MEDICINE

Cytoprotective effects of nitrite during in vivo ischemia-reperfusion of the heart and liver

Mark R. Duranski1, James J.M. Greer1, Andre Dejarn1,2, Sathya Jagannathan1, Neil Hogg3, William Langston1, Rakesh P. Patel1, Shaw-Fang Yet1, Xunde Wang1, Christopher G. Kevit4, Mark T. Gladwin7,8 and David J. Lefer1

The Journal of Clinical Investigation http://www.jci.org Volume 115 Number 5 May 2005

Deoxymyoglobin Is a Nitrite Reductase That Generates Nitric Oxide and Regulates Mitochondrial Respiration

Sruti Shiva,1* Zhi Huang,1* Rozalina Grubina, Junhui Sun, Lorna A. Ringwood, Peter H. MacArthur, Xiuli Xu, Elizabeth Murphy, Victor M. Darley-Usmar, Mark T. Gladwin

Circ Res. 2007;100:654-661

Nitrite Reductase Function of Deoxymyoglobin

Oxygen Sensor and Regulator of Cardiac Energetics and Function

Tienush Rassaf,1* Ulrich Flögel,1* Christine Drexhage, Ulrike Hendgen-Cotta, Malte Kelm, Jürgen Schröder

Circ Res. 2007;100:1749-1754;

http://www.vmi.pitt.edu/Gladwin.html
Globins are Nitrite Reductases At Low Oxygen

\[
\text{Deoxy-Heme}^+ + \text{NO}_2^- + \text{H}^+ \rightarrow \text{Met-Heme} + \text{NO} + \text{OH}^- 
\]

Hemoglobin
Blood

Myoglobin
Heart

Endothelium
Blood

Deoxy-Hb

GTP
sGC

NO

GMP

RELAXATION

NO

Cardiac Muscle Cell

Mitochondrion

Deoxy-Mb

Oxy-Mb

Cytoprotection
Globins evolution

Hemoglobin -> Myoglobin -> Cytoglobin -> Neuroglobin -> Globin X

Erythrocytes -> Muscles -> Heart -> Fibroblasts -> Neurons -> Endocrine -> ??? Brain?
GbX is found in zebrafish blood
GbX is a fast nitrite reductase

Protein | Nitrite reduction rate (M$^{-1}$s$^{-1}$)
--- | ---
Zf Ngb | 0.68 ± 0.04
Zf Cgb1 | 28.3 ± 3.1
Zf Cgb2 | 0.94 ± 0.18
Zf GbX | 26.7 ± 2.0
hNgb | 0.52 ± 0.19
hCgb | 1.14 ± 0.07

(Corti et al, submitted to *Nitric Oxide*)
GbX in RBCs produces NO from nitrite

Nitric Oxide Analyzer (NOA)

Scramble siRNA | GbX siRNA

Nitrite production (pM/s)

0.0 | 0.2 | 0.4 | 0.6 | 0.8

Scramble siRNA vs. GbX siRNA
GbX in RBCs and nitrite inhibit platelets activation

\[
\text{deoxyHb} + \text{NO}_2^- \rightarrow \text{methHb} + \text{NO}
\]

(Corti et al., FRBM 2014)
GbX and nitrite inhibit platelet activation

Nitrite reduction

\[ \text{NO}_2^- \]

GbX is 6-coordinate

NO production

\[ \text{NO}^\bullet \]

platelets activation

NO signaling
What is the Effect of Hypoxic Nitrite during Heart Regeneration?

(Chablais et al., *Dev Biol* 2011)
Effect of Nitrite at 5dpC on Injured Area

**Hypoxia**

**Hyp + NO₂**

- Control
- NO₂
- Hypoxia
- Hyp + NO₂

Injured area (%)

- 0
- 10
- 20
- 30
- 40

****

***

****
Effect of Nitrite at 5dpC on Cell Proliferation

Hyp+NO2

5 dpC

PCNA+/Mef2+ cardiomyocytes (%)

n=7-10
Nitrite has anticoagulant effects

$P<0.01$
Future Plans

- Investigate the role of GbX in the blood coagulation process during heart regeneration

- Generate Talen and CRISPr knockout

- Evaluate the possible involvement of other globins in mediating the effect of nitrite. Cytoglobin 1 is found in the fish epicardium: important during regeneration?

- Initiate studies of cryoinjury in mouse. Effect of Nitrite on mouse cryoinjury
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Bin Sun

Michael Tsang
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Daniel Kim-Shapiro
Nadeem Wajia

Mr Wholey
Titolo slide
GbX is a six-coordinate globin

(Myoglobin)

(Absorbance (AU) vs Wavelength (nm))

(Jesus Tejero)
GbX in RBCs produces NO from nitrite

D

1.2 μM GbX

1.2 μM HbA

mV

0 2 4 6 8 10

Time (min)

Nitric Oxide Analyzer (NOA)

Scramble co
30μM heme

GbX siRNA
30μM heme

Nitric oxide production (pM/s)

Scramble siRNA

GbX siRNA

*
GbX and nitrite inhibit platelets activation

(Corti et al., 2014)
GbX is found in blood

**TRANSCRIPT**

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**PROTEIN**

23 kDa