Oxidative Inhibition of Erythrocyte Na\(^+\)-K\(^+\) Pump: A Functionally Relevant Circulating Marker of Oxidative Stress

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Outline

• Cardiovascular disease (CVD)
• CVD and Na pump
• Background of Na pump
• Oxidative regulation of the pump
• Measurement of oxidative damage of Na pump- a potential biomarker
Cardiovascular disease (CVD)

- the number 1 cause of death globally
- accounting for 17.3 million deaths per year
- a number that is expected to grow to >23.6 million by 2030

http://www.who.int/cardiovascular_diseases/en/
Overview

- Reactive oxygen species: key feature of cardiovascular disease
- Antioxidants fail to prevent cardiovascular morbidity and mortality
- Complexity of ROS signalling

Myung et al. BMJ 2013;346:f10
Raised levels of cardiac myocyte Na$^+$

DM Bers. Cardiac E-C Coupling. 2001
• Plasma membrane ion transporter
• Transports $3\text{Na}^+$ out- and $2\text{K}^+$ into cells
• Uses $\sim20\%$ of all energy (ATP) in body
• Crucial for normal function and survival of all cells, especially for cardiac myocytes
3D structure of the Na\(^+\)-K\(^+\) pump

Na\textsuperscript{+}-K\textsuperscript{+} pump Regulation

- **Kinases** mediate Na\textsuperscript{+}-K\textsuperscript{+} pump regulation
  - *however*, kinases have **poor access** to phosphorylation sites on the pump molecule


- **Chemical oxidants** decrease Na\textsuperscript{+}-K\textsuperscript{+} pump activity

  - Ellis DZ, Rabe J, Sweadner KJ. J Neurosci 2003
Protein Glutathionylation

• Protein glutathionylation -
  – adduct with -ve charge
  – is stable but reversible

Liu, C.C. Circ Res 105, 693-700; 2009
Glutathionylation of Na\(^+\)-K\(^+\) pump

**Total Cell Protein**
- GSS- Protein
- -ve control

**Baseline**
- Streptavidin
- Biotin-GSS
- Protein

**GSS-β₁**
- Control
- ONOO⁻

**IP: β₁**
- Control
- 0.2 mM ONOO⁻
- 0.5 mM ONOO⁻

**IB: β₁**
- Control
- 0.2 mM ONOO⁻
- 0.5 mM ONOO⁻

**IB: GSH**
- Control
- 0.2 mM ONOO⁻
- 0.5 mM ONOO⁻

**Liu, C.C. Circ Res 105, 693-700; 2009**
Mutation of Cys45 in \( \beta_1 \) subunit

Cys→Trp mutation eliminates ONOO- induced inhibition of Xenopus \( \alpha_1/\beta_1 \) by cRNA injection in oocytes.

Cys→Trp mutation eliminates ONOO- induced inhibition.

Cys45→Trp mutation eliminates ONOO- induced inhibition.

Liu, C.C. Circ Res 105, 693-700; 2009
Only Cys 45 in $\beta_1$ subunit
Would knowing the degree of oxidative inhibition of the Na\(^+\)-K\(^+\) pump in erythrocytes help to monitor the heart disease progress?
β1 subunit is detectable in erythrocytes, and is glutathionylated.
Enzyme Linked Immunosorbent Assay - to quantify eβ1-GSS

A. Method

B. Plate assay

Patient A

Patient B
\[ e\beta_1\text{-GSS in HF vs sham rabbits} \]
How does eβ1-GSS relate to what’s happening in the heart?

Correlation of eβ1-GSS with β1 subunit glutathionylation in cardiac myocytes

\[ V_m = E_{Cl} = -14 \text{ mV} \]

Liu, Unpublished, 2014

\[ r = 0.851; p < 0.001 \]
How does eβ1-GSS relate to what’s happening in the heart?

B-type Natriuretic Peptide (BNP)

LV systolic function

Natasha Fry PhD in progress; Unpublished, 2014
What about in humans?

Detection of eβ1-GSS in humans

Human

TL | IP: β1 | + DTT IP: β1 | IP: IgG

IB: β1

IB: GSH

55 kDa
What about in humans?......eβ1-GSS

$e\beta_1$-GSS HF patients vs. control

$3167 \pm 164 \text{ U vs } 1018 \pm 20 \text{ U; n=16; p}<0.001$

Independent of age, gender, bmi.
What about in humans?......$\text{Na}^+\text{-K}^+\text{-ATPase activity}$

$\text{Na-K ATPase activity in erythrocytes from HF patients vs. control}$
What about in humans? BNP
Diabetes and eb1-GSS in animal models

Animal Model: Rabbit

A.

Na-K ATPase Activity (%)

120
100
80
60
40
20
0

N  DM

NaK ATPase activity decreased in DM erythrocytes.

B.

TL IP: β1 + DTT IP: β1 IP: IgG

IB: β1
IB: GSH

S-glutathionylation of erythrocyte β1 subunits detected.
Diabetes and animal models

C. Western blot
   - Slow
   - Small sample sizes

D. ELISA
   - Rapid
   - Screen large sample size in one go

S-glutathionylation of erythrocyte \( \beta_1 \) subunits detected by CO-IP. S-glutathionylation was significantly increased in DM erythrocytes.
Diabetes and eb1-GSS in humans

Human Model:

S-glutathionylation of erythrocyte β1 subunits detected in human.

S-glutathionylation of erythrocyte β1 subunits detected by ELISA. S-glutathionylation of erythrocytes was significantly increased in DM patients compared to Normal.
Summary: eβ1-GSS

• occurs and is detectable!
• ELISA assay is rapid and quantitative
• parallels oxidative inhibition of cardiac Na⁺-K⁺ pump
• increases in patients with HF and reflects severity
• increases in diabetics

Liu, Unpublished, 2014
Potential prognostic value of eβ1-GSS?

• For HF: important if being used as diagnostic tool, but less so if combined with clinical and laboratory biomarkers for prognostic purposes

• Ongoing work:
  – Prognostic significance of eβ1-GSS over conventional biomarkers and risk factors:
    • in hospitalized HF/DM
    • in community subjects at high risk of HF (SCREEN-HF ~ 4000 subjects)
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