The role of neutrophils in corneal wound healing in HO-2 null mice

Giovanni Li Volti, MD, PhD
HEME OXYGENASE

a potent and efficient scavenger of reactive oxygen species and is involved in proliferation and neurons survival

anti-inflammatory
✓ suppresses pro-inflammatory cytokines
✓ increases anti-inflammatory IL-10 expression

decreases LPS-induced proinflammatory transcription factors (NF-kB) and cytokines (IL-6, TNF-α)
**HEME OXYGENASE**

**Heme oxygenase 1 (HO-1)**
(288 amino acids; 33 kDa)
contains **no cysteine residues** is an **inducible isoform** in response to stress such as oxidative stress, hypoxia, heavy metals, cytokines, etc.

**Heme oxygenase 2 (HO-2)**
(316 amino acids; 36 kDa) contains **three cysteine residues** is a **constitutive isoform** which is expressed under homeostatic conditions.
Today's presentation

**FIRST PART:** Evaluation of the role of heme oxygenase and its metabolites to the wound healing process using an in vitro model of epithelial scratch injury in primary and immortalized HCE cells

**SECOND PART:** Examination of the possible relationship between HO-2 and the recruitment of neutrophils following a corneal surface injury in wild type (WT) and HO-2 knockout (HO-2−/−) mice treated with Gr-1 monoclonal antibody to deplete peripheral neutrophils

**THIRD PART:** Evaluation of a possible correlation between heme oxygenase and phagocytic activity of the macrophages
**A**

Ho-1 Merged DAPI

HO-2 DAPI Merged

control

1h

24h

B

**B**

Fluorescence intensity (relative to control)

* p<0.05 to control; ** p<0.05 to 8h wound edge

Halilovic A; Patil K; Bellner L; Marrazzo G et al, J Cell Physiol. 2011 Jul;226(7):1732-40. doi: 10.1002/jcp.22502
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BACKGROUND

The HO-2 (-/-) mice vs. WT when injured showed:

• Impaired and delayed wound healing

• Exaggerated inflammatory response

• Consistent increase of corneal neovascularization

• 4- Fold higher number of inflammatory cells that infiltrate the corneal stroma

• Impaired induction on HO-1 under mechanical injury stimulus
EXPERIMENTAL PROTOCOL:

Control B6129SF2/J (WT)  HO-2 null (HO-2/-/-)

control

Gr-1 treated

Isolation of neutrophils from peripheral blood

Corneal epithelium removal using Algerbrush II corneal rust ring remover

Monitoring wound closure 48 hours after injury

Corneas collected for assessment of mRNA levels and histology

Confluent mouse aortic endothelial cells (mAEC) isolated from WT and HO-2 null mice
A

Day 0

Control Neutrophil-depleted

WT (injured)

HO-2-/-(injured)

Day 2

Control Neutrophil-depleted

B

\[ \text{% Re-epithelialization} \]

\[ \text{WT Control} \]

\[ \text{WT N-depleted} \]

\[ \text{HO-2-/ Control} \]

\[ \text{HO-2-/ N-depleted} \]

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**Control TNF-α α α α**

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<tr>
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<th>WT mAEC</th>
<th>HO-2/- mAEC</th>
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<td>100</td>
<td>120</td>
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<td><strong>160</strong></td>
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**Mdk mRNA (Relative Expression)**

- WT mAEC
- HO-2-/- mAEC

**VE-Cadherin mRNA (Relative Expression)**

- WT mAEC
- HO-2-/- mAEC

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EXPERIMENTAL PROTOCOL:

RAW 264.7 CELLS
MURINE MACROPHAGES

✓ INDUCED BY SnCl₂
✓ TREATED WITH shRNA AGAINST HO-2

INCUBATED 30 min WITH TEXAS RED-LABELED ZYMOSAN

✓ EVALUATION OF THE PHAGOCITIC ACTIVITY
✓ Q-PCR

**Transiently transfected RAW cells**

A

![Bar graph showing % of phagocytosis](image)

- no plasmid
- Scramble
- ShRNA HO-2

B

![Bar graph showing HO-2 mRNA expression](image)

- no plasmid
- Scramble
- ShRNA HO-2

**Cornea**
RAW cells treated with different concentration of SnCl$_2$}

A

% of phagocytosis

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<th></th>
<th>control</th>
<th>induced (1uM)</th>
<th>induced (5uM)</th>
<th>induced (10 uM)</th>
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<td>control</td>
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B

HO-1; HO-2 mRNA (Relative Expression)

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<tr>
<th></th>
<th>control</th>
<th>1 uM</th>
<th>5 uM</th>
<th>10 uM</th>
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<td>0.8</td>
<td>1.2</td>
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<tr>
<td>HO-2</td>
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* indicates significance.
CONCLUSIONS

- HO-2 is critical for a self-resolving inflammatory and repair response in the cornea.

- Epithelial injury in HO-2 null mice leads to impaired wound closure and chronic inflammation in the cornea.

- Systemic and corneal neutrophil depletion worsened rather than improved the wound healing process in both WT and HO-2/-/- mice.

- The absence of HO-2 gene within corneal cells contributed to the impaired corneal healing in the HO-2 null mice.
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