Biliverdin and Mesobiliverdin: 
Gold from Green
Erythrocyte senescence

- Hemoglobin
- Heme
- Toxic amino acids
Conjugated Bilirubin

Heme Oxygenase

Biliverdin Reductase

Biliverdin

Bilirubin
bilirubin \rightarrow \text{BVR} \rightarrow \text{biliverdin}

also anti-oxidants
Bilirubin $\xrightarrow{\text{BVR}}$ Biliverdin

Reactive oxygen species

$\text{Bilirubin}$

$\text{Biliverdin}$

$\text{H}_2\text{O}_2 \cdot \text{HO} \cdot$

$\text{O}_2 \cdot \text{RO} \cdot$

etc.
Bilirubin and biliverdin are interconvertible via the BVR (biliverdin reductase) pathway.

Toxins, injury, trauma, and disease can lead to the production of reactive oxygen species (ROS) such as H$_2$O$_2$, HO·, O$_2$·, RO·, etc., which contribute to oxidative stress. Oxidative stress is associated with cell death, necroses, acute and chronic inflammation.
bilirubin

BVR

biliverdin

toxins, injury, trauma, disease

 Reactive oxygen species

oxidative stress (cell death, necroses, acute and chronic inflammation)
Bilirubin → BVR → Biliverdin

Bilirubin

Biliverdin

Oxidative stress (cell death, necroces, acute and chronic inflammation)
Bilirubin & biliverdin are powerful anti-oxidants.

- As low as 10 nM protects against H$_2$O$_2$ at $10^4$ times higher concentrations.
- Provides better protection against lipid peroxidation than α-tocopherol.
- Abundant natural anti-oxidants in mammalian tissues.
Biliverdin activates anti-inflammatory mechanisms through BVR.

Anti-inflammatory response (lowers IL-1, TNFα, IL-6)

Biliverdin suppresses pro-inflammatory mechanisms through BVR.
Biliverdin has dual anti-inflammatory mechanisms:

- scavenges ROS directly
- anti-inflammatory/pro-inflammatory pathway regulation by BVR (biliverdin reductase)

Biliverdin cytoprotective effects:

e.g. vascular injuries (intimal hyperplasia, vascular endothelial dysfunction), organ (liver, kidney, cardiac, small bowel, lung) transplantation, ischemia/reperfusion injuries, graft rejection, corneal epithelial injury, hepatitis C infection, endotoxic shock, type 2 diabetes, pancreatic islet β-cell apoptosis,

Biliverdin sources:

• derived from animal bile bilirubin
  • possibly contaminated with TSE prions
  • contains isomers

  *(Biliverdin IXα is the major, active isomer)*

• supply is limited
Engineering *E. coli* to produce biliverdin IXα

**E. coli**

**HemA**

**heme** → **HO-1** → **BVIXα**

**Dong Chen (SBC)**
Biliverdin IXα bioproduction
• High quality (>98% purity) BVIXα
  (Currently available animal-derived BVIXα is < 90% purity)
• Consistent production (~40 mg per L E. coli culture)
• Substrate for human biliverdin reductase
• Requires endotoxin (E. coli lipopolysaccharide) clean-up
• Expensive ($275 per 50 mg)
Another path to biliverdin IX$_\alpha$

Photosynthetic cyanobacteria

phycocyanin

chromophore: **phycocyanobilin**
Tom Chang

phycocyanobilin

biliverdin IXα

mesobiliverdin IXα
Mesobiliverdin IXα is a substrate for biliverdin reductase.

biliverdin IXα → biliverdin IXα reductase → bilirubin IXα

phycocyanobilin
Mesobiliverdin IXα protects rat pancreatic islet β-cells from oxidative stress

Pancreatic islet allograft transplantation for type 1 diabetes
Mesobiliverdin IXα protects rat pancreatic islet β-cells from oxidative stress

Dithizone staining (red) of viable, insulin producing islets

E. coli BV

MesoBV

Glucose tolerance test

Blood glucose (mg per dL⁻¹) vs. min

Control

MesoBV

Viable islet yield after pancreatic ductal administration of biliverdin IXα and mesobiliverdin IXα

<table>
<thead>
<tr>
<th>Treatment</th>
<th># Viable Islets</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1µM BV_{FS}</td>
<td>1328 (358)</td>
<td>11.3</td>
</tr>
<tr>
<td>100µM BV_{FS} control</td>
<td>1527 (403)</td>
<td>28</td>
</tr>
<tr>
<td>1µM BV_{EC}</td>
<td>1345 (629)</td>
<td>4.3</td>
</tr>
<tr>
<td>100µM BV_{EC} control</td>
<td>1759 (703)</td>
<td>36.5</td>
</tr>
<tr>
<td>1µM mesoBV</td>
<td>1599 (475)</td>
<td>86.8</td>
</tr>
<tr>
<td>100µM mesoBV control</td>
<td>1535 (287)</td>
<td>79.3</td>
</tr>
<tr>
<td>p38IH control</td>
<td>2100</td>
<td>39.1</td>
</tr>
</tbody>
</table>

- 7 to 9 organs per infusion treatment
- # viable islets g⁻¹ pancreatic tissue
Conclusion

**Hypothesis:** Heme/HO-derived metabolites and analogs that are substrate oxidants for BVR are powerful anti-inflammatories.

**Reasoning:** They have dual and complementary anti-inflammatory mechanisms:

1) Directly scavenge ROS

2) Activate anti-inflammatory and/or suppress pro-inflammatory cell signaling pathways
<table>
<thead>
<tr>
<th>Reactants</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{O}_2 + e^- \rightarrow \text{O}_2^-$</td>
<td>Superoxide</td>
</tr>
<tr>
<td>$\text{O}_2^- + e^- + 2 \text{H}^+ \rightarrow \text{H}_2\text{O}_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}_2 + e^- + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{OH}^\cdot$</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>$\text{OH}^\cdot + e^- + \text{H}^+ \rightarrow \text{H}_2\text{O}$</td>
<td>Water</td>
</tr>
</tbody>
</table>

Outcome:

$\text{O}_2 + 4 e^- + 4 \text{H}^+ \rightarrow 2 \text{H}_2\text{O}$
**Catalase**

\[
\text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2
\]

*(a)* Catalase

**Peroxidase**

\[
\text{H}_2\text{O}_2 + \text{NADH} + \text{H}^+ \rightarrow 2 \text{H}_2\text{O} + \text{NAD}^+
\]

*(b)* Peroxidase

**Superoxide dismutase**

\[
\text{O}_2^- + \text{O}_2^- + 2 \text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\]

*(c)* Superoxide dismutase

**Superoxide dismutase/catalase in combination**

\[
4 \text{O}_2^- + 4 \text{H}^+ \rightarrow 2 \text{H}_2\text{O} + 3 \text{O}_2
\]

*(d)* Superoxide dismutase/catalase in combination

**Superoxide reductase**

\[
\text{O}_2^- + 2 \text{H}^+ + \text{rubredoxin}_{\text{reduced}} \rightarrow \text{H}_2\text{O}_2 + \text{rubredoxin}_{\text{oxidized}}
\]

*(e)* Superoxide reductase