BNP signal peptide protects the heart from ischemia-reperfusion injury

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Mechanism of ischemia/injury mediated cell death

Cardio-protection pathways

There is a clear need for novel ischemia/reperfusion therapies

Signal peptides are generally thought to be destroyed at translation. We discovered the signal peptide from BNP to be present in the circulation

*Siriwardena et al. 2010 Circulation 122: 255*
BNPsp is elevated in blood very early after ACS

Siriwardena et al. 2010 Circulation 122: 255-264
Liebetrau et al. 2015 Clin Chem 61: 1532-1539

**Quaeritur:** is BNPsp passive or active during its ACS release - 1?

Investigated *ex vivo* potential of human BNPsp to be protective in cardiac ischemia

**Rat ex vivo isolated heart ischemia model**
(n=35 pre-condition, n= 28 at reperfusion)

- Haemodynamics
- Perfusate sampling – cTnI, myoglobin
- TUNEL staining, Caspase 3 staining
- Western Blot analysis of signalling proteins

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Troponin I Release

-50 0 50 100

0.5 1.0 1.5

control (n=9)

0.3nmol (n=10)

10nmol (n=8)

0.1nmol (n=8)

Time from Reperfusion (minutes)

Troponin I (μg/L)

ᵠᵠᵠᵠ = P<0.01 vs control

₀ᵠᵠᵠᵠ = P<0.01 vs control

₀.₃nmol/L preconditioning 1 nmol/L at reperfusion

₁₀nmol/L preconditioning Control

TUNEL positive cell

% apoptotic cells

*P<0.05

₀*₀*₀*₀*₀*₀

₀.₃nmol (pre)

₁₀nmol (IDR)

Control

Caspase-3 staining tended to reduce

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% pERK 1/Total ERK is upregulated by lower BNPsp doses

- Significant difference from Sham p< 0.05 for pERK1
  - Control n=6, sham n=6, 0.3nM n=5, 1nM n=6, 3nM n=5
pAkt is unaltered by low, but inhibited by higher BNPsp

* p< 0.01 vs. control
Φ p<0.01 vs. sham

Control n=6, sham n=6, 0.3nM n=5, 1nM n=6, 3nM n=5
**Quaeritur**: is BNPsp passive or active during its ACS release - 2?

Investigated *in vivo* potential of human BNPsp to be protective in cardiac ischemia

- Ovine *in vivo* normal animal infusions (n=6)
  - LCA (angiocath), jugular (polyeth and PA Swan ganz) and Foley urinary cannulation

- Ovine *in vivo* pre-conditioning ischemia model (control n=7, BNPsp Tx n= 8)
  - Continuous 150min BNPsp infusion across ischemia
  - Reversible ischemia via 90min snare
  - Haemodynamics
  - Echo to determine LVEF & AAR
  - Blood sampling – cTnI, BNPsp
  - TUNEL staining, Caspase 3 staining
  - Infarct size/AAR determination by sectioning on day 6/7 after euthanasia
### Hemodynamics

- **Infusion of BNPsp or vehicle**
- **Anesthesia**
- **Ischemia - Reperfusion**

### Echocardiography

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BNPsp -</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of segments of LV (AAR)</strong></td>
<td>2.63 (±0.460)</td>
<td>2.86 (±0.690)</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Pre-op LV vol diast (ml)</strong></td>
<td>69.8 (±14.9)</td>
<td>75.2 (±16.2)</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>Pre-op LV vol systol (ml)</strong></td>
<td>31.7 (±6.9)</td>
<td>32.5 (±9.0)</td>
<td>0.85</td>
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<tr>
<td><strong>Pre-op LVEF(%)</strong></td>
<td>56.4 (±5.5)</td>
<td>55.9 (±6.4)</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Post-op LV vol diast (ml)</strong></td>
<td>72.8 (±21.9)</td>
<td>75.5 (±23.4)</td>
<td>0.72</td>
</tr>
<tr>
<td><strong>Post-op LV vol systol (ml)</strong></td>
<td>44.1 (±13.1)</td>
<td>50.2 (±15.9)</td>
<td>0.77</td>
</tr>
<tr>
<td><strong>Post-op LVEF(%)</strong></td>
<td>40.3 (±5.2)</td>
<td>44.2 (±6.9)</td>
<td>0.38</td>
</tr>
</tbody>
</table>
Large variation in achieved levels of human BNPsp in vivo

Achieved hBNPsp levels - Sheep Infarction Study

Troponin I Release in Sheep Infarction Study

Cumulative Troponin I Release - Sheep Infarction Study Adjusted for Area at Risk

Study hearts

Control hearts

Infused at 1mcg/kg/min

r=0.77
p<0.001

r=0.62
p=0.02
Like ex vivo rat heart, trend to reduced Caspase-3.
Conclusions

• BNPsp does not appear to have any major biological actions in the setting of normal cardiac function or health

• However, BNPsp has cardio-protective actions in the setting of I/R injury

• Ex vivo cardiac function is improved by BNPsp post-I/R, with concomitant reductions in troponin release and DNA fragmentation (TUNEL). Caspase-3 activation trended towards a reduction whereas pERK1 is significantly activated at the dose range 0.3-1nM; pAkt activation is inhibited at higher doses (>3nM).

• In vivo, BNPsp reduces infarct size/AAR by a remarkable ~50%, troponin release by ~25%, DNA fragmentation and may improve cardiac function.

• The actions of BNPsp appear dose dependent and look likely to follow the U-shaped curve well known for other Tx agents. Any effect on receptor actions is unknown. The half-life/clearance mechanism of BNPsp is unknown, but may involve the liver (Siriwardena et al. Circulation 2010 122:255-264).
Research group and funding sources

Maithri Siriwardena

Chris Charles

Prisca Mbikou

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