The Prohormone of B-Type Natriuretic Peptide, proBNP\textsubscript{1-108}, is Secreted by the Failing and non-Failing Human Heart

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1 Mayo Clinic, Rochester, MN, USA
2 Helios Clinic Erfurt, Germany
3 Helios Clinic Wuppertal, Germany
4 Bio-Rad, Marnes-la-Cotquette, France
Disclosures

• Catherine Larue is an employee of BioRad
• John C. Burnett, Jr received a research grant from BioRad
Background

- Pro-B-type natriuretic peptide (proBNP<sub>1-108</sub>) is a 108-amino acid peptide produced primarily by the heart.
- This prohormone with limited bioactivity is cleaved into bioactive BNP<sub>1-32</sub> and biologically inactive NTproBNP<sub>1-76</sub>.
- Assays developed to measure BNP<sub>1-32</sub> and NTproBNP<sub>1-76</sub> show increased plasma immunoreactivity in conditions of cardiac overload such as heart failure.
- Importantly, assays with antibodies directed against BNP<sub>1-32</sub> and NTproBNP can crossreact with proBNP<sub>1-108</sub>.
- Due to the lack of specificity there is limited information regarding secretion and peripheral processing of proBNP<sub>1-108</sub>.

ProBNP\textsubscript{1-108}

proBNP\textsubscript{1-108}

NTproBNP\textsubscript{1-76}

BNP\textsubscript{1-32}

Corin? Furin?
ProBNP\textsubscript{1-108}

proBNP\textsubscript{1-108}

NTproBNP\textsubscript{1-76}

BNP\textsubscript{1-32}

mAb Hinge 76

(Capture antibody for Bio-Rad proBNP assay)

Corin? Furin?
Background

- Using this specific proBNP\textsubscript{1-108} assay (Bio-Rad, Hercules, CA, USA) we and others reported that proBNP\textsubscript{1-108} circulates in healthy subjects’ plasma and is elevated in subjects with cardiovascular disease\textsuperscript{1,2}

- We also reported that proBNP\textsubscript{1-108} is cleaved into BNP\textsubscript{1-32} and NTproBNP\textsubscript{1-76} in plasma\textsuperscript{3}

- However, it is unknown whether
  - proBNP\textsubscript{1-108} is secreted by the human heart (as opposed to an extracardiac source?)
  - secretion of proBNP\textsubscript{1-108} differs between the non-failing and failing human heart; e.g. are the increased proBNP\textsubscript{1-108} plasma levels due to increased secretion or decreased peripheral conversion or both?

\textsuperscript{1} Giuliani I et al, Clin Chem 2006;52:1054-61
\textsuperscript{2} Macheret F et al, JACC 2011;95:1386-95
\textsuperscript{3} Ichiki T et al, Clin Chem 2011;57:40-7
Hypothesis

• We hypothesized that proBNP$_{1-108}$ is secreted by both the non-failing and failing human heart and that cardiac secretion of proBNP$_{1-108}$ is increased in failing hearts
Methods

- Arterial blood samples and blood samples from the coronary sinus (CS) were collected in EDTA tubes from two groups of subjects:
  - Subjects without overt heart disease who underwent cardiac catheterization, which excluded coronary artery disease (Mayo Clinic, Rochester, MN)
  - Patients with HF and LV dysfunction during implantation of a biventricular pacemaker for cardiac resynchronization therapy (Helios Clinic Wuppertal, Germany)
- Plasma samples were stored at -80°C until analysis
Methods

• ProBNP\textsubscript{1-108} was measured using a specific assay (Bio-Rad, Hercules, CA, USA) on an automated analyzer not yet commercially available\textsuperscript{1,2,3}

• Interassay and intra-assay variabilities were 10.3\% and 11.6\%, respectively; lower limit of detection 9 pg/mL\textsuperscript{3}

• <0.05\% crossreactivity with BNP\textsubscript{1-32} or NTproBNP\textsuperscript{1}

• Within the two groups the gradient from the coronary sinus to the arterial blood was assessed by paired t-test using log-transformed values

• ProBNP\textsubscript{1-108} gradients were compared between groups with unpaired t-test

\textsuperscript{1}Wu AHB et al, Clin Chim Acta 2009;408:143-144
\textsuperscript{2}Giuliani I et al, Clin Chem 2006;52:1054-61
\textsuperscript{3}Macheret F et al, JACC 2011;57:1386-95
Results
## Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Non-HF (n=9)</th>
<th>HF (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female, %</strong></td>
<td>78</td>
<td>14</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>46 ± 12</td>
<td>69 ± 9</td>
</tr>
<tr>
<td><strong>Ischemic etiology, %</strong></td>
<td>NA</td>
<td>62</td>
</tr>
<tr>
<td><strong>Ejection fraction, %</strong></td>
<td>(&gt;50%)</td>
<td>24 ± 7</td>
</tr>
<tr>
<td><strong>Diabetes, %</strong></td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td><strong>Atrial fibrillation, %</strong></td>
<td>0</td>
<td>29</td>
</tr>
</tbody>
</table>

Values are percentage or mean ± SD
ProBNP\textsubscript{1-108} in the Non-Failing Heart

Median (25\textsuperscript{th}/75\textsuperscript{th} percentile)

\begin{itemize}
  \item Arterial
  \item Coronary Sinus
\end{itemize}

Lower limit of detection

\[ p = 0.009 \]
ProBNP<sub>1-108</sub> in the Failing Heart

**Graphs**

- **ProBNP<sub>1-108</sub> (pmg/mL)**
  - Arterial
  - Coronary Sinus

- **ProBNP<sub>1-108</sub> (pg/mL)**
  - Arterial
  - Coronary Sinus

**Statistics**

- Median (25<sup>th</sup>/75<sup>th</sup> percentile)
- p<0.001
Transcardiac Gradients:
$\Delta \text{ProBNP}_{1-108}^{\text{CS-art}}$ in Non-HF vs. HF

$p<0.001$

Median (25th/75th percentile)
Summary

• In both non-failing and failing human hearts there is a transcardiac gradient from the arterial blood to the coronary sinus of the prohormone proBNP$_{1-108}$, consistent with net secretion

• The transcardiac gradient of proBNP$_{1-108}$ is higher in failing hearts as compared to non-failing hearts
Conclusions

• Cardiac secretion of proBNP$_{1-108}$ occurs in non-failing and failing hearts and is increased in failing hearts

• The pathophysiological and prognostic relevance of the peripheral processing of proBNP$_{1-108}$ to bioactive BNP$_{1-32}$ remains to be characterized
Thank You