Serelaxin

The recombinant form of Human Relaxin-2 for the treatment of patients with acute heart failure

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Patients have a poor prognosis following heart failure hospitalization

Analysis of HF data from the Atherosclerosis Risk in Communities (ARIC) population-based study from four communities in the USA (1987–2002)

Mortality (%) over time following hospitalization for HF:
- 30 days: 10.4%
- 1 year: 22.0%
- 5 years: 42.3%

Loehr et al. Am J Cardiol 2008;101:1016–22
The pathophysiology of heart failure results in an increasingly downward spiral

- Acute decompensated HF is associated with frequent hospitalizations\(^1\)
  - after initial stabilization, there are high rates of mortality and rehospitalizations\(^2\)
  - with each hospitalization, there is likely myocardial and renal damage that contributes to progressive LV or renal dysfunction, leading to an inevitable downward spiral\(^3\)
  - current therapies only treat symptoms and do not target the underlying mechanisms leading to cardiac and renal injury

LV=left ventricular

The pathophysiology of acute heart failure remains poorly understood

Acute HF involves a complex set of interactions between systems

Heart
Peripheral vasculature
Kidney

The exact mechanisms and the relative contribution of each is uncertain and is likely to vary between patients

Facing the challenges of AHF
A complex syndrome with three main pathophysiological components

1. Myocardial and renal overload
   - Impaired cardiac function coupled with rapid increases in afterload or preload due to:
     - vasoconstriction or uncontrolled BP
     - acute rise in systemic vascular resistance
     - fluid overload due to renal impairment, dietary indiscretion, medications, etc
     - fluid redistribution to pulmonary circulation

2. Myocardial and renal cell death
   - Accelerated cardiomyocyte and renal cell death during AHF episodes due to:
     - ischemia due to sudden O$_2$ supply-demand mismatch
     - apoptosis induced by stretch, norepinephrine, Ang II, inflammatory cytokines or oxidative stress

3. Myocardial remodeling and renal dysfunction
   - Activation of matrix metalloproteinases (MMP-2, TIMP-1)
   - Stimulation of cardiac fibroblasts (CF) with increased collagen deposition
   - Nephron loss and decrease GFR

AHF=acute heart failure; Ang II=angiotensin II; BP=blood pressure; GFR=glomerular filtration rate; MMP-2=matrix metalloproteinase-2; NSAIDS=non-steroidal anti-inflammatory drugs; TIMP-1=tissue inhibitors of matrix metalloproteinase-1
Acute heart failure syndrome episodes are associated with transient increases in NT-proBNP and troponin I, a marker of cell injury/death.  

- Troponin, a marker of myocyte injury and/or death, is known to be elevated in patients with HF without evidence of acute myocardial infarction.  
- Episodes of AHFS are associated with non-ischemic processes known to cause myocyte death, including mechanical strain and oxidative stress.  
- These data suggest that episodes of acute HF decompensation may be associated with cardiac myocyte injury and/or death.

AHFS=acute heart failure syndrome; NT-pro-BNP=N-terminal pro-B-type natriuretic peptide.  
Cell death and injury in heart failure: multiple mechanisms and evolving concepts

**Mechanical stress**
- Apoptosis
  - Mitochondrial or intracellular signal cascade
  - Activation of caspases
  - DNA fragmentation
  - No inflammation
- Necrosis
  - Energy exhaustion
  - Excessive free radicals
  - Membrane disintegration
  - Cell swelling and spillage
  - Pro-inflammatory
- Necroptosis
  - Alternative mode for cell to die under chronic stress
  - Activated in presence of innate immune or mitochondrial stress
  - Promoted by oxidative stress
  - RIPK1 / RIPK3 complex key to execution

**Oxidative stress**
- Apoptosis
  - Mitochondrial or intracellular signal cascade
  - Activation of caspases
  - DNA fragmentation
  - No inflammation
- Necrosis
  - Energy exhaustion
  - Excessive free radicals
  - Membrane disintegration
  - Cell swelling and spillage
  - Pro-inflammatory
- Necroptosis
  - Alternative mode for cell to die under chronic stress
  - Activated in presence of innate immune or mitochondrial stress
  - Promoted by oxidative stress
  - RIPK1 / RIPK3 complex key to execution

**Growth or death signals**

DNA=deoxyribonucleic acid; RIPK=receptor-interacting serine/threonine-protein kinase

Episodes of acute heart failure are associated with increased oxidative stress and collagen synthesis

Myocardial remodeling involves rebuilding of the myocardial extracellular matrix, which is predominantly composed of collagen fibers.

Free radical generation has also been linked with myocardial remodeling.

These data from a study in 43 patients with acute HF demonstrate increased markers of collagen type I synthesis and oxidative stress, and reduced collagen type I degradation and total antioxidant status during the acute HF phase compared with ~2 weeks after admission.

CITP=carboxy-terminal telopeptide of collagen type I; 8-iso-PGF$_{2\alpha}$=8-iso-prostaglandin F$_{2\alpha}$; PIP=procollagen type I carboxy-terminal peptide

Large randomized controlled trials in acute heart failure have failed to demonstrate outcome benefits

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Patient population</th>
<th>Intervention</th>
<th>Primary endpoint</th>
<th>Significant effect?</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPTIME-CHF¹</td>
<td>951 patients admitted with exacerbation of systolic HF</td>
<td>i.v. milrinone vs pbo for 48 hours</td>
<td>Length of hospitalization for CV causes</td>
<td>×</td>
</tr>
<tr>
<td>VERITAS²</td>
<td>1,448 patients hospitalized with AHF</td>
<td>i.v. tezosentan vs pbo for 24–72 hours</td>
<td>Change in dyspnea, incidence of death and worsening HF at 7 days</td>
<td>×</td>
</tr>
<tr>
<td>SURVIVE³</td>
<td>1,327 patients hospitalized with AHF</td>
<td>i.v. levosimendan vs dobutamine</td>
<td>All-cause mortality at 180 days</td>
<td>×</td>
</tr>
<tr>
<td>EVEREST⁴</td>
<td>4,133 patients hospitalized with AHF</td>
<td>Tolvaptan 30 mg once-daily vs pbo for 60 days</td>
<td>All-cause mortality and CV death or hospitalization for HF</td>
<td>×</td>
</tr>
<tr>
<td>ASCEND-HF⁵</td>
<td>7,141 patients hospitalized for AHF</td>
<td>i.v. nesiritide vs pbo for 24 hours–7 days</td>
<td>Change in dyspnea and 30-day all-cause mortality or HF hospitalization</td>
<td>×</td>
</tr>
<tr>
<td>PROTECT⁶</td>
<td>2,033 patients hospitalized for AHF</td>
<td>i.v. rolofylline vs pbo for up to 3 days</td>
<td>Composite of survival, HF status and renal function</td>
<td>×</td>
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pbo=placebo
Serelaxin
Mechanism of action and clinical data
Serelaxin is a recombinant form of the human hormone relaxin-2 that acts directly on CV tissues

- Naturally occurring peptide hormone, discovered in 1929
- Human relaxin-2 is one of seven peptides in the relaxin family of hormones
- Structure of human relaxin-2: 53 amino acids (2 chains connected by 2 disulphide bonds)
- Relaxin-2 mediates its effects via specific G-protein-coupled receptors: RXFP1 (LGR7) and RXFP2 (LGR8)
- Cardiovascular tissues are equipped with relaxin receptors that are activated by circulating or regionally generated relaxin-2 to mediate diverse signaling pathways

**Structure of native and manufactured human relaxin-2**

**Relaxin acts directly on CV tissues**

Positive immunostaining for relaxin and precursor forms in both the endothelium and vascular smooth muscle of a small renal artery from a virgin female rat

Serelaxin triggers multiple pathways following binding to its receptor

NOS=nitric oxide synthase; ET$_B$R=endothelin type B receptor; MMP=matrix metalloproteinase; VEGF=vascular endothelial growth factor

Adapted from Du et al. Nat Rev Cardiol 2009;7:48–58
Relaxin mediates maternal hemodynamic improvements during pregnancy

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>PREGNANCY</th>
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<tbody>
<tr>
<td>Systemic vascular resistance (dyn.s.cm²)</td>
<td>↓</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>↑</td>
</tr>
<tr>
<td>Global arterial compliance (mL/mm Hg)</td>
<td>↑</td>
</tr>
<tr>
<td>Renal vascular resistance (dyn.s.cm²)</td>
<td>↓</td>
</tr>
<tr>
<td>Renal blood flow (mL/min/1.73m²)</td>
<td>↑</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min/1.73m²)</td>
<td>↑</td>
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- Onset of hemodynamic changes coincident with relaxin elevation during 1st trimester of pregnancy; similar but smaller changes observed during the luteal phase of menstrual cycle
- The beneficial effects of relaxin see in pregnancy may be beneficial in acute heart failure

Relaxin is expressed in myocardial tissues and levels are elevated in patients with heart failure

- Relaxin is constitutively expressed in human myocardial tissues
- Expression of relaxin is elevated in patients with CHF compared with individuals with no evidence of structural cardiovascular disease

CHF=congestive heart failure; ELISA=enzyme-linked immunosorbent assay; GAPDH=glyceraldehyde-3-phosphate dehydrogenase; mRNA=messenger ribnucleic acid; RT-PCR=reverse transcriptase polymerase chain reaction; vs=versus

Dschietzig et al. FASEB J 2001;15:2187–95
Serelaxin dilates isolated human small resistance arteries *in vitro*

- Resistance vessels from gluteal biopsies were preconstricted with norepinephrine (10^{-5} mol/L)
- Serelaxin was as potent as prostacyclin (PGI_2) in causing vessel relaxation

![Graph showing relaxation (%)]

There is an inverse correlation between plasma endothelin-1 and relaxin in patients with severe heart failure.

Among patients with severe CHF, individuals with the highest plasma levels of relaxin had the lowest circulating levels of ET-1.

CHF=congestive heart failure; ET-1=endothelin-1; vs=versus
Dschietzig et al. FASEB J 2001;15:2187–95
Serelaxin demonstrated favorable hemodynamic effects in a pilot study in patients with chronic heart failure

- Hemodynamic effects of serelaxin during a continuous 24-hour, dose escalating infusion (colored bars) and during the 24-hour post-infusion (white bars)
- Hemodynamic measurements, including cardiac Index (by thermodilution method), systemic vascular resistance and pulmonary capillary wedge pressure, were serially performed using Swan–Ganz and arterial catheters

Serelaxin increases GFR and renal plasma flow and reduces renal vascular resistance in rats compared with time control.

* * *  

Serelaxin increases renal plasma flow in healthy volunteers

- Administration of serelaxin increased RPF in male and female healthy volunteers without significant effects on GFR. Other effects included significant natriuresis.

MAP=mean arterial pressure; PR=pulse rate; RPF=renal plasma flow; SEM=standard error of the mean

Serelaxin reduces markers of myocardial damage in an in vivo porcine model of ischemia/reperfusion

- Serelaxin (1.25, 2.5 and 5.0 μg/kg body weight) was administered following a 30-minute ischemia
- Serelaxin caused a dose-related reduction in key markers of myocardial damage (serum myoglobin, CK-MB, troponin T)
- The greatest reductions were observed with the highest two doses of serelaxin

I-R=ischemia-reperfusion with no serelaxin (vehicle alone)
*p<0.05; **p<0.01; ***p<0.001 vs vehicle alone
Serelaxin reduces inflammatory leukocyte recruitment and oxygen free radical-mediated cardiomyocyte injury in vivo

- In an in vivo porcine model of myocardial ischemia/reperfusion, three increasing doses of serelaxin (1.25, 2.5 and 5.0 μg/kg body weight) were administered following 30 minutes of ischemia.

- Serelaxin caused a dose-dependent reduction in inflammatory leukocyte recruitment (measured by myeloperoxidase concentration) and oxygen free radical-mediated cardiomyocyte injury (measured by malondialdehyde concentration and tissue calcium overload).

Sham-operated = negative control; I-R = ischemia-reperfusion = positive control
*p < 0.05; ***p < 0.001 vs sham-operated; a = p < 0.05 vs I-R; b = p < 0.01 vs I-R; c = p < 0.001 vs I-R
Serelaxin reduces myocardial apoptosis in an *in vivo* porcine model of ischemia/reperfusion

- Three increasing doses of serelaxin (1.25, 2.5 and 5.0 μg/kg body weight) were administered following a 30-minute ischemia

- Serelaxin caused a dose-related reduction of key markers of cardiomyocyte apoptosis (caspase 3, TUNEL assay)

Sham-operated = negative control; I-R = ischemia-reperfusion = positive control (vehicle only)

***p<0.001 vs sham-operated; b=p<0.01 vs I-R; c=p<0.001 vs I-R

Serelaxin modulates collagen deposition *in vitro* and fibrosis *in vivo*

Collagen content of fibroblasts, untreated/treated with serelaxin (100 ng/mL) alone or with TGF-β (2 ng/mL), for 72 h of culture. Results are mean data from 3–4 separate experiments.

Collagen content/dry weight ventricular tissue was determined from 12-month-old relaxin wild-type (RLX+/+) mice (n=8), relaxin knockout (RLX-/-) mice treated with vehicle alone (n=4), and RLX-/- mice treated with 500 µg/kg/day serelaxin (n=4) for 14 days.

* *p<0.05, **p<0.01 vs untreated cells
† *p<0.05 vs TGF-β--treated cells
* *p<0.05 vs RLX+/+
† *p<0.05 vs RLX-/- mice treated with vehicle alone
Serelaxin has potential multi-mechanistic effects which may address the pathophysiology of AHF

1. ↓ Myocardial overload; ↑ Renal function
   - Vasorelaxation*
     - ↑ Endothelial NO*
     - ↓ SVR, ↑ RBF, ↑ GFR
     - ↓ ET-1
     - Volume redistribution

2. ↑ Cell preservation
   - ↓ Inflammation
     - ↓ Inflammatory cell infiltration
     - ↓ Oxidative stress

3. ↑ ECM remodeling
   - ↑ Remodeling
     - ↑ Matrix metalloproteinases
     - ↓ Vessel stiffness
   - ↓ Fibrosis
     - ↓ Collagen synthesis
     - ↑ Collagen breakdown
   - ↑ Cell survival
     - ↓ Oxidative stress
     - ↓ Apoptosis
     - ↓ Ca\(^{2+}\) overload
     - ↓ Infarct size

*Selective dilation of pre-constricted vessels; AHF=acute heart failure; ECM=extracellular matrix; ET-1=endothelin-1; GFR=glomerular filtration rate; NO=nitric oxide; RBF=renal blood flow; SVR-systemic vascular resistance

Adapted from Du et al. Nat Rev Cardiol 2010;7:48–58