Prolactin and its cleaved 16-kDa subform enhance myocardial injury after ischemia/reperfusion

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There is no conflict of interest
Prolactin is a member of the growth hormone family responsible for the coordination of a wide range of biological processes: Fertility, lactation, tumor growth, immunity and inflammation, hemodynamics.
Pro- and anti-angiogenetic effects of the pregnancy and nursing hormone prolactin

**Prolactin promotes:**

- Proliferation, Survival
- Motility
- Angiogenesis

**In vitro**

- Inhibition of extracellular matrix degradation
- Stimulation of EC apoptosis
- Activation of caspases & inactivation of Bcl-2
- Inhibition of EC proliferation
- Res/Ras/MEK/ERK pathway inhibition

**In vivo**

- Inhibition of angiogenesis in the chicken chorioallantoic membrane
- Inhibition of tumor growth and angiogenesis
- Inhibition of the neovascularization of the cornea

16-kDa prolactin acts as a potent inhibitor of angiogenesis

Linda Schuler; Corbacho A.M. et al., J Endocrinol 2002
16-kDa Prolactin decreases cardiac capillary density, increases cardiac apoptosis, attenuates cardiac function, and promotes LV dilatation

16-kDa Adenovirus

LacZ Adenovirus

Capillaries/Cardiomyocytes

%FS

LVESD

*P<0.05

Evidence for Prolactin cleavage in the heart?

- Pituitary gland
  - 23 kDa Prolactin
  - 16-kDa Prolactin

- Mitochondrium
  - MnSOD
  - ROS
  - active CD
  - inactive CD

- Fibroblast
  - MMPs↑

- Cardiomyocyte
  - STAT3

- Endothelial cell
  - Apoptosis
  - Dissociation of capillary structures
  - Vasocstriction
  - Inflammation

- PPCM
  - Reduced metabolism
  - Affected function

Yamac and Hilfiker-Kleiner, Heart 2010
Does 16-kDa Prolactin play a role in cardiac ischemia reperfusion injury?

- Oxidative Stress
- Cathepsin D ↑
- 16-kDa Prolactin
  - Inflammation
  - Reduced Metabolism
  - Endothelial cell apoptosis
Enhanced generation of 16-kDa Prolactin in patients with acute MI (AMI)

**P<0.01 vs Co

##P<0.01 pd vs AMI

Serum total Prolactin

Serum Cathepsin D activity

**P<0.01 vs Co

##P<0.01 pd vs AMI

Immunoprecipitation of serum 16-kDa prolactin

16-kDa Prl

Controls AMI/IGG AMI

Fold induction

Controls AMI

§ P<0.05
Similar to patient data: serum Prolactin, Cathepsin D activity and 16-kDa Prolactin are increased in acute MI followed by reperfusion (I/R)

**Serum total Prolactin**

- CO
- Sh
- I/R

**Serum Cathepsin D activity**

- CO
- Sh
- I/R

**IP of serum 16-kDa Prolactin**

Time of ischemia by LAD occlusion: 50 min
Cathepsin D activity and 16-kDa Prolactin levels are increased in borderzone and ischemic zone after I/R

**P<0.01 vs Co

**P<0.01 vs CO
I/R promotes binding of Prolactin to cardiomyocytes and enhances its cleavage into the 16-kDa subform.
Hypothesis

The generation of 16kDa Prolactin from 23kDa full length Prolactin after oxidative stress (Mouse Ischemia/reperfusion model) might responsible for adverse cardiac effects.

Inhibition of Prolactin secretion by the dopamine D2 receptor agonist Bromocriptine should eliminate both Prolactin forms and subsequently I/R injury should be reduced.

Treatment of SV129 male mice with Bromocriptine, a pharmacological inhibitor of Prolactin secretion. 4 mg/kg/d, 5 days before till 24 h to 14 d after ischemia/reperfusion
Bromocriptine prevents increase of full-length and 16-kDa Prolactin levels in serum but has no effect on Cathepsin D activity after I/R.

**Serum total Prolactin**

- **CO**
- **Sh**
- **I/R**
- **I/R;BR**

*P<0.05 vs Co

#P<0.05 vs I/R

**Serum Cathepsin D activity**

- **CO**
- **CO/BR**
- **Sh**
- **Sh/BR**
- **I/R**
- **I/R;BR**

§P<0.05 vs CO

**Immunoprecipitation of serum Prolactin**

- 23-kDa Prl
- 16-kDa Prl

I/R, I/R;BR, I/R;IgG, Sh
Bromocriptine prevents increase 16-kDa Prolactin levels but has no effect on Cathepsin D activity in cardiac tissue after I/R.
Blockage of Prolactin release by bromocriptine attenuates I/R induced myocardial injury

Infarction size/Area at risk

**P<0.01

Cardiac Troponin I

*P<0.05
Smaller infarct sizes persist and are associated with better cardiac function 14 days after reperfusion in the bromocriptine treated group.

### Infarction size/heart 14d

- **Control-group baseline**: 1,12 ± 0,18
- **Control-group after I/R**: 1,2 ± 0,17
- **Bromocriptine-group baseline**: 1,24 ± 0,17
- **Bromocriptine group after I/R**: 1,24 ± 0,13

**IVSD** 20,95 ± 4,5 23,6 ± 6,25 20,6 ± 3,1 19,8 ± 4,3

**LVEAD** 9,6 ± 2,5 15,1 ± 6,1 8,9 ± 18 9,0 ± 2,9

**LVEAS** 54,3 ± 4,2 37,6 ± 9,5 # 57,2 ± 3,6 54,9 ± 6,3 §

**FAC** 445 ± 54 492 ± 36 444 ± 52 475 ± 34

**HR**

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*P<0.05 vs baseline  
§P<0.05 vs Co after I/R
Which Prolactin form is mainly responsible for I/R injury?

Adapted from Yamac and Hilfiker-Kleiner, Heart 2010
Mutation of Cathepsin D cleavage site in Prolactin prevents its efficient cleavage into the 16-kDa subform

Piwnica, Endocrinology 2004

Prolactin cleavage at pH 5

Prolactin cleavage in BZ at pH 7

RM: REMOTE MYOCARDIUM, BZ: BORDERZONE
IS: ISCHEMIC MYOCARDIUM, P: PEPSTATIN A
Experimental setting

- L146P Prl: Non-cleavable prolactin
- rWT Prl: Prolactin effectively processed into 16-kDa form

- BR 5 days in drinking water
- Osmotic minipumps with recombinant Prl
- Ischemia reperfusion
- Evans blue/TTC

Day 1  Day 5  Day 8  Day 9

16 kDa
Enhanced 16-kDa Prolactin cleavage promotes myocardial injury after I/R

Infarction size/Area at risk

§P<0.05 vs rWT
**P<0.01 vs I/R

Infarction size (%)

I/R;BR, rWT
I/R;BR, rL146P
I/R;BR
I/R

L146P Prl
Non-cleavable prolactin

rWT Prl
Prolactin effectively processed into 16-kDa form

Cardiac Troponin I

*P<0.05

cTnl (%)

I/R;BR, rWT
I/R;BR, rL146P

I/R;BR; rWT
I/R;BR; rL146P
How does 16-kDa Prolactin promote I/R injury of the heart?

Pituitary gland

23 kDa Prolactin

Fibroblast

Oxidative stress

Cardiomyocyte

16 kDa Prolactin

- Apoptosis
- Inflammation

• Reduced metabolism
• Affected function

Adapted from Yamac and Hilfiker-Kleiner, Heart 2010
The Antiangiogenic Factor 16K Human Prolactin Induces Caspase-Dependent Apoptosis by a Mechanism that Requires Activation of Nuclear Factor-κB

SÉBASTIEN P. TABRUYN, CATHERINE M. SORLET, FRANÇOISE RENTIER-DELRUE, VINCENT BOURS, RICHARD I. WEINER, JOSEPH A. MARTIAL, AND INGRID STRUMAN

Laboratoire de Biologie Moléculaire et de Génie Génétique (S.P.T., C.M.S., F.R.-D., J.A.M., I.S.) and Génétique Humaine (V.B.), Université de Liège, B-4000 Liège, Belgium; and Center for Reproductive Sciences (R.I.W.), Department of Obstetrics, Gynecology and Reproductive Sciences, University of California School of Medicine, San Francisco, California 94143
........ but not in cardiomyocytes

**P<0.01 vs NOX
Reduced Myocardial Ischemia-Reperfusion Injury in Toll-Like Receptor 4-Deficient Mice
Jun-ichi Oyama, MD; Charles Blais, Jr, PhD; Xiaoli Liu, MD; Minying Pu, MD; Lester Kobzik, MD; Ralph A. Kelly, MD; Todd Bourcier, PhD. Circulation 2004.

Endothelial Cell Overexpression of Fas Ligand Attenuates Ischemia-Reperfusion Injury in the Heart
Jiang Yang, Steven P. Jones, Toshimitsu Suhara, James J. M. Greer, Paul D. Ware, Nhan P. Nguyen, Harris Perlman, David P. Nelson, David J. Lefer and Kenneth Walsh. JBC 2003.

Anti-tumor necrosis factor-alpha improves myocardial recovery after ischemia and reperfusion
Prolactin and 16-kDa Prolactin enhance cardiac inflammation after I/R

CD45

**P<0.01 vs NaCl

∞∞ P<0.01 vs rWT

MOMA

∞∞ P<0.01 vs rWT

*P<0.05 vs NaCl
Prolactin and 16-kDa Prolactin enhance inflammatory cytokines and chemokines expression after I/R

**P<0.01 vs Sh  #P<0.05 vs I/R  ##P<0.01 vs I/R  § P<0.05 vs rWT
16-kDa Prolactin induces expression of pro-inflammatory cytokines and chemokines in cultured cardiomyocytes

**P<0.01 vs CO
16-kDa Prolactin up-regulates the pro-inflammatory CCL2 via activation of NFκB

![Western blot images showing IkB and Actin levels at 1h and 2h.

Bar graph showing CCL2 fold induction with CO, BAY, r16-kDa, and BAY + r16-kDa conditions.

**P<0.01 vs CO

§§P<0.01 vs r16-kDa
Summary and Conclusion

• Transiently elevated prolactin levels and cathepsin D activity in sera of patients with acute myocardial infarction are associated with the generation of the cleaved anti-angiogenic and pro-inflammatory 16-kDa prolactin.

• Systemic prolactin blockage by the dopamin D2 receptor agonist bromocriptine reduces infarction size and improves cardiac function after I/R in mice.

• Prolactin dependent myocardial damage in I/R mainly derives from the cleaved 16-kDa prolactin fragment.

• 16-kDa prolactin promotes cardiac inflammation by up-regulation of cytokine and chemokine expression in cardiomyocytes in vitro and in the heart in vivo.

• 16-kDa prolactin promotes up-regulation of pro-inflammatory CCL2 via activation of NFκB dependent in cardiomyocytes.
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BACKUPS
Do cardiomyocytes generate prolactin and its subform?

Hilfiker-Kleiner et al. Future Cardiology 2009
Cardiomyocytes produce 16-kDa Prolactin
GST/PRL

23-kDa Prl
16-kDa Prl

Supernatant

Whole cell-lysate

I/R NOX I/R+Prl NOX+Prl

16-kDa Prl
23-kDa Prl
16-kDa Prl

I/R NOX I/R+GST NOX+GST

GST
MCP1

Fold induction

Normoxia  I/R

CO  r16-kDa  r146 Prl  rWT Prl  CO  r16-kDa  r146 Prl  rWT Prl

*  §  #
16kDa Prolactin interferes with endothelial Ca\(^{2+}\) transients and disturbs vasorelaxation

AND what about effects of 16-kDa prolactin on cardiomyocytes?