AT1 RECEPTOR BLOCKADE ATTENUATES INSULIN RESISTANCE AND MYOCARDIAL REMODELING IN RATS WITH DIET-INDUCED OBESITY


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There is not any conflict of interest regarding the current presentation.
BACKGROUND
Cardiac remodeling

- Adaptive process to maintain myocardial performance in response to stress conditions

- Cardiac remodeling involves myocyte hypertrophy, interstitial fibrosis and molecular modifications

Cohn JN, Ferrari R, Sharpe N. J Am Coll Cardiol, 2000;35(3):569-82
Cardiac remodeling

• Differential molecular mechanisms can interact in multiple points of the cytosol in cardiac remodeling

• Important connection (cross-talk) between the signal transduction pathways that mediate insulin and angiotensin II actions in the heart

Cardiac Remodeling and Insulin Resistance

Regulation of insulin signaling

- Insulin actions are highly regulated by several factors.

- Regulatory mechanisms attenuate metabolic signaling from insulin stimuli by decreasing Tyrosine phosphorylation of proteins members from insulin pathway, as insulin receptor (IR), insulin receptor substrate (IRS) and phosphatidilinositol 3-kinase (PI3K) messenger.

- **Insulin resistance** is a common pathological state in which target cells fail to respond to normal stimuli from circulating insulin.
Molecular mechanisms of angiotensin II signaling

- Elevations in angiotensin II (Ang-II) contribute to stimulation of Ang-II type 1 (AT1) receptor: remodeling and insulin resistance in the heart

Cross-talk between Ang-II and insulin in **Obesity**

- Clinical studies/ genetic models of **obesity** (*Zucker rats)*:
  - Interventions with ACE inhibitors or AT1 receptor blockade:
    - attenuation of metabolic and endocrine disorders;
    - normalization of arterial pressure as well as remodeling and insulin resistance in the heart

- Ang-II and insulin interaction in models of diet-induced obesity

  *Ernsberger et al.; Am J Hypertens. 2007;20(8):866-74*
  *Carvalheira et al.; Endocrinology. 2003;144(12):5604-14*
HYPOTHESIS

- Hypercaloric diet-induced obesity has been associated with metabolic and cardiovascular disorders, including remodeling and insulin resistance in the myocardium.
OBJECTIVE
OBJECTIVE

• To evaluate the influence of angiotensin-II type I (AT1) receptor blocker losartan on insulin receptor/ phosphatidylinositol 3-kinase pathway and myocardial remodeling in rats with diet-induced obesity
METHODS
Animals and experimental design

Wistar-Kyoto rats (60 days-old)

Control group (C)

Obese group (OB)

• 30 Weeks

• 5 Weeks

Experimental period:

- C groups: animals submitted to commercial rat chow (3.2 kcal/g)
- OB groups: animals submitted to hypercaloric diet (4.6 kcal/g)
- L groups: groups treated with Losartan (30 mg/kg/day)
Nutritional and metabolic parameters

- Body weight (g)
- Adiposity (AD):
  \[ AD = \left[ \text{epididymal fat (EF) + retroperitoneal fat (RPF)} \right] \times 100 \]
  \( (\text{body weight - sum of fat pads}) \)
- Glycemia was obtained from glucose tolerance test
- Insulin concentration (ELISA)

Cardiovascular parameters

- Systolic blood pressure (SBP) was assessed, using a noninvasive tail-cuff method (plethismography)
- Morphological study integrated myocyte cross-sectional area and collagen interstitial fraction determination
Cardiovascular parameters

• Molecular expression of following proteins (Western blot):
  - **β subunit of insulin receptor (βRI)**
    - antibodies against βRI (sc-711) and phospho-\(Tyr^{1162}\)-βRI (sc-25103)
  - **p85 subunit of phosphatidylinositol 3-kinase (p85/PI3-K)**
    - antibodies against p85/PI3K (sc-1637) and phospho-\(Tyr^{508}\)-p85/PI3K (sc-12929)
  - Protein levels were normalized to those of GAPDH (6C5, sc-32233, Santa Cruz Biotechnology)

*Martinez et al.; Med Sci Monit 2010; 16(12):BR374-83*
Results were evaluated by two-way analysis of variance (ANOVA).

When significant differences were found (p<0.05), the post hoc Tukey’s multiple comparisons test was carried out.

The level of significance was considered to be 5%.

Bayley; J Am Stat Assoc 1977; 72: 469-78

Norman & Streiner; Biostatistics: the bare essentials. 1994
RESULTS
## Nutritional, metabolic and cardiovascular profile

### Table 1. Nutritional, metabolic and cardiovascular results according groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>C</th>
<th>OB</th>
<th>CL</th>
<th>OBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>553 ± 40</td>
<td>644 ± 45 *</td>
<td>552 ± 44</td>
<td>645 ± 63 †</td>
</tr>
<tr>
<td>Adiposity (%)</td>
<td>6.0 ± 1.0</td>
<td>12.0 ± 2.3 *</td>
<td>6.4 ± 1.3</td>
<td>11.3 ± 2.3 †</td>
</tr>
<tr>
<td>Glycemia (AUC)</td>
<td>26,345±1,935</td>
<td>34,841±1,836 *</td>
<td>26,520±1,840</td>
<td>31,300±1,836</td>
</tr>
<tr>
<td>Insulin (ng/dL)</td>
<td>1.64 ± 0.41</td>
<td>2.89 ± 0.37 *</td>
<td>1.03 ± 0.21 *</td>
<td>2.06 ± 0.20 # †</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115.3 ± 4.5</td>
<td>124.0 ± 9.7 *</td>
<td>108.7 ± 7.6 *</td>
<td>112.2 ± 13.9 #</td>
</tr>
</tbody>
</table>

Results in mean±standard error; AUC: area under curve of responses to glycemic tolerance test; SBP: systolic blood pressure; * p<0.05 vs C; # p<0.05 vs OB group; † p<0.05 vs CL group; ANOVA and Tukey’s test.
Histological parameters of the heart

Figure 1. Morphometric analysis of left ventricle; (A) histological sections stained with hematoxylin-eosin; (B) myocyte cross-sectional area, according group; results in mean and standard-error; * p<0.05 vs C group; # p<0.05 vs OB group; ANOVA and Tukey’s test
Figure 2. Morphometric analysis of left ventricle; (A) histological sections stained with picro-sirius red; (B) interstitial collagen fraction according groups; results in mean and standard-deviation; * p<0.05 vs C; # p<0.05 vs OB; † p<0.05 vs CL; ANOVA and Tukey’s test
**Figure 3.** Protein levels of insulin receptor in cardiac muscle analyzed by Western blotting. (A) \( \beta \)-subunit of insulin receptor (\( \beta Im \)); \( \beta Im \) protein levels normalized to the GAPDH levels. (B) phospho-Tyr\(^{1162} \)-\( \beta Im \) expression; protein levels normalized to the \( \beta Im \) total levels; results in mean and standard-deviation; * \( p < 0.05 \) vs C; # \( p < 0.05 \) vs OB; † \( p < 0.05 \) vs CL; ANOVA and Tukey’s test.
Figure 4. Protein levels of p85 subunit of phosphatidylinositol 3-kinase (PI3K) in cardiac muscle analyzed by Western blotting. (A) PI3K expression; PI3K protein levels normalized to the GAPDH levels. (B) phospho-Tyr508-PI3K expression; protein levels normalized to the PI3K total levels; results in mean and standard-error; * p<0.05 vs C; # p<0.05 vs OB; † p<0.05 vs CL; ANOVA and Tukey’s test.
CONCLUSION

• Losartan attenuates insulin resistance and myocardial remodeling in obese rats.

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