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Letters to the editor: Effects of Nebivolol on Myocardial Gene Expression during N2-stimulated Ischemia in Human Atrial Myocardium

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Letters to the Editor

Effects of Nebivolol on Myocardial Gene Expression during N2-stimulated Ischemia in Human Atrial Myocardium

To the editors:

Introduction

Pharmacologically, nebivolol constitutes a new type of beta-blocker, which shows properties different from those of other beta-blockers [1, 2]. It exerts various additional effects, such as acting on the endothelial NO-pathway [3, 4] and an extremely high affinity to the beta1-adrenoceptor. It also shows a high affinity to serotonin receptors of the 5-$HT1_A$ subtype and has been attributed a considerable cardioprotective effect [5, 6]. During and after myocardial ischemia, beta-blockers support the preservation of myocardial integrity. Since nebivolol shows numerous pharmacological properties which may entail anti-ischemic protection, we have analysed the expression of 27184 genes in human atrial myocardium under ischemic conditions in the presence and absence of nebivolol. However, little is known about the molecular effects of nebivolol upon myocardial ischemia [7, 8]. In the present work, we have identified the specific molecular signature of myocardial ischemia, which, on one hand equals the severity and type of tissue damage produced. On the other hand, it demonstrates the activation of repair mechanisms and changes in the metabolic state of the cell. Doing so, in the presence and absence of nebivolol, will allow for conclusions, in which direction cardioprotective mechanisms of the drug should be further investigated.

Methods

Material

Myocardial tissue probes derive from the right auricle of patients undergoing cardiac surgery. Cardiac tissue was instantly placed in well-oxygenated, cooled Tyrode solution and cut in two pieces of about equal size. Each of the preparations was then put into an individual chamber as done in earlier experiments [9–11]. Experimental ischemia was brought about by switching 100 % oxygen to 100 % nitrogen (hypoxia) in one of the chambers [10]. After 30 minutes of simulated ischemia, tissue samples were snap-frozen using liquid nitrogen.

Solutions

The preparations were continuously perfused with Tyrode solution [10] at 37 °C.

RNA-Isolation and cDNA Transcription

Total RNA was extracted using the Trizol® method (Invitrogen Corporation, Carlsbad, CA, USA) and further purified using RNeasy Mini Kit (Qiagen Inc., Hilden, GER). The quality of the isolated RNA was analysed on Agilent's Bioanalyzer 2100 system. Either 20–40 µg or 0,2–1 µg total RNA, when only reduced amounts of material were available, was then directly or indirectly, via *in-vitro* transcription, transcribed into DIG-labelled cDNA.

Microarray [12–14]

The labelled probes were hybridised onto the array for 16 hrs. Subsequently, arrays were washed and detection was carried out using alkaline-phosphatase-conjugated anti-DIG antibodies and the appropriate substrate according to a highly standardized protocol. Arrays were scanned with the AB1700 Chemiluminescence Array Reader and images, raw data and tissue information were stored in a MIAME-compliant ORACLE dat AB1700 Microarray Analyzer System. In each experiment, we used pooled data of four experiments as well as one individual single experiment (n = 4 + 1).

Results

Preliminary results from molecular profiling during myocardial ischemia: after 30 minutes of myocardial ischemia we found that, in the presence of nebivolol, the following pathways were significantly down-regulated:

- B-cell- and antibody-mediated immunity
- Immunity and defense
- Cell structure and motility
- MHCII-mediated immunity
- Signal transduction
- Cell surface receptor-mediated signal transduction
- Cell motility
- Developmental processes
- JNK cascade
- Protein modification
- Other receptor-mediated signalling pathways
- Mesoderm development
- T-cell-mediated immunity
- Receptor protein tyrosine kinase signalling pathway
- Cell structure
- Protein phosphorylation
- Extracellular matrix protein-mediated signalling
- Other signal transduction
- Receptor-mediated endocytosis
- Protein-metabolism and modification
- Ion transport
- Other polysaccharide metabolism
- NF-kappaB cascade
- Proteolysis
- Cytokine- and chemokine-mediated signalling pathway
- Angiogenesis
- Ectoderm development
- Cell proliferation and differentiation
- Cation transport
- Protein glycosylation
- Calcium ion homeostasis

and the following up-regulated:

- Induction of apoptosis
- Other blood circulation and gas exchange activities
- Protein-targeting and localization
- Blood circulation and gas exchange
- Glucose homeostasis
- Receptor-mediated endocytosis
- Miscellaneous
- Meiosis
- Cell structure and motility
- Cell structure
- Other oncogenesis

See Figures 1–3.

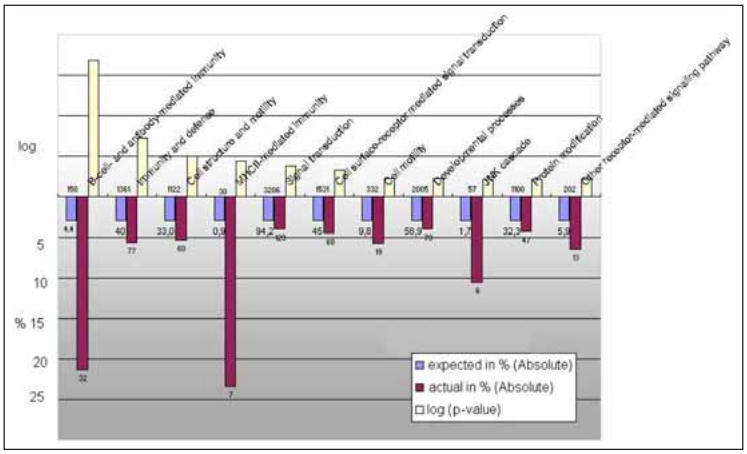

Figure 1: Myocardial cellular gene expression significantly down-regulated during experimental ischemia under nebivolol compared to control-ischemia experiments without nebivolol. Yellow bars indicate significance expressed as log p-value: left – highest significance.

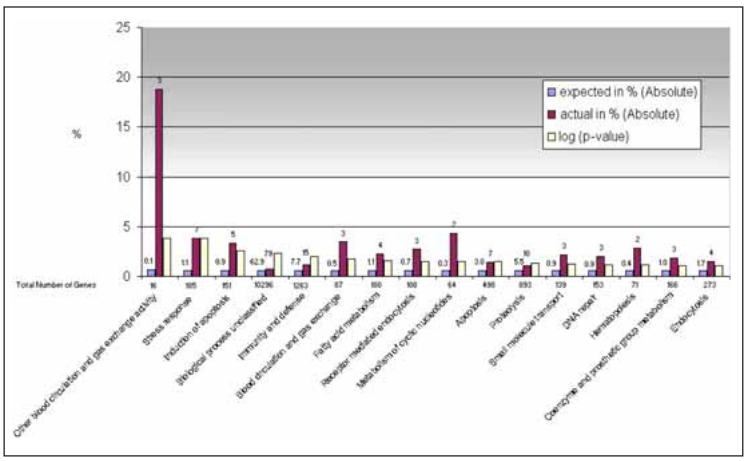

Figure 2: Myocardial cellular gene-expression significantly up-regulated during experimental ischemia under nebivolol compared to control-ischemia experiments without nebivolol. Yellow bars indicate significance expressed as log p-value: left – highest significance.

Discussion

Quality as well as quantity of isolated mRNA were double-checked using both spectrophotometry as well as Agilent's Bioanalyzer 2100 system. The AB1700 microarray system available at the Core Facility Molecular Biology (CF-MB) is operated by a team consisting of four members with many years of experience in various microarray technologies and platforms (Affymetrix, cDNA- and Oligonucleotide Arrays). To evaluate the performance of the novel Applied Biosystems microarray technology a multicenter proof-of-principle study was conducted by the CF-MB, which included Affymetrix, cDNA- and oligonucleotide-based platforms and that was performed at approved international facilities. The novel AB1700 chemiluminescence microarray system turned out to be superior with respect to sensitivity and reliability. Hence, we believe that data are highly reliable.

One can see, that, in the presence of nebivolol, processes that are majorly involved in myocardial ischemia, damage and inflammation are down-regulated, while processes involved in structural integrity, circulation, gas exchange activity, glucose homeostasis and other vital cellular mechanisms expressing myocardial anti-ischemic protection are significantly more expressed. From these data one can conclude that nebivolol exerts an important cardioprotective action on a variety of intracellular pathways.

Further studies should verify the more important pathways by Real Time PCR and, where possible, on the molecular level. Nebivolol should be compared with another standard beta-blockers commonly used in the treatment of myocardial ischemia and find differences in the expression profiles between different types of betablockers during myocardial ischemia. By doing so, we intend to investigate whether or not nebivolol unfolds cardioprotective actions different from other betablockers.

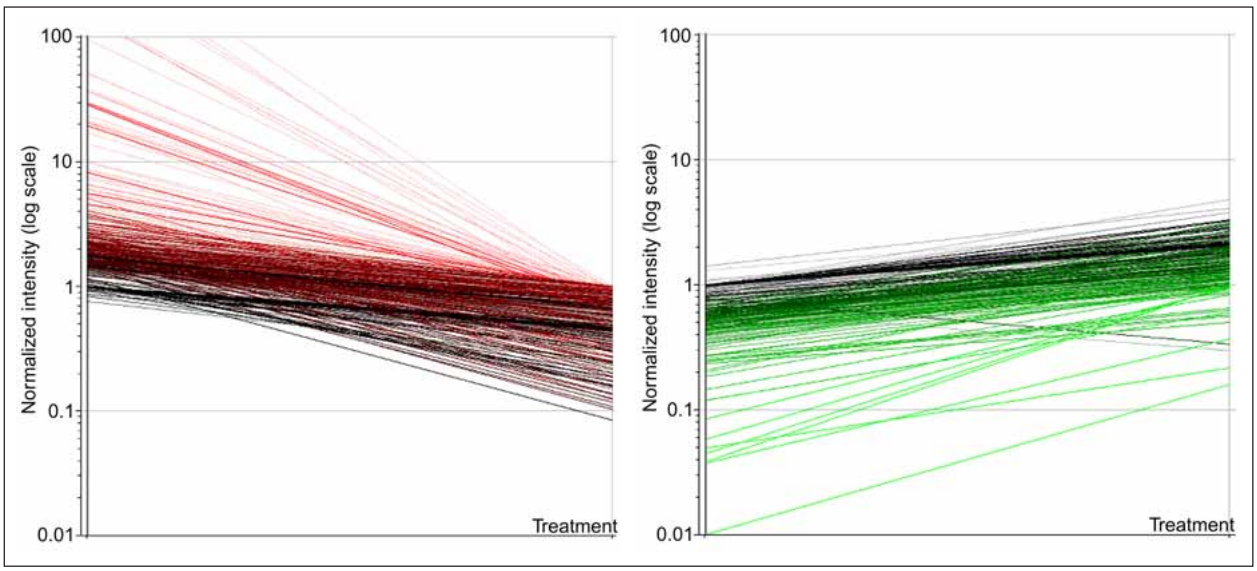

Figure 3: Graphic display of gene de-regulation in myocardial ischemia under nebivolol compared to ischemia under control conditions. One can clearly see a distinct difference between treatment (right side of each penal) and no-treatment (left side of each penal). Red: genes down-regulated under nebivolol, green: genes up-regulated under nebivolol.

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