Volume 53(Suppl.1) 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Biglycan protects cardiomyocytes against simulated ischemia/ reoxygenation injury via an NO-dependent mechanism

A Görbe[1], A Szunyog[1], T Csont[1], E Aypar[1], E Bereczki[2], M Sántha[2], P Ferdinandy[1]

[1]Cardiovascular Research Group, Department of Biochemistry, University of Szeged and Pharmahungary Group Szeged, Szeged, Hungary, [2]Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Although biglycan, a proteoglycan component of extracellular matrix, has been suspected to contribute to the development of atherosclerosis, overexpression of biglycan has been shown to induce cardioprotective genes including nitric oxide (NO) synthases in the heart of a transgenic mouse model.

The aim of the present study was to test whether biglycan is cardioprotective against hypoxia/reoxygenation injury in cardiomyocytes and if an NO-dependent mechanism is involved in the cytoprotection.

Therefore, primary cardiomyocytes were prepared from newborn Wistar rats and kept in growing medium (90% DMEM, 10% fetal calf serum) under normoxic conditions (37°C, 5% CO_2). Two days old cultures were treated with 1, 3, 10, 30 and 100 nM biglycan. In separate experiments, biglycan (30 nM) was combined with the NO synthase inhibitor L-nitro-arginin-methyl-ester (L-NAME, 100 μM). After a 20-hour pretreatment, media of the cultures were replaced with a "hypoxic" solution and plates were kept in a hypoxic chamber (gased with 95% N_2 and 5% CO_2 at 37°C) for 150 minutes, which was followed by 120 minutes of reoxygenation. All treatments were continued throughout hypoxia and reoxygenation. Finally, viability tests were done in all groups with Trypane blue staining. In order to check the effect of biglycan on NO synthase (NOS) expression, in separate experiments, normoxic cells were treated with 30 nM biglycan for 20 hours and then mRNA and total protein were isolated.

After simulated ischemia and reoxygenation, 41.8±1.0% of the cells died in control cultures. Biglycan significantly decreased cell death at 3, 10, 30 and 100 nM concentrations. Protection was the strongest at 30 nM (17.3±2.4%). Biglycan enhanced expression of mRNA of endothelial NOS, but not inducible NOS. Endothelial NOS expression at protein level was also significantly elevated after biglycan treatment. The L-NAME abolished the cytoprotective effect of biglycan (36.3±1.6%).

The proteoglycan biglycan exerts a cytoprotective effect against hypoxia/reoxygenation injury via at least in part an NO-dependent mechanism.

Antioxidant characterization of perspective apricot hybrids

A Hegedűs[1], N Papp[2], P Pfeiffer[1], L Abrankó[2], R Hermán[1], A Pedryc[1], A Blázovics[3], É Stefanovits-Bányai[2]

[1]Department of Genetics and Plant Breeding, Corvinus University of Budapest, Budapest, Hungary, [2]Department of Applied Chemistry, Corvinus University of Budapest, Budapest, Hungary, [3]Department of Medicine, Semmelweis University of Medicine 2nd, Budapest, Hungary

Health-promoting effects of fruits are at least partially attributed to the antioxidant compounds accumulating in fruit flesh. Apricot fruit contains three major types of antioxidant compounds: water-soluble ascorbic acid (vitamin C), lipid-soluble carotenoids and polyphenolics encompassing both hydro- and lipophilic components. To survey the potential health-effects of apricot it is important to know about the variations in quantity of the antioxidant compounds present in fruit. Studies on parents and their progeny may help to shed light on the inheritance of fruit antioxidant properties and to clarify if the increase in fruit antioxidant capacity may be possible in a carefully designed breeding program.

The measurements were carried out on the apricot cultivars maintained in the germplasm collection of the Department of Genetics and Plant Breeding, CUB and 18 hybrids obtained from a breeding program of the Department of Genetics and Plant Breeding, CUB. The following parameters were studied in apricot fresh fruits: colour values (lightness factor, hue angle and chroma colour); ferric reducing ability (FRAP); DPPH-radical scavenging activity; total radical scavenging capacity measured with chemiluminescence methods; as well as total phenol (TPC) and vitamin C contents measured with spectrophotometer and HPLC-DAD, respectively.

The FRAP and TPC assays revealed 22- and 21-fold differences, respectively, between the lowest and the highest values, indicating a great diversity in the antioxidant power of apricot fresh fruits. A perspective hybrid produced outstanding values in all of the antioxidant assays, exceeding 2.5-times the same parameters determined for the best commercial cultivar. The

FRAP values of twelve hybrids resulting from the cross 'Bergeron' ×'Baneasa 4/11' varied between the values determined for the parents ('Bergeron': 3.57 mmolAA/L and 'Baneasa 4/11': 1.12 mmol AA/L). Three hybrids showed FRAP values very similar to that of the 'Baneasa 4/11', while two others almost reached the level measured in 'Bergeron'.

The closest correlation occurred between the FRAP and DPPH-radical scavenging capacity. Close correlations were also obtained between FRAP, TPC, DPPH and vitamin C content data. Colour values did not show significant correlations with any of the measured parameters of water-soluble antioxidants, since colour values were correlated exclusively with the lipid-soluble carotenoids.

Our results indicate that several valuable genotypes can be selected from a progeny obtained from crosses where at least one of the parents is characterized by enhanced fruit antioxidant properties.

This work was co-financed by the Ányos Jedlik programme NKFP06A2-BCETKA06 and the NKTH-OTKA K68921 grant.

Regulation of metal responsive transcription factor MTF-1 expression in common carp

E Hermesz, Á Ferencz

Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary

Metal responsive control of gene expression allows organisms to adjust the concentration of essential metal ions such as Zn^{2+} and Cu^{2+}, within an acceptable range and cope with detoxification of heavy metals (Cd^{2+}, Pb^{2+} and As^{3+}) with no biological function. Metallothioneins (MTs) are widely inducible at transcriptional level by a variety of metals and other stress conditions such as accumulation of reactive oxygen species, hormones and cytokines. Transactivation of metallothionein genes involves the Metal-responsive Transcription Factor (MTF-1) a metal responsive element (MRE) binding, zinc sensitive protein.

In this study we present the first evidence for an *mtf-1* splicing variant (*mtf-1.1a*), originated from the brain of unstressed common carp. We have follow the level of *mtf-1.1a* mRNA in the liver, kidney, heart, muscle and brain of unstressed animals and the effect of heavy metal loading (Cd and As) on the alternative splicing of *mtf-1.1* transcript. For the detection and semiquantitative determination, an *mtf-1.1*-specific primer pair was designed. This primer pair has the potential to amplify a segment from both *mtf-1.1* and *mtf-1.1a* in the same PCR reaction, with a well-distinguishable size difference.

The splice variant of *mtf-1.1* mRNA codes for a truncated MTF-1.1 protein. The lack of a 103 nucleotides internally in the *mtf-1.1a* transcript, between positions 1047-1149, results in a frame shift causing an early termination of translation. The putative MTF-1.1a protein consists of the first 349 amino acids of MTF-1.1 followed by an additional 64 amino acids, which don't resemble at all to the corresponding region of MTF-1.1. The 349 amino acid covers the six Zn-finger DNA binding domains, the nuclear localization (NLS) and the nuclear exporting (NES) signals and the first 12 amino acid of the acidic region. Under unstressed conditions *mtf-1.1a* was detected in all tissues examined, but the liver, with the highest level in the brain. Arsenic alters the level of both *mtf-1.1* and *mtf-1.1a* transcripts in an isoform- and tissue-specific manner. Cadmium had no measurable effect on the alternative splicing of *mtf-1.1* in the liver, while the amount of both *mtf-1.1* transcripts gradually decreased in the brain.

The above observations suggest a tissue- and stressor-specific function of the predicted MTF-1.1a protein. In addition, we have already identified another MTF-1-coding gene, *mtf-1.2*, exclusively expressed in the brain of unstressed animals. The possible presence of 3 MTF-1 protein variants in the brain suggests a crucial role of the MTF-1s in this organ. MTF-1.1a might function as a negative regulator of MRE-controlled expressions. However, it is also possible that the introduction of a new C-terminal domain might result in a new level of regulation by recruiting new proteins to MTF-1.1-controlled promoters.