Summary of the Ph.D. Thesis

Investigating the neuroprotective role of Hsp27 in transgenic mice

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Introduction

Heat shock proteins are ubiquitously expressed evolutionary conserved proteins which are critical regulators of cellular homeostasis. Heat shock proteins can be induced by various stresses such as ethanol, infection, hypoxia, ischaemia and heavy metals. They are also upregulated in several pathological conditions. Hsp27 belongs to the small heat shock protein family, which are ATP-independent chaperones. The most important function of Hsp27 is based on its ability to bind non-native proteins and inhibit the aggregation of incorrectly folded proteins, maintaining them in a refolding-competent state. Moreover, it also has anti-apoptotic and antioxidant activities and it binds to the actin cytosceleton and stabilizes it.

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases which prevalence is strongly correlated with aging. Alzheimer's disease is characterized by progressive loss of memory and cognitive functions, senile plaque deposition, formation of neurofibrillary tangles, and finally neuronal cell death. The major component of senile plaques is the amyloid-β peptide (Aβ) which is a 4 kDa polypeptide and it is generated from the amyloid precursor protein (APP) by proteolytic cleavage of β- and γ-secretases. Conformational changes of soluble Aβ may lead to aggregation into oligomers, protofibrils and mature fibrils. Aβ aggregation may cause neurodegeneration through multiple pathways, therefore cellular factors that affect the production, clearance or oligomerization of Aβ may be good targets for drug

development to prevent or treat AD. Several reports demonstrated that heat shock proteins are upregulated in different neurodegenerative diseases and protect brain cells against free radical injury, oxidative stress and misfolded proteins. In AD Hsp protein is associated with Aβ deposition and neurofibrillary tangles, and recent findings suggest that Hsps might prevent the accumulation and aggregation of Aβ.

Ethanol affects actually all body organs including brain. Ethanol has several cytotoxic effects and most of them are independent of cell type. Ethanol treatment leads to the physical disruption of lipid membranes in the cells. The interaction of ethanol with membranes causes a decreased membrane order, increased membrane fluidity and changes in the membrane protein composition. It can also interact directly with membrane proteins causing conformational changes thus influencing their function. The increase of oxidative stress may be also involved in neurodegenerative action of ethanol. The heat shock and ethanol stress responses exhibit extensive similarities. Both heat shock and ethanol have membrane disruptive effects and they denaturate proteins and cause similar changes in the plasma membrane protein composition. As heat shock and ethanol stress provoke similar responses, it is likely that heat shock protein activaton also has a protective effect during ethanol treatment. Indeed it has been suggested that ethanol induced expression of stress proteins is associated with the enhanced ethanol tolerance in bacteria.

Aim of the study

The aim of our studies was 1) to investigate the neuroprotective effect of Hsp27 protein after acute and chronic ethanol administration, and 2) to study the effect of the small heat shock protein Hsp27 on Aβ accumulation and related pathological features of Alzheimer's disease. Therefore the following experiments were performed:

1a) Generation of the human Hsp27 overexpressing transgenic mouse strain.

1b) Acute ethanol treatment of transgenic mice.

1c) Investigating ataxia and motor coordination using behavioral tests.

1d) Chronic ethanol treatment.

1e) Detection of degenerating neurons using Fluoro-JadeC staining.

2a) Crossing the Hsp27 overexpressing mouse strain with the validated mouse model of Alzheimer's disease (APPswexPSE1dE9).

2b) Investigating learning and memory using behavioral tests.

2c) Staining amyloid depositions using immunohistochemistry.

2d) Performing electrophysiology on hippocampal slices.

2e) Investigating apoptosis and neurodegeneration using immunhistochemistry and Fluoro-JadeC staining.

Methods

-Recombinant DNA technology

-Pronucleus microinjection

-DNA extraction and PCR analysis

-Protein extraction and Western blot analysis

-Preparing frozen sections

-Immunohistochemistry

-Fluoro-JadeC staining

-Behavioral tests

-Electrophysiology on hippocampal slices

Results

In our lab we have generated a transgenic mouse line overexpressing the human Hsp27 protein in order to study the neuroprotective effects of the small heat shock protein. First, we investigated the neuroprotective effect of Hsp27 after acute ethanol administration. After intraperitoneal injection of ethanol, different behavioral tests were performed to monitor motor coordination, imbalance and ataxia. We found that after ethanol treatment wild-type mice had impaired motor coordination, while Hsp27 overexpressing mice performed better in the behaviour tests. In a separate set of experiments drinking water of mice was replaced by a 20% ethanol solution for five weeks to study the effect of chronic ethanol treatment. Brain sections of treated transgenic and non-transgenic mice were stained with Fluoro-JadeC staining. We found significantly lesser amount of degenerated neurons in the brain of ethanol-treated transgenic mice compared to wild type mice. We can conclude, that Hsp27 can protect neurons against the acute and chronic toxic effects of ethanol.

In order to study the protective role of Hsp27 during the development of Alzheimer's disease we crossed Hsp27 transgenic strain with APPswe/PS1dE9 mice, which is the validated model of Alzheimer's disease. Spatial learning and memory which depends on the hippocampal function were tested using Barnes maze and Morris water maze. In both tests we found that the spatial learning of APPswe/PS1dE9 mice was impaired. In contrast, wild type and APPswe/PS1dE9/Hsp27 mice learned to find the escape hole or platform during the training period as indicated by the progressive reduction in escape latency. After behavioral testing amyloid plaque formation was studied using Aβ immunostaining on frozen sections. We found that the number of the plaques was significantly less in the Hsp27 overexpressing mice compared to the APPswe/PS1dE9 group indicating that Hsp27 might prevent amyloid aggregation and plaque-formation or enhanced amyloid clearance in the brain. To monitor neuronal apoptosis in 7 and 14 months old transgenic mice, caspase-3 immunostaining and Fluoro-JadeC staining were used. We detected an increased number of apoptotic cells and degenerated neurons in both transgenic strains compared to wild type animals. The number of degenerated neurons was markedly increased during aging. Although less degenerated neuron was counted in APPswe/PS1dE9/Hsp27 transgenic mice, than in APPswe/PS1dE9 mice, however the difference was not significant. Electrophysiological recordings revealed, that the excitability of neurons was significantly increased and long term potentiation was impaired in the APPswe/PS1dE9 group, but this was rescued in the

APPswe/PS1dE9/Hsp27 mice. These results suggest that the overexpression of Hsp27 protein might ameliorate symptoms of Alzheimer's disease.

Summary of the results

1. We have generated a human Hsp27 overexpressing transgenic mouse strain.
2. Using this strain we have demonstrated that Hsp27 has protective role against the harmful effect of ethanol on motor coordination.
3. We have also demonstrated that Hsp27 could moderate neurodegeneration during chronic ethanol treatment.
4. Crossing the Hsp27 overexpressing mouse strain with the validated mouse model of Alzheimer's disease, we have generated the APPswe/PS1dE9/Hsp27 multiple transgenic mouse strain.
5. Using behavioral tests we found an improved spatial learning and memory in the APPswe/PS1dE9/Hsp27 mice compared to AD model mice.
6. Using hippocampal slice electrophysiology it was demonstrated that the excitability of neurons was increased and long term potentiation was impaired in the APPswe/PS1dE9 group, but this was rescued in transgenics overexpressing Hsp27.
7. It was also demontrated, that number of amyloid depositions was significantly less in the Hsp27 overexpressing AD model mice.
8. In the number of degenerated neurons we did not found significant difference between the APPswe/PS1dE9 and APPswe/PS1dE9/Hsp27 groups.

List of publications

Toth ME, Gonda S, Vigh L, Santha M. (2010) Neuroprotective effect of small heat shock protein, Hsp27, after acute and chronic alcohol administration. Cell Stress Chaperones.15 (6):807-17.

(IF: 3.162)

Toth ME, Szegedi V, Varga E, Juhasz G, Horvath J, Borbely E, Lenart N, Penke B and Santha M. (2012) Overexpression of Small Heat Shock Protein, Hsp27 ameliorates symptoms of Alzheimer's Disease. *Manuscript in preparation*

Wang S, **Toth ME,** Bereczki E, Santha M, Guan ZZ, Winblad B, Pei JJ. (2011) Interplay between glycogen synthase kinase-3β and tau in the cerebellum of Hsp27 transgenic mouse. J Neurosci Res. 89(8):1267-75.

(IF: 2.985)

Csont T, Gorbe A, Bereczki E, Szunyog A, Aypar E, **Toth ME,** Varga ZV, Csonka C, Fulop F, Santha M, Ferdinandy P. (2010) Biglycan protects cardiomyocytes against hypoxia/reoxygenation injury: role of nitric oxide. J Mol Cell Cardiol.48(4):649-52.

(IF: 5.499)

Poster presentation:

Toth ME, Gonda S, és Santha M. (2009). *A Hsp27 neuroprotekív szerepe akut és krónikus etanoladagolás után*. MBKE 2009. évi Vándorgyűlése, Budapest