The Role of Inducible Hsp70 Protein in Modulation of Neurodegenerative Pathology in the Nigrostriatal System Typical to Parkinson’s Disease

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Proteins are major products of the operation of the genome. Without them, the evolution of life on Earth is hardly imaginable. These macromolecules are by far most universal and structurally complicated. Being involved in almost every biological process, they are essential for activities of cells and the whole organism. Maintenance of intracellular proteostasis (i.e., protein levels and qualities) is achieved due to integrated cooperation of the two most important and evolutionarily ancient systems, molecular chaperones and protein degradation. The intracellular systems of selective protein degradation (ubiquitin–proteasome and chaperone–mediated autophagy) ensure the preservation of healthy cell populations by means of immediate removal of damaged, aged or mutant proteins, accumulation of which may lead to cell death [1]. The chaperone system of heat shock proteins (HSPs) is responsible for the cell proteome quality, as this system carries out the fundamental process of folding and refolding of proteins and maintenance of their structural integrity [2]. Malfunctioning of these systems may play a part in the pathogenesis of some human diseases, specifically, neurodegenerative. Thus, protein misfolding may lead to fatal defects, for example, the synthesis of toxic oligomers of the vesicular protein α-synuclein which cause neuronal cell death in the brain during the development of Parkinson’s disease (PD) [3, 4]. There is published evidence that the inducible heat shock protein Hsp70 may be an important “player” in the pathogenesis of PD, since this chaperone is able to prevent oligomerization of α-synuclein and formation of its aggregates in experimental models of PD [3, 4]. However, PD patients have a lower Hsp70 level in the nigrostriatal system, probably, reflecting the attenuation of the mechanisms that control neuronal protein conformation and cell defense [4]. These data provided a basis for our hypothesis that the decreased Hsp70 level in neurons of the substantia nigra pars compacta (SNpc) may serve as one of the factors that accelerate neurodegeneration in the nigrostriatal system during PD.

The study was performed on male Wistar rats (n = 28) aged 6 months and weighing 280–300 g. The hypothesis was tested by using a lentiviral construct that carried the gene of hairpin RNA-Hsp70 (shRNA-Hsp70) allowing local reduction
Changes in the expression level of the inducible Hsp70 protein and in the number of dopaminergic neurons in the substantia nigra pars compacta (SNpc) after administration of a shRNA-Hsp70 lentivirus construct in the lactacystin model of Parkinson’s disease in rats. (a) Immunoblotting with anti-Hsp70 antibodies carried out 14 days after administration of a shRNA-Hsp70 lentivirus construct and control solutions; PB—phosphate buffer; GFP—lentivirus expressing green fluorescent protein; (b) tyrosine hydroxylase immunopositive neurons in the SNpc under control conditions (GFP) and after lactacystin (LC) administration to rats infected with lentiviruses GFP (GFP + LC) and shRNA-Hsp70 (shRNA-Hsp70 + LC); (c) the number of dopaminergic neurons in the SNpc under control conditions (GFP) and following lactacystin (LC) administration to rats infected with lentiviruses GFP and shRNA-Hsp70. Significance of differences from the control: *—$p<0.05$; **—$p<0.01$; relative to the LC effect: #—$p<0.05$. The efficacy of transfection of neuronal cells in the SNpc was assessed with LVTHM lentivirus that carried the green fluorescent protein (GFP) gene. The lentiviral particles were produced according to a previously published protocol [5]. Concentrated suspensions of lentiviral particles and the control solution (phosphate buffer with pH 7.4) were injected bilaterally using a stereotactic technique into the SNpc of narcotized rats. The efficacy of lentiviral transfection was assessed by confocal microscopy. Changes in the Hsp70 level in the SNpc following transfection were determined using the immunoblotting technique [5]. PD-specific pathological changes in the nigrostriatal system were modeled by bilateral microinjections of lactacystin (LC), which is an inhibitor of proteasome enzyme activity, into the SNpc. The microinjections of LC dissolved in apyrogenic phosphate buffered saline (pH 7.4) were carried out 14 days after transfection of the lentiviral construct (LVTHM-GFP or shRNA-Hsp70), twice with a one-week interval. LC was administered through delivering cannulae at concentrations of 0.4 µg/1 µL (first injection) and 4.0 µg/1 µL (second injection). Observation of the one-week interval between LC microinjections prevented acute toxic effects and enabled tracing of the neurodegenerative process dynamics [6]. All manipulations with experimental animals were in compliance with the bioethical standards; surgical procedures were performed under general anesthesia.

The comparative analysis of how the morphological signs of neurodegeneration in the nigrostriatal system and motor behavior parameters changed was conducted 21 days after the first LC administration. This implied the use of the traditional immunohistochemical methods, primary polyclonal rabbit anti-tyrosine hydroxylase antibodies (Abcam, UK) and motor dysfunction tests. Disturbances of the forelimb, mouth and tongue manipulation abilities were detected by the sun-