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Exercise Training Upregulates the Expression of HSP70 in Brain of Mice with Induced Parkinson Disease

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Abstract: Parkinson disease is a common neurodegenerative disease. Deficiency of dopamine is thought to be responsible for development of Parkinson disease. Exercise training has been associated with improvements in patients with Parkinson disease. The objective of the present study is to explore the effect of exercise training on the expression of HSP70 in brains of mice with induced Parkinson disease. Forty albino mice were selected and assigned into four groups: Sedentary Control (SC, N = 10), Exercised Control (EC, N = 10), Parkinson Disease (PD, N = 10) and Exercised Parkinson Disease (EPD, N = 10). MPTP protocol was used to induce Parkinson disease by injections of 10 doses of MPTP (25 mg kg^{-1}) and probenecid (250 mg kg^{-1}) over 5 weeks. After the protocols treadmill exercise training had been finished, samples from the brain tissues were assessed by immunohistochemistry to examine the expression of HSP70 in the four groups of animals. The results of the present study showed that the expression of HSP70 was reduced in the brain of mice with Parkinson disease significantly ($p \leq 0.05$) compared with control groups. The results also showed that exercise training increased the expression of HSP70 in EC significantly ($p \leq 0.05$) compared with control group and insignificantly ($p > 0.05$) in EPD compared with PD. Although, the increased expression of HSP70 in exercised Parkinson disease was not significant, it has a potential role in improved the status of mice with Parkinson disease and it may have a potential therapeutic role.

Key words: Parkinson disease, neurodegenerative disease, chaperon, heat shock protein, therapeutic role

INTRODUCTION

Parkinson Disease (PD) is characterized by being a neurodegenerative disorder and the deficiency of dopamine is considered the reason for developing PD (Erekat *et al*., 2013), leading to abnormal voluntary movements in skeletal muscles (Tinazzi *et al*., 2010). Several studies have shown that the 1-Methyl-4-1, 2, 3, 6-Tetrahydro Pyridine (MPTP) leads to PD through the production of reactive oxygen species, peroxynitrite, a process causes the nitration of tyrosine residues and deficiencies of dopamine (Vodovotz *et al*., 1996; Vajdovich, 2008; Tinazzi *et al*., 2010).

Heat Shock Proteins (HSPs) have been studied extensively in literature and proved to provide an intrinsic mechanism to defend the cell against external diverse physiological stress that may initiate a cascade of events affecting cell structure and function. It has been assumed that due to the high conservation of HSPs throughout the evolution these proteins may have a vital role in protecting cells from injury. HSPs are composed of several classes of proteins according to their molecular weight which include high molecular mass HSPs (≥100 kD), HSP90 (81-99 kD), HSP70 (65-80 kD), HSP60 (55-64 kD), HSP40 (35-54 kD) and small HSPs (≤34 kD) (Hart, 1996).

It has been indicated that different classes of HSPs to play a diversity role in governing proper protein assembly, folding and translocation (Hightower, 1991; Hartl, 1996). Furthermore, regulation of these HSPs synthesis has been found to create a unique defense system to maintain cellular protein homeostasis and to ensure cell survival (Hightower, 1991).

The understanding of HSPs' function is based on two main lines of evidence: the clearance of waste proteins requires protein folding machinery called chaperones (Hartl, 1996) and HSPs chaperones bind to denatured proteins to promote their degradation (Hightower, 1991). Other evidence suggests that HSPs may actively participate in an array of cellular processes including cytoprotection (Benn and Woolf, 2004) and HSPs dysfunction may contribute to the pathogenesis of PD, a disease characterized by conformational changes in proteins that result in misfolding, aggregation and intracellular Lewy Body formation (Meriin and Sherman, 2005).

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It has been reported that HSP70 is associated with αSN, Dopamine Transporter (DAT), parkin, proteasome subunits, ubiquitin and UCH-L1 (Cuervo *et al*., 2004). HSP70 is believed not only to protect cells from rotenone-mediated cytotoxicity but also to decrease soluble αSN aggregation (Zhou *et al*., 2004). Furthermore, HSP70 can work as a putative anti-apoptotic factor to protect against neuronal cell death in PD (Benn and Woolf, 2004; Meriin and Sherman, 2005). These results highlight the possibility of using HSP70 as a potential therapy for PD. Recent studies of function and inducer of HSP90 also indicate its potential therapy for PD (Uryu *et al*., 2006; Waza *et al*., 2006).

It has been reported that *HSP70* gene transferred to dopaminergic neurons by a recombinant adeno-associated virus significantly protected the mouse against MPTP-induced nigral dopaminergic neuron loss and striatal dopamine levels decline (Dong *et al*., 2005). HSP70 attenuated MPTP induced apoptosis in the SNpc and increased amphetamine-induced rotation (Dong *et al*., 2005). Collectively, these results demonstrate that increasing chaperone activity may be beneficial for the treatment of PD.

Several studies have pointed to protective roles of HSPs to involve two major pathways besides their own chaperon activity: reducing mitochondrial dysfunction and oxidative stress and preventing UPS impairment. Mitochondrial dysfunction is probably the leading cause of increased oxidative stress and apoptosis in PD. Dopaminergic neurons are more vulnerable to oxidative stress than other neurons because of the special substrate dopamine (Jenner, 2003). In general, apoptotic process can be divided into the three phases: induction (or triggering), transduction of signal and execution. Theoretically, HSPs may modulate any of these apoptotic phases to rescue the cells (Beere and Green, 2001). In addition, it has been reported that stable expression of wild-type αB-crystallin protects cancer cells from caspase-3 activation *in vitro*, indicating that small HSPs αB-crystallin is a novel inhibitor of the activation of apoptosis (Kamradt *et al*., 2005). Other gene products linked to monogenic forms of PD also appear to be implicated in mitochondrial dysfunction. Parkin can interact with Leucine-rich repeat kinase 2 (Lrrk2) which is part of the mitochondrial outer membrane (Smith *et al*., 2005; West *et al*., 2005). Thus, Parkin may have an unexpected role in the regulation of normal mitochondrial function in an indirect way (Winklhofer *et al*., 2003; Palacino *et al*., 2004).

MATERIALS AND METHODS

Protocols published before for induction of PD were followed. Forty Albino mice were selected randomly and assigned into 4 groups: control group (N = 10), exercise group (N = 10), PD group (N = 10) and exercise PD group (N = 10).

The animals were housed in individual cages under identical conditions (22±1°C, free access to standard chow and water, 12 h dark/light cycle). PD was induced by injecting mice with 10 doses of MPTP (25 mg kg^{-1}) and probenecid (250 mg kg^{-1}) (chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA)). Protocols were run for 5 weeks, 3 days and half apart. Mice in control group were injected by saline (25 mg kg^{-1}).

Exercise protocol: A modified human treadmill as described in previous studies was designed. Animals were introduced and run in separate chambers which were divided by glass so running animals can see each other. Ten chambers were attached on the belt of human treadmill to let 10 animals to exercise each time of training. Exercise program implied that animals to exercise for 40 min day^{-1}, 5 days $week^{-1}$ for 4 weeks at a speed of 18 m min^{-1}.

HSP70 immunostaining of brain tissue: The mice were sacrificed and their brains were removed and fixed in 10% formalin, embedded in paraffin and sliced into 3 μm thick sections. Then, the 3 μm thick sections were processed via immuno-histo-chemistry using an antibody to HSP70 (Santa Cruz biotechnology). So, the 3 micron thick paraffin-embedded sections mounted on glass slides were deparaffinized in xylene for 2 min twice and subsequently rehydrated through serially descending dilutions of alcohol (starting with 100% and ended with 70%) followed by water (2 min for each step). After that sections were processed for antigen retrieval in the Reveal Solution (RV 1000M, Biocare Medical, Concord, CA) under pressure in the Decloaking chamber (Biocare medical) for 2 min. Tissue sections were then cooled down to room temperature and incubated with 3% hydrogen peroxidase in methanol for 5 min. After washing the sections in Phosphate Buffered Saline (PBS) they were incubated with HSP70 antibody (Santa Cruz Biotechnology) with the dilution recommended by the vendor at room temperature for 1 h. Next, the sections were washed in PBS and treated with secondary antibodies and Streptavidin using ImmunoCruz™ goat ABC staining system (sc-2023). Diaminobenzidine (DAB) was applied for 2 min or longer until the desired intensity was developed and then the slides were washed with tap water to stop the reaction. Negative control sections were processed without the primary antibody. All sections were then counterstained with hematoxylin and viewed under the light microscope. Ten slides of brain tissues from each animal group were evaluated for HSP70 expression by immunohistochemistry.

Data analysis: The sections were photographed with digital camera. Photoshop software was used. The slides from each group were analyzed by counting the total pixels area occupied by positive staining. HSP70 expression was analyzed in the different brain tissues and statistically compared among the 4 different groups using paired Student t-test. Differences in HSP70 expression were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results for Heat Shock Protein (HSP70): The expression of HSP70 was compared among four groups in the present study. The average expression rate of HSP70 was 0.045% in SC group. This rate was decreased in PD group to 0.0029%. This decreased rate was statistically significant as shown by t-test ($p \leq 0.05$). Exercise was able to increase to the expression rate of HSP70 to 0.0122% in EPD but this was not statistically significant ($p > 0.05$). Exercise was also able to increase significantly the expression rate of HSP70 in EC group compared with SC group to 0.106% ($p \leq 0.05$) (Fig. 1 and 2).

The findings of the present study showed significant decrease in the level of HSP70 in PD group significantly ($p < 0.05$). The findings confirm the hypothesis that chaperone action is a crucial aspect in protecting against the otherwise damaging consequences of protein misfolding (Dedmon *et al*., 2005). The findings can also explain the other reported considerations in which there is declining level of heat shock protein with ageing, the level of HSPs is decreased insufficiently to keep the cellular proteins homeostasis which may give rise to certain diseases such as PD (Berke and Paulson, 2003; Meriin and Sherman, 2005).

Other studies showed that HSP70 is believed not only to protect cells from rotenone-mediated cytotoxicity but also to decrease soluble αSN aggregation (Zhou *et al*., 2004). Furthermore, HSP70 can work as a putative anti-apoptotic factor to protect against neuronal cell death in PD (Benn and Woolf, 2004; Meriin and Sherman, 2005). These results highlight the possibility of using HSP70 as a potential therapy for PD. Recent studies of function and inducer of HSP90 also indicate its potential therapy for PD (Uryu *et al*., 2006; Waza *et al*., 2006).

The findings of the study also showed that exercise increased the level of HSP70 in EPD group insignificantly ($p > 0.05$). Researchers believed that in part exercise can benefit patients with PD and this is attributed to increase HSP70 levels. The results of the present study have also revealed significant expression of HSP70 attributed to exercise in EC group compared with SC group ($p < 0.05$).

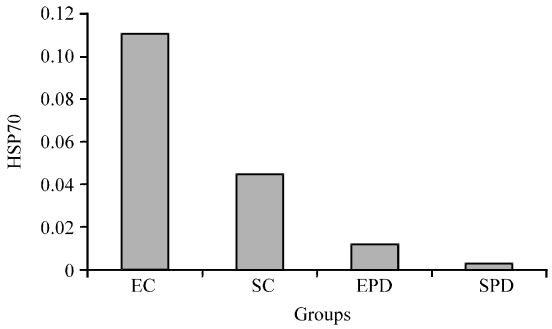

Fig. 1: A histogram represents the average expression of HSP70 in the brain of rats among study groups

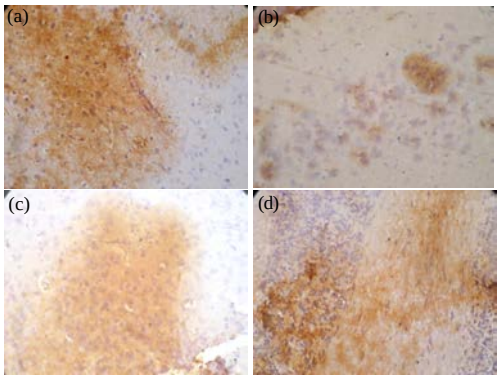

Fig. 2: Expression of HSP70 in study groups; a) good expression of HSP70 in brain of mouse in control group; b) low expression of HSP70 in brain of mouse with PD; c) increased expression of HSP70 in brain of excercised mouse; d) increased expression of HSP70 in brain of EPD excercised mouse

CONCLUSION

These findings explain how exercise can benefit normal people from oxidative damage and how exercise training becomes crucial with age to compensate for deficiency of HSP with ageing (Berke and Paulson, 2003; Meriin and Sherman, 2005). Taken together, the possibility of using HSP70 for targeting PD has been supported by findings of the present study.

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