

Heat shock protein 90 is a potential therapeutic target for ameliorating skeletal muscle abnormalities in Parkinson's disease

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Abstract

Previous studies have confirmed that heat shock protein 90 overexpression can lead to dopaminergic neuronal death. This study was designed to further investigate what effects are produced by heat shock protein 90 after endurance exercise training. Immunohistochemistry results showed that exercise training significantly inhibited heat shock protein 90 overexpression in the soleus and gastrocnemius in Parkinson's disease rats, which is a potential therapeutic target for ameliorating skeletal muscle abnormalities in Parkinson's disease.

Key Words: nerve regeneration; heat shock protein 90; Parkinson's disease; exercise; soleus muscle; gastrocnemius muscle; skeletal muscle; treadmill exercise; neural regeneration

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Introduction

Heat shock protein (HSP90) is an abundant molecular chaperone in the cell, and it is involved in cytoprotection against stress (Benjamin and McMillan, 1998; Sreedhar et al., 2003; Conte et al., 2011). HSP90 has been implicated in the pathogenesis of Parkinson's disease (PD) (Uryu et al., 2006; Luo et al., 2010). HSP90 expression is notably increased in PD brains, and is correlated with raised levels of insoluble α -synuclein (Uryu et al., 2006; Falsone et al., 2009; Luo et al., 2010). HSP90 has been suggested to be involved in neuronal protein misfolding and accumulation in PD brains leading to dopaminergic neuronal death and the eventual dopamine depletion (Luo et al., 2010; Ebrahimi-Fakhari et al., 2011; Dimant et al., 2012). Indeed, HSP90 inhibition by Geldanamycin has been shown to protect against 1-methyl-4-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity preventing the induction of PD (Shen et al., 2005; Hurtado-Lorenzo and Anand, 2008), therefore, HSP90 has been suggested as a therapeutic target in PD (Hurtado-Lorenzo and Anand, 2008).

HSP90 overexpression has been demonstrated in many pathological conditions (Hojlund et al., 2003). Increased HSP90 expression has been shown in skeletal muscles of type 2 diabetic patients (Hojlund et al., 2003), and elevated levels of HSP90 have been found in the heart muscle of streptozin-induced diabetic (SID) rats (Atalay et al., 2004). HSP90 is also overexpressed in inflammatory diseases such

as atherosclerosis (Kilic and Mandal, 2012) and upregulation has been reported in idiopathic inflammatory myopathies including sporadic inclusion body myositis and polymyositis (De Paepe et al., 2009). HSP90 inhibition, indeed, has been shown to decrease levels of inflammatory cytokines (Madrigal-Matute et al., 2010).

PD is a common neurodegenerative disease that is characterized by resting tremor, bradykinesia and akinesia due to lack of coordination of voluntary movements (Horowski et al., 1995; Parkinson, 2002; Kempster et al., 2007), which appear to be associated with skeletal muscle abnormalities. PD skeletal muscle abnormalities have been grouped into inflammatory myopathies, necrotizing myopathies and myopathies associated with mitochondrial abnormalities (Gdynia et al., 2009). The pathogenesis of PD skeletal muscle abnormalities, which can be very debilitating, has never been studied before.

HSP90 inhibits the production of cytoprotective HSPs such as HSP40, HSP70 and HSP90, by binding to heat shock factor 1 (HSF-1) (Ali et al., 1998; Zou et al., 1998; Kim et al., 1999). HSPs increase in different tissues following exercise (Naito et al., 2001; Walsh et al., 2001; Febbraio et al., 2004; Paulsen et al., 2007; Noble et al., 2008; Chen and Noble, 2009; Harkins, 2009; Hu et al., 2009; Krause and Rodrigues-Krause Jda, 2011; Noble and Shen, 2012; Fittipaldi et al., 2013), but very few studies have investigated alterations in HSP90 in response to exercise (Atalay et al., 2004; Harris

et al., 2008). Exercise training has been shown to increase HSP90 expression in the slow-twitch rat soleus (Harris et al., 2008), but exercise training has not resulted in altered HSP90 expression in fast-twitch gastrocnemius and vastus lateralis muscles of normal and diabetic rats (Atalay et al., 2004).

Exercise has been suggested as an adjunct treatment in PD patients (Fisher et al., 2008; David et al., 2012; Konerth and Childers, 2013), since exercise training has been shown to improve motor performance and corticomotor excitability in people with early PD (Bergen et al., 2002; Dibble et al., 2006; Cakit et al., 2007; Herman et al., 2007; Fisher et al., 2008; Li et al., 2012; Ridgel et al., 2012; Petzinger et al., 2013). In addition, exercise training caused an increase in stimulus-evoked dopamine release and a decrease in decay of dopamine in the dorsal striatum of MPTP-lesioned mice (Petzinger et al., 2007).

HSP90 expression has never been investigated in PD skeletal muscles before, therefore, the purpose of this study was to test the impact of chronic MPTP/probencid-induced PD on HSP90 expression in slow-twitch (soleus) and fast-twitch (gastrocnemius) skeletal muscles, and to examine the effect of endurance exercise training on HSP90 expression in both types of PD skeletal muscles. The ultimate goal was to ascertain whether HSP90 could potentially be used as a therapeutic target to ameliorate PD skeletal muscle abnormalities.

Materials and Methods

Animals

Forty normal Albino mice provided by the Animal House at Jordan University of Science and Technology, Jordan were used in this study. The animals were housed in individual cages under identical conditions ($22 \pm 1^\circ\text{C}$, free access to standard chow and water, 12 hour dark/light cycle). Animal-related protocols were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Jordan University of Science and Technology, Jordan.

The 40 mice were equally and randomly divided into four groups: sedentary control (SC), exercised control (EC), sedentary Parkinson's disease mice (SPD), and exercised Parkinson's disease mice (EPD).

Induction of PD mouse models

PD was induced according to the protocol described in our previous publication (Al-Jarrah et al., 2007). Briefly, mice in both SPD and EPD groups were intraperitoneally injected with 10 doses of MPTP (25 mg/kg) and probencid (250 mg/kg) over 5 weeks, 3 days and half apart. Simultaneously, control mice received 10 doses of intraperitoneal (25 mg/kg) saline injections.

Exercise protocol

All 40 mice were transferred to the training room daily, in order to be exposed to the same environment. However, only mice in the EC and EPD groups were trained on a modified human treadmill as described in our previous publication (Al-Jarrah and Jamous, 2011) purchased from Amman (Co-

lumbus Instruments, Columbus, OH, USA) for 40 minutes per day, 5 days per week for 4 weeks at a speed of 18 m/min.

HSP90 immunohistochemistry

The mice were sacrificed following light ether anesthesia, and the skeletal muscles of interest (gastrocnemius muscle/soleus muscle) were dissected from the right lower limb and fixed in 4% paraformaldehyde. Subsequently, 5 μm thick paraffin-embedded sections were prepared and processed via immunohistochemistry using an antibody to HSP90 (Biocare medical, San Antonio, TX, USA). Following this, the 5 μm thick paraffin-embedded sections were deparaffinized in xylene for 2 minutes twice, and subsequently rehydrated through serially descending dilutions of alcohol (starting with 100%, and ending with 70%) followed by water (2 minutes for each step). The reveal solution (RV 1000M, Biocare Medical, Concord, CA, USA) was used under pressure in the Decloaking chamber (Biocare medical) for 2 minutes for antigen retrieval. After being cooled down to room temperature, tissue sections were incubated with 3% hydrogen peroxidase in methanol for 5 minutes. After washing the sections in phosphate buffered saline (PBS), they were incubated with the dilution recommended by the vendor, at room temperature for 1 hour. Subsequent to washing in PBS, the sections were incubated with biotinylated secondary antibody (LSAB kit, Dako Carpinteria, CA, USA) for 15 minutes at room temperature, and washed with PBS. According to the vendor, biotinylated secondary antibody is reactive with both the constitutive and the inducible forms of HSP90. However, it does not bind to the native form of HSP90. Then, sections were incubated with streptavidin horse radish peroxidase (LSAB kit, Dako) for 15 minutes at room temperature and washed with PBS. 3'-Diaminobenzidine (DAB) was applied for 2 minutes or longer, until the desired staining intensity was developed. Finally, 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. (NewYork Microscope Company, USA)

Data collection and analysis

Ten sections from each animal in each of the four groups were examined microscopically and photographed with digital camera. Ten random areas from each section were analyzed by counting the total pixel area occupied by positive HSP90 staining in relation to the total pixels in each field in the sections examined, using Adobe Photoshop software, as described previously in the literature (Al-Jarrah et al., 2010; Al-Jarrah and Jamous, 2011).

Statistical analysis

HSP90 expression was analyzed, in the different skeletal muscles, and statistically compared among the four different groups using one-way analysis of variance followed by independent samples *t*-test using SPSS software version 19.0, (SPSS Inc., USA). Differences in HSP90 expression were

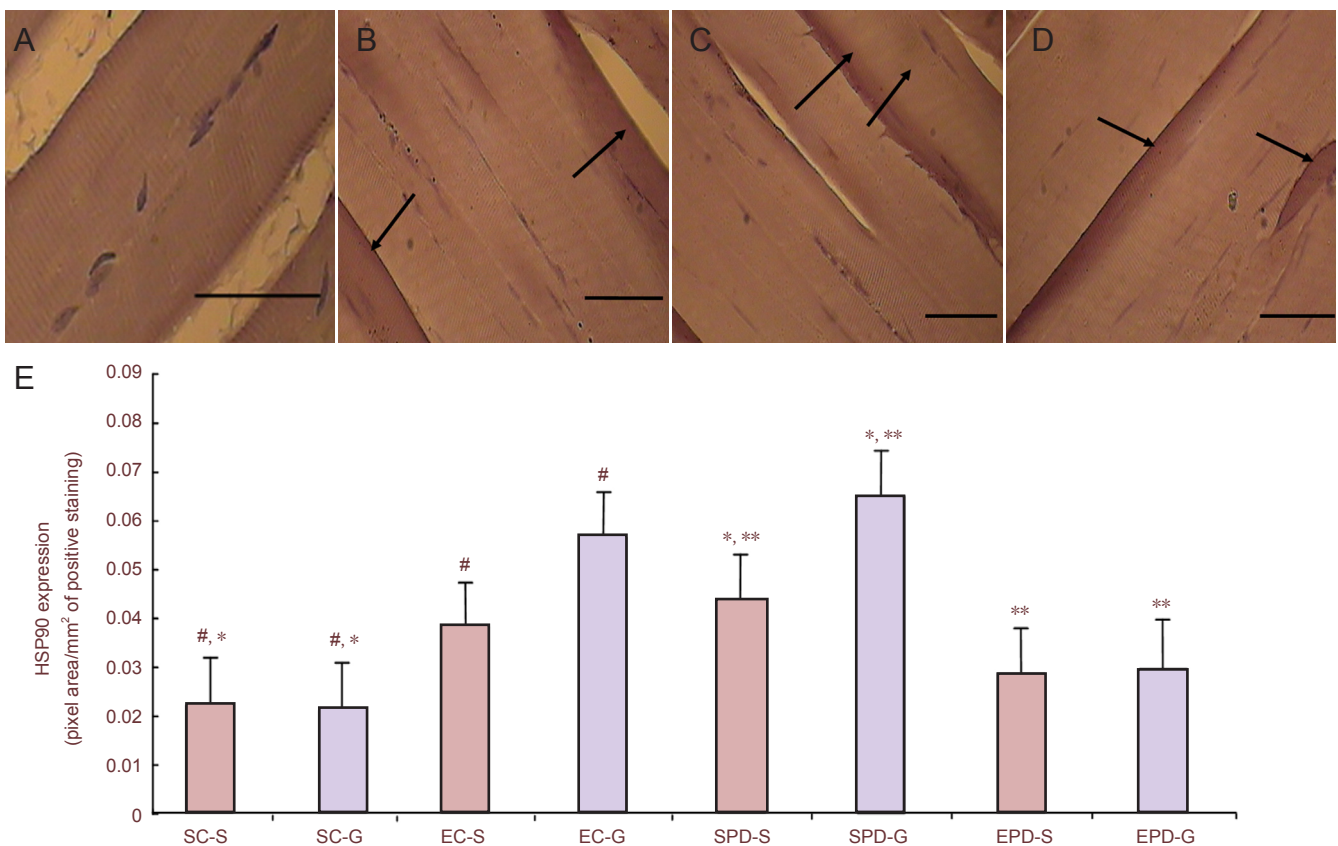


Figure 1 HSP90 expression level in soleus and gastrocnemius of PD mice following endurance exercise training.

(A–D) HSP90 immunostaining in 5- μ m-thick longitudinal paraffin-embedded gastrocnemius sections. (A) From sedentary control. (B) From exercised control. (C) From sedentary PD. (D) From Exercised PD. Scale bars: 50 μ m. HSP90 immunoreactivity (at the tip of the arrows) is strong in the sedentary PD gastrocnemius (C) and weak in the sedentary control gastrocnemius (A). Exercise training decreased HSP90 immunoreactivity (at the tip of the arrows) in the PD gastrocnemius (D) and increased it in the control gastrocnemius (B). The quantitative results are shown in E. Soleus results followed the same trend. (E) HSP90 expression in the soleus and gastrocnemius skeletal muscles. The level of HSP90 expression increased significantly in the sedentary PD soleus and gastrocnemius compared with the sedentary control soleus ($*P < 0.01$). Exercise training significantly increased HSP90 expression level in the control soleus ($\#P < 0.01$). However, exercise training significantly decreased HSP90 expression level in the PD soleus ($**P < 0.01$). PD: Parkinson's disease; HSP90: heat shock protein 90; SC-S: sedentary control soleus; SC-G: sedentary control gastrocnemius; EC-S: exercised control soleus; EC-G: exercised control gastrocnemius. SPD-S: sedentary Parkinson's disease soleus; SPD-G: sedentary Parkinson's disease gastrocnemius; EPD-S: exercised Parkinson's disease soleus; EPD-G: exercised Parkinson's disease gastrocnemius.

considered statistically significant at a P value less than 0.05.

Results

HSP90 immunostaining appeared to be associated with the striations in both soleus and gastrocnemius sections from all four groups: SC, EC, SPD, and EPD (Figure 1A–D). HSP90 was viewed to upregulate mainly at the sarcolemma of both types of skeletal muscles from EC, SPD, and EPD (Figure 1A–D). HSP90 expression in the PD soleus and gastrocnemius muscles was statistically significantly higher ($P < 0.01$) than in the control soleus (Figure 1E). However, HSP90 expression was significantly ($P < 0.01$) reduced in the exercised PD group when compared with the sedentary PD group (Figure 1E).

Discussion

This is the first study to report the impact of PD on HSP90 expression in both slow- and fast-twitch skeletal muscles and the resulting analysis reveals HSP90 upregulation in both slow- and fast-twitch skeletal muscles. Moreover, the present

study is the first one to show the effect of endurance exercise training on the slow- and fast-twitch PD skeletal muscles. Endurance exercise training decreased HSP90 expression in both slow- and fast-twitch skeletal muscles of PD mice.

HSP90 was detected in both slow- and fast-twitch skeletal muscles, and HSP90 expression appears to associate with the striations in both slow- and fast-twitch skeletal muscles. These observations may be consistent with the previous findings in both human and zebrafish skeletal muscles (Donlin et al., 2012), however, further ultrastructural investigation is required to confirm this observation. Very few studies have examined the effect of exercise training on skeletal muscles (Atalay et al., 2004; Harris et al., 2008), with results of this study revealing a significant increase in the HSP90 expression in both fast- and slow-twitch skeletal muscles following endurance exercise training, which are consistent with the findings demonstrated in the rat slow-twitch soleus after endurance exercise training (Harris et al., 2008). On the other hand, the study results are inconsistent with the findings shown in the rat fast-twitch gastrocnemius and vastus later-

alis muscles in response to endurance exercise training (Atalay et al., 2004), and consistent with the previous findings in both human and zebrafish skeletal muscles (Donlin et al., 2012). A previous study has shown that thermal therapy, namely Waon therapy, augmented ischemia-induced angiogenesis in a mouse model of hindlimb ischemia by upregulating HSP90 and endothelial nitric oxide synthase (eNOS; Miyauchi et al., 2012). HSP90 binds to eNOS, enhancing it and resulting in angiogenesis (Miyauchi et al., 2012), which is one of the skeletal muscle adaptations to exercise (Wagner, 2001; Yan et al., 2011) in agreement with our observation. The endurance exercise-induced HSP90 upregulation in the skeletal muscles revealed by our results may constitute skeletal muscle adaptations to exercise leading to the desired angiogenesis similar to that described for the Waon therapy (Miyauchi et al., 2012).

Based on a review of current literature, this study is the first to show alterations in HSP90 expression as a consequence of PD. The present study suggests that the induction of chronic PD by MPTP/probencid increased HSP90 expression in both slow- and fast-twitch skeletal muscles. HSP90 overexpression has been demonstrated in skeletal muscles of type 2 diabetic patients (Hojlund et al., 2003). It has been suggested that increased reactive oxygen species production and oxidative stress due to mitochondrial abnormalities and dysfunction in type 2 diabetic skeletal muscles have caused HSP90 overexpression (Hojlund et al., 2003). In addition to that, both mitochondrial abnormalities and HSP90 overexpression have been shown in PD brains (Keeney et al., 2006; Uryu et al., 2006). Indeed, HSP90 inhibition prior to treatment with MPTP prevents the induction of PD (Shen et al., 2005). Consistent with previous reports (Keeney et al., 2006; Uryu et al., 2006), the present results reveal HSP90 overexpression in PD skeletal muscles, with a potential link to myopathies associated with mitochondrial abnormalities that have been reported in PD patients (Gdynia et al., 2009). Mitochondrial abnormalities have been reported to increase reactive oxygen species and oxidative stress (Zuin et al., 2008). Thus, it could be inferred that HSP90 upregulation in the PD skeletal muscles as revealed by our results, is probably caused by the high levels of reactive oxygen species and oxidative stress resulting from the mitochondrial abnormalities present in PD skeletal muscles. HSP90 overexpression has been shown to be involved in inflammatory responses and diseases (Madrigal-Matute et al., 2010), whereas HSP90 inhibition has been shown to decrease inflammatory cytokines and inflammation (Madrigal-Matute et al., 2010, 2012). Thus, HSP90 overexpression revealed by current results is consistent with the finding of inflammatory myopathies in PD skeletal muscles (Gdynia et al., 2009), and also consistent with the HSP90 overexpression demonstrated in idiopathic inflammatory myopathies including sporadic inclusion body myositis and polymyositis (De Paepe et al., 2009).

Exercise has been suggested as an adjunct treatment in PD patients (Fisher et al., 2008; David et al., 2012; Konerth and Childers, 2013). To examine the mechanism by which

exercise ameliorates PD patients' motor symptoms, this study investigated the effect of endurance exercise training on HSP90 expression in PD skeletal muscles. HSP90 expression has been insignificantly altered in PD skeletal muscles following endurance exercise training (Sreedhar et al., 2003), with impaired heat shock protein response in PD skeletal muscles being suggested (Sreedhar et al., 2003). In contrast, current results reveal intact HSP90 response in the PD skeletal muscles, leading to postulation that endurance exercise training decreased HSP90 expression by promoting mitochondrial biogenesis. Exercise training has been reported to promote mitochondrial biogenesis by increasing mitochondrial content, volume and number (Menshikova et al., 2006; Palmer, 2010; Phielix et al., 2010; Safdar et al., 2011) which could account for reduced reactive oxygen species and oxidative stress, and the consequent HSP90 downregulation following endurance exercise training. Moreover, endurance exercise-induced HSP90 downregulation may decrease the inflammatory cytokines and inflammation in PD skeletal muscles. Therefore, results from this study are consistent with the report that exercise training is beneficial to PD skeletal muscles (Fisher et al., 2008; David et al., 2012; Konerth and Childers, 2013).

In conclusion, this study may be one of the first to report the impact of PD on HSP90 expression in skeletal muscles. In summary, HSP90 expression increased in both slow- (soleus) and fast-twitch (gastrocnemius) PD skeletal muscles, however, HSP90 overexpression in PD skeletal muscles is attenuated following endurance exercise training. Thus, HSP90 is probably implicated in skeletal muscle abnormalities seen in PD, and could be considered as a potential therapeutic target to ameliorate skeletal muscle abnormalities caused by PD.

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Conflicts of interest: *None declared.*

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