Acta Biochim Biophys Sin 2011, 43: 448–454 | © The Author 2011. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmr029.
Advance Access Publication 9 May 2011

Original Article

Cold water stress attenuates dopaminergic neurotoxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice

Mingfeng Xia[1†], Minjuan Bian[1†], Qian Yu[1], Jie Liu[1], Yufang Huang[1], Xueting Jin[1], Shiduo Lu[1], Mei Yu[1], and Fang Huang[1,2,3*]

[1]State Key Laboratory of Medical Neurobiology, Shanghai Medical College, Fudan University, Shanghai 200032, China
[2]Department of Neurobiology, Shanghai Medical College, Fudan University, Shanghai 200032, China
[3]Institutes of Brain Science, Fudan University, Shanghai 200032, China
[†]These authors contributed equally to this work.
*Correspondence address. Tel: +86-21-54237856; Fax: +86-21-64174579; E-mail: huangf@shmu.edu.cn

In the present study, we tested the effect of cold water stress (CWS) on dopaminergic neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's disease (PD) mouse model, and found that CWS pretreatment elicited less MPTP neurotoxicity. To understand the molecular mechanism underlying this phenomenon, we detected the expression of heat shock protein 70 (Hsp70) in the striatum of the experimental mice, and found that CWS pretreatment could significantly increase striatal Hsp70 in MPTP-treated mice. Furthermore, in parallel with the induction of Hsp70, the MPTP-induced increase of striatal α-synuclein was inhibited in the CWS + MPTP-treated mice. CWS pretreatment also significantly inhibited the reduction of anti-apoptotic molecule Bcl-2 expression in the striatum and enhanced *Bcl-2* transcription in the substantia nigra of MPTP-treated mice. Taken together, these data indicated that Hsp70 might be an important intermediate for the neuroprotective effect of CWS against MPTP-induced dopaminergic toxicity.

Keywords cold water stress; MPTP; Hsp70; α-synuclein; bcl-2

Received: October 21, 2010 Accepted: February 23, 2011

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra (SN) and the presence of Lewy bodies in residual neurons. The precise molecular mechanisms underlying PD pathogenesis remain unclear. Mitochondrial dysfunction, α-synuclein toxicity, oxidative stress, and ubiquitin–proteasome system insufficiency have been proved to contribute to PD [1–3]. The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in intravenous illicit drug users and its specific damage to the nigrostriatal dopaminergic pathway have made MPTP widely used in producing experimental PD models [4]. In the MPTP-induced PD model, oxidative stress is an early event that can kill some of the dopaminergic neurons directly, but it seems that the intracellular cell death-related molecular pathways activated by the oxidative stress play greater roles in the death of dopaminergic neurons, such as c-Jun N-terminal kinases and cellular apoptotic pathway [3]. The toxicity of MPTP depends on not only the regimen of MPTP administration [5], but also the microenvironment of the dopaminergic neurons. Cellular stress response has presented great potential in inhibiting experimental PD progress. Recent study has shown that mild thermal stress could partially protect striatal field potential against rotenone-induced neurotoxicity through up-regulation of heat shock protein (Hsp70) [6]. Growing evidence has also revealed that the continuous presence of a small stimulus could induce defense mechanisms, including heat shock proteins (HSPs), and protect brain cells from deleterious insults [7].

Hsps belong to the family of chaperone proteins that facilitate refolding misfolded proteins and directing proteins toward proteasomal degradation. Hsp70 is an inducible Hsp in cells after exposure to stress. A wide variety of stressors could induce the expression of Hsp70, such as hyperthermia, hypoxia, exercise, viral infection, reactive oxygen species, and ischemia [8]. It has been reported that overexpression of Hsp70 or induction of Hsp70 by pharmacological methods can prevent α-synuclein aggregation and toxicity both *in vitro* and *in vivo* [9,10]. On the other hand, the suppression of Hsp70 in a *Drosophila* PD model increases the toxicity of α-synuclein [11]. Furthermore,

stress-induced Hsp70 also inhibits neuronal apoptosis through increasing the expression of anti-apoptotic molecule bcl-2, and decreasing the release of cytochrome c [12]. All these findings indicated that Hsp70 may play an important role in protecting dopaminergic neurons against the MPTP neurotoxicity.

In the present study, we studied the effects of cold water stress (CWS) on MPTP-treated mouse brain to explore whether CWS could play a neuroprotective role. Furthermore, we also detected the mechanisms that might involve in the neuroprotective function of CWS.

Materials and Methods

Animals and treatments

Male C57BL/6 mice (11−13 weeks old, Shanghai Slac Laboratory Animal Company, China) weighing 25.0 ± 5.0 g were housed at 24°C under 12 h light/dark cycle with free access to food and water. All animal experiments were performed under the guidelines for animal experiments in Fudan University. These mice were randomly divided into four groups: saline, CWS, MPTP, and CWS + MPTP. CWS was performed once a day between 12:00 and 13:00 for 5 consecutive days. Mice were put in a ceramic container, which was 16 cm in diameter and 4 cm in depth, and filled with ice-chilled water for 5 min, and then they were gently wiped dry and returned to the cages. Mice of CWS and CWS + MPTP group were injected intraperitoneally with saline or MPTP at 15 min after CWS, respectively. Mice of saline or MPTP group were also injected intraperitoneally with saline or MPTP without treatment of CWS. The dosage of MPTP was 30 mg/kg/day for 5 consecutive days. The rectal temperatures were determined using a temperature monitor (69000 style, RWD Life Science Co. Ltd, China). Rectal temperatures were recorded before and 15 min after CWS, 15 and 60 min after saline or MPTP injection in CWS and CWS + MPTP groups. In saline and MPTP groups, rectal temperatures were measured before treatment, as well as 15 and 60 min after saline or MPTP injection. Then the rectal temperatures of mice in the MPTP, CWS, and CWS + MPTP groups were compared with that in the saline group at each time point, respectively.

Preparation of mouse brain tissue samples

Mice were sacrificed 24 h after last MPTP administration. They were anesthetized with 10% chloral hydrate to minimize suffering. After being perfused intracardially with 0.9% saline solution, brains were carefully removed. The right cerebral hemispheres of mice were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for section preparation, the striatum and SN of the left cerebral hemispheres were isolated on the ice for protein preparation and total RNA extraction, respectively.

Western blot analysis

The method for protein extraction and western blot analysis has been described elsewhere [13]. The primary antibodies used here were as followed: mouse anti-tyrosine hydroxylase (TH) antibody (1:4000; Sigma, St. Louis, USA); mouse anti-β-actin (C4) antibody (1:1000; Santa Cruz, Santa Cruz, USA); mouse monoclonal anti-Hsp70 antibody (1:400; Santa Cruz); rabbit anti-α-synuclein antibody (1:2000; Sigma); mouse anti-phosphorylated α-synuclein (1:400; Epitomics, Burlingame, USA); rabbit anti-bcl-2 antibody (1:1000; Beyotime, Haimen, China); rabbit anti-bax antibody (1:1000; Cell Signaling Technology, Beverly, USA). Signals were detected with a chemiluminescence detection system (Santa Cruz). The protein levels were quantified by densitometry analysis using Quantity One 4.5.2 software (Bio-Rad, Hercules, USA).

Immunohistochemistry

The right cerebral hemisphere was post-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Frozen sections were then prepared at 30 μm thick on the freezing microtome (Leica, Germany). Sections were selected every 120 μm per mouse ($n = 9-13$ per group). Immunohistochemistry was carried out according to the previously published methods [13]. As controls, adjacent sections were treated with the same procedure except for incubation with the primary antibody. Four sections (30 μm) were captured under light microscope per mouse. For measurement of the density of TH positive cells in the SN pars compacta (SNpc), we performed total cell counting and stereological counting based on the previous description [14]. Cell counting was performed under light microscope in a double-blind fashion.

Real-time PCR

Total RNA was extracted from SN of the left hemispheres using Trizol reagent (Invitrogen, Carlsbad, USA). Reverse transcription was carried out using random primer and Moloney murine leukemia virus reverse transcriptase (Promega, Madison, USA). Real-time PCR was performed for quantification of *Bcl-2*, *α-Synuclein*, *Hsp90*, *Hsp27*, *Hsp105*, and *Timm22* mRNA on ABI 7300 PCR machine (Applied Biosystems, Foster City, USA). For plotting a standard curve, serial diluted cDNA fragments were used in each experiment. The expression of target genes or *GAPDH* was quantified to the standard curve, and the relative expression value was calculated as the ratio of target cDNA to *GAPDH*. The primers used in the real-time PCR were as follows:

α-Synuclein forward: 5'-GTGGTTCATGGAGTGACAAC-3';
α-Synuclein reverse: 5'-AGGCTTCAGGCTCATAGTCT-3';
Bcl-2 forward: 5'-GGATTGTGGCCTTCTTTGAGTTCGG-3';
Bcl-2 reverse: 5'-CATATTTGTTTGGGGCAGGTTTGTC-3';

Hsp90 forward: 5'-CACCCTGCTCTGTACTACTACTCGG-3';
Hsp90 reverse: 5'-GCCAATGCCTGTGTCCACCAAAGTC-3';
Hsp27 forward: 5'-TCCGGAGGAGCTCACAGTGAAGACC-3';
Hsp27 reverse: 5'-CAGACTGTTCAGACTTCCCAGCTTC-3';
Hsp105 forward: 5'-TCTATTCTGACCCTCAAGGAGTTCC-3';
Hsp105 reverse: 5'-TGTTCCAGCTTCACTGTTGTCTTGC-3';
Timm22 forward: 5'-CGAGGAGCAGAAGATGATCGAGAGG-3';
Timm22 reverse: 5'-GATGACGCTGTTCTTCCAGTCCGAC-3';
GAPDH forward: 5'-GTAGACAAAATGGTGAAGGTCGGTG-3';
GAPDH reverse: 5'-CTCGCTCCTGGAAGATGGTGATGGG-3'.

Statistical analysis

Data were analyzed using SPSS software (version 11.5; SPSS, Chicago, USA). All values were expressed as means ± SE. Statistical analysis of group differences was assessed by ANOVA followed by multiple comparisons with the LSD *post-hoc* test. $P < 0.05$ was considered as the significant difference.

Results

MPTP significantly defers the recovery of mouse rectal temperature after CWS

CWS could rapidly decrease the mouse rectal temperature to ~25°C in 5 min. For mice treated with saline, the rectal temperature recovered rapidly and returned to the normal level in 1 h. However, for mice treated with MPTP after CWS, the rectal temperature recovery speed was significantly inhibited, especially during the first 15 min after MPTP injection (**Fig. 1**).

CWS attenuates MPTP-induced depletion of tyrosine hydroxylase protein and TH positive neurons in the nigrostriatal system of mice

MPTP induced significant depletion of striatal TH protein 24 h after the last injection. However, the pretreatment of CWS significantly inhibited the reduction of striatal TH caused by MPTP neurotoxicity [**Fig. 2(A,B)**]. Meanwhile, MPTP administration also reduced the number of TH-positive neurons in the SNpc of mice, while CWS pretreatment could inhibit the loss of TH-positive neurons in the SNpc of mice in the CWS + MPTP group [**Fig. 2(C,D)**].

Hsp70 expression is increased in the striatum of CWS + MPTP-treated mice

As one of the most important molecule chaperons induced by cellular stress response, Hsp70 has been shown to protect against α-synuclein toxicity and inhibit the loss of dopaminergic neurons. Here, we investigated the effect of

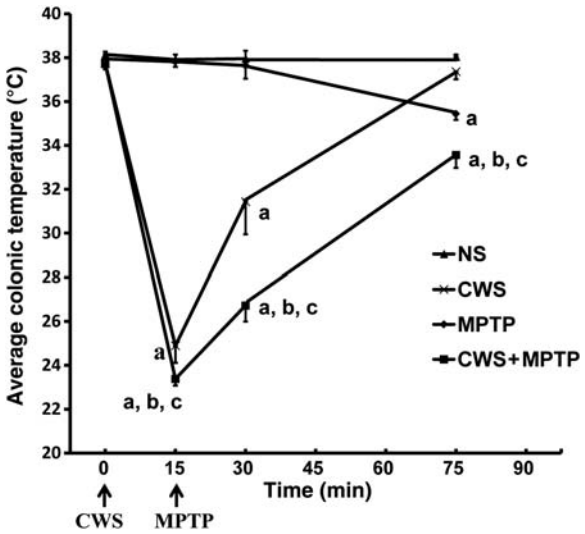

Figure 1 Alteration of average colonic temperatures of mice from four experimental groups during the CWS treatment and MPTP administration in five consecutive days (a) $P < 0.05$, significant differences compared with saline control group; (b) $P < 0.05$, significant differences between the groups of MPTP and CWS + MPTP; (c) $P < 0.05$, significant difference between the groups of CWS and CWS + MPTP ($n = 3$ per group).

CWS on the striatal Hsp70 expression in MPTP-treated mice. CWS or MPTP treatment alone could not increase the expression of striatal Hsp70. However, a combination treatment of CWS and MPTP remarkably increased the expression of Hsp70 in the mouse striatum compared with the other three groups [**Fig. 3(A,B)**].

CWS inhibits the MPTP-induced increase of α-synuclein protein level in the mouse striatum

Since Hsp70 has been shown to protect against α-synuclein toxicity, we examined the level of α-synuclein protein in the mouse striatum. MPTP injection significantly increased the expression of α-synuclein, while CWS pretreatment restored the amount of α-synuclein to normal level in the mice striatum exposed to MPTP [**Fig. 4(A,B)**]. Further, the level of phosphorylated α-synuclein in the mouse striatum was evaluated. CWS treatment, MPTP treatment, or the combination did not alter the expression of phosphorylated α-synuclein significantly [**Fig. 4(C,D)**].

CWS pretreatment inhibits the reduction of striatal Bcl-2 expression and enhanced *Bcl-2* transcription in the SN of CWS + MPTP-treated mice

One of the protective functions of Hsp70 is inhibiting neuronal apoptosis. We found that the pretreatment of CWS significantly inhibited the reduction of Bcl-2 expression in the striatum after MPTP treatment and upregulated *Bcl-2* transcription in the SN of CWS + MPTP-treated mice [**Figs. 5(A,B)** and **6(A)**]. However, the expression of pro-apoptotic molecule bax showed no significant difference among the four groups [**Fig. 5(C)**].

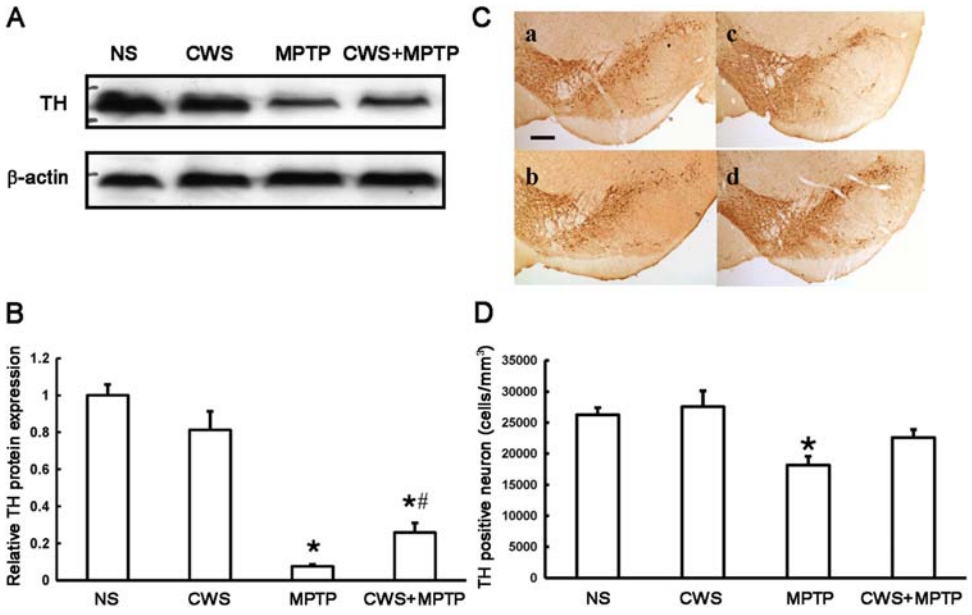

Figure 2 Levels of striatal TH protein and numbers of TH positive neurons in the SNpc (A) Western blot analysis of TH protein in the striatum of mice treated with saline, CWS, MPTP, or CWS + MPTP. β-Actin served as the loading control. (B) Quantification of relative TH protein levels. Data presented are the means ± SE. $^{*}P < 0.05$, significant differences between groups of saline or CWS and groups of MPTP or CWS + MPTP; $^{\#}P \leq 0.05$, significant differences between groups of MPTP and CWS + MPTP. $n = 9–13$ per group. (C) Immunohistochemical staining of TH in the SN. (a) Saline (NS), (b) CWS, (c) MPTP, (d) and CWS + MPTP. Scale bar, 20 μm. (D) Statistical data of the number of TH positive neurons in the SNpc. Bars represent means ± SE; $^{*}P < 0.05$, significant differences between group of saline and group of MPTP. $n = 9–13$ per group.

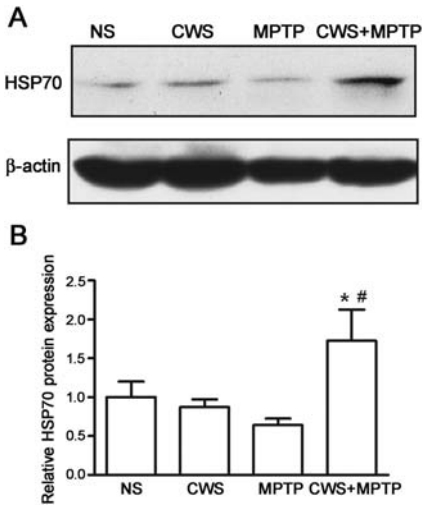

Figure 3 Levels of striatal Hsp70 protein expression (A) Western blot analysis of Hsp70 protein in the striatum of mice. β-Actin served as the loading control. (B) Quantification of relative Hsp70 protein. Data presented are the means ± SE. $^{*}P < 0.05$, significant differences between group of saline and group of CWS + MPTP; $^{\#}P < 0.05$, significant differences between groups of MPTP and CWS + MPTP. $n = 9–13$ per group.

CWS pretreatment or MPTP treatment does not affect the transcriptions of *Hsp90*, *Hsp105*, *Hsp27*, and *α-Synuclein* in the SN

We also detected the transcriptional levels of other members in the Hsp family, including *Hsp90*, *Hsp105*, and *Hsp27*, as well as *α-Synuclein* in the SN. The mRNA levels of *Hsp90*, *Hsp105*, *Hsp27*, and *α-Synuclein* did not show significant alterations within the experimental groups [**Fig. 6(B–E)**]. *Timm22*, a non-affected gene, also showed no changes [**Fig. 6(F)**].

Discussion

Our study showed that short-time CWS pretreatment before MPTP administration inhibited the depletion of TH protein and TH-positive neurons in MPTP-induced PD mouse model. To the best of our knowledge, this study is the first time to show the potential of CWS to alleviate the MPTP toxicity to dopaminergic neurons.

The previous study has shown that lowering ambient temperature actually elevated striatal 1-methyl-4-phenylpyridinium (MPP^{+}) levels and enhanced toxicity to dopamine neurons in PD mice under subacute MPTP regimen [15], which seems contradictory to our conclusion. But it is noticeable that they lowered mouse body temperature by placing the mice in a 4°C room, and mildly decreased mouse rectal temperature to ~35°C in 2 h. The CWS used in our study was an acute and much stronger stress for mice compared with mere cold environment and reduced the mouse rectal temperature to ~20°C in 5 min. Thus, we suppose a neuroprotective system against MPTP toxicity was probably activated by CWS treatment and MPTP injection. Recently, it has been reported that physiological stressors, such as mild hyperthermia or exercise, could

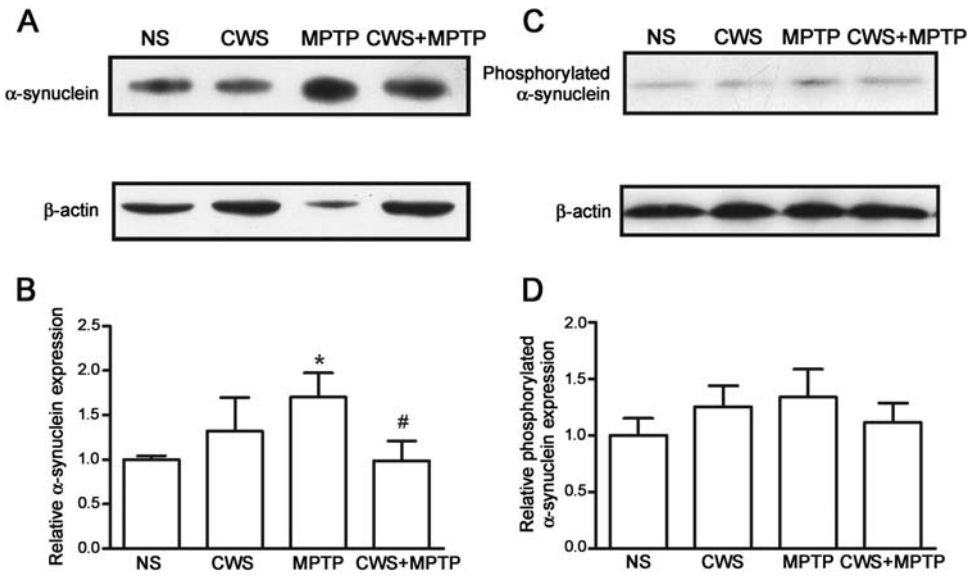

Figure 4 Levels of striatal α-synuclein and phosphorylated α-synuclein protein (A) Western blot analysis of α-synuclein protein in the striatum of mice. β-Actin served as the loading control. (B) Quantification of relative α-synuclein protein. Data presented are the means ± SE. $^{*}P < 0.05$, significant differences between group of saline and group of MPTP; $^{\#}P < 0.05$, significant differences between groups of MPTP and CWS + MPTP; $n = 9–13$ per group. (C) Western blot analysis of striatal phosphorylated α-synuclein in experimental mice. β-Actin served as the loading control. (D) Quantification of relative phosphorylated α-synuclein protein. Data presented are the means ± SE. $n = 9–13$ per group.

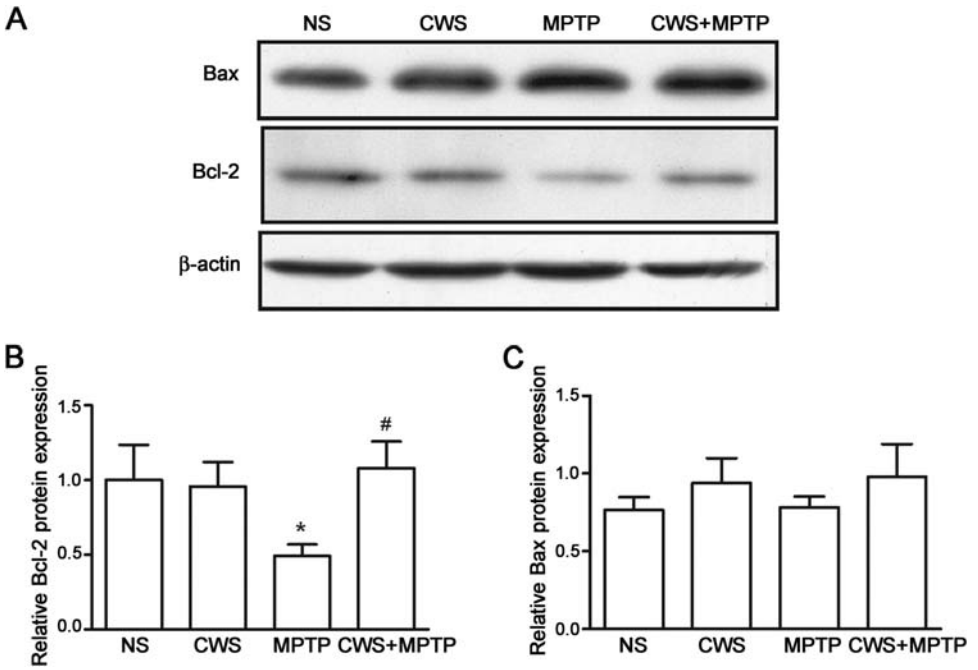

Figure 5 Levels of striatal Bcl-2 and Bax protein (A) Western blot analysis of Bcl-2 and Bax protein in the striatum of mice. β-Actin was served as the loading control. (B) Quantification of relative Bcl-2 protein. Data presented are the means ± SE. $^{*}P < 0.05$, significant differences between group of saline and group of MPTP; $^{\#}P < 0.05$, significant differences between groups of MPTP and CWS + MPTP. $n = 9–13$ per group. (C) Quantification of relative Bax protein. Data presented are the means ± SE. $n = 9–13$ per group.

inhibit the loss of neurons induced by MPTP or cerebral ischemia through upregulation of Hsp70 [16,17], so we detect the Hsp70 expression in CWS- and MPTP-treated mouse striatum in this study.

Hsp70 is mainly induced when the cells are exposed to environmental and physiological stressors. As a major molecular chaperone, Hsp70 may prevent protein aggregation and mediate the proper refolding of proteins in cells under a variety of conditions [18]. We found that CWS or MPTP treatment alone could not induce Hsp70 expression in the striatum, which was in accordance with previous studies [19,20]. However, when the CWS and MPTP

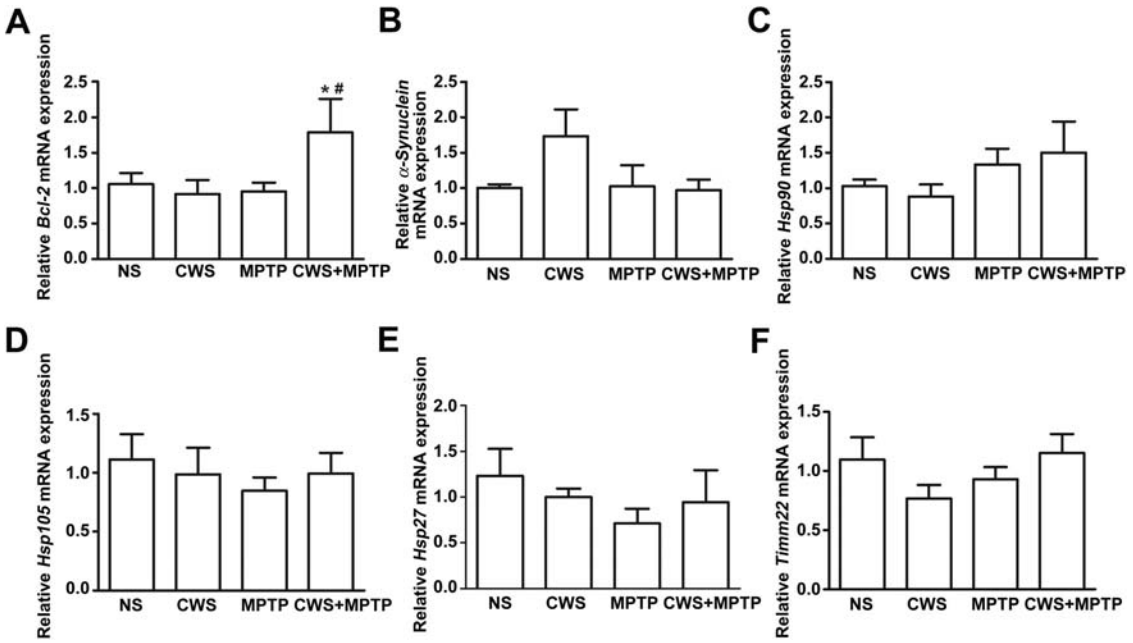

Figure 6 Transcriptions of *Bcl-2*, *α-Synuclein*, *Hsp90*, *Hsp27*, *Hsp105*, and *Timm22* in the SN determined by real-time PCR All samples were normalized to *GAPDH*. Values are shown as means ± SE. (A) *Bcl-2*, $^{*}P < 0.05$, significant differences between groups of saline and CWS + MPTP; $^{\#}P < 0.05$, significant differences between groups of MPTP and CWS + MPTP. (B) *α-Synuclein*; (C) *Hsp90*; (D) *Hsp105*; (E) *Hsp27*; and (F) *Timm22*. Values are shown as means ± E. $n = 7–13$ per group.

treatment were combined, we observed a significant elevation of striatal Hsp70 protein level. This finding indicated that the elevation of Hsp70 might be one of the mechanisms underlying the neuroprotective function of CWS against MPTP toxicity. However, the reason why Hsp70 was elevated in CWS + MPTP group but not in CWS or MPTP group was still unclear. One possible explanation is that there is a stress–strength threshold in activating Hsp70 expression. Neither CWS nor MPTP treatment alone was strong enough to induce Hsp70 upregulation. But when the two stresses combined together, CWS pretreatment could pre-excite the cellular stress response system, and MPTP administration as the second stress, thus activated Hsp70 expression. Previous studies have reported that the upregulation of Hsp70 protein depends on the strength, duration, type, and interval of environmental stresses [20,21], which also support the existence of such a strength threshold for Hsp70 induction.

α-Synuclein is known to be one of the main substrates of Hsp70 and an important molecule involving in PD process. Homeostasis of α-synuclein is important for normal neural physiological functions, and overexpression of α-synuclein in the mouse SN leads to a 25% reduction in dopaminergic neurons [22]. Upregulation of Hsp70 could not only facilitate stabilizing the native conformation of α-synuclein, but also promote α-synuclein degradation via chaperone-mediated autophagy and/or the proteasome [23]. In the present study, we found that MPTP intoxication alone significantly enhanced the expression of α-synuclein, which was in accordance with previous studies [24]. However, CWS pretreatment followed by MPTP administration restored the amount of α-synuclein protein to its normal level in the mouse striatum. Since excessive α-synuclein protein is prone to aggregate and form filamentous fibrils in PD, Hsp70 induced by the combination of CWS and MPTP possibly maintains the striatal α-synuclein by accelerating its degradation, thus protecting neurons in the nigrostriatal system [25].

In addition, the toxicity of α-synuclein is also enhanced by phosphorylation at serine 129 of this protein [26]. CWS could rapidly decrease the phosphorylation of α-synuclein in the mouse striatum, and recover thereafter [27]. Though we did not find significant depletion of α-synuclein phosphorylation at our experimental time point, a transitory dephosphorylation of α-synuclein induced by CWS might still contribute to the protective function of CWS in our model.

The current study also showed an inhibition of the decrease of Bcl-2 protein in the mouse striatum and an upregulation of *Bcl-2* transcription within the mouse SN of CWS + MPTP group, which could partially contribute to the neuroprotective function of CWS against MPTP toxicity. It has been reported that overexpressing Hsp70 causes less apoptotic cell death and increases the expression of the anti-apoptotic molecule, Bcl-2 [12], so the elevation of *Bcl-2* transcription in the present study might also correlate with the induction of Hsp70 by CWS and MPTP treatment.

Overall, CWS reduces dopaminergic neurotoxicity induced by MPTP. The elevation of Hsp70 protein might partially explain CWS neuroprotective function, and be associated with the alteration of α-synuclein and Bcl-2 expression in the CWS- and MPTP-treated mice. This study is the first to show the potential of CWS to alleviate the injury to dopaminergic neurons and defer PD process.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (30770659, 90919004), College Science Foundation of Fudan University, and Program of IOBS and Program for NCET to F.H.

References

1 Meredith GE, Totterdell S, Petroske E, Santa Cruz K, Callison RC, Jr and Lau YS. Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of Parkinson's disease. Brain Res 2002, 956: 156–165.

2 Shadrina MI and Slominskii PA. Mitochondrial dysfunction and oxidative damages in the molecular pathology of Parkinson's disease. Mol Biol (Mosk) 2008, 42: 809–819.

3 Zhou C, Huang Y and Przedborski S. Oxidative stress in Parkinson's disease: a mechanism of pathogenic and therapeutic significance. Ann NY Acad Sci 2008, 1147: 93–104.

4 Langston JW, Ballard P, Tetrud JW and Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 1983, 219: 979–980.

5 Petroske E, Meredith GE, Callen S, Totterdell S and Lau YS. Mouse model of Parkinsonism: a comparison between subacute MPTP and chronic MPTP/probenecid treatment. Neuroscience 2001, 106: 589–601.

6 Tantucci M, Mariucci G, Taha E, Spaccatini C, Tozzi A, Luchetti E and Calabresi P, *et al*. Induction of heat shock protein 70 reduces the alteration of striatal electrical activity caused by mitochondrial impairment. Neuroscience 2009, 163: 735–740.

7 Calabrese V, Cornelius C, Mancuso C, Pennisi G, Calafato S, Bellia F and Bates TE, *et al*. Cellular stress response: a novel target for chemoprevention and nutritional neuroprotection in aging, neurodegenerative disorders and longevity. Neurochem Res 2008, 33: 2444–2471.

8 Madden LA, Sandström ME, Lovell RJ and McNaughton L. Inducible heat shock protein 70 and its role in preconditioning and exercise. Amino Acids 2008, 34: 511–516.

9 McLean PJ, Klucken J, Shin Y and Hyman BT. Geldanamycin induces Hsp70 and prevents alpha-synuclein aggregation and toxicity in vitro. Biochem Biophys Res Commun 2004, 321: 665–669.

10 Dong Z, Wolfer DP, Lipp HP and Bueler H. Hsp70 gene transfer by adeno-associated virus inhibits MPTP-induced nigrostriatal degeneration in the mouse model of Parkinson disease. Mol Ther 2005, 11: 80–88.

11 Auluck PK, Chan HY, Trojanowski JQ, Lee VM and Bonini NM. Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. Science 2002, 295: 865–868.

12 Yenari MA, Liu J, Zheng Z, Vexler ZS, Lee JE and Giffard RG. Antiapoptotic and anti-inflammatory mechanisms of heat-shock protein protection. Ann NY Acad Sci 2005, 1053: 74–83.

13 Bian M, Yu M, Yang S, Gao H, Huang Y and Huang F. Expression of Cbl-interacting protein of 85 kDa in MPTP mouse model of Parkinson's disease and 1-methyl-4-phenyl-pyridinium ion-treated dopaminergic SH-SY5Y cells. Acta Biochim Biophys Sin 2008, 40: 505–512.

14 Zhang R, Zhang Z, Wang L, Wang Y, Gousev A, Zhang L and Ho KL, *et al*. Activated neural stem cells contribute to stroke-induced neurogenesis and neuroblast migration toward the infarct boundary in adult rats. J Cereb Blood Flow Metab 2004, 24: 441–448.

15 Moy LY, Albers DS and Sonsalla PK. Lowering ambient or core body temperature elevates striatal MPP^+ levels and enhances toxicity to dopamine neurons in MPTP-treated mice. Brain Res 1998, 790: 264–269.

16 Chen YW, Chen SH, Chou W, Lo YM, Hung CH and Lin MT. Exercise pretraining protects against cerebral ischaemia induced by heat stroke in rats. Br J Sports Med 2007, 41: 597–602.

17 Quigney DJ, Gorman AM and Samali A. Heat shock protects PC12 cells against MPP^+ toxicity. Brain Res 2003, 993: 133–139.

18 Ben-Zvi A, De Los Rios P, Dietler G and Goloubinoff P. Active solubilization and refolding of stable protein aggregates by cooperative unfolding action of individual hsp70 chaperones. J Biol Chem 2004, 279: 37298–37303.

19 Shen HY, He JC, Wang Y, Huang QY and Chen JF. Geldanamycin induces heat shock protein 70 and protects against MPTP-induced dopaminergic neurotoxicity in mice. J Biol Chem 2005, 280: 39962–39969.

20 Tolson JK and Roberts SM. Manipulating heat shock protein expression in laboratory animals. Methods 2005, 35: 149–157.

21 Freyaldenhoven TE and Ali SF. Role of heat shock proteins in MPTP-induced neurotoxicity. Ann N Y Acad Sci 1997, 825: 167–78.

22 St Martin JL, Klucken J, Outeiro TF, Nguyen P, Keller-McGandy C, Cantuti-Castelvetri I and Grammatopoulos TN, *et al*. Dopaminergic neuron loss and up-regulation of chaperone protein mRNA induced by targeted over-expression of alpha-synuclein in mouse substantia nigra. J Neurochem 2007, 100: 1449–1457.

23 Witt SN. Hsp70 molecular chaperones and Parkinson's disease. Biopolymers 2010, 93: 218–228.

24 Vila M, Vukosavic S, Jackson-Lewis V, Neystat M, Jakowec M and Przedborski S. α-synuclein up-regulation in substantia nigra dopaminergic neurons following administration of the parkinsonian toxin MPTP. J Neurochem 2000, 74: 721–729.

25 Klucken J, Shin Y, Masliah E, Hyman BT and McLean PJ. Hsp70 reduces α-synuclein aggregation and toxicity. J Biol Chem 2004, 279: 25497–25502.

26 Chen L and Feany MB. Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. Nat Neurosci 2005, 8: 657–663.

27 Hirai Y, Fujita SC, Iwatsubob T and Hasegawac M. Phosphorylated α-synuclein in normal mouse brain. FEBS Lett 2004, 572: 227–232.