Geldanamycin Induces Heat Shock Protein 70 and Protects against MPTP-induced Dopaminergic Neurotoxicity in Mice*

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As key molecular chaperone proteins, heat shock proteins (HSPs) represent an important cellular protective mechanism against neuronal cell death in various models of neurological disorders. In this study, we investigated the effects of geldanamycin (GA), an inhibitor of Hsp90, on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity, a mouse model of Parkinson disease. Neurochemical analysis showed that pretreatment with GA (via intracerebral ventricular injection 24 h prior to MPTP treatment) increased residual dopamine content and tyrosine hydroxylase immunoreactivity in the striatum 24 h after MPTP treatment. To dissect out the molecular mechanism underlying this neuroprotection, we showed that the GA-mediated protection against MPTP was associated with a reduction of cytosolic Hsp90 and an increase in Hsp70, with no significant changes in Hsp40 and Hsp25 levels. Furthermore, in parallel with the induction of Hsp70, striatal nuclear HSF1 levels and HSF1 binding to heat shock element sites in the Hsp70 promoter were significantly enhanced by the GA pretreatment. Together these results suggested that the molecular cascade leading to the induction of Hsp70 is critical to the neuroprotection afforded by GA against MPTP-induced neurotoxicity in the brain and that pharmacological inhibition of Hsp90 may represent a potential therapeutic strategy for Parkinson disease.

PD* is the second most common human neurodegenerative disorder, affecting 1–3% of people over 65 years old and ~10% of those over 80 years old (1–4). The hallmark of PD pathology is the progressive loss of dopaminergic neurons in substantia nigra, leading to profound depletion of dopamine in the striatum, and consequently severe movement abnormalities (1, 3, 4). These pathological changes of PD are associated with formation of Lewy bodies (enriched for α-synuclein) in neurites of dopaminergic and non-dopaminergic neurons (1, 5). For the last three decades, despite the symptomatic relief offered by L-3,4-dihydroxyphenylalanine, no effective neuroprotective strategy for PD has emerged to halt or slow down dopaminergic neurodegeneration (1, 3–6).

Heat shock proteins (HSPs) represent an important cellular protective mechanism against a variety of stresses and insults (7–9). HSPs are a large family of evolutionarily conserved proteins consisting of subfamilies with molecular masses of ~110, 90, 70, 60, 40, and 15–30 kDa (8, 10, 11). Some HSPs (such as Hsp40 and Hsp90) are constitutively expressed, whereas others (such as Hsp70 and Hsp27) are mainly induced after exposure of cells to environmental and physiological stressors (12–15). A large body of evidence supports a critical role for HSPs in cellular protection against a variety of stressors and insults, including heat, hypoxia, ischemia, excitotoxicity, glucose deprivation, cancer, and aging (13, 16–24). The cellular protection of HSPs is attributed to their molecular chaperone function by facilitating nascent protein folding, refolding, or degradation of abnormally folded protein (8, 10). Given the critical role of HSPs in protein stabilization, folding, and assembly, it is not surprising that HSPs are involved in the pathogenesis of a variety of neurodegenerative disorders characterized by abnormal protein aggregation. For example, HSPs have been implicated in several neurodegenerative disorders such as Huntington disease (20, 21, 25), amyotrophic lateral sclerosis (18), spinocerebellar ataxia type 1 (26), spinal and bulbar muscular atrophy (27), and Tau neuropathology in the brain (28, 29). Recent evidence suggests that HSPs may also be involved in neuronal cell death in PD (30–35). Genetic studies in Drosophila showed that overexpression of Hsp70 attenuated the death of dopaminergic neurons induced by transgene expression of α-synuclein (30, 35). Moreover, induction of Hsp70 by prior exposure to heat shock protects against 1-methyl-4-phenylpyridinium ion (MPP+)–induced neuronal cell death in cultured PC12 cells (36) and against α-synuclein aggregation in human H4 neuroglioma cells (32, 33). Similarly, overexpression of Hsp70 in dopaminergic neurons using adeno-associated virus inhibits MPTP-induced nigrostriatal degeneration in mice (37). Hsp70 was found in the Lewy bodies of the postmortem tissues of a sporadic PD patient (30, 38). These experimental findings are further supported by a recent epidemiological study showing that the susceptibility to PD may be associated with a potential polymorphism in the α promoter region of the hsp70-1 gene (39). Thus, HSPs may represent an important molecular target for neuroprotective strategies in PD treatment.

Pharmacological manipulation of HSPs in the brain may provide a potential approach to halt or slow down dopaminergic neurodegeneration in PD. Geldanamycin (GA) is a naturally occurring benzoquinone ansamycin that specifically binds to and inhibits the molecular chaperone Hsp90 (40, 41). GA has been shown to protect against neuronal damage induced by ischemia and huntingtin aggregates, through the induction of Hsp70 in the brain (17, 25). GA also prevents dopaminergic neuron death caused by overexpression of α-synuclein in Drosophila (35) and attenuates α-synuclein aggregation and neurotoxicity in human H4 neuroglioma cells (32, 33). However, these studies have been limited to cultured cell models and Drosophila, and it is not known whether GA can offer a neuroprotective effect in an in vivo rodent...
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MATERIALS AND METHODS

Animals and Drug Treatments—All experiments were performed in accordance with the Boston University School of Medicine and National Institutes of Health guidelines on the ethical use of animals. Male C57BL/6 mice (purchased from The Jackson Laboratory, Bar Harbor, ME, weight 25–30 g) were housed in plastic cages and provided free access to food and water. Animals were maintained in temperature- and humidity-controlled rooms with a 12-h light-dark cycle (light from 6:00 a.m. to 6:00 p.m.). Mice were randomly divided into four treatment groups (as described in the legend for Fig. 1): S+S, saline pretreatment followed by saline treatment; S+M, saline pretreatment followed by MPTP treatment; G+S, GA pretreatment followed by saline treatment; G+M, GA pretreatment followed by MPTP treatment. Pretreatment with GA (Sigma) or saline was perfused by stereotaxical (intracerebral ventricular) injection, under anesthesia, into the lateral cerebral ventricles in a dosage of 1 or 10 μg/kg at a rate of 1 μl/min using a micro syringe pump. GA was administered at 72 or 24 h prior to, or co-administered with, or 6 h after the MPTP treatment (40 mg/kg, intraperitoneal) or saline treatment. Mice were sacrificed at 24 h or 7 days after the MPTP treatment.

Dopamine Measurements—The striatum was dissected out from the right cerebral hemisphere, frozen on dry ice, and stored at −80 °C until used. Each striatum was weighed and homogenized in 150 mM phosphoric acid and 0.2 mM EDTA and placed on ice for 1 h. The homogenate then was centrifuged at 12,000 × g for 15 min at 4 °C. Supernatant was analyzed for dopamine content using standard reverse-phase HPLC with electrochemical detection as described previously (42). Briefly, biogenic amines were separated on a C-18, 5-

Western Immunoblotting—For Western blots, the whole cell extracts (30 μg) or nuclear proteins (10–20 μg) were resuspended in Laemmli buffer and applied to a 10–20% gradient Tris-glycine gel (Invtrogen). After blotting, the membranes were incubated with antibodies against Hsp90 (dilution 1:2,000), Hsp70 (1:4,000), Hsp40 (1:2,000), Hsp25 (1:1,000), and HSF1 (1:4,000) (all HSP antibodies from Stressgen, Victoria, BC Canada). The specific HSP bands were developed by incubating the blot with peroxidase-conjugated anti-rabbit antibody or anti-mouse antibody (1:5,000; Vector Laboratories, Burlingame, CA). Quantification of the immunoblot was performed by optical densitometric (OD) scanning using a UVP BioImaging acquisition and image analysis system (Lab Works). To normalize HSP immunoreactivity, β-actin protein was measured on the same blot with a mouse monoclonal anti-β-actin antibody (1:5,000; Sigma), and the OD ratio of the HSPs/β-actin was calculated.

Statistical Analysis—Data from all experiments were analyzed using parametric statistics with Student’s t test or one-way ANOVA followed by post hoc Bonferroni test, as appropriate.

RESULTS

Intraventricular Administration of GA Attenuates MPTP-induced Depletion of Dopamine and Tyrosine Hydroxylase Immunoreactivity in Mouse Striatum—Since GA is a large molecule (molecular weight 156 to 117 bp of the Hsp70 promoter (46) and its complementary strand). After incubating in binding buffer (20 μM HEPES, pH 7.9, 40 mM KCl, 1.0 mM MgCl2, 0.1 mM EDTA, 0.5 mM dithiothreitol) for 20 min at room temperature, the samples were loaded onto non-denaturing 4% polyacrylamide gels and electrophoresed for 2 h at 200 V. The gel was dried under vacuum and exposed to Hyperfilm (Amersham Biosciences) at −80 °C. Supershift studies were carried out using an antibody against HSF1 (Stressgene, Victoria, BC Canada). The protein sample was incubated with HSF1 antibody at 1:10 dilution for 30 min prior to incubate with HSE oligonucleotides.

Statistical Analysis—Data from all experiments were analyzed using parametric statistics with Student’s t test or one-way ANOVA followed by post hoc Bonferroni test, as appropriate.

RESULTS

Intraventricular Administration of GA Attenuates MPTP-induced Depletion of Dopamine and Tyrosine Hydroxylase Immunoreactivity in Mouse Striatum—Since GA is a large molecule (molecular weight 560.64) with poor blood-brain barrier permeability, we employed intracerebral ventricular injection to deliver GA into the brain to test its ability to protect against MPTP-induced dopaminergic toxicity in mice. Twenty-four hours after pretreatment of GA (1 or 10 μg/kg of body weight = 0.03–0.3 μg/mice), mice were treated with a single dose of MPTP and examined at 24 h after the MPTP treatment (Fig. 1A). HPLC analysis showed that striatal dopamine content in the GA-saline group

model of PD. In this study, we examined the effect of GA on MPTP-induced dopaminergic neurotoxicity in mice. We further investigated the molecular mechanism underlying the potential neuroprotective actions of GA in the MPTP model of PD.
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Intraventricular injection of GA protected against MPTP-induced dopaminergic neurotoxicity in mouse striatum. Adult male mice were injected stereotaxically with GA (1 or 10 μg/kg) or saline (SAL) into the left cerebral ventricle (intracerebral ventricular) at 72 or 24 h prior to, co-administered with, or 6 h after the MPTP (40 mg/kg) or saline treatment (intraperitoneal) (A and C). Mice were randomly assigned to six different treatment groups (n = 6–8 per group in A; n = 6–7 in C) as follows: S+S, saline pretreatment followed by saline treatment; S+M, saline pretreatment followed by MPTP treatment; G+S, GA pretreatment followed by saline treatment; G+M, GA pretreatment followed by MPTP treatment; M+S, MPTP treatment followed by saline after treatment; M+G, MPTP treatment followed by GA after treatment. At 24 h or 7 days after the MPTP treatment, the mouse striatum was dissected out from one hemisphere for HPLC analysis of dopamine content (Fig. 1A), and the other hemisphere was used for striatal TH-IR immunohistochemistry (B). *

was slightly higher than the saline-saline group but did not reach statistical significance (Fig. 1A). MPTP treatment reduced striatal dopamine content by ~50% when compared with the saline-treated mice (n = 6, p < 0.001, one-way ANOVA, post hoc Bonferroni). Importantly, pretreatment with GA at the dose of 10 μg/kg completely prevented the loss of striatal dopamine content by MPTP treatment (n = 9 for Fig. 1A, p < 0.05, one-way ANOVA, post hoc Bonferroni), whereas GA at the dose of 1 μg/kg was not effective (Fig. 1A).

Furthermore, GA pretreatment increased not only residual dopamine content but also striatal tyrosine hydroxylase immunoreactivity (TH-IR, a marker of dopaminergic terminals) in the striatum (Fig. 1B) and substantia nigra (data not shown). Representative striatal sections of TH-IR are shown in Fig. 1B (left panel). Although MPTP treatment markedly reduced TH-IR in the striatum, intracerebral ventricular injection of GA (24 h prior to MPTP treatment) significantly increased striatal TH-IR levels when compared with the vehicle-pretreated group (Fig. 1B). Densitometric analysis of TH-IR showed that GA pretreatment significantly attenuated the reduction of TH-IR by MPTP in the striatum (Fig. 1B, right panel, n = 4, p < 0.05, one-way ANOVA, post hoc Bonferroni). Together, these data clearly demonstrate that intraventricular pretreatment with GA protects against MPTP-induced dopaminergic neurotoxicity (i.e. depletion of striatal dopamine content as well as dopaminergic terminals).

Moreover, we further examined the effect of pretreatment with GA (10 μg/kg) when delivered 72 and 24 h prior to, as well as co-administration with, or 6 h after the MPTP treatment on residual dopamine content in the striatum (Fig. 1C). GA pretreatment alone did not alter dopamine content in the striatum after the saline treatment. Again, the neuroprotective effect of GA was observed when delivered 24 h prior to the MPTP (Fig. 1C, n = 6, p < 0.05, one-way ANOVA, post hoc Bonferroni). The experiment was repeated for the third time and showed similar neuroprotective effect by GA (10 μg/kg) pretreatment at 24 h prior to the MPTP injection in the striatum (data not shown). In contrast, all the other three time points examined (i.e. treatment with GA at 72 h prior to, or co-administration with, or 6 h after the MPTP treatment) were not effective in protecting against striatal dopamine depletion (Fig. 1C, n = 6–9, p > 0.05, one-way ANOVA, post hoc Bonferroni). Finally, we also examined the effect of GA pretreatment (24 h prior to MPTP treatment) on striatal dopamine content at 7 days after the MPTP treatment. Although GA pretreatment tended to enhance striatal dopamine content at 7 days after the MPTP treatment (Fig. 1C, right panel), the residual dopamine contents between the saline-pre-
treated group and the GA-pretreated group did not reach statistical significance ($p = 0.08$, $n = 6$, one-way ANOVA, post hoc Bonferroni).

**Intraventricular Injection of GA Decreased Hsp90 Protein Level in the Striatum of Control and MPTP-treated Mice**—To understand the molecular mechanism underlying GA-mediated neuroprotection in MPTP-treated mice, we examined the effect of GA pretreatment on Hsp90 levels in the striatum of MPTP-treated mice. Twenty-four hours after the saline or MPTP treatment, the Hsp90 levels were significantly lower in the G+S group when compared with the S+S group (Fig. 2A, $n = 6$, $p < 0.05$). Similarly, striatal Hsp90 levels in the G+M group were also lower than the S+M group (Fig. 2A, $n = 6$, $p < 0.05$). However, MPTP treatment alone did not affect striatal Hsp90 level (Fig. 2A, comparing the S+M group with the S+S group). The combined GA pretreatment and MPTP treatment did not further reduce Hsp90 when compared with the G+S group (Fig. 2A). Furthermore, the reduction of Hsp90 level following GA pretreatment (either in saline- or MPTP-treated mice) was observed only when GA was delivered 24 h prior to the MPTP treatment. When GA was delivered 72 h prior to the MPTP treatment, Hsp90 levels in GA-pretreated mice were not different from the saline-treated group.

GA Pretreatment Induces Expression of Hsp70 in the Striatum of MPTP-treated Mice—Since the expression of Hsp70 has been shown to protect against $\alpha$-synuclein toxicity in *Drosophila* (31, 35) as well as cultured cells (32, 33), and since GA alteration can induce the expression of Hsp70 in tumor (47) and neuronal cells (17), we investigated the effect of GA pretreatment on the expression of Hsp70 in the striatum of MPTP-treated mice. GA pretreatment alone tended to increase the expression of Hsp70 but did not reach statistical significance (Fig. 3A). MPTP treatment alone also did not alter the expression of Hsp70 in the

**FIGURE 2.** GA pretreatment reduced Hsp90 levels in mouse striatum. Four different groups of mice were pretreated with GA 24 (A) or 72 h (B) prior to the MPTP or saline treatment as described in the legend for Fig. 1. Mouse striatum was dissected for Western blot analysis of Hsp90. The images show representative Western blots of Hsp90 protein levels ($n = 3$ per group), and the bar graph shows quantitative densitometric analysis of Hsp90 level in the striatum ($n = 6$ per group). The data are presented as OD after normalization with actin level. *, $p < 0.05$, one-way ANOVA, post hoc Bonferroni, compared the S+S with the G+S group. #, $p < 0.05$, one-way ANOVA, post hoc Bonferroni, compared the S+M with the G+M group.

**FIGURE 3.** GA pretreatment or combined GA and MPTP treatment induced Hsp70 expression in mouse striatum. Four different groups of mice were pretreated with GA 24 (A) or 72 h (B) prior to the MPTP or saline treatment as described in the legend for Fig. 1. Mouse striatum was dissected for Western blot analysis of Hsp70. The image shows representative Western blot analysis of Hsp70 levels ($n = 3$ per group), and the bar graph shows densitometric analysis of Hsp70 levels in the striatum ($n = 6$ per group). The data are presented as OD after normalization with actin level. *, $p < 0.05$, one-way ANOVA, post hoc Bonferroni, compared the S+S with the G+M group. #, $p < 0.05$, one-way ANOVA, post hoc Bonferroni, compared the S+M with the G+M group.
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FIGURE 4. GA pretreatment and MPTP treatment has no effect on Hsp40 and Hsp25 in mouse striatum. Four different groups of mice were pretreated with GA 24 h prior to the MPTP or saline treatment as described in the legend for Fig. 1. Mouse striatum was dissected for Western blot analysis of Hsp25 and Hsp40. This figure shows representative Western blot of Hsp40 (top) and Hsp25 (middle) (n = 3 per group) as well as densitometric analysis of Hsp40 and Hsp25 levels in the striatum (n = 6 per group). The data are presented as OD after normalization with actin level (bottom).

FIGURE 5. GA pretreatment and MPTP treatment increased nuclear HSF1 levels in mouse striatum. Four different groups of mice were pretreated with GA or saline (intracerebral ventricular) 24 h prior to MPTP or saline treatment (intraperitoneally) as described in the legend for Fig. 1. Whole cell extracts and nuclear proteins were isolated from the dissected striatum for Western blot analysis of HSF1. This figure shows representative Western blot analysis of whole cell extract (WCE) (A) and nuclear (B) HSF1 levels in the striatum (n = 3 per group). Bar graphs represent densitometric analysis of HSF1 in whole cell extract and nuclear fractions (n = 6 per group). Separation of whole cell extract versus nuclear protein was indicated by detection of actin in whole cell extract but not nuclear fractions. *, p < 0.05, one-way ANOVA, post hoc Bonferroni, compared the S + S with the S + M or the G + S group. #, p < 0.05, one-way ANOVA, post hoc Bonferroni, compared the G + M with the S + M or the G + S group.

striatum (Fig. 3A). However, the combined GA pretreatment and MPTP treatment significantly increased the expression of Hsp70 in the striatum when compared with the S + S or S + M group (Fig. 3A, n = 6, p = 0.015 and 0.008, respectively, one-way ANOVA) when GA was administered 24 h prior to the MPTP treatment. When GA was delivered 2 h prior to the MPTP treatment, the combined GA pretreatment and MPTP treatment did not produce an increase in Hsp70 expression in the striatum when compared with the S + S strain. MPTP treatment significantly increased the expression of Hsp70 in the striatum when compared with the saline treated group. The combined treatment of GA and MPTP further increased Hsp70-HSE binding in the striatum when compared with the saline pretreatment group. Similarly, the expression of Hsp25 in the striatum was not affected by the GA pretreatment or MPTP treatment or both (Fig. 4).

GA Pretreatment and MPTP Treatment Did Not Affect Expression of Hsp40 and Hsp25 in the Striatum—We also evaluated the expression levels of other members of the HSP family including Hsp40 and Hsp25 in the striatum after GA pretreatment, MPTP treatment, or both. Western blot analysis showed that neither GA pretreatment, MPTP treatment, nor the combined treatment altered the expression of Hsp40 in the striatum when GA was administered 24 h prior to the MPTP (Fig. 4). Similarly, the expression of Hsp25 in the striatum was not affected by the GA pretreatment or MPTP treatment or both (Fig. 4).

GA Pretreatment and MPTP Treatment Increased Cytosolic as Well as Nuclear HSF1 Levels in Striatum—To dissect out the molecular events underlying the induction of Hsp70, we tested the hypothesis that GA binding to Hsp90 results in the dissociation of HSF1 from the Hsp90-HSF1 complex, leading to the translocation of HSF1 from the cytosol to the nucleus and subsequently acting on the Hsp70 promoter. We first evaluated whether the intracerebral ventricular pretreatment with GA alters HSF1 subcellular distribution in the striatum by determining whole cell and nuclear levels of HSF1 proteins in the striatum after the treatment with GA, MPTP, or both. Separation of the whole cell protein and nuclear fraction was supported by strong staining of actin (a cytosol protein) in the whole cell extracts but not in nuclear fractions (Fig. 5). GA pretreatment or MPTP treatment alone significantly increased both whole cell extract and nuclear HSF1 protein levels in the striatum (Fig. 5, A and B, p < 0.01, n = 6). The combined GA pretreatment and MPTP treatment produced the largest increase in both whole cell and nuclear HSF1 protein levels (Fig. 5, A and B, p < 0.001, n = 6).

GA Pretreatment and MPTP Treatment Enhanced HSE Binding to the Hsp70 Promoter in Mouse Striatum—Next, we used a gel shift mobility assay to determine the HSF1 binding activity at the HSE site of the Hsp70 promoter in the GA-pretreated and MPTP-treated striatum using the double oligonucleotide corresponding to the Hsp70 promoter (called Hsp70-HSE below, corresponding to the HSE site at −156 to −117 bp upstream of the transcription start site of the mouse Hsp70) (46). The specificity of Hsp70-HSE binding was confirmed by the appearance of a supershifted band using the specific HSF1 antibody (Fig. 6, Super shift) and by the reduction of HSE binding after incubating excessive (50×) unlabeled (cold) Hsp70-HSE double strand oligonucleotides (Fig. 6, 50X cold probe). Pretreatment with GA increased Hsp70-HSE binding in the striatum when compared with the saline pretreatment group. Similarly, MPTP treatment also increased Hsp70-HSE binding in the striatum when compared with the saline-treated group. The combined treatment of GA and MPTP further increased Hsp70-HSE binding in the striatum when compared with groups pretreated with GA alone or treated with MPTP alone. The HSE binding, using the self-complementary HSE consensus oligonucleotide (5′-CTAGAAGCTTTCTAGAAGCTTTCTAG-3′) (48), also showed a similar pattern of enhancement of HSE binding in the striatum of GA pretreated, MPTP-treated, or combined treated mice (data not shown).
DISCUSSION

This study reveals two main findings. First, pharmacological inhibition of Hsp90 by GA reduced the depletion of dopamine content and dopaminergic terminals in a mouse MPTP model of PD. These results extend the previous findings in Drosophila (31, 35), cultured neuroblastoma, and neuroglioma cells as well as PC12 cells (33, 36, 49) by providing in vivo evidence for GA-mediated neuroprotection against MPTP-induced neurotoxicity in a mouse model of PD. Second, GA-mediated neuroprotection against MPTP neurotoxicity was associated with the reduction of Hsp90 and induction of Hsp70, with a parallel increase of cytosolic and nuclear HSF1 protein levels as well as an increased HSE binding of the Hsp70 promoter in the striatum. These results supported the view that the dopaminergic system may be particularly sensitive to compromised chaperone levels and that the pharmacological manipulation of chaperon activity might be a therapeutic strategy for delaying or preventing the progressive degeneration of dopaminergic neurons in PD (31, 35, 37).

In this study, we employed intracerebral ventricular injection to deliver GA into the mouse brain to circumvent poor central nervous system permeability due to the large molecular size of GA. The neurochemical analysis showed that neuroprotection offered by GA is both dose- and time-dependent. First, the protection by GA was observed after pretreatment with 10 μg/kg but not 1 μg/kg. This result is consistent with the previous reports of dose-dependent neuroprotection by GA in models of Huntington disease and stroke. In a cell culture model of Huntington disease, the dose-dependent induction of Hsp70 induced by GA, at the concentration range of 18–360 nM, was closely correlated with its ability to reduce aggregates by ∼30–80% (25). During ischemic brain injury, GA induced Hsp70 and Hsp25 expression in the brain in a dose-dependent manner (10 ng–1 μg/kg), with neuroprotection correlating with the highest concentration (17). Second, we selected a specific GA pretreatment paradigm (i.e. 24 or 72 h prior to the MPTP injection) because the previous study suggests that pretreatment with GA (16–18 h prior), but not co-administration, confers neuroprotection against α-synuclein-induced neurotoxicity in cultured H4 cells (33). Interestingly, we noted that the GA-mediated protection was only detected when GA was administrated 24 h prior to MPTP treatment. At all the other three time points tested (72 h prior, co-administration, 6 h after MPTP treatment), GA failed to reduce MPTP-induced neurotoxicity in the striatum (Fig. 1C). Thus, the pretreatment interval (24 h prior) may be critical for GA-mediated neuroprotection, suggesting that GA-mediated neuroprotection in mouse striatum may due to the alteration of target gene expression induced by GA.

The exact mechanism underlying the neuroprotection provided by GA in the PD model is not clear. Since its discovery in 1994, benziquinone ansamycins, and GA in particular, have been shown to bind specifically to Hsp90 and inhibit the association of this chaperone with client proteins including HSF1, resulting in the destabilization of protein complex usually mediated by the proteasome (40, 41, 50). The major consequence of GA-mediated inhibition of Hsp90 is the induction of Hsp70. Indeed, our results strongly supported the assertion that GA exerts neuroprotection against MPTP-induced dopaminergic neu-
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rototoxicity by the destabilization of the Hsp90-HSF1 complex, nuclear translocation of HSF1, and induction of Hsp70 expression due to HSF1 binding with the HSE site in the Hsp70 promoter (Fig. 7). This proposed mechanism of GA-mediated neuroprotection was supported by the following observations. First, GA pretreatment destabilized the Hsp90 protein, resulting in the decreased level of Hsp90 in the striatum as detected by Western blot. This result suggested that GA destabilizes Hsp90 itself and results in Hsp90 dissociation from its partner protein, HSF1. This finding is different from previous reports that suggested that GA or its analog, GA-analog (17DMAG), induces or has no effect on the expression of Hsp90 in liver or cultured cells (25, 51, 52). The reason for this discrepancy is not clear but may be due to the different model systems examined, i.e. in vivo mouse striatum (in this study) versus liver or in vitro cultured cells (25) Second, GA pretreatment increased HSF1 levels in cytosolic as well as nuclear fractions assessed by Western blot. A similar induction of nuclear HSF1 was noted in the ischemic brain in vitro or in vivo or MPTP-induced dopaminergic neurotoxicity is not yet clear. Since chaperones Hsp70 and α-synuclein are found to be the major components of Lewy bodies in human PD postmortem tissues (30, 38, 53), it is possible that Hsp70 may mitigate α-synuclein toxicity by influencing the conformation of α-synuclein and thus preventing protein aggregations (30). The ability of Hsp70 to prevent neurotoxicity may be due to its activity of refolding misfolded proteins and its interaction with co-chaperones such as C-terminal Hsp70-interacting protein and parkin-associated ubiquitinylation pathway (32). A recent report suggests that dysfunction of the ubiquitin-proteasome system results in the sequestration and formation of the Hsp70-parkin complex in Lewy bodies, contributing to the pathogenesis of sporadic PD (34). In addition, the induction of Hsp70 and tissue protection has been associated with inhibition of protein synthesis (13), interference with caspase activation (22), and regulation of cellular redox balance following ATP depletion (54). However, it should be noted that neuroprotection by Hsp70 induction is not universal since several studies using overexpression models of Hsp70 showed a lack of neuroprotection in a mouse model of global (55) or permanent (19) cerebral ischemia and an animal model of Huntington disease (56). Thus, the protective effect by induction or overexpression of Hsp70 may be related to the nature and severity of the insults, as well as subpopulations of brain cells (19). It also remains possible that Hsp70 requires co-factors or helper proteins such that it alone is insufficient, but activation of the entire stress response, as with GA or other drugs, may prove protective.

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