

Multiple Molecular Pathways Are Involved in the Neuroprotection of GDNF Against Proteasome Inhibitor Induced Dopamine Neuron Degeneration In Vivo

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The impairment of ubiquitin-proteasome system (UPS) is a cellular mechanism underlying the neurodegenerative process in Parkinson's disease (PD). Glial cell line-derived neurotrophic factor (GDNF) is one of the most potent neurotrophic factors promoting the growth and survival of mesencephalic dopamine (DA) neurons. To investigate whether GDNF has neuroprotective effects in a PD model induced by UPS impairment we administered GDNF by osmotic pump in C57BL/6 mice after nigrostriatal lesions with stereotactic injection of proteasome inhibitor lactacystin in the middle forebrain bundle. We found that lactacystin injection severely injured the nigral DA neurons and reduced the striatal levels of DA and its metabolites, while prolonged administration of GDNF at a sustained moderate dose for two weeks can significantly attenuate the lactacystin-induced loss of nigral DA neurons and striatal DA levels by 31% and 40%, respectively. We also investigated the molecular mechanisms for the neuroprotective effects of GDNF showing that lactacystin administration can cause the phosphorylation of extracellular signal-regulated kinase (ERK), p38MAPK (p38), and the c-Jun N-terminal kinase (JNK), whereas GDNF treatment can further enhance the phosphorylation of ERK and Akt but reduce the levels of JNK and p38. These results indicate that prolonged treatment with GDNF can protect the nigral DA neurons from the UPS impairment-induced degeneration. Several signaling path-

ways including p38, JNK, Akt and ERK molecules seem to play an important role in this neuroprotection by GDNF. *Exp Biol Med* 233:881–890, 2008

Key words: glial cell line-derived neurotrophic factor; proteasome inhibitor; mitogen-activated protein kinase; Parkinson's disease

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by loss of dopamine (DA) neurons and the formation of Lewy bodies (LBs). Mutations in the genes encoding α -synuclein, parkin and ubiquitin C-terminal hydrolase L1, have been identified in familial forms of PD and are thought to cause nigral degeneration through protein handling dysfunction (1–3). There is a wide range of proteins aggregated in LBs, which include α -synuclein, ubiquitin, neurofilament and other oxidatively damaged or nitrated proteins (4–6). Increasing evidences have suggested that failure of the ubiquitin-proteasome system (UPS) to clear misfolded proteins may play a major role in the etiopathogenesis of PD (7–9). Therefore, animal models induced by selective proteasome inhibitors may be valuable in studying etiopathogenic mechanisms and putative neuroprotective therapies for this disease. Importantly, recent studies showed that microinjection of the proteasome inhibitors into the nigrostriatal pathway of mice could cause progressive nigral DA neuron loss and α -synuclein-positive inclusions (10, 11). In this report, therefore, we established the animal model of PD with administration of a selective proteasome inhibitor lactacystin in the middle forebrain bundle (MFB) of mice.

One of the features in the PD nigrostriatal region is a severe deficiency of various neurotrophic factors (NTFs) (12). Glial cell line-derived neurotrophic factor (GDNF) has

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been shown to protect the DA neurons and stimulate axonal sprouting in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (13, 14) and 6-hydroxy-dopamine (6-OHDA) treated animal models (15–17). It is of interest to study whether GDNF has neuroprotective effects against the proteasome inhibitor induced nigral DA neuron degeneration, a model of which may represent progressive condition of PD. In this report, the neuroprotection of GDNF was tested in lactacystin-lesioned mouse model of PD and GDNF was given in a sustained fashion with a moderate dose in order to avoid its potential side effects (18, 19).

The neuroprotective mechanisms of neurotrophic factors are still a matter of controversy, despite the evidence indicating that GDNF acts by binding to its receptor complex, which consists of the GDNF family receptor α (GFR α) and a membrane Ret protein tyrosine kinase (Ret). Ret can activate several intracellular signaling pathways including PI3K/Akt and members of the MAPK superfamily (20). These intracellular signaling pathways play different roles in regulating cell growth, differentiation, survival, and death (21). ERK activation appears to antagonize apoptotic pathways in some cell systems, while JNK and p38 are likely the candidates for mediating cell death (22–24). The PI3K/Akt pathway is an important regulator of neuronal survival initiated by the activation of PI3K, which in turn activates a cascade of downstream effectors including the serine/threonine kinase Akt (25–27). The dynamic changes of the MAPK and PI3K/Akt family and the delicate balance between the two pathways will determine the fate of neurons in response to different insults (28, 29). Whether these signaling pathways are involved in the protective effects of GDNF on proteasome inhibition-induced DA neuron degeneration has not been elucidated. Therefore, we explore several potential signaling pathways and determine whether they mediate the protection against nigral DA neuron degeneration in a UPS impairment animal model of PD.

Materials and Methods

Animals and Proteasome Inhibitor Induced Lesions. Male C57BL/6 mice (8 weeks old) provided by Shanghai Experimental Animals Centre of Chinese Academy of Sciences were used in this study. These animals were housed pre- and post-surgery in a temperature and humidity controlled room with a 12 hr light-dark cycle (lights on 07:00–19:00). Food and water were freely available. Animal handlings were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals were randomly divided into 4 groups with 4–5 mice in each group. Mice in group A and B receiving one dose stereotaxical injection of lactacystin or phosphate-buffered saline (PBS) respectively, were sacrificed 3 weeks after the injections. Mice in group C and D receiving one dose stereotaxical injection of lactacystin were given one

week later with sub-chronic striatal infusion of GDNF or artificial cerebrospinal fluid (aCSF) via Alzet brain infusion mini osmotic pump for 2 weeks (Alzet brain infusion kit, ALZA Corporation, CA) before sacrificed. To produce a unilateral nigro-striatal lesion, the experimental mice received a stereotaxical microinjection of selective proteasome inhibitor lactacystin into the right MFB. The microinjection was performed using a 10 μ l Hamilton syringe with a 33-gauge blunt-tip needle mounted on a David Kopf stereotaxic frame (Tujunga, CA, USA). Under chloral hydrate anesthesia (380 mg/kg, intraperitoneally), 2 μ l of either lactacystin (1.25 μ g dissolved in 0.1% DMSO, Calbiochem, San Diego, CA) or vehicle (0.1 M PBS contains 0.1% DMSO) was injected into the MFB (1.30 mm posterior, 1.10 mm lateral, and 5.25 mm ventral from bregma) (10, 30). The injections were given at a rate of 0.4 μ l/min and the needle tip was left in place for an additional 5 min before slowly withdrawing. The wounds were sealed with suture.

Mini-Osmotic Pump Implantation and GDNF Infusion. In order to give a sustained infusion of GDNF, brain infusion cannula (28-gauge), connected to a mini osmotic pump via 4-cm-long catheter tubing was used. Tip of the infusion cannula was stereotaxically placed in the right striatum (0.8 mm posterior, –2.0 mm lateral, and 3.0 mm ventral from bregma). The pump was designed for sustained infusion over 14 days at a rate of 0.25 μ l/hr. The recombinant GDNF protein (R&D System, USA) in 100 μ l aCSF (150 mM NaCl, 1.8 mM CaCl₂, 1.2 mM MgSO₄, 2.0 mM K₂HPO₄, 10.0 mM glucose, pH 7.4) at a concentration of 0.08 μ g/ μ l was stored in each pump. The same volume of aCSF stored in mini osmotic pump was used as vehicle control (31). The pump was inserted into a subcutaneous pocket slightly posterior to scapulae. Acrylic cement was used to fix the cannula in the skull. Mice were sacrificed after 2 weeks' infusion.

Immunohistochemistry Analysis. Animals were deeply anaesthetized with chloral hydrate (400 mg/kg, intraperitoneally) and perfused through the ascending aorta with 50 ml of isotonic saline, followed by 100 ml of 4% paraformaldehyde in 0.1 M PBS. Brains were postfixed for 3 hr in the same solution and dehydrated in 20% sucrose for 24 hr then immersed in 30% sucrose for additional 24 hr before being stored in optimal cutting temperature compound (OCT, Sakura, USA) at –80°C. A serial of cryostat-cut (LEICA CM 1850, Germany) sections encompassing the whole SN and striatum regions were sliced according to the coordination landmarks described by Muthane et al (32) and Bove et al (33). Nigral and striatal sections collected from the cryostat-cut were immunostained by antibodies against tyrosine hydroxylase (TH), α -synuclein or ubiquitin.

Sections were rinsed three times in PBS between each incubation period. After quenching for 30 min in 3% peroxide, the sections were precoated with 3% normal goat serum for 1 hr and incubated overnight at 4°C with rabbit anti-TH (1:500), rabbit anti- α -synuclein antibody (1:1000),

or mouse anti-ubiquitin antibody (1:500) (Chemicon, USA). For TH immunostaining, a second biotinylated anti-rabbit IgG (1:200) was used and the reaction was visualized with avidin-biotin-peroxidase complex, using 3,3-diaminobenzidine as a chromogen (Vector Laboratories, CA, USA). For immunofluorescent stainings of α -synuclein and ubiquitin, a donkey anti-rabbit TRITC-conjugated IgG (1:100) or horse anti-mouse FITC conjugated IgG (1:100) (Chemicon, USA) was used. The sections were counterstained with Hoechst (1:330, Molecular Probes, USA) to visualize cell nuclei and observed under fluorescent microscope.

Morphological Analysis. The number of TH-positive cells in the SN was determined as described previously (34, 35). Every fifth section (12 μ m) and its neighboring section (the test and reference slides) were sampled on separate glass slides. To ensure that the estimates of the total number of nigral neurons would be unbiased, the first section pair was randomly selected from within the first five section pairs. Thereafter, every fifth section pair was studied. The numbers of pigmented neurons present in the test field, but not in the reference field, were counted. The studied section pairs were viewed at a final magnification of $\times 100$ using $\times 20$ objectives under projection microscope (Olympus IX 81, Japan). The number of α -synuclein- or ubiquitin-positive neurons in the SN region was counted with the same method under the light microscope equipped for fluorescence by an observer blinded to the experimental conditions. The injected side of the vehicle group was used as a control. Data were expressed as percentage of total cells and were representative of at least 2 independent experiments.

The optical density of the TH-immunoreactive fibres in the striatum was measured using the Image-pro plus 5.1 program. To estimate the specific TH staining density, the optical density reading was corrected for nonspecific background density, as measured from the completely denervated area of the striatum. Striatal images converted to gray scale were then delineated and the intensity of staining was thus assessed for the entire region of the selected sections sampled, subsequently averaged for each animal. The data was expressed as integrated optical density (IOD).

Biochemical Analysis. The whole right striatum was dissected and homogenized (10% wt/vol) by sonication in ice-cold 0.2 M perchloric acid with 3, 4-dihydroxybenzylamine (DHBA) as internal standard. Homogenate was centrifuged at 20,000 *g* for 15 min at 4°C and the supernatant was collected. The levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindolacetic acid (5-HIAA) were determined by high-pressure liquid chromatography (HPLC; EPC-300, Eicom, Japan) equipped with a column of 5 μ m spherical C18 particles and detected with an electrochemical detector. The mobile phase (previously filtered and degassed) consisted of 0.042 M citric acid monohydrate, 0.038 M sodium acetate trihydrate, 0.940 mM

sodium octane sulfonate and 0.013 mM EDTA-2Na (pH 3.8).

Immunoprecipitation and Western Blot Analysis. The SN tissues were removed from the right site midbrain in the anatomical region of the SN (30). Briefly, a 2-mm coronal slab of the midbrain region was dissected by using a mouse brain matrix (Roboz, Maryland, USA). SN tissue extracts were isolated from the mouse brain section with a 1-mm-diameter needle. The tissue extracts were lysed in modified radioimmunoprecipitation (RIPA) buffer containing 25 mM NaF and 1 mM Na₃VO₄. The protein content was measured using the bicinchoninic acid method. Protein extracts (50 μ g) were loaded in 12% SDS-PAGE gel for electrophoresis and then transferred to nitrocellulose membranes. After blocked in 5% non-fat dry milk buffer, the membrane was incubated overnight at 4°C with rabbit polyclonal antibody to ERK, pERK, Akt, pAkt, p38, p p38, pJNK, and β -actin (Cell Signaling, USA), followed by 1 hr incubation with an appropriate secondary antibody. After several washes, immunoreactive bands were visualized with the ECL chemiluminescence detection system (Promega, USA).

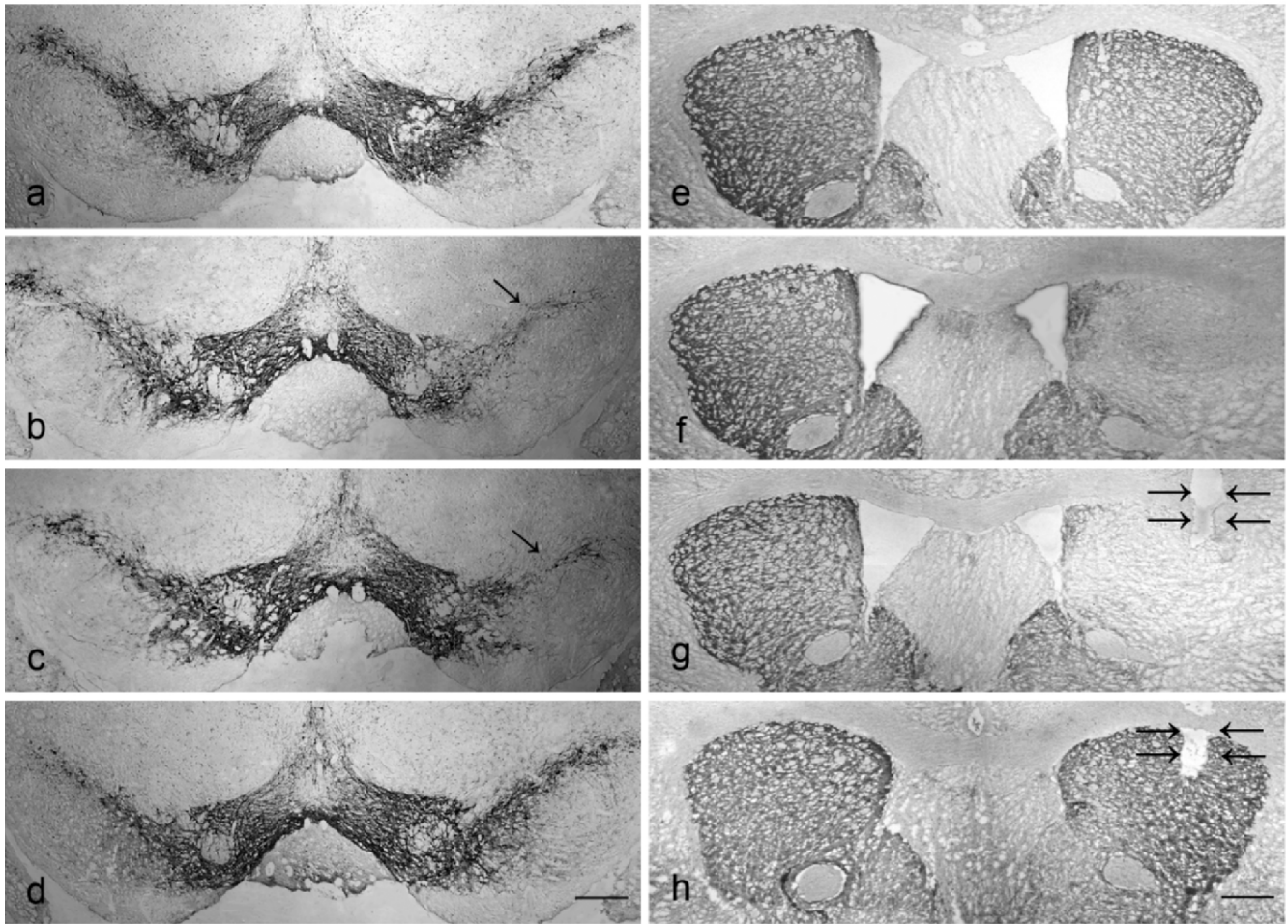
Statistical Analysis. All data derived from the immunostaining and western blot analysis in SN and DA measurement in striatum were expressed as mean $\pm$ SEM. Inter-group differences between the various dependent variables were assessed using one-way ANOVA, followed by the Dunnett post hoc multiple comparisons test. Statistical analyses were performed by SAS 6.12 software. *P* values lower than 0.05 were considered significant.

Results

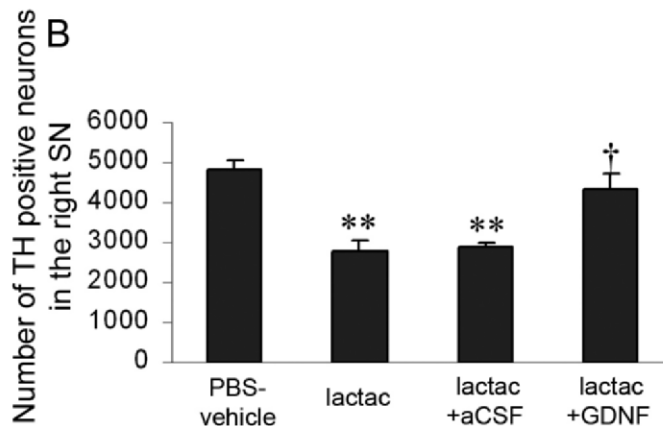
Proteasome Impairment Causes Nigrostriatal DA Neuron Degeneration. Microinjection of lactacystin, a streptomyces metabolite that irreversibly blocks the activity of mammalian proteasome (36), is able to interrupt proteasome system and then result in DA neuron degeneration. Microinjection of 1.25 μ g/2 μ l lactacystin into MFB significantly caused DA neuron loss in the ipsilateral SN (Fig. 1A). Quantitative analysis of nigral TH-immunoreactive cells showed a 42% decrease of DA neurons compared with the PBS-vehicle control (*P* < 0.01, Fig. 1B). In parallel with nigral DA neuron loss, there was a 79% decline of the density of TH-immunoreactive nerve terminals in the striatum (Fig. 1A and 1C). Biochemical analysis of catecholamine in the striatal tissues revealed that lactacystin injection reduced the levels of DA and its metabolites DOPAC and HVA by 59%, 55%, and 45%, respectively (*P* < 0.01, Fig. 2A), while 5-HT and 5-HIAA levels were not significantly affected (*P* > 0.05, Fig. 2B).

Lactacystin Induces Protein Aggregation in Nigral Neurons. Intracellular inclusion body-like protein aggregations were found in about 3–6% neurons in mice injected with lactacystin (Fig. 3B and 3C). These inclusions shared some features of LBs associated with PD, showing

A



B



C

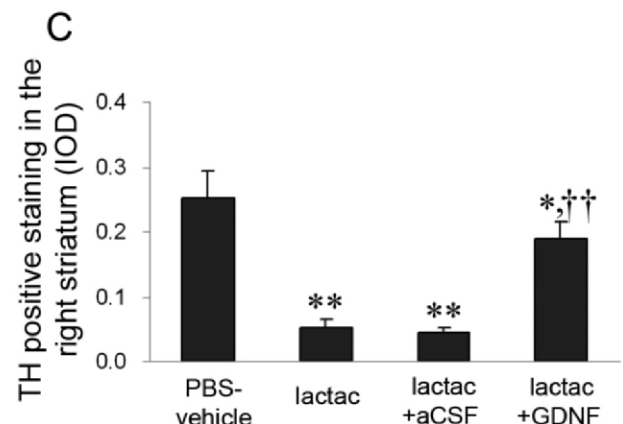


Figure 1. Neuroprotective effects of GDNF in the lactacystin-injected mouse model. (A) Photomicrographs of TH-immunostained sections through SN and striatum of the mice treated with PBS-vehicle (a, e), lactacystin (b, f), lactacystin+aCSF (c, g) and lactacystin+GDNF (d, h). Scale bar: 450 μ m at left; 1000 μ m at right. (B–C) Quantitative analysis of TH-immunoreactive neurons or fibers in the right nigra (B) or striatum (C). Values are reported as mean $\pm$ SEM based on the number of TH-immunoreactive neurons or optical density of striatal TH-immunoreactive staining (each group consists of 4 mice). * P < 0.05 and ** P < 0.01 vs PBS-vehicle control; † P < 0.05 and †† P < 0.01 vs aCSF-vehicle control. lactac, lactacystin.

prominent immunoreactivity for α -synuclein and ubiquitin with spherical or elliptical shape in the cytoplasm (Fig. 3A). Under the examination of fluorescence microscope, α -synuclein- and ubiquitin-immunoreactive aggregates in some

nigral neurons were 4–10 μ m in diameter. Some of the ubiquitin-immunoreactive inclusions seemed to have a more granular appearance than those of α -synuclein-immunoreactive aggregates. There were no α -synuclein or ubiquitin-

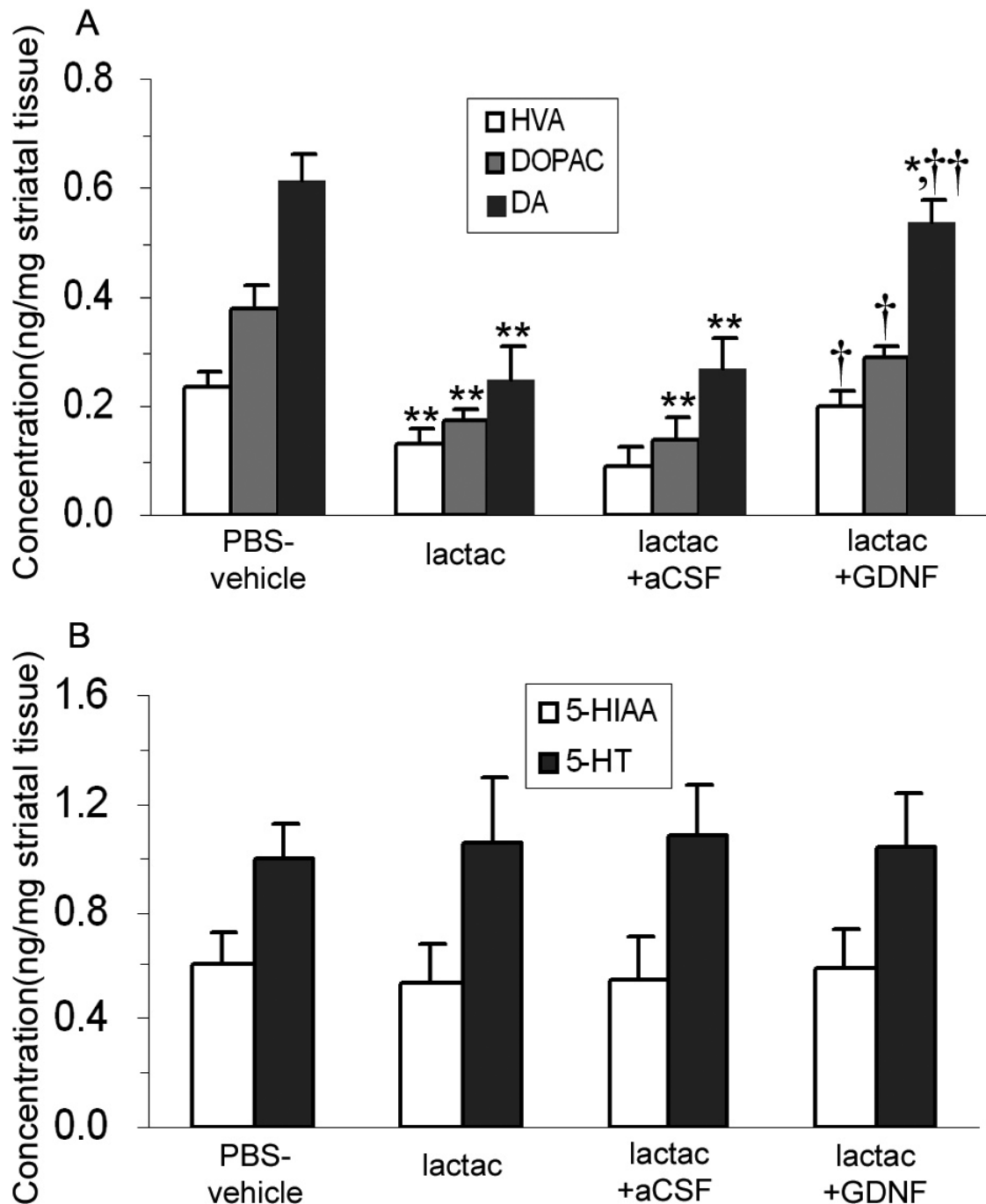


Figure 2. Effects of GDNF on nigro-striatal catecholamine levels in the lactacystin-treated mouse model. (A) Striatal DA and its metabolites, DOPAC and HVA. (B) Striatal 5-HT and 5-HIAA. The results are expressed as means $\pm$ SEM of 4 mice in each group. * $P < 0.05$ and ** $P < 0.01$ vs PBS-vehicle control; † $P < 0.05$ and †† $P < 0.01$ vs aCSF-vehicle control.

positive protein aggregates observed in the PBS-vehicle injected mice.

GDNF Protects DA Neurons Degeneration Induced by Lactacystin. In mice that received GDNF infusion in the striatum for two weeks, there was a 31% more TH-immunoreactive cells in the SN as compared with aCSF-vehicle control ($P < 0.05$, Fig. 1A and 1B). TH-positive terminals in the striatum were also increased by

40% as compared with the same control ($P < 0.01$, Fig. 1C). In the striatum, DA and its metabolites also showed partial but significant recovery. The levels of DA, DOPAC and HVA were 44% ($P < 0.01$), 40% ($P < 0.05$) and 45% ($P < 0.05$) higher respectively in GDNF-treated mice as compared with aCSF-vehicle control (Fig. 2A). Striatal 5-HT and 5-HIAA levels were found no significant difference

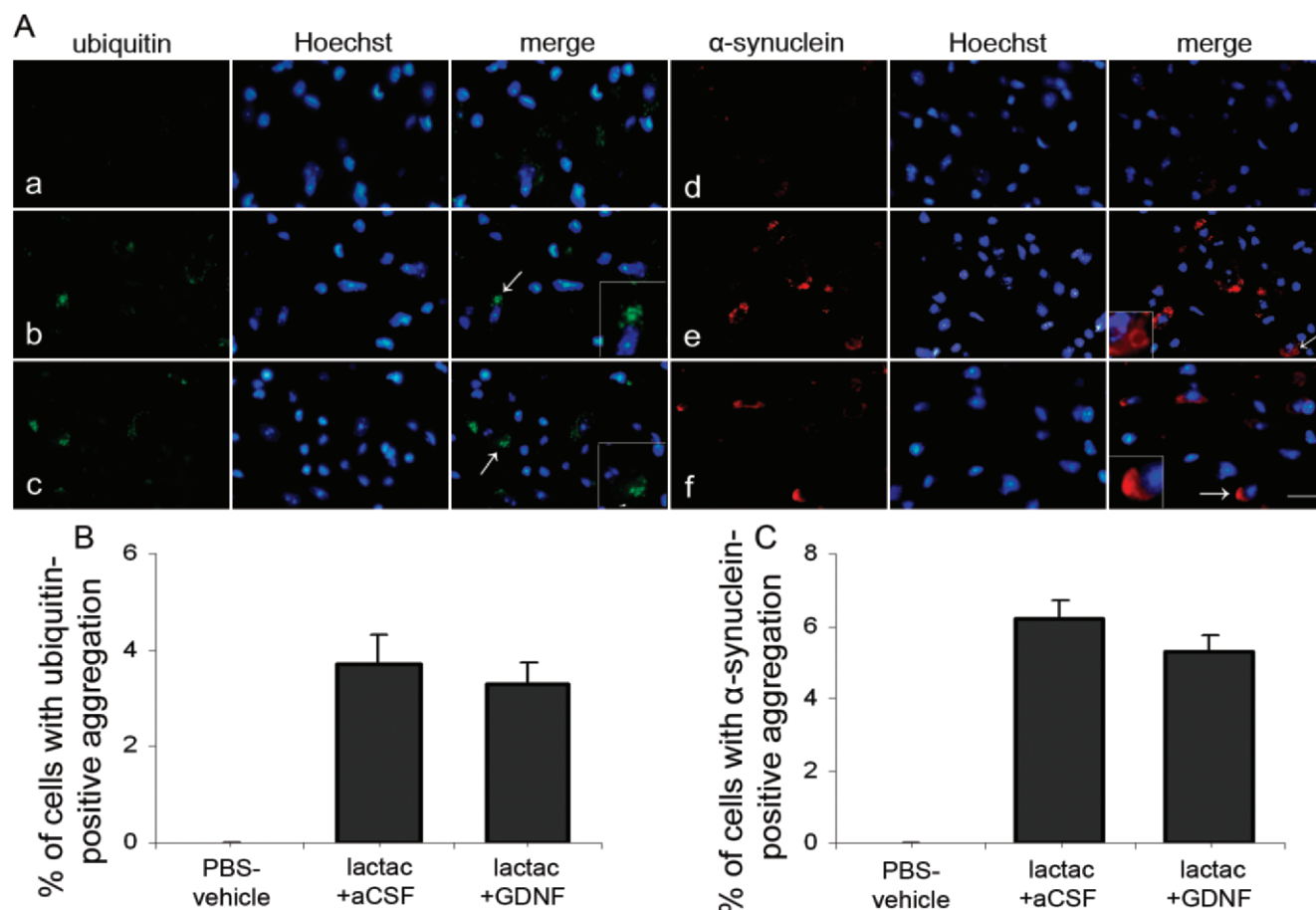


Figure 3. Double immunostaining of nigral pigmented neurons in the right SN (A). Fluorochrome-linked secondary antibodies were used to show ubiquitin immunoreactivity (green) or α -synuclein immunoreactivity (red). Nuclei were counterstained with hoechst dye (blue) on coronal mesencephalon sections. (a, d) PBS-vehicle control; (b, e) aCSF administration after lactacystin injection; (c, f) GDNF treatment after lactacystin injection. White arrows in the column show immunoreactive inclusion bodies. Scale bar: 30 μ m (inset: 15 μ m). Quantitative cell counting of ubiquitin (B) or α -synuclein-positive cells (C) in the SN, which was expressed as the percentage of immunoreactive cells in total number of examined nigral neurons.

between the GDNF-treated group and the aCSF-vehicle control ($P > 0.05$, Fig. 2B).

Interestingly, the number of neurons containing α -synuclein- or ubiquitin-immunoreactive aggregates was not significantly changed after GDNF treatment ($P > 0.05$, Fig. 3) as compared with aCSF-treated control.

Lactacystin Induces Changes in MAPK Pathway. The western blotting assay showed that JNK had two immunoreactive bands with the size of 46 and 54 kDa, p38 had a 40 kDa band, ERK1, ERK2 and Akt has 44-, 42- and 60-kDa bands, respectively. The levels of total JNK, p38, Akt were similar between the lactacystin-lesioned and the PBS-injected mice. However, the levels of phosphorylated ERK, p38 and 54-kDa JNK in mice received lactacystin microinjection were increased by 86%, 88% and 80%, respectively (Fig. 4), although 46-kDa JNK was not significantly changed as compared with the PBS control. There was also no significant difference in phosphorylated Akt between the two groups.

GDNF Treatment Further Enhances MAPK and PI3K/Akt Levels. GDNF infusion over two weeks

increased the levels of phosphorylated MAPK and PI3K/Akt signaling pathways (Fig. 4). In lactacystin-lesioned mice, GDNF treatment markedly elevated the levels of phosphorylated ERK and Akt by 68% and 56%, respectively, but reduced the levels of p38 and 54-kDa JNK by 45% and 34%, respectively, as compared with aCSF-treated control. There was no significant change in phosphorylated MAPK and PI3K/Akt in aCSF-vehicle control mice.

Discussion

The present study generates several interesting results and new findings. First, microinjection of lactacystin in MFB can produce severe nigral degeneration replicating some of the biochemical and pathological features of PD, which is in agreement with other reports (10, 11). Second, lactacystin administration can cause the phosphorylation of ERK, p38, and JNK, while phosphorylated Akt is not obviously altered. Third, striatum infusion of GDNF over a short period of time may provide adequate dose for the therapeutic benefits against proteasome inhibitor induced

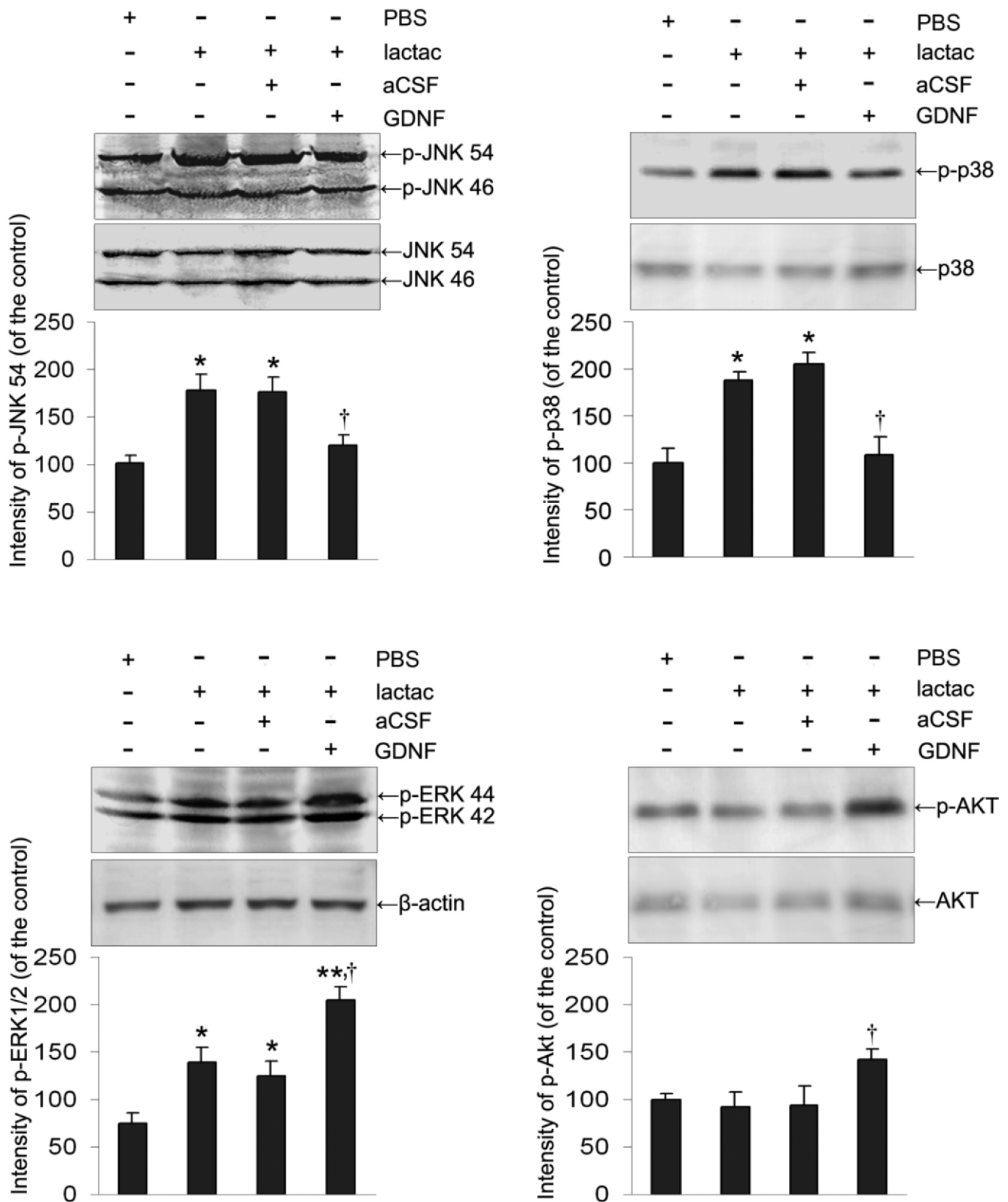


Figure 4. Effects of GDNF on lactacystin-induced MAPK and Akt phosphorylation in SN. Statistical analysis of fold induction of p54-kDa-JNK, pp38, pERK, pAkt was performed based on the densitometric scan of immunoblots normalized for total JNK, p38, Akt, β -actin. Data represent mean $\pm$ SEM of at least three independent experiments and analyzed by one-way ANOVA followed by Dunnett's multiple comparison test for post-hoc significance testing. * $P < 0.05$ and ** $P < 0.01$ vs PBS-vehicle control; † $P < 0.05$ vs aCSF-vehicle control.

DA neuron degeneration. Finally, molecular mechanisms for the protective effect of GDNF are believed to be related to the enhancement of the phosphorylation of ERK and Akt, and the reduction of JNK and p38 levels.

We have previously shown *in vitro* that lactacystin treatment in ventral mesencephalon cultures can impair DA neurons, which can be attenuated through GDNF administration (37). In the present study, we further demonstrate the protective effects of GDNF in the nigro-striatal pathway *in vivo* and reveal a novel pathological mechanism for the UPS impairment-induced DA neuron degeneration.

To determine whether targeting administration of proteasome inhibitor lactacystin can induce DA neuron injury in SN, we stereotactically injected lactacystin into MFB of C57BL/6 mice. The dose of lactacystin we used was based upon the dose-response curves from our animal study *in vivo* (37). We found that a 1.25- μ g lactacystin injection into MFB resulted in a significant loss of nigral TH-positive cells and striatal DA content. The neuro-degeneration induced by lactacystin was further confirmed in some of the nigral neurons with cytoplasmic inclusions with spherical or elliptical shape. These inclusions were positively stained for α -synuclein, ubiquitin and most intense in perinuclear regions. This intense structure can represent the sequestration and aggregation of excessive levels of proteins and components of the UPS in aggresomes due to impaired degradation and clearance following inhibition of proteasome function (38, 39). Our study clearly demonstrates that injection of lactacystin in SN can produce nigral degeneration replicating some of the biochemical and pathological features of PD.

Using this model we examined whether GDNF infusion at 0.48 μ g/day, a dose when converted as body surface area ratio is equivalent to that used in clinical patients (18, 19), could reverse the pathological and biochemical changes induced by lactacystin. This infusion of GDNF started one week after the lactacystin lesions and continued for two weeks. We found that constant infusion of GDNF can significantly rescue the nigral DA neurons and striatal terminal loss and reverse the deficit of striatal DA content following lactacystin lesions. It is interesting to note that GDNF infusion does not reduce the number of cells with intracellular protein aggregations in the nigra of GDNF-treated mice as compared with aCSF-vehicle control. The exact mechanisms by which GDNF protects DA neurons but fails to reduce the burden of α -synuclein aggregation are not clear. The possible explanations are: (1) GDNF may protect DA neurons through multiple molecular pathways to block the α -synuclein oligomers-induced neuron degeneration rather than to act on the clearance of α -synuclein and ubiquitin containing inclusions. This finding may support the current belief that mature formed protein aggregations may not be the major culprit for the DA neuron degeneration (40). (2) In the present study, GDNF was used for two weeks, which may not be long enough to show its biological effect on the clearance of mature formed α -

synuclein aggregation. Further systematic investigation to examine the effects of GDNF on proteasome activities and autophagic function appears to be warranted. Nevertheless, these findings underline the fact that the striatal infusion at such concentration of GDNF over a relative short period of time may provide adequate therapeutic benefits.

Multiple lines of evidence have indicated the activation of JNK, ERK and p38 MAPK is critical for the GDNF-induced cell migration and differentiation on DA cells whereas little studies have been performed on these molecules involved in the protective effects of GDNF against different toxins, especially, proteasome inhibitors. In this study we focus on elucidating the potential signal pathways by which GDNF exerts its protective role on DA neurons. Our results show that the phosphorylation of ERK, p38 and JNK, especially 54-kDa JNK, was significantly increased in DA neurons after exposed to lactacystin. Phosphorylated Akt, however, was not obviously altered. Application of GDNF markedly enhanced phosphorylation status of pro-survival molecules ERK and Akt while attenuated the levels of pro-apoptotic molecules p38 and JNK, especially 54-kDa JNK. These data suggest that GDNF may promote neurons to survival through enhancing or reducing the activities of these signaling pathways. However, we found that ERK phosphorylation is under biphasic regulation in response to the selective proteasome inhibitor. The mechanism behind the effects of ERK on DA neurons remains unclear but our findings are consistent with other studies, where sequential activation of ERK contributes to peroxynitrite-induced apoptosis and inhibition of DNA synthesis while a sustained increase in TH phosphorylation at Ser31 by GDNF treatment is thought to enhance ERK activity (41, 42). Thus, our finding extends the hypothesis that molecular mechanisms for the protective effect of GDNF are related to multiple signal pathways including p38, JNK, Akt and ERK molecules.

A recent study has demonstrated that degeneration of DA neurons induced by selective proteasome inhibitor is associated with increased levels of the tumor suppressor gene p53 (43). p53 is a sequence-specific transcription factor that can be upregulated in response to a variety of cellular stresses and is essential for the neuronal death (44). It has been reported that triggering p53-dependent neuronal apoptosis involves the activation of the JNK or p38 pathway (45, 46). However, the regulation of GDNF on p53-mediated pathway and the effect of p53 on MAPK in the animals exposed to proteasome inhibitors have not been explored. Further studies are yet to be conducted to unveil how these pathways are integrated for the regulation of DA neurons survival.

In conclusion, our data show that in the animal model of PD induced by the selective proteasome inhibitor, sustained delivery of GDNF with a moderate dose can be a viable neuroprotective approach to rescue the ongoing degenerated DA neurons. Multiple molecular pathways are involved in neuroprotection of GDNF, which include the

activation of the PI3K/Akt and ERK with concomitant reduction of the p38 and JNK. These results may provide inside information into the molecular basis of GDNF-mediated neuroprotective therapy for PD.

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