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Myricitrin alleviates MPP$^+$-induced mitochondrial dysfunction in a DJ-1-dependent manner in SN4741 cells

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ABSTRACT

Oxidative stress and mitochondrial dysfunction have been linked to Parkinson's disease. DJ-1 is a recessive familial PD gene involved in antioxidative function and mitochondrial maintenance. Myricitrin, a flavanoid isolated from the root bark of *Myrica cerifera*, has potent antioxidative properties. In the present study, we investigated the protective effects of myricitrin against MPP$^+$-induced mitochondrial dysfunction in SN4741 cells and attempted to elucidate the mechanisms underlying this protection. The results showed that incubating SN4741 cells with myricitrin significantly reduced cell death induced by the neurotoxin MPP$^+$. Furthermore, myricitrin protected cells from MPP$^+$-induced effects on mitochondrial morphology and function. However, these protective effects were lost under DJ-1-deficient conditions. Thus, our results suggest that myricitrin alleviates MPP$^+$-induced mitochondrial dysfunction and increases cell viability via DJ-1, indicating that myricitrin is a potential beneficial agent for age-related neurodegenerative diseases, particularly Parkinson's disease.

1. Introduction

Parkinson's disease is a common neurodegenerative disorder that is clinically characterised by bradykinesia, rigidity, tremors, and gait dysfunction with postural instability [1]. The pathological hallmark of PD is a loss of dopamine neurons in the substantia nigra pars compacta, causing subsequent depletion of striatal dopamine [2]. Although the aetiology of PD remains elusive, increasing evidence indicates that oxidative stress and mitochondrial dysfunction are important mediators of its pathogenesis [3,4].

DJ-1, which was originally identified as an oncogene product, has been reported to have many biological functions, including transcriptional regulation, antioxidative defence and mitochondrial maintenance [5–7]. Recent studies show that the DJ-1 gene is one of the causative genes associated with a familial form of PD, namely, PARK7 [8]. Mutations in PARK7 can cause autosomal recessive parkinsonism [9]. DJ-1 is sensitive to the oxidative stress triggered by environmental toxins, such as MPP$^+$, and oxidative damage could lead to DJ-1 inactivation [10]. Thus, it is crucial to identify a drug that can protect DJ-1 from oxidative stress.

Myricitrin (Myr), which is extracted from Chinese bayberry bark and fruit and from other medicinal plants, has been reported to have many pharmacological effects [11]. These effects include anti-inflammatory [12], antioxidant [13,14], and anti-allodynia activity [15], as well as a neuroprotective action through the antagonism of 6-OHDA-induced neurotoxicity [16].

However, few studies have investigated whether myricitrin is able to exert protective effects against MPP$^+$-induced mitochondrial function in SN4741 cells. Therefore, the aim of this study was to investigate the protective effects of myricitrin against MPP$^+$-induced mitochondrial dysfunction and the possible mechanisms underlying these effects.

2. Materials and methods

2.1. Chemicals and antibodies

Anti-DJ-1 and anti-β-actin antibodies were obtained from Abcam. MTT and MPP$^+$ were purchased from Sigma. Lipofectamine 2000, TMRE, MitoTracker and CM-H2DCFDA were obtained from Life Technologies. Myricitrin was purchased from the Chinese

Abbreviations: Myr, myricitrin; MPP$^+$, 1-methyl-4-phenylpyridinium; PD, Parkinson's disease; MMP, mitochondrial membrane potential.

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National Institutes for Food and Drug Control. All the other chemicals were obtained from Sigma–Aldrich unless otherwise stated.

2.2. Cell culture

SN4741 cells, a mouse embryonic substantia nigra-derived cell line, were cultured at 33 °C with 5% CO_2 in complete Dulbecco's minimum essential medium containing 10% heat-inactivated foetal bovine serum, 1% D-glucose, 1% penicillin-streptomycin, and 2 mM L-glutamine [17]. Most of the experiments were performed when cells reached 50–60% confluence.

2.3. Measurement of cell viability

Cell viability was determined by the conventional 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the instructions of the manufacturer. Briefly, MTT labelling reagents were added to SN4741 cells grown in the wells of a 96-well microplate, and the microplate was then incubated for 2–4 h in a humidified incubator at 37 °C. The absorbance was read at 570 nm using a microplate reader (Bio-Rad, Hercules, CA, USA), and the absorbance values were expressed as a percentage of the values of the untreated control cells (control = 100%).

2.4. Immunocytochemistry

For immunocytochemistry, SN4741 cells were grown on poly-L-lysine-coated slides. After treatment, the cells were washed with PBS three times, fixed with 4% paraformaldehyde (adjusted to pH 7.4) for 15 min and permeabilised with 0.1% Triton X-100 for 30 min. After being washed with PBS, the cells were incubated at 4 °C overnight with a DJ-1 antibody. The next day, the cells were washed with PBS and labelled with a combination of FITC-conjugated anti-rabbit secondary antibodies, and the nuclei were labelled with DAPI. Fluorescence images were captured using a confocal microscope (C2; Nikon, Japan).

2.5. Western blot analysis

Cells were washed with PBS three times and suspended in lysis buffer. After lysis, the supernatant was separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% non-fat milk for 2 h at room temperature and incubated with the indicated antibodies with gentle shaking at 4 °C overnight. The membranes were incubated with an HRP-conjugated secondary antibody, and the protein bands were visualised using ECL.

2.6. Semiquantitative RT-PCR and real-time qRT-PCR

Semiquantitative RT-PCR was performed according to the manufacturer's protocol for TRIzol reagent. Briefly, total cellular RNA was extracted using TRIzol reagent. Single-strand cDNA synthesis was performed using Brilliant II SYBR green single-step quantitative RT-PCR master mix. The products were used as a template to amplify the specific DNA, and 25-cycle regular PCR was performed for a semiquantitative determination of the original amount of RNA. The PCR products were separated using 1.2% agarose gels and visualised under a light-emitting diode light source. The abundance of each specific mRNA was normalised to β-actin mRNA. Real-time quantitative RT-PCR (qRT-PCR) was performed using an Mx3005 or Mx4000 multiplex quantitative PCR system (Stratagene). The expression of the target gene (DJ-1) was normalised to that of β-actin mRNA. The primer sequences were as follows: DJ-1 forward, 5′-AGCCGGGATCAAAGTCACTG-3′; DJ-1 reverse, 5′-GGTCCCTGCGTTTTTGCATC-3′; β-actin forward, 5′-AAGGACTCCTATAGTGGGTGACGA-3′; and β-actin reverse, 5′-ATCTTCTCCATGTCGTCCCAGTTG-3′.

2.7. DJ-1 siRNA transfection

Cells were transfected with DJ-1 siRNA (5′-AGGCGCGGCTG-CAGTCTTTAA-3′) or the control siRNA (5′-UUCUCCGAACGUGU-CACGUTT-3′) for 48 h using Lipofectamine 2000 according to the manufacturer's instructions.

2.8. Mitochondrial staining

Mitochondrial staining was performed according to the manufacturer's instructions. Briefly, SN4741 cells were grown on poly-L-lysine-coated slides. After treatment, the cells were stained for 20 min with 10 nM MitoTracker Red CMXRos (Life Technologies) at 33 °C. Mitochondria were imaged under a laser scanning confocal microscope (C2 Si; Nikon, Japan). Quantification of the mitochondria morphology was performed as described previously [18].

2.9. ROS detection

CM-H2DCFDA (Life Technologies) was used to measure the ROS production. For each designated treatment, cells were incubated with 5 μM dye for 30 min at 33 °C. The cells were washed three times with prewarmed PBS at 33 °C to remove excess dye. SN4741 cells at the same density were harvested by cell counting, and the relative levels of fluorescence were quantified using a multi-detection microplate reader (485 nm excitation and 535 nm emission).

2.10. Mitochondrial membrane potential assay

The mitochondrial membrane potential was measured according to the incorporation of TMRE. For each designated treatment, cells were incubated with TMRE (200 nM) for 20 min at 33 °C. After the cells were washed with PBS to remove excess TMRE, the cells were collected, and the fluorescence intensity was analysed using flow cytometry (BD).

2.11. TUNEL staining

The TdT-mediated dUTP nick end labelling (TUNEL) assay was performed using a One Step TUNEL Apoptosis Kit (Beyotime, JS, China). SN4741 cells were grown on poly-L-lysine-coated slides. For each designated treatment, cells were fixed, permeabilised, and incubated with the TUNEL reaction mixture at 37 °C for 1 h as described in the manufacturer's protocol. After three times washes with PBS (pH 7.4), the slides were stained using DAPI and observed under a laser scanning confocal microscope (C2 Si; Nikon, Japan).

2.12. Statistical analysis

Statistical analysis was performed using one-way ANOVA. All values are reported as the mean ± SEM of three independent experiments. P values <0.05 were considered statistically significant.

3. Results

3.1. Effect of Myr on the MPP^+-induced reduction in cell viability

To investigate the effect of MPP^+ on SN4741 cells, we treated cells with a range of MPP^+ concentrations for 24 h and then

evaluated the cell viability using MTT. The results showed that MPP^+ reduced the cell viability in a dose-dependent manner (Fig. 1B). In the group exposed to 80 μM MPP^+ for 24 h, the viability of the SN4741 cells was significantly decreased (48.33 ± 5.8%) compared with that of the control group ($P < 0.05$). An 80-μM concentration of MPP^+ and a 24-h incubation period were therefore used in subsequent experiments. To determine whether Myr was solely responsible for the difference in cell viability, SN4741 cells were treated with 40 μM Myr alone for 24 h. The results showed no obvious effect on cell viability. However, compared with MPP^+-treated group, the cell viability increased at Myr concentrations of 10, 20, and 40 μM when the cells were pretreated with various concentrations of Myr for 24 h before adding MPP^+ (Fig. 1C). These results demonstrated that Myr could protect SN4741 cells from the MPP^+-induced decrease in cell viability in a dose-dependent manner.

3.2. Myr effectively rescued DJ-1 from MPP^+-induced decline in SN4741 cells

DJ-1, an antioxidant protein, plays a central role in limiting mitochondrial damage in response to oxidative stress [19]. DJ-1 deficiency leads to multiple mitochondrial abnormalities [20]. To test whether Myr may affect the levels of DJ-1 protein, SN4741 cells were pretreated with Myr for 24 h before adding MPP^+, and the levels of DJ-1 protein were measured using immunocytochemistry assays and immunoblots (Fig. 2A and B). The results showed that MPP^+ caused a decrease in the protein levels of DJ-1. However, DJ-1 levels increased when SN4741 cells were pretreated with Myr compared with cells treated with MPP^+ alone. To test whether MPP^+ affected DJ-1 mRNA, we employed the same treatment paradigm and measured the DJ-1 mRNA levels by semi-quantitative PCR and quantitative real-time PCR (Fig. 2C and D). These data showed that DJ-1 mRNA transcription was not affected by MPP^+ or Myr. Thus, MPP^+ induced the decrease in the levels of DJ-1 without decreasing the level of DJ-1 mRNA, and Myr was able to inhibit the MPP^+-induced decline of DJ-1.

3.3. Myr protected SN4741 cells from MPP^+-induced mitochondrial dysfunction via DJ-1

SN4741 cells were transfected with siRNA targeting the mouse DJ-1 gene or with control siRNA. At 48 h after transfection, the expression levels of DJ-1 in SN4741 cells were examined by western blotting (Fig. 3A). The results showed that approximately 80% of the baseline DJ-1 expression was knocked down in the siDJ-1-transfected SN4741 cells. These cells were pretreated with Myr for 24 h before the addition of MPP^+. The level of ROS, mitochondrial membrane potential (MMP) and mitochondrial morphology were determined using CM-H2DCFDA, TMRE and MitoTracker, respectively (Fig. 3B, C and D). The results showed that MPP^+ caused significant mitochondrial fragmentation, an increase in the level of ROS and a decrease in MMP. After pretreatment with Myr for 24 h before the addition of MPP^+, the MPP^+-induced mitochondrial fragmentation was partially alleviated, as indicated by the appearance of longer mitochondrial tubules; additionally, the MPP^+-induced ROS production decreased, and MMP increased. Conversely, silencing DJ-1 largely abrogated the protection of Myr both against the fragmented mitochondria phenotype under MPP^+-induced stress and against the impairment of MMP and ROS production.

3.4. Myr attenuated the MPP^+-induced apoptosis in SN4741 cells by DJ-1-mitochondrial pathway in SN4741 cells

TUNEL staining was used to investigate the effect of Myr on MPP^+-induced apoptosis in SN4741 cells. The results showed that MPP^+ caused an increase in the number of TUNEL-positive cells (Fig. 4A). Significantly fewer TUNEL-positive cells were observed when SN4741 cells were pretreated with Myr for 24 h before the addition of MPP^+ compared with treatment with MPP^+ alone.

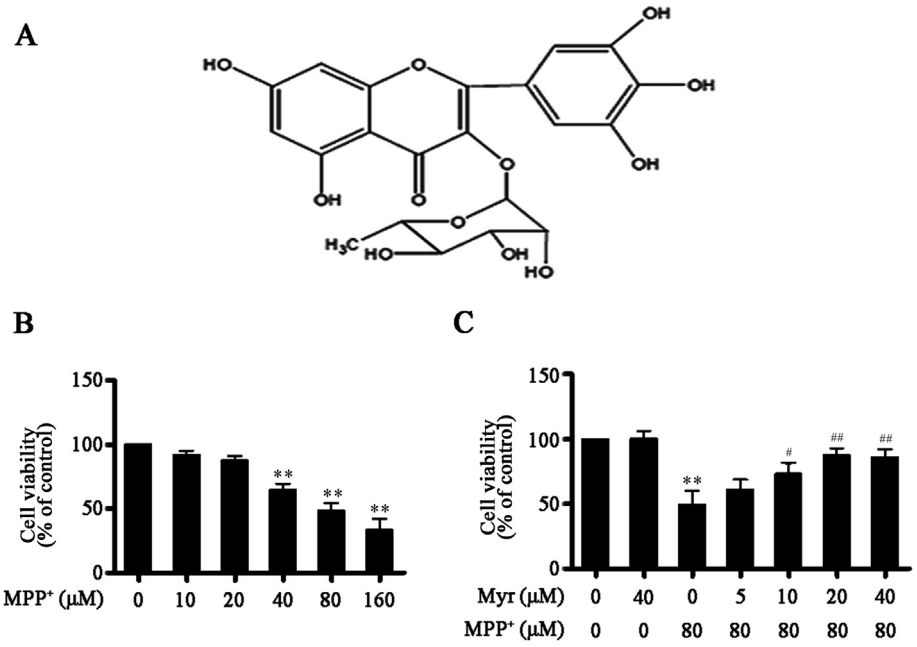

Fig. 1. Protective effect of Myr against MPP^+-induced cytotoxicity in SN4741 cells. **A**: Chemical structure of Myr. **B**: Effects of various concentrations of MPP^+ on the cell viability of SN4741 cells. SN4741 cells were incubated with various concentrations of MPP^+ for 24 h, and the cell viability was assayed using MTT. **C**: The effect of Myr on MPP^+-induced cytotoxicity in SN4741 cells. Cells were pretreated with various concentrations of Myr for 24 h, and MPP^+ (80 μM) was added for an additional 24 h. The cell viability was measured using MTT. Data are shown as the mean ± SEM (n = 3). $^{**}P < 0.01$ compared with the control group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the MPP^+-treated group.

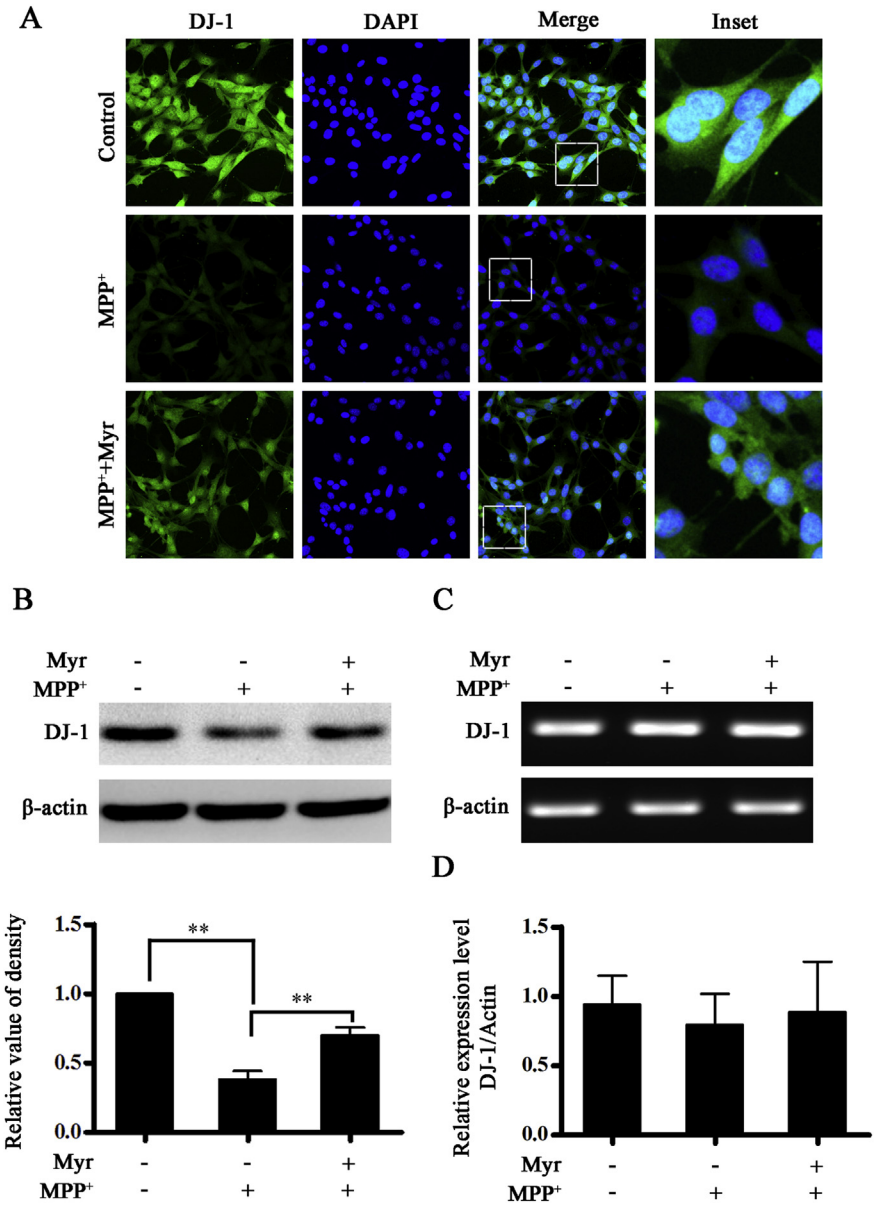

Fig. 2. Effect of Myr on the decline of DJ-1 induced by the neurotoxin MPP^+ in SN4741 cells. **A**: Immunofluorescence analysis of DJ-1 in SN4741 cells. Cells were pretreated with Myr for 24 h, and MPP^+ (80 μM) was added for an additional 24 h. The cells were then analysed using immunofluorescence (green: DJ-1; blue: DAPI; the insets represent the boxed areas). **B**: Western blot analysis of the DJ-1 protein level in SN4741 cells. SN4741 cells were treated as described in (**A**), and the DJ-1 protein level was measured (n = 3). The bottom graph shows the quantification of DJ-1 levels (mean ± SEM; n = 3; $^{**}p < 0.01$). **C** and **D**: Analysis of mRNA in SN4741 cells treated with MPP^+ in the presence of Myr or not. SN4741 cells were treated with MPP^+ as described in (**A**). Total RNA was extracted from SN4741 cells and analysed using semi-quantitative reverse-transcription PCR (**C**) and real-time quantitative PCR (**D**). The values presented were normalised to the levels of β-actin (mean ± SEM; n = 3; $^{*}p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

However, the protective effect of Myr was abrogated when DJ-1 was knocked down. These findings were further supported by determining the cell viability using an MTT assay in SN4741 cells (Fig. 4C). As described above, Myr was able to reduce the MPP^+-induced loss of cell viability. However, the protective effect was abrogated when DJ-1 was knocked down in SN4741 cells.

4. Discussion

PD is the second most common neurodegenerative disorder and is characterised by bradykinesia, rigidity, tremors, and gait dysfunction with postural instability. Currently, only symptomatic treatments of PD are efficacious. Most treatments do not halt or retard the degeneration of dopaminergic neurons [21]. Thus, drugs that slow or stop the neurodegeneration associated with PD are expected to have a major impact [22]. Myr, a flavanoid isolated from the root bark of *Myrica cerifera*, has been shown to protect SH-SY5Y cells from 6-OHDA-induced cell apoptosis. However, whether Myr provides neuroprotection against MPP^+-induced apoptosis was unknown prior to this study.

MPP^+, which acts as a selective toxin for DA neurons and causes parkinsonism in humans and nonhuman primates, has been extensively studied using in vivo and in vitro experimental models of PD [23]. MPP^+ inhibits complex I and stimulates superoxide formation, causing oxidative damage to proteins. In the current study, the results showed that treating SN4741 cells with MPP^+ resulted in a dose-dependent decrease in viability, as indicated by the MTT assay. Moreover, MPP^+ caused an increase in the number

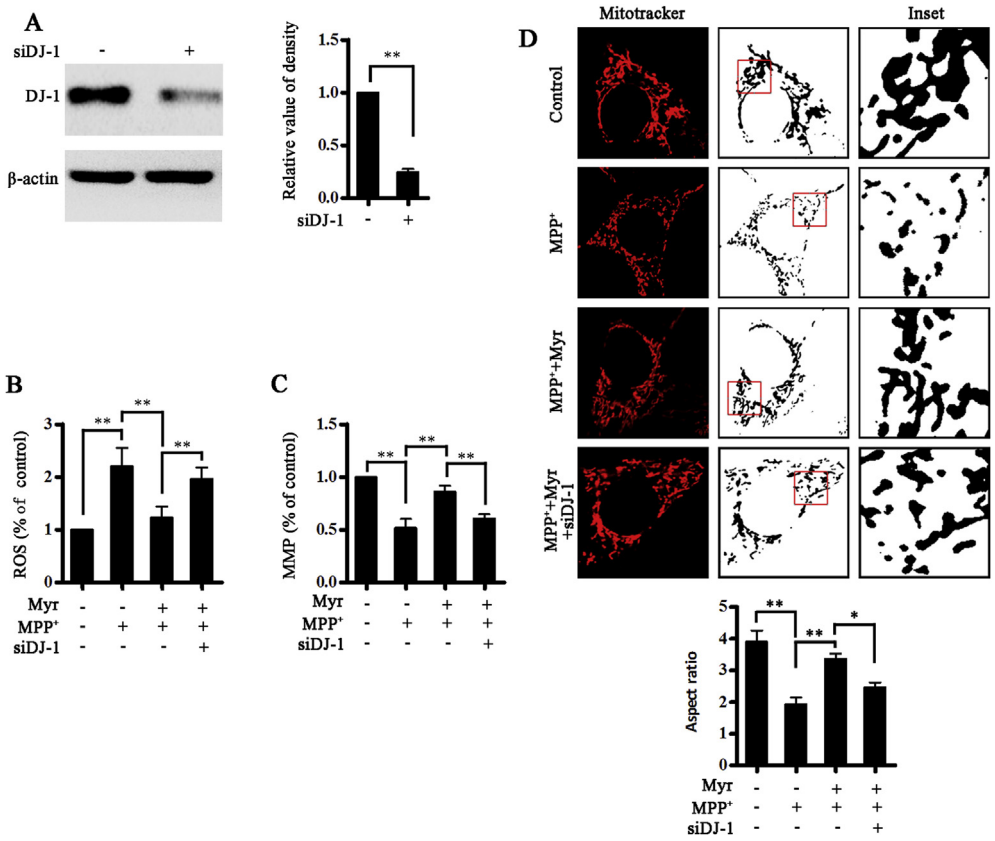

Fig. 3. Myr exerts a DJ-1-dependent protective effect against mitochondrial dysfunction induced by the neurotoxin MPP^+ in SN4741 cells. **A**: SN4741 cells were transfected with siRNA targeting the mouse DJ-1. At 48 h after transfection, cell extracts were prepared, and the expression levels of DJ-1 and actin were analysed using western blotting with the appropriate antibodies. The right graph shows a quantification of the DJ-1 levels (mean ± SEM, n = 4, **P < 0.01). **B**: The effect of DJ-1 downregulation on Myr-induced changes in the ROS levels. SN4741 cells transfected with siRNA targeting DJ-1 or control siRNA were treated as described in Fig. 2A and then incubated with CM-H2DCFDA at 33 °C for 30 min. Ten thousand cells were assayed for CM-H2DCFDA fluorescence by flow cytometry (mean ± SEM; n = 3; **p < 0.01). **C**: The effect of DJ-1 downregulation on the Myr-induced protection of MMP. SN4741 cells treated as described in (B) were incubated with TMRE at 33 °C for 30 min. Ten thousand cells were assayed for TMRE fluorescence by flow cytometry (mean ± SEM; n = 3; **p < 0.01). **D**: The effect of DJ-1 downregulation on the Myr-induced protection of mitochondrial morphology. Cells were treated as described in (**B**) and incubated with MitoTracker (10 nM, MitoTracker Red CMXRos from Life Technologies) for 20 min at 33 °C. Mitochondrial morphology in living SN4741 cells was analysed using live cell imaging (C2 Si; Nikon, Japan) (red: MitoTracker; insets represent the boxed areas). The bottom graph shows quantification of the mitochondria morphology (the aspect ratio of mitochondria is the ratio between the major and minor axes of an ellipse equivalent to the shape of mitochondria) (mean ± SEM; n = 3; *p < 0.05; **p < 0.01). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of TUNEL-positive cells. In contrast, after pretreatment with Myr, the cell viability increased compared with that in response to MPP^+ alone, indicating that Myr does protect SN4741 cells from MPP^+-induced apoptosis.

Mitochondria are important organelles in eukaryotes and supply energy for almost all cellular activities through oxidative phosphorylation, which is accompanied by the production of ROS [24]. Under physiological conditions, a low level of intracellular ROS is important for maintaining redox balance and promoting cell proliferation. Excess ROS is highly toxic to key cellular macromolecules, including DNA, proteins, and lipids, and ROS cause damage to mitochondria and may eventually lead to cell death [25,26]. Increasing evidence from PD patients, animal toxin models, and genetic models indicates that mitochondrial dysfunction plays a central role in the pathophysiology of PD. Drugs that are able to reduce mitochondrial dysfunction hold great promise for interventions. Mitochondrial dysfunctions, including the accumulation of fragmented mitochondria, a loss of the mitochondrial membrane, and an increase in ROS, are associated with apoptosis, and these three processes were observed in MPP^+-treated cells. These events were all found to be alleviated in a dose-dependent manner by pretreatment with Myr. Therefore, the neuroprotection mediated by Myr occurs through a ROS-related mitochondrial pathway.

DJ-1, which is composed of 189 amino acids and forms a dimer, is expressed in almost all cells and tissues, including the brain. In 2003, Bonifati et al. reported a large deletion and missense mutation in the DJ-1 gene in Italian and Dutch PD patients, identifying the DJ-1 gene as a causative gene for familial PD [8]. DJ-1 is a multifunctional protein involved in transcriptional regulation, chaperone activity, antioxidative stress, protease functions, and mitochondrial regulation. DJ-1 plays an important role in maintaining mitochondrial function, and fragmented mitochondria are observed in DJ-1-knockout mice and cells. Excess oxidation renders DJ-1 inactive, and highly oxidised DJ-1 has been observed in patients with PD and AD [10]. These results suggest that blocking the inactivation of DJ-1 could be used for therapeutic purposes for the oxidative stress-related diseases mentioned previously. In this study, Myr could reversed the decline of DJ-1 induced by MPP^+ but not *DJ-1* genetic transcription. Rescuing DJ-1 from MPP + -induced decline may be involved in the regulation of mitochondrial functions. Thus, we measured mitochondrial function with DJ-1 silenced by siRNA in SN4741 cells. The results showed that the accumulation of fragmented mitochondria, the increase in the level of ROS and the MMP decrease induced by MPP^+ could not be rescued by Myr when DJ-1 was knocked down in SN4741 cells. These results suggest that Myr rescues mitochondrial function in a DJ-1-dependent manner. More importantly, in siDJ-1-transfected

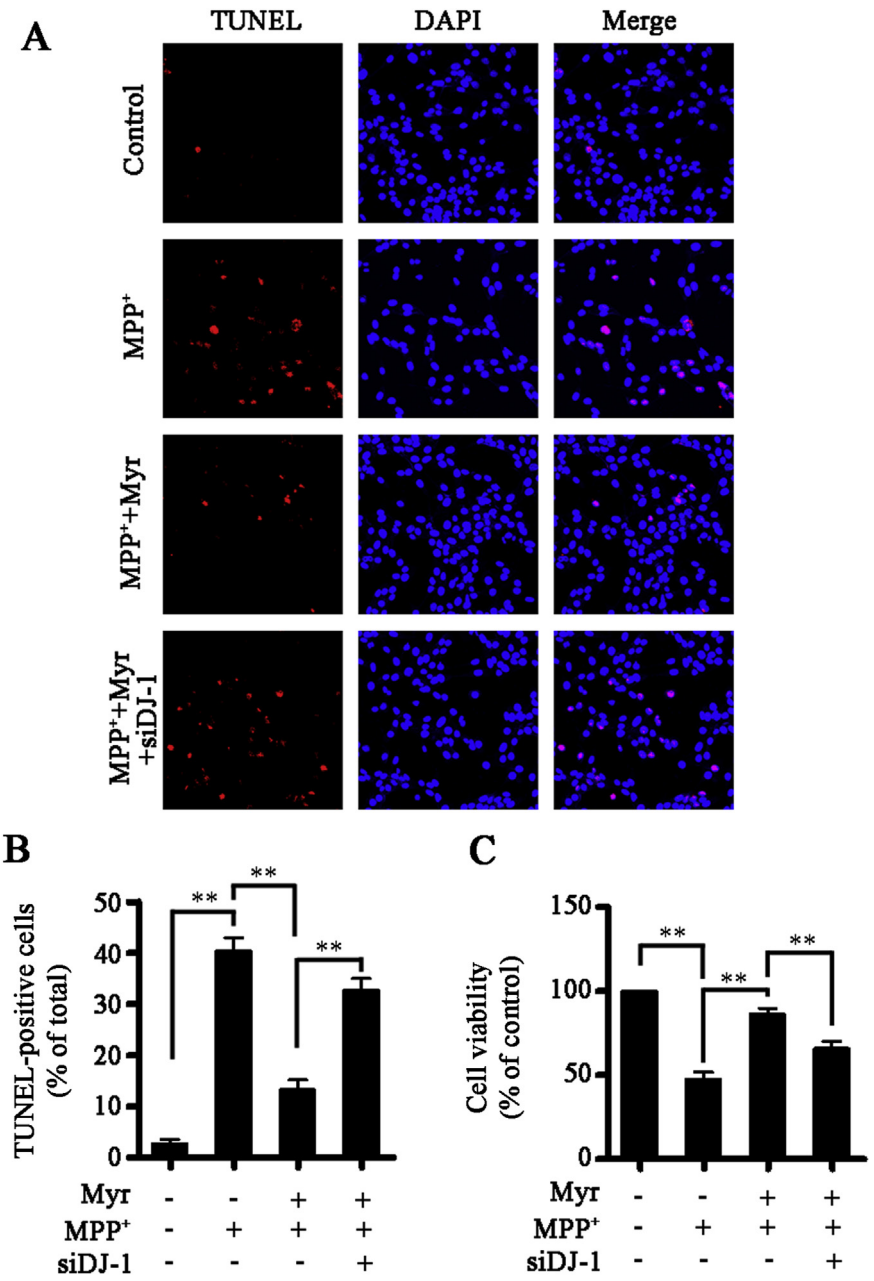

Fig. 4. Myr exerts a DJ-1-dependent protective effect against cell death induced by the neurotoxin MPP^+ in SN4741 cells. SN4741 cells were treated as described in Fig. 3B. **A**:Apoptosis was assayed by the TUNEL assay. **B**: Quantitative analysis of the number of TUNEL-positive cells (mean ± SEM; n = 3; $^{**}p < 0.01$). **C**: The cell viability was measured using the MTT assay (mean ± SEM; n = 3; $^{**}p < 0.01$).

cells, Myr lost its protective activity against MPP^+-induced cell death, indicating that Myr functions in a DJ-1-dependent manner.

In conclusion, our study indicates that Myr inhibits the apoptosis induced by MPP^+ in SN4741 cells. The protective effects of Myr are associated with an increase in the levels of DJ-1 to rescue mitochondrial dysfunction. Those results show that the protective effects of Myr on SN4741 cells are mediated, at least in part, by the DJ-1-mitochondrial pathway. Myr should next be tested in animal models that mimic the progression of Parkinson's disease before being considered as a candidate for a clinical trial.

Conflicts of interest

None.

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References

[1] J.M. Fearnley, A.J. Lees, Ageing and Parkinson's disease: substantia nigra regional selectivity, Brain J. Neurol. 114 (Pt 5) (1991) 2283–2301.
[2] O. Hornykiewicz, S.J. Kish, Biochemical pathophysiology of Parkinson's disease, Adv. Neurol. 45 (1987) 19–34.
[3] C. Henchcliffe, M.F. Beal, Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis, Nat. Clin. Pract. Neurol. 4 (2008) 600–609.
[4] M.P. Mattson, M. Gleichmann, A. Cheng, Mitochondria in neuroplasticity and neurological disorders, Neuron 60 (2008) 748–766.
[5] X. Tao, L. Tong, Crystal structure of human DJ-1, a protein associated with early onset Parkinson's disease, J. Biol. Chem. 278 (2003) 31372–31379.
[6] P.J. Kahle, J. Waak, T. Gasser, DJ-1 and prevention of oxidative stress in Parkinson's disease and other age-related disorders, Free Radic. Biol. Med. 47 (2009) 1354–1361.
[7] M.K. McCoy, M.R. Cookson, DJ-1 regulation of mitochondrial function and autophagy through oxidative stress, Autophagy 7 (2011) 531–532.
[8] V. Bonifati, P. Rizzu, M.J. van Baren, O. Schaap, G.J. Breedveld, E. Krieger, M.C. Dekker, F. Squitieri, P. Ibanez, M. Joosse, J.W. van Dongen, N. Vanacore, J.C. van Swieten, A. Brice, G. Meco, C.M. van Duijn, B.A. Oostra, P. Heutink, Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism, Sci. (New York N.Y.) 299 (2003) 256–259.
[9] D.W. Miller, R. Ahmad, S. Hague, M.J. Baptista, R. Canet-Aviles, C. McLendon, D.M. Carter, P.P. Zhu, J. Stadler, J. Chandran, G.R. Klinefelter, C. Blackstone, M.R. Cookson, L166P mutant DJ-1, causative for recessive Parkinson's disease, is degraded through the ubiquitin-proteasome system, J. Biol. Chem. 278 (2003) 36588–36595.
[10] J. Choi, M.C. Sullards, J.A. Olzmann, H.D. Rees, S.T. Weintraub, D.E. Bostwick, M. Gearing, A.I. Levey, L.S. Chin, L. Li, Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases, J. Biol. Chem. 281 (2006) 10816–10824.
[11] B.D. Paul, G.S. Rao, G.J. Kapadia, Isolation of myricadiol, myricitrin, taraxerol, and taraxerone from *Myrica cerifera* L. root bark, J. Pharm. Sci. 63 (1974) 958–959.
[12] S.J. Wang, Y. Tong, S. Lu, R. Yang, X. Liao, Y.F. Xu, X. Li, Anti-inflammatory activity of myricetin isolated from *Myrica rubra* Sieb. et Zucc. leaves, Planta Medica 76 (2010) 1492–1496.
[13] J. Bao, Y. Cai, M. Sun, G. Wang, H. Corke, Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability, J. Agric. Food Chem. 53 (2005) 2327–2332.
[14] G.B. Sun, M. Qin, J.X. Ye, R.L. Pan, X.B. Meng, M. Wang, Y. Luo, Z.Y. Li, H.W. Wang, X.B. Sun, Inhibitory effects of myricitrin on oxidative stress-induced endothelial damage and early atherosclerosis in ApoE−/− mice, Toxicol. Appl. Pharmacol. 271 (2013) 114–126.
[15] F.C. Meotti, F.C. Missau, J. Ferreira, M.G. Pizzolatti, C. Mizuzaki, C.W. Nogueira, A.R. Santos, Anti-allodynic property of flavonoid myricitrin in models of persistent inflammatory and neuropathic pain in mice, Biochem.. Pharm. 72 (2006) 1707–1713.
[16] Y.-H. Wang, Z.-H. Xuan, S. Tian, G.-R. He, G.-H. Du, Myricitrin attenuates 6-hydroxydopamine-induced mitochondrial damage and apoptosis in PC12 cells via inhibition of mitochondrial oxidation, J. Funct. Foods 5 (2013) 337–345.
[17] J.H. Son, H.S. Chun, T.H. Joh, S. Cho, B. Conti, J.W. Lee, Neuroprotection and neuronal differentiation studies using substantia nigra dopaminergic cells derived from transgenic mouse embryos, J. Neurosci. Off. J. Soc. Neurosci. 19 (1999) 10–20.
[18] W.J. Koopman, F. Distelmaier, J.J. Esseling, J.A. Smeitink, P.H. Willems, Computer-assisted live cell analysis of mitochondrial membrane potential, morphology and calcium handling, Methods (San Diego Calif.) 46 (2008) 304–311.
[19] D.J. Moore, L. Zhang, J. Troncoso, M.K. Lee, N. Hattori, Y. Mizuno, T.M. Dawson, V.L. Dawson, Association of DJ-1 and parkin mediated by pathogenic DJ-1 mutations and oxidative stress, Hum. Mol. Gen. 14 (2005) 71–84.
[20] I. Irrcher, H. Aleyasin, E.L. Seifert, S.J. Hewitt, S. Chhabra, M. Phillips, A.K. Lutz, M.W. Rousseaux, L. Bevilacqua, A. Jahani-Asl, S. Callaghan, J.G. MacLaurin, K.F. Winklhofer, P. Rizzu, P. Rippstein, R.H. Kim, C.X. Chen, E.A. Fon, R.S. Slack, M.E. Harper, H.M. McBride, T.W. Mak, D.S. Park, Loss of the Parkinson's disease-linked gene DJ-1 perturbs mitochondrial dynamics, Hum. Mol. Gen. 19 (2010) 3734–3746.
[21] A. Toulouse, A.M. Sullivan, Progress in Parkinson's disease – where do we stand? Prog. Neurobiol. 85 (2008) 376–392.
[22] M. Fukui, H.J. Choi, B.T. Zhu, Mechanism for the protective effect of resveratrol against oxidative stress-induced neuronal death, Free Radic. Biol. Med. 49 (2010) 800–813.
[23] D. Blum, S. Torch, N. Lambeng, M. Nissou, A.L. Benabid, R. Sadoul, J.M. Verna, Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease, Prog. Neurobiol. 65 (2001) 135–172.
[24] K. Schmitt, A. Grimm, A. Kazmierczak, J.B. Strosznajder, J. Gotz, A. Eckert, Insights into mitochondrial dysfunction: aging, amyloid-beta, and tau-A deleterious trio, Antioxid. Redox Signal. 16 (2012) 1456–1466.
[25] A.H. Schapira, Mitochondrial disease, Lancet 368 (2006) 70–82.
[26] S. Orrenius, V. Gogvadze, B. Zhivotovsky, Mitochondrial oxidative stress: implications for cell death, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 143–183.