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Effects of the Flavonoid Luteolin on Oxidative Stress in Parkinson's Disease Model

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ABSTRACT

Luteolin is one of the flavonoids compound of numerous traditional medicines. Luteolin has been reported to provide protective effects against apoptosis and oxidative stress. Oxidative stress plays an important role in physiology and pathological conditions. Increasing evidences indicate that the generation of reactive oxygen species (ROS) contributes to oxidative stress, which plays a crucial role in neurodegenerative diseases, including Parkinson's disease (PD). Previous studies demonstrated that neurotoxins induced oxidative stress resulted in cell death. Among them, 1-Methyl4-phenylpyridinium ion (MPP^+) has been widely used to study the role of oxidative stress, leading to cell death in PD. Therefore, optimization of ROS production is considered to be target to prevent PD. However, the effect of luteolin on PD via ROS generation is still obscure. In this study, human neuroblastoma SH-SY5Y cells were pre-treated with luteolin and NAC for 1 h, following treatment with 100 µM MPP^+. After treatment, cell viability was measured using MTT assay. Apoptotic cell death was observed by Hoechst 33342 staining. Intracellular ROS was measured using DCFH-DA. The results demonstrated that luteolin inhibited MPP^+-induced cell death. In addition, luteolin and NAC reduced ROS generation in MPP^+-treated SH-SY5Y cells. This study provides evidence that luteolin has a potent neuroprotective effect against MPP^+-induced cell death via the reduction of oxidative stress.

Keywords: Luteolin, MPP^+, Oxidative stress, ROS, SH-SY5Y

INTRODUCTION

Luteolin (3,4,5,7-tetrahydroxylflavone) is a common flavonoid abundant in fruits, vegetables and medicinal herbs (Williams and Spencer, 2012). Luteolin is known for its anti-inflammatory, anti-apoptosis activity, and anti-oxidative activity (Lin and Beal, 2006; Nabavi et al., 2015).

Oxidative stress occurs when there is an imbalanced redox state due to either excessive oxidants (ROS) or dysfunction of the antioxidant system (Lu et al., 2019). ROS include free radicals such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) (D'Autréaux and Toledano, 2007). The major sites of ROS generation is mitochondria (Tsang and Chung, 2009). ROS generation is initiated by the electron leak primarily at complex I of mitochondrial electron transport chains (ETC) (Lin and Beal, 2006). In the normal physiological state, cells have their own antioxidant defense mechanisms required for survival. The failure of antioxidant defense system can cause oxidative damage to biomolecules, eventually leading to neurodegenerative disease, including Parkinsin's disease (PD) (Turrens, 2003; Singh and Dikshit, 2007).

PD is the second most common neurodegenerative disease associated with progressive loss of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc) (Singh and Dikshit, 2007). The cause of PD is still unclear (Abushouk et al., 2017). However, it has been believed mitochondrial dysfunction, oxidative stress and neurotoxins are involved (Mounsey and Teismann, 2011). 1-Methyl4-phenylpyridinium ion (MPP^+) is a neurotoxin (Zhong et al., 2018). It causes symptom of PD in both *in vivo* and *in vitro* models by selectively destroys DAergic neurons in SNc (Hu et al., 2015; Zhong et al., 2018). MPP^+ has been shown to inhibit complex I of the mitochondrial ETC, subsequently, increased ROS, oxidative stress, and finally induced DAergic neuron death (More and Choi, 2017; Zhong et al., 2018). Thus, appropriate level of ROS have been shown to be indispensible to cell survival. Recently, luteolin has been documented to use in pharmacological studies against ROS which leads to activation of cell apoptosis (Lin and Beal, 2006). However, the protective effect of luteolin on MPP^+-induced ROS generation is still unclear. The present study investigated whether luteolin has a protective effect against MPP^+-induced cell death in human neuroblastoma SH-SY5Y cells via inhibition of ROS production.

MATERIAL AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and other supplement for cell culture were purchased from Gibco (Island, NY, USA). 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), N-acetylcyteine (NAC) and MPP^+ were purchased from Sigma-Aldrich (St. Louis, MO, USA). Luteolin, dimethyl sulfoxide (DMSO), 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide dye (MTT), were purchased from Merck Millipore (Darmstadt, Germany). Hoechst 33342 was purchased from Invitrogen by Thermo Fisher Scientific Incorporation (Oregon, USA).

Cell culture

SH-SY5Y cells were cultured in DMEM medium supplemented with 10% FBS, 1% penicillin/streptomycin and 1% L-alanyl-L-glutamine. The cells were maintained at 37 °C in 5% of CO_2 and 95% humidified incubator for 24 h.

Cell viability assay

Cell viability was measured using MTT assay (Zhou et al., 2013). The cells were seeded in 96-well plate (SPL Life Sciences, Gyeonggi-do, Korea). The cells were incubated with 1, 10 and 100 µM MPP^+ for 3, 9, 18 and 24 h. The cells were treated with luteolin (5, 10 and 20 µM) and MPP^+, with or without luteolin for 24 h. After indicated treatment, the cells were incubated with 5 mg/ml MTT at 37 °C for 4 h. The absorbance was determined using a microplate reader (ASYS UVM 340, Biochrom Ltd.).

Hoechst 33342 staining

Cell death was detected by Hoechst 33342 staining (Han et al., 2019). The cells were pre-treated with luteolin for 1 h, followed by treatment with 100 µM MPP^+ for 24 h. After treatment, the cells were incubated with 10 µg/ml Hoechst 33342 for 20 min and images were captured under inverted fluorescent microscope IX73 (model ULH100L-3). Apoptotic cells were considered to be those with cell nuclear condensation and/or fragmentation.

Intracellular ROS measurement

Intracellular ROS was measured using DCFH-DA (Zhang et al., 2013).

The cells were incubated with 100 µM MPP^+, with or without 20 µM luteolin, 10 mM antioxidant NAC was used as a positive control. After treatment, DCFH-DA was added and incubated at 37 °C for 1 h. The cells were irradiated by a fluorescence microscope using excitation and emission wavelengths 485 and 535 nm, respectively.

Statistical analysis

Experimental data were presented as means ± standard deviation (SD). Student's t-test was used to determine the significance of different between groups. The *p value* < 0.05 was considered to indicate statistical significance.

RESULTS

Effect of MPP^+ on cell viability

To investigate whether MPP^+ could induce cell death, SH-SY5Y cells were incubated with different concentrations of MPP^+ (1, 10 and 100 µM) for 3, 9, 18 and 24 h. The cell viability was determined by MTT assay. As shown in Figure 1, treatment with MPP^+ at concentration of 100 µM was significantly reduced in the percentage of cell viability compared with control to 89.57 ± 4.74 %, 83.66 ± 6.12 % 81.76 ± 8.60 % and 80.02 ± 6.85 %, at 3, 9, 18 and 24 h, respectively. Our results were in agreement with previous finding that 100 µM MPP^+ could decrease the viability of the SH-SY5Y cells in a concentration and time dependent manner (Choi et al., 1999; Chakraborty et al., 2016; Wu et al., 2017). Therefore, the concentration of MPP^+ at 100 µM was chosen as the experimental concentration in further experiments.

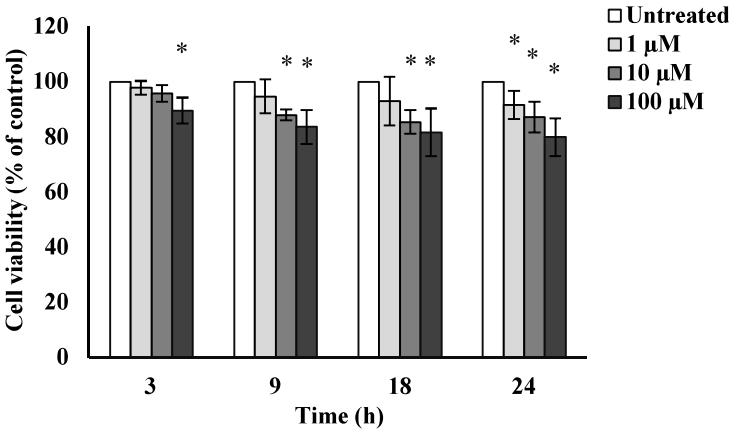

Figure 1. Effect of MPP^+ on cell viability in SH-SY5Y cells. The cells were exposed to MPP^+ at various concentrations (1, 10 and 100 µM) for 3, 9, 18 and 24 h. Cell viability was measured by MTT assay. Results were presented as means ± standard deviation (SD) (n = 3). * indicated p value < 0.05 significant differences compared with control group.

Luteolin attenuate MPP^+-induced cell death

To examine whether neuroprotective effect of luteolin on MPP^+ induced cell death, the cells were pre-treated with 5-20 µM luteolin for 1 h following treatment with 100 µM MPP^+ for 24 h. As shown in Figure 2, cell viabilities were significantly increased by luteolin in a concentration-dependent manner. Pre-treatment with 20 µM luteolin for 1 h prior exposure to 100 µM MPP^+ for 24 h was significantly increased cell viability to 97.05 ± 2.19 % compared with the MPP^+ treated group. In contrast, 20 µM luteolin did not show a significant difference of cell viability compared to the control group. Therefore, 20 µM luteolin was chosen as the experimental concentration in further experiments.

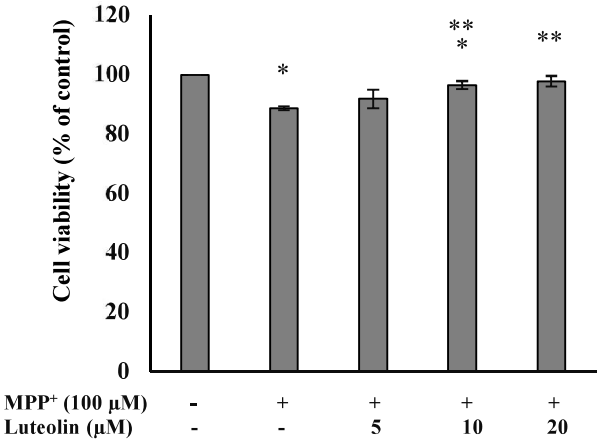

Figure 2. Anti-cytotoxicity effect of luteolin on MPP^+-induced cell death in SH-SY5Y cells. The cells were pre-treated with 5-20 µM luteolin for 1 h prior exposure to MPP^+ for 24 h. Cell viability was analyzed by MTT assay. Results were presented as means ± standard deviation (SD) ($n = 3$). * and ** represented $p < 0.05$, compared with the control and MPP^+ treated group, respectively.

Luteolin attenuated MPP^+-induced apoptosis

To investigate whether luteolin has neuroprotective effect against MPP^+-induced cell apoptosis, the cells were pre-treated with 20 µM luteolin for 1 h prior exposure to 100 µM MPP^+ for 24 h. Hoechst 33342 staining showed that cells exposed to MPP^+ exhibited a marked increase in nuclear condensation and DNA fragmentation. In contrast, pre-treatment with 20 µM luteolin displayed a reduction in nuclear condensation and DNA fragmentation (Figure 3). Hence, luteolin showed a remarkable protective effect against MPP^+-induced cell apoptosis.

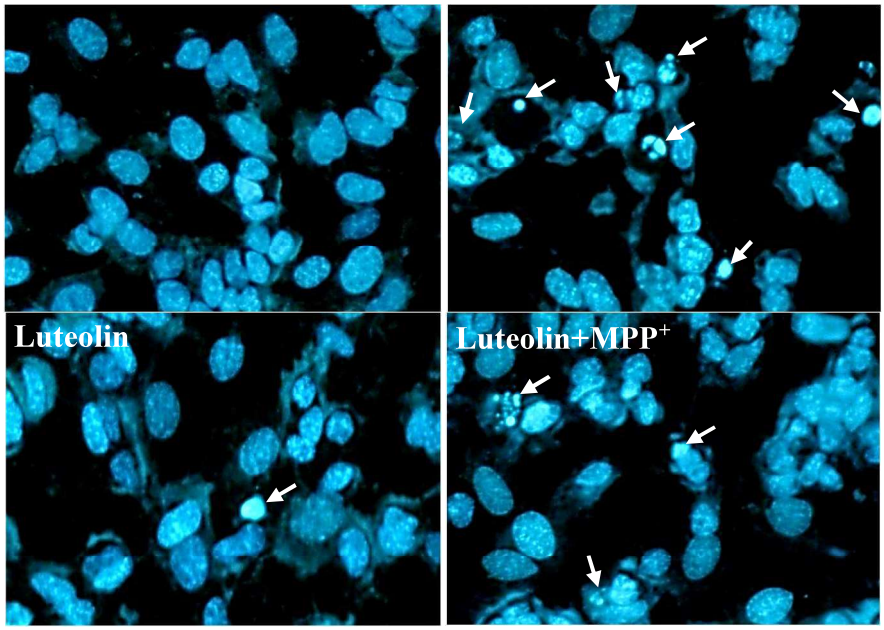

Figure 3. Luteolin attenuated MPP^+-induced cell apoptosis in SH-SY5Y cells. The cells were pre-treated with 20 μM luteolin for 1 h prior exposure to MPP^+ for 24 h. Cell apoptosis observed using Hoechst 33342 staining. Photographs were taken under a fluorescence microscope (20x). Arrows represented apoptotic cells.

Luteolin suppressed MPP^+-induced intracellular ROS generation

To evaluate whether antioxidant activity of luteolin againsts MPP^+-induced ROS formation, the cells were pre-treated with 20 μM luteolin and 10 mM ROS scavenger NAC for 1 h prior exposure to MPP^+ for 3 h. The intracellular ROS level was evaluated by DCFH-DA. As shown in Figure 4, exposure to MPP^+ significantly increased the intracellular ROS. In contrast, pre-treatment with 20 μM luteolin and 10 mM NAC for 1 h following treatment with 100 μM MPP^+ for 3 h significantly decreased ROS levels compared with the MPP^+ treated group. Therefore, the results indicated that luteolin exhibited anti-oxidative properties.

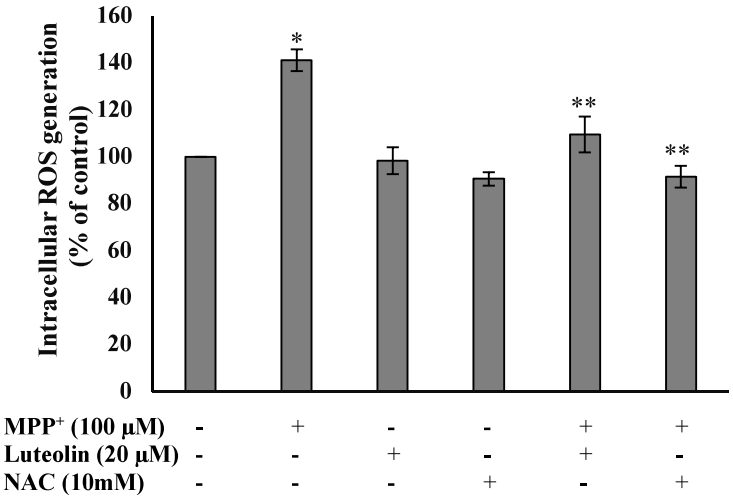

Figure 4. Protective effect of luteolin on MPP^+-induced ROS generation. Intracellular ROS levels were detected by DCFH-DA analysis. Cells were pre-treated with 20 µM luteolin for 1 h. Intracellular ROS was detected after exposure to 100 µM MPP^+ for 3 h, and expressed as percentage of control. The results were presented as mean ± SD ($n = 3$). * and ** indicated p value < 0.05, compared with control and the MPP^+ group, respectively.

DISCUSSION

In the present study, the effects of luteolin on MPP^+-induced oxidative stress was investigated in SH-SY5Y cells. The results demonstrated that pre-treatment with luteolin increased cell viability compared with cells treated with MPP^+ alone. Furthermore, luteolin reduced intracellular ROS generation and attenuated cell apoptosis in a model of MPP^+-induced PD.

Parkinson's disease (PD) is a progressive neurodegenerative disorder. PD involves progressive degeneration of DAergic neurons and depletion of dopamine (Singh and Dikshit, 2007; Rangel-Barajas et al., 2015).

Although the cause of Parkinson's disease is unknown, it is believed that oxidative stress and neurotoxins are involved (Lin and Beal, 2006; Zhong et al., 2018). MPP^+ is a neurotoxin that acts by interfering with oxidative phosphorylation by inhibiting complex I, leading to ROS generation and

finally induced neuron death (Rangel-Barajas et al., 2015). Our results agree with the previous finding that 100 μM MPP^+ could decrease the viability of cells in a concentration and time dependent (Yu et al., 2015; Zhang et al., 2016). Previous studies indicated that MPP^+ caused neuronal apoptosis via ROS generation (Zhang et al., 2016; More and Choi, 2017). Our results consistent with the previous findings that MPP^+ increased the intracellular ROS (Hu et al., 2015; More and Choi, 2017). Many preclinical studies have reported that luteolin has been used in pharmacological studies against ROS involved neurodegenerative disease (Lin et al., 2015). NAC, an effective scavenger of *free radicals reagent* was used to investigate the effect of luteolin on MPP^+-induced ROS generation (Ali et al., 2017; Markoutsa and Xu, 2017). These results showed that pre-treatment with luteolin and NAC increased cell viability compared with MPP^+ treated group. In contrast, luteolin and NAC showed a significant decrease MPP^+-induced ROS generation. Consistent with the previous study that revealed luteolin plays an important role in protecting biological system from ROS generation (Lin et al., 2015; Yu et al., 2015). Taken together, our study demonstrated that luteolin protect MPP^+-induced DAergic cell death through the mitochondria-mediated pathway.

CONCLUSION

In conclusion, the current study demonstrated that luteolin protected SH-SY5Y cells from MPP^+-induced apoptosis via the reduction of ROS generation. Therefore, luteolin may have a protective effect against oxidative stress. However, the mechanisms underlying the protective effect of luteolin against MPP^+-induced DAergic cell death should be further investigated.

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