行政院國家科學委員會補助專題研究計畫 ☑ 成 果 報 告
☐ 期中進度報告

芍藥甙對神經退化性疾病保護作用之研究

計畫類別：☑ 個別型計畫　　☐ 整合型計畫
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計畫參與人員：

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執行單位：中國醫藥大學
中　華　民　國　96　年　12　月 12　日

Content

Introduction

Paeoniae radix has been used to relieve spasmodic abdominal pain in traditional Chinese medicine. Paeoniflorine (PF), a major component from *paeoniae radix*, has several pharmacological effects including anti-allergic, antinociceptive, antispasmodic and anti-inflammatory action. In our previous studies, we found that paeoniflorin inhibited nociceptive behavior-induced by glutamate and NMDA etc. Additionally, PF showed protective effects on NMDA-induced neuronal cell damage and on ischemia-reperfusion injury in our previous study. Moreover, we found that the antinociceptive and neuronal protective effects of PF is via the interaction with ionotropic glutamatergic receptors, specifically mediated by the inactivation of NMDA receptor. Over excitation of NMDA receptor results in several neurological diseases, such as Parkinson disease, epilepsy, schizophrenia, dementia etc. It is also well known that dopaminergic/GABAergic/ glutamatergic neurons are closely interacted in the control of motor. One debatable issue is raised for consideration: dose the inhibitory property of PF on ionotropic glutamatergic receptors makes it available in the amelioration of Parkinson disease? In order to evaluate the effect of paeoniflorin on Parkinsonism, the effect of PF on neurotoxin-induced neuronal cell damage and on MPTP-treated MOR-KO mice will be investigated in this study.

Methods & Results

Effect of paeonilforin on MPP^+-induced neuronal cell damage

Neuronal cell line (SK-N-SH) was purchased from ATCC. The passage number of SK-N-SH cells used in this study was kept in 8 – 10. The cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and antibiotics and were plated at 1×10^4 cells per well in 96-well plastic tissue culture plates. Cells were placed in a humidified atmosphere of 95% air and 5% CO_2 for 24 h, and then cells were treated with 1mM MPP^+ for 48 h. Paeoniflorin (10 or 20 μM) was added 30 min prior to MPP^+. After 48 hr, each culture well was incubated in cultured medium containing 0.05 mg/ml MTT for 2 hr in 5% CO_2 at 37°C. The cells were lysed thoroughly with DMSO and the absorbance at 570 nm was measured. MTT reduction activity is expressed as a percentage of the control.

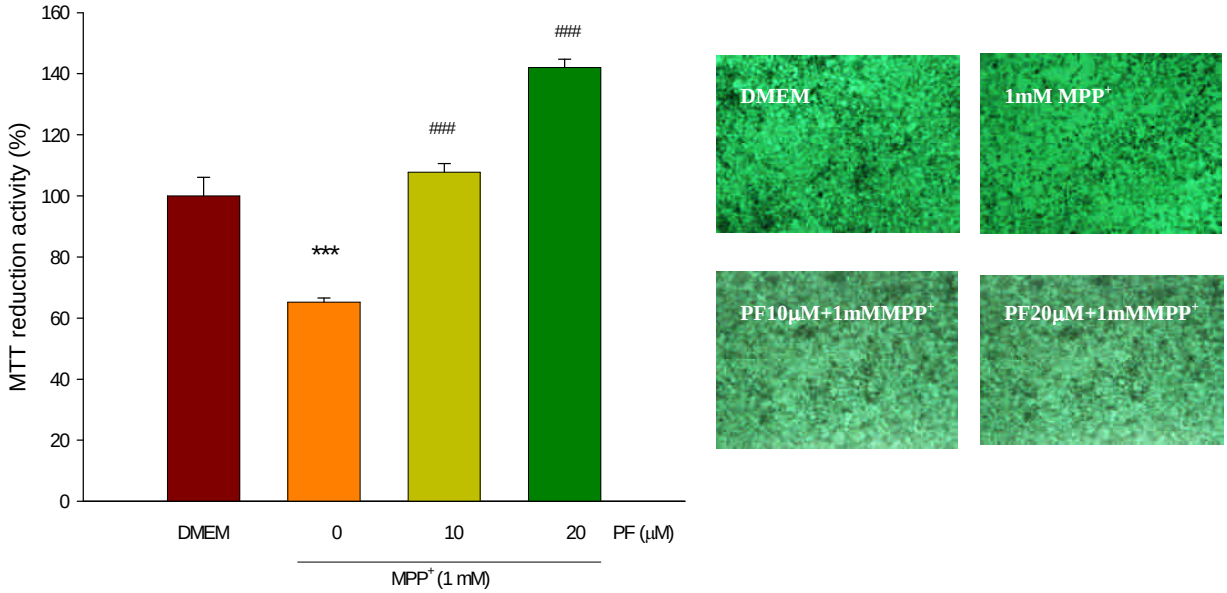

Figure 1. Effect of paeoniflorin on MPP^+-treated neuronal cells (SK-N-SH). Paeoniflorin (PF 10, 20 μM) and MPP^+(1 mM) was prepared in DMEM. Forty-eight hr after incubated with MPP^+ or PF, MTT was added to each well for detecting of cell viability. Results are means±S.E. of 24 wells and were obtained from 3 separate experiments. ***P < 0.001, compared with group without MPP^+; ###P<0.001, compared with MPP^+- treated group.

Effect of paeonilforin on 6-OHDA-induced neuronal cell damage

Neuronal cells (SHSY-5Y) were cultured in modified Eagle medium containing 10% fetal bovine serum and antibiotics and were plated at 1×10^4 cells per well in 96-well plastic tissue culture plates. Cells were placed in a humidified atmosphere of 95% air and 5% CO_2 for 24 h, and then cells were treated with 50μM 6-OHDA for 24 and 36 h. Paeoniflorin (PF 10, 20 or 30 μM) was added 30 min prior to 6-OHDA. After 24 or 36 hr, each culture well was incubated in cultured medium containing 0.05 mg/ml MTT for 2 hr in 5% CO_2 at 37°C. The cells were lysed thoroughly with DMSO and the absorbance at 570 nm was measured. MTT reduction activity is expressed as a percentage of the control.

(A) (B)

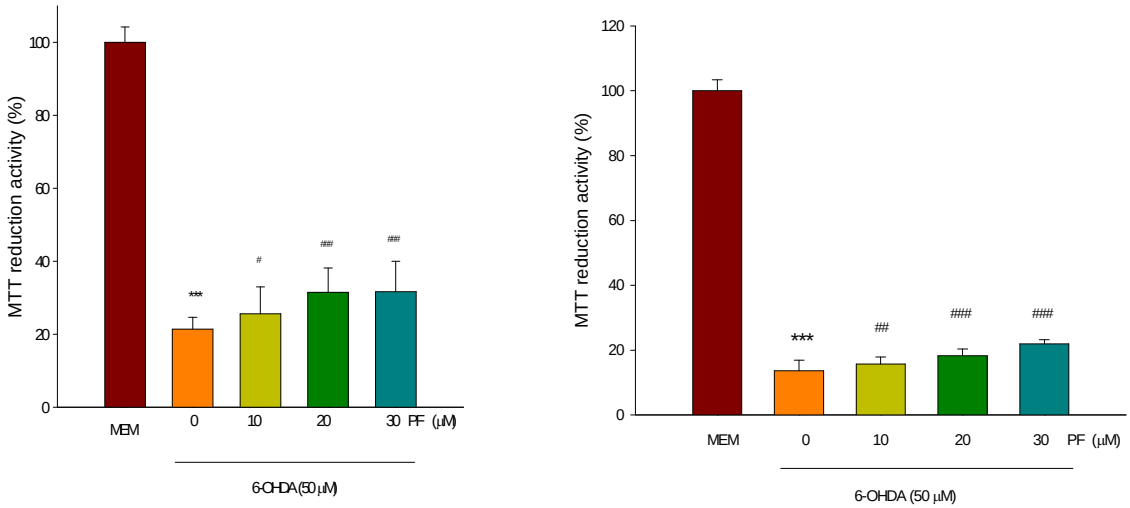

Figure 2. Effect of paeoniflorin (PF) on 6-OHDA treated neuronal cells (SHSY-5Y). PF (10, 20, 30 μM) or 50 μM 6-OHDA was prepared in MEM. (A) Twenty-four hr, (B) thirty-six hr after incubated with 6-OHDA or PF, MTT was added to each well for detecting of cell viability. Results are means±S.E. of 16-24 wells and were obtained from 3 separate experiments. *P<0.05, ***P<0.001, compared with MEM-control group; #P<0.05, ###P<0.001, compared with 6-OHDA-treated group.

Effect of paeonilforin on serum-, glucose-deprivation induced neuronal cell damage

Neuronal cells (SK-N-SH) were cultured in serum-, and glucose-free DMEM containing antibiotics and were plated at 1×10^4 cells per well in 96-well plastic tissue culture plates. Cells were placed in a humidified atmosphere of 95% air and 5% CO_2 for 6 h, 12 h, 24 h, 36 h. Paeoniflorin (PF 10, 20 or 30 μM) was added 30 min prior to serum-, glucose-deprivation. After 6, 12, 24 or 36 hr, 0.05 mg/ml MTT was added to each culture well for 2 hr in 5% CO_2 at 37°C. The cells were lysed thoroughly with DMSO and the absorbance at 570 nm was measured. MTT reduction activity is expressed as a percentage of the control.

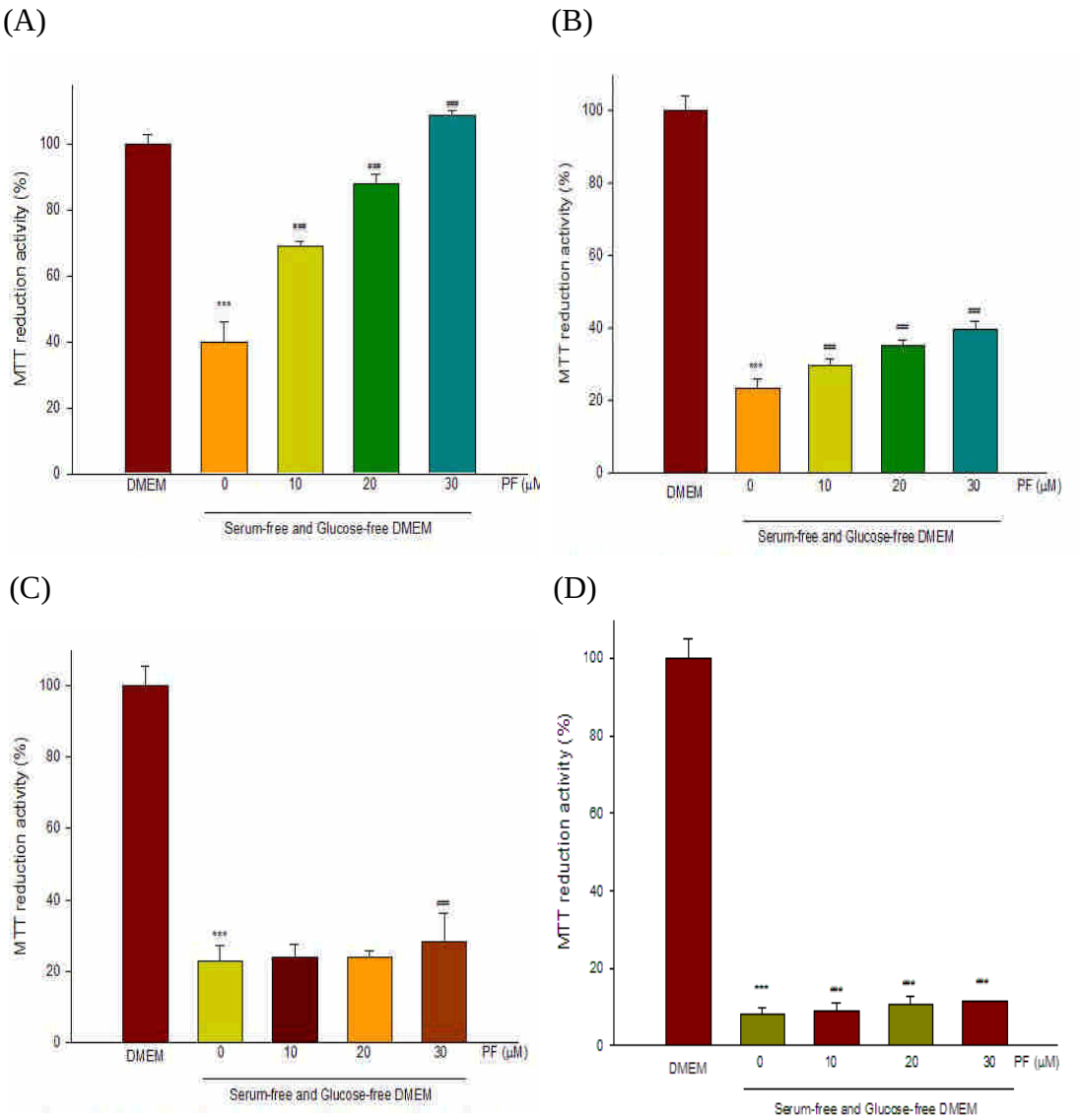

Figure 3. Effects of paeoniflorin (PF) on serum- and glucose-deprivation on cultured neuronal cells (SK-N-SH cells). PF (10, 20, 30 μm) was prepared in serum- and glucose-free DMEM. Neuronal cells were incubated in DMEM, serum- and glucose-free DMEM and PF for (A) 6 hr, (B) 12 hr, (C) 24 hr, (D) 36 hr. Vertical bars represented mean ± S.E. ***P<0.001 compared with DMEM group, ###P<0.001 compared with serum- and glucose-free DMEM group.

Effect of paeonilforin on H_2O_2-induced neuronal cell damage

Neuronal cells (SK-N-SH) were cultured in DMEM containing 10% fetal bovine serum and antibiotics and were plated at 1×10^4 cells per well in 96-well plastic tissue culture plates. Cells were placed in a humidified atmosphere of 95% air and 5% CO_2 for 24 h, and

then cells were treated with 0.1 ~ 0.5 mM H_2O_2 for 2 h. Paeoniflorin (PF 10 μM) was added 30 min prior to H_2O_2. After 2hr, each culture well was incubated in cultured medium containing 0.05 mg/ml MTT for 2 hr in 5% CO_2 at 37˚C. The cells were lysed thoroughly with DMSO and the absorbance at 570 nm was measured. MTT reduction activity is expressed as a percentage of the control.

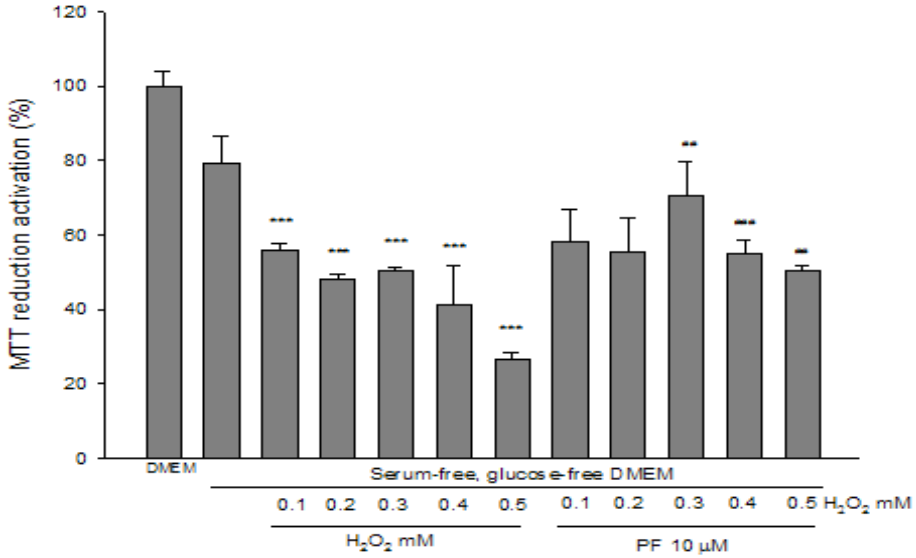

Figure 4. Effects of paeoniflorin (PF) and H_2O_2 on serum- and glucose-deprivation DMEM In cultured neuronal cells (SK-N-SH). PF (10μM) and H_2O_2 (0.1-0.5mM) was prepared in serum- and glucose-free DMEM. Cells were cultured in above medium for 2 hr. Vertical bars represented mean±S.E. ***P<0.001 compared with serum-, glucose-free DMEM group. ##P<0.01, ###P<0.001 compared with H_2O_2 group.

Effect of paeonilforin on the locomotion of MPTP-induced experimental Parkinsonism in MOR-KO mice

Wild-type or mu-opioid receptor knockout (MOR-KO) mice were treated with saline, or 1 mM MPTP s.c. everyday for 5 days. Paeoniflorin (PF 10 μM, i.c.v.) was administered 15 min prior to MPTP. The locomotor activity of mice was taken on the 3rd day after the final administration of MPTP. The locomotion of MOR-KO mice treated with saline is higher than that of wild-type treated with saline group. There's no significant difference between these two groups. Whereas, the locomotion was significantly decreased in MPTP-treated MOR-KO mice (**P<0.01). However, the locomotion of MOR-KO mice pretreated with PF was not significantly different from MPTP-treated MOR-KO mice.

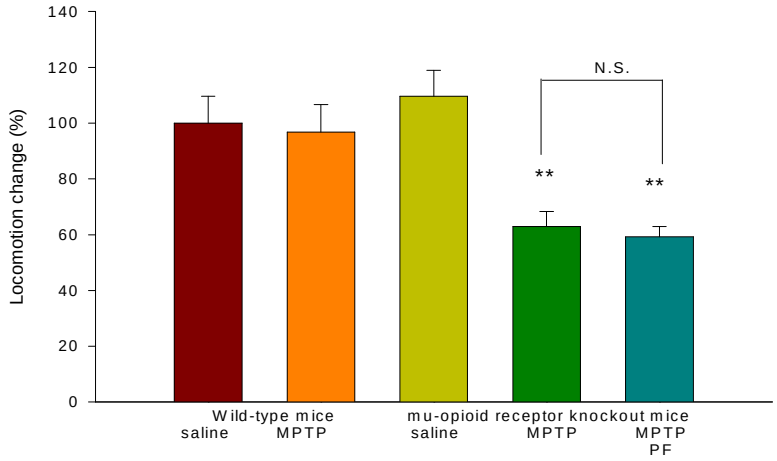

Figure 5. Effect of paeonilforin on the locomotion of MPTP-induced experimental Parkinsonism in MOR-KO mice. Paeoniflorin (PF, 10 μM, i.c.v.) 15 min prior to MPTP s.c. injection. MPTP (25 mk/kg, s.c.) was administered once daily for 5 days. Locomotion was taken on the 3rd day after the final MPTP administration. Data were represented as mean± S.E. **P<0.01 compared with MPTP-treated

Effect of paeonilforin on the striatum dopamine contents of MPTP-induced experimental Parkinsonism in MOR-KO mice

Wild-type or mu-opioid receptor knockout (MOR-KO) mice were treated with saline, or 1 mM MPTP i.p. everyday for 5 days. Paeoniflorin (PF 10 μM, i.c.v.) was administered 15 min prior to MPTP. Mice were sacrificed on the 3rd day after the final administration of MPTP and the striatum was subjected to homogenize. The extracts from striatum homogenization were filtered and the DOPAC, DOAP and DA contents were measured by using HPLC-ECD. DA contents in MOR-KO mice were higher than that of wild-type. The DA dramatically decreased in MOR-KO mice after MPTP treated for 5 days.

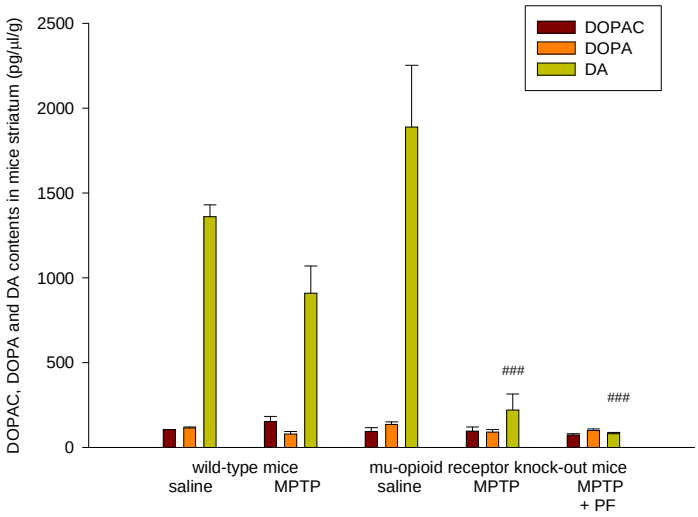

Figure 6. Effect of paeonilforin on the striatum dopamine contents of MPTP-induced experimental Parkinsonism in MOR-KO mice. Paeoniflorin (PF, 10 μM, i.c.v.) 15 min prior to MPTP i.p. injection. MPTP (25 mk/kg, i.p.) was administered once daily for 5 days. Animals were sacrificed on the 3rd day after the final MPTP administration, and the striatum DOPAC, DOPA and DA contents were measured by using HPLC-ECD. Data were represented as mean±S.E. *P<0.05 compared with wild-type mice; ###P<0.001 compared with MPTP-treated MOR-KO mice.

Conclusions

Results from the present study, our conclusions are as follows,

1. Paeoniflorin (10 or 20 μM) significantly protected SK-N-SH cells from 1 mM MPP^+-induced neuronal cell damage.
2. Paeoniflorin (20 or 30 μM) significantly protected SHSY-5Y cells from 50 μM 6-OHDA-induced neuronal cell damage.
3. MOR-KO mice are more sensitive to MPTP (25 mg/kg) than wild-type mice did. There's a significant decrease of locomotion and DA content in MPTP-treated MOR-KO mice than MPTP-treated wild-type mice.
4. Paeoniflorin (10 μM) was not able to reverse the MPTP-induced DAergic neuron damage in vivo.