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The anti-apoptosis effect of curcumin I on bax/bcl-2 ratio against 6-OHDA induced SH-SH5Y toxicity

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Abstract

Curcumin is a naturally occurring polyphenolic compound. It has been reported that it exerts anti-oxidative and anti-apoptotic activities. 6-Hydroxydopamine (6-OHDA) is used as a neurotoxin to generate a model of Parkinson's disease. In the present study, we investigate whether curcumin I (diferuloylmethane) could protect SH-SY5Y, dopaminergic cells from 6-OHDA-induced neurotoxicity. The results showed that pretreatment with curcumin I significantly prevented 6-OHDA induced cell viability reduction. Further experiments indicated that curcumin I could protect 6-OHDA-induced apoptosis signaling cascades activation. The results showed that pretreatment with curcumin I could prevent 6-OHDA-induced increasing of Bax/Bcl-2 ratio. This study suggested that curcumin I exerts its protective effects against 6-OHDA induced neurotoxicity. Therefore, curcumin I may be used as a potential compound for preventing oxidative stress-induced neurodegeneration.

Keywords: 6-OHDA, Curcumin I, Bax, Bcl-2, Phospho-P38.

Introduction

The generation of reactive oxygen species (ROS) has been well known to play a pivotal role in the pathogenesis of neurodegenerative diseases including Parkinson's disease (PD). In particular, 6-OHDA, a hydroxylated analogue of dopamine, is known to produce ROS [3] and widely used to mimic a model of PD both *in vivo* and *in vitro* studies[1]. Curcumin has been well known of its anti-oxidative, anti-inflammatory and anti-apoptotic properties. It has been reported that curcumin could prevent MPP^+- induced neurotoxicity in PC-12 cells via its anti-oxidative activity [2]. Also, another study reported that pretreatment with curcumin could protect 6-OHDA-induced cytotoxicity by anti-oxidative modulation in MES23.5 cells[4]. However, little is known about the cytoprotective effects of pure compound isolated from *Curcuma longa* on preventing 6-OHDA-induced dopaminergic cell death. Therefore, this study was taken to investigate whether a pure compound isolated from *Curcuma longa* which is curcumin I has a neuroprotective effect for preventing 6-OHDA-induced neurotoxicity in SH-SY5Y cells.

Methods

Cell cultures and cell viability assay

SH-SY5Y cells were maintained in MEM and Ham F-12 supplemented with 10% fetal bovine serum (FBS), 25 mg/ml of penicillin, 25 U/ml of streptomycin, 1 mM sodium pyruvate and 1 mM non-essential amino acids at 37 °C in an atmosphere of 5 % CO_2. 6-OHDA was dissolved in 0.01 % ascorbic acid in iced cold sterile water to give a stock

solution of 10 mM. Curcumin I was also dissolved in dimethylsulfoxide (DMSO) as a stock solution of 10 mM and the final concentration of DMSO was always less than 0.1 %. MTT (3-(4,5-dimethylthiazol-2,yl)2,5 diphenyltetrazolium bromide) assay was performed in order to determine the protective effect of curcumin I on cell viability in 6-OHDA treated cells. Cells were plated at a density of 3×10^4 cells/well in 96-well plate. After 24 h, cells were pretreated with curcumin I at the concentrations of 1, 5, 10 and 20 μM for 30 min before exposure to 6-OHDA 25 μM for 24 h. Then, 50 μl of 10 mg/ml MTT solution was added to each well and incubated for 4 h at 37 °C.

Western blot analysis

After treatment, cells were lysed in freshly prepared lysis buffer solution. Lysates were centrifuged at 12,000 rpm for 15 min at 4°C and the equal amounts of protein were separated by 12.5 and 10 % SDS-PAGE and transferred to hybond ECL nitrocellulose membranes. Membranes were blocked with 5 % skimmed milk in TBST for 1 h and then incubated with primary anti-Bax, Bcl-2 and actin antibodies in TBST containing 3 % BSA. After incubation with HRP-conjugated anti-IgG antibody, detected proteins were visualized using an enhanced chemiluminescence assay kit. Protein bands were measured using densitometry analysis program.

Data analysis

All data are expressed as mean ± SEM and analyzed using one-way ANOVA. Tukey's multiple comparison post-test was used to calculate the statistical significance. Differences were considered statistically significant when $p < 0.05$.

Results

Pretreatment with curcumin I could significantly prevent 6-OHDA induced cell death in a concentration dependent manner (Figure 1). The present study also found that curcumin I prevented 6-OHDA-induced increase in Bax/Bcl-2 ratio.

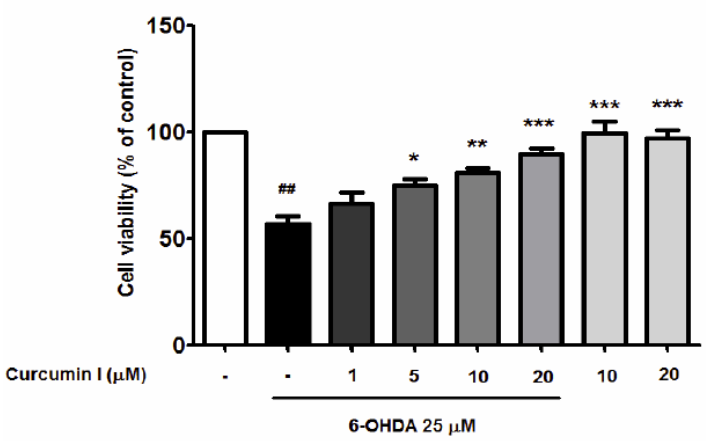

Figure 1 The protective effect of curcumin I on 6-OHDA- induced cell death. Values represent mean ± SEM of at least three separate determinations. * = $p<0.05$, ** = $p<0.01$, and *** = $p<0.001$ significant difference when compared with only 6-OHDA group, ##$P<0.001$ significant difference when compared with control group.

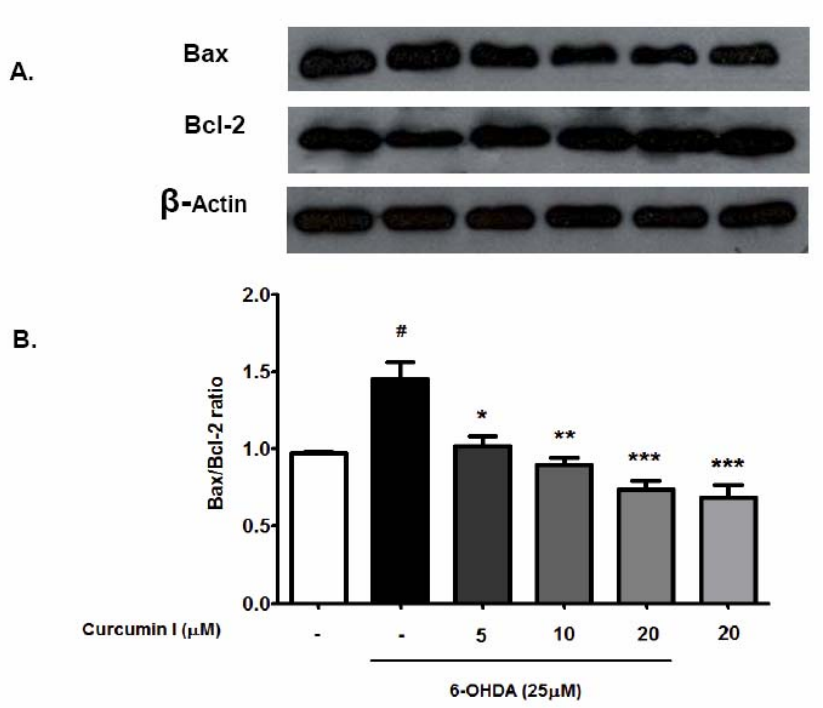

Figure 2. Curcumin I reduces Bax/Bcl-2 ratio in 6-OHDA-treated cells. A. Levels of Bax, Bcl-2 and β-Actin expression. B. Values represent mean ± SEM of Bax/Bcl-2 of at least three separate determinations. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ significant difference when compared with only 6-OHDA group, # = $p<0.001$ significant difference when compared with control group.

Discussion

6-OHDA-treated human neuroblastoma SH-SY5Y cells is a useful *in vitro* model for studying neurodegenerative events that may occur in PD. In this study, we demonstrated that curcumin I protects SH-SY5Y cells against 6-OHDA-induced cytotoxicity in apoptosis signaling pathway. The ratio between Bax and Bcl-2 has been used as an indicator for determining cell undergoing apoptosis. The ratio of the pro-apoptotic Bax to the anti-apoptotic Bcl-2 increases significantly upon treatment with 6-OHDA whereas curcumin I reduced the expression of Bax and increased the expression of Bcl-2 significantly, thereby ameliorating the 6-OHDA-induced Bax/Bcl-2 ratio elevation in SH-SY5Y cells. The present study showed that curcumin I has significant cytoprotection against 6-OHDA-induced apoptosis SH-SY5Y cells. The cytoprotection of curcumin I may be attributed from its inhibitory effect on the apoptotic signaling as evident from the decrease in Bax/Bcl-2 ratio. The antioxidant property of curcumin I may be one of the major mechanisms participated in its neuroprotection against cell death.

Conclusion

Our results show that curcumin I protects SH-SY5Y cells against 6-OHDA-induced cytotoxicity. Its anti-apoptotic activity of curcumin I may be useful as a potential compound for preventing oxidative stress-induced Parkinson's disease.

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