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Research article

Curcumin attenuates paraquat-induced cell death in human neuroblastoma cells through modulating oxidative stress and autophagy

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HIGHLIGHTS

- Curcumin attenuated APP accumulation in the paraquat-induced SH-SY5Ycells.
- Curcumin alleviated paraquat-induced SH-SY5Y cell death and suppressed excess ROS production.
- Curcumin enhanced autophagy and rescued chloroquine-treated SH-SY5Y cells.

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ABSTRACT

Paraquat is a neurotoxic agent, and oxidative stress plays an important role in neuronal cell death after paraquat exposure. In this study, we assessed the neuroprotective effect of curcumin against paraquat and explored the underlying mechanisms of curcumin *in vitro*. Curcumin treatment prevented paraquat-induced reactive oxygen species (ROS) and apoptotic cell death. Curcumin also exerted a neuroprotective effect by increasing the expression of anti-apoptotic and antioxidant genes. The pretreatment of curcumin significantly decreased gene expression and protein production of amyloid precursor protein. The activation of autophagy process was found defective in paraquat-induced cells, indicated by the accumulation and reduction of LC3I/II. Noteworthy, curcumin restored LC3I/II expression after the pretreatment. Collectively, curcumin demonstrated as a prominent suppressor of ROS, and could reverse autophagy induction in SH-SY5Y cells. The consequences of this were the reduction of APP production and prevention of SH-SY5Y cells from apoptosis. Altogether, curcumin potentially serves as a therapeutic agent of neurodegenerative diseases, associated with ROS overproduction and autophagy dysfunction.

1. Introduction

Alzheimerís disease (AD) pathology is characterized by the accumulation of extracellular plaques, including aggregated amyloid-β (Aβ) peptide, and intracellular tangles in the brain. Genetic evidence implicates deregulated Aβ homeostasis as an early event of AD pathology [29]. For this reason, most AD therapies have targeted at Aβ production. Autophagy is involved in the intracellular turnover of proteins and cell organelles and has a key role in regulating cell fates in response to stress [22,25]. Alteration of endocytic pathway can impede turnover of autophagic vacuoles and lead to diseases [33]. The modulation of autophagy was highlighted as a sophisticated mechanism in AD, since the induction and activation of autophagy can promote Aβ degradation and consequently reduce AD pathology [37]. Disrupted autophagy would lead to the accumulation of intracellular proteins, and the aberration of the lysosomal pathway might lead to neurodegeneration. Reportedly, the impaired clearance of autophagic vacuoles was observed in AD animal models and also AD patients [4,24].

Nowadays, therapies of AD patients are mostly palliative cares and rarely improve the disease symptoms. For this reason, the AD aetiology is attracted scientist attentions to investigate the causative molecular mechanisms of the disease, and develop novel therapeutic options. Curcumin is a naturally-occurring polyphenolic compound, extracted from turmeric, the powered rhizome of the plant *Curcuma longa*. Curcumin comprises 3 to 5% of turmeric and

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functions as pharmacologically active component [40]. Several *in vitro*, animal and human studies demonstrated potent antioxidant and anti-inflammatory properties of curcuminoids [3,6,32,38]. The ability of curcumin to reduce Aβ production, inflammation, and oxidative stress highlights its potential use for AD treatment.

In the present study, the exposure of SH-SY5Y cells to paraquat resulted in the increase of APP production/accumulation, exerted oxidative stress, and eventually led to cell death. Curcumin exhibited the protective roles on paraquat-induced neurotoxicity and its mechanisms of action involved in the activation of autophagy in human neuroblastoma SH-SY5Y cells.

2. Materials and methods

2.1. Chemical and reagents

Curcumin (Sigma, USA) was dissolved in 100% DMSO (Dimethyl sulfoxide) to prepare a stock solution (10 mM), aliquoted, and stored at −20 °C. Paraquat was reconstituted with DMSO, aliquoted, and stored at −20 °C. Chloroquine, N-acetylcysteine (NAC), and 2′,7′- dichlorofluorescin diacetate (DCFDA) were supplied by Sigma (Sigma, USA). The following antibodies were used: mouse anti-Alzheimer's Precursor Protein A4, a.a. 66–81 of APP (N-terminal), clone 22C11, rabbit anti-LC3-I/II antibody, goat anti-mouse IgG (H + L) antibody, goat anti-rabbit IgG (H + L), ALEXA-FLUOR (Merck KGaA, Germany).

2.2. Cell culture

SH-SY5Y cells were maintained in DMEM supplemented with heat-inactivated fetal bovine serum (FBS, 10% V/V), L-glutamine (1% V/V), penicillin/streptomycin (1% V/V), and 0.05% trypsin-EDTA at 37 °C in 5% CO_2.

2.3. MTT assay

Cell viability was measured by MTT assay. Briefly, MTT solution was added to cell culture media at 0.5 mg/mL and incubated for 3 h at 37 °C in the dark. Then, cells were solubilised with 100 μL of DMSO upon medium removal and absorbance was measured at 570 nm using a microplate reader (BMG Labtech, Germany).

2.4. Trypan blue staining

Cell death was evaluated by trypan blue staining. After incubation, cells were stained with trypan blue for 1 min, and stained cells were photographed by using an inverted microscope (Olympus IX41, Japan).

2.5. Annexin V and 7-AAD staining

Dual staining with Annexin V conjugated to fluorescein-isothiocyanate (FITC) and 7-amino-actinomysin (7-AAD) was performed using the commercial kit (Muse Annexin V and Dead Cell Kit, Merck KGaA, Germany). Staining was performed according to the manufacturer's instructions.

2.6. Measurement of intracellular ROS

The production of intracellular ROS was assessed by DCFH-DA oxidation. After the treatment with curcumin and paraquat for 24 h, cells were incubated with 10 μM DCFH-DA at 37 °C for 1 h and washed twice with PBS. The fluorescence intensity of DCFH-DA was measured by a fluorescence microplate-reader (Thermo Scientific, USA) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Results were expressed as percentage of controls.

2.7. Semiquantitative PCR for mRNAs

Total RNA was extracted from SH-SY5Y cells using NucleoSpin RNA kit (Macherey-Nagel, Dueren Germany), according to the manufacturer's protocol. For gene expression analysis, RNA was reverse transcribed using 2-steps RT-PCR Kit (Vivantis Technologies, Malaysia) according to the manufacturer's instructions. RT-PCR was performed using the Biorad/C1000Touch Thermocycle (Biorad, USA). The relative expression levels of mRNAs were quantified by normalization with the internal control *β-actin* gene.

2.8. Immunofluorescence staining

Cells were plated at 2×10^5 cells/cm^2 on 22 × 22 mm coverslips in 6-well plates for 24 h. Briefly, following the respective treatments, cells were fixed with 4.0% paraformaldehyde/PBS for 20 min at room temperature, washed 3 times for 5 min each, permeabilized with 0.2% Triton X-100 in PBS for 5 min and blocked in 10% FBS in PBS for 1 h. The coverslips were then immunostained using the antibodies diluted in blocking buffer, followed by fluorescently-conjugated secondary antibody. The nuclei were counterstained with DAPI (Vector Laboratories) and the stained cells were observed under a laser scanning confocal microscope (Nikon A1). Images were analysed using the ImageJ program (NIH).

2.9. Statistical analysis

Results were expressed as mean ± standard error (mean ± SE). All analysis was performed with GraphPad Prism 7 for Windows (GraphPad Software, USA). Significant differences between treatment effects were determined by one-way ANOVA, with statistical significance of $P < 0.05$.

3. Results

3.1. Effects of Curcumin on the mRNA Expression Levels of APP, PSI and PSII in paraquat-induced SH-SY5Y cells

To determine whether paraquat and curcumin have an effect on genes involving in AD pathology (*APP, PS1* and *PSII*), the expression of these genes were examined. The exposure to 0.5 mM paraquat for 24 h caused a significant increase of *APP* and *PSI* mRNA (Fig. 1A–C). The pretreatment of 5 μM curcumin for 24 h significantly reduced *APP* expression (Fig. 1B), while the direct treatment of curcumin alone to SH-SY5Y cells significantly downregulated *PsiI* (Fig. 1D). Thus, curcumin could reduce paraquat-induced *APP* expression, which is primarily characterized as an early sign of APP production/accumulation in AD pathology.

3.2. Curcumin altered the levels of APP accumulation in paraquat-induced SH-SY5Y cells

As previously shown, paraquat affected on the upregulation of *APP* expression in paraquat-induced SH-SY5Y cells, and the reduction was observed in the presence of curcumin (Fig. 1A, B). To confirm the production/accumulation of APP, confocal imaging was performed to detect changes in the production of APP protein in the SH-SY5Y cells. SH-SY5Y cells were pre-exposed to various concentrations of curcumin (5 and 10 μM) for 24 h, followed by the presence or absence of 0.5 mM paraquat for another 24 h (Fig. 2A). The results appeared that the treatment of paraquat significantly