Resveratrol protects Neuro-2a cells against Thapsigrin-induced damage

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Abstract

Alteration of the calcium homeostasis of endoplasmic reticulum (ER) results in excessive calcium accumulation in cytoplasm, which is one the main causes of neuronal cell death in neurodegenerative disorders. Thapsigargin (TG) is a drug that widely used to induce ER stress by specific inhibition of the ER Ca2+-ATPase. In this study, we investigated the neuroprotective potential of resveratrol, a polyphenol phytoalexin compound extracted from spermatophytes, against TG-induced damage in the neuronal-like Neuro-2a cell lines. Cells were treated with DMSO or TG in the presence or absence of resveratrol. Cell viability was detected by methyl-thiazolyl-tetrazolium (MTT) assay. The results showed that 20 μM resveratrol attenuated TG-induced decrease of cell viability. Thus, resveratrol may play a neuroprotective role in neuronal cells upon ER stress.

Keywords

resveratrol, neuroprotection, thapsigargin, endoplasmic reticulum

1. Introduction

Thapsigargin (TG) is a specific inhibitor of calcium-ATPase of endoplasmic reticulum (ER), which leads to excessive Ca^{2+} entering into cytoplasm and thus causing ER stress and oxidative stress [1,2]. Previous studies have found that TG induces apoptosis of primary rat cortical neurons through triggering ER stress, which was demonstrated by the loss of mitochondrial function and the activation of caspase [3,4]. Moreover, a variety of evidence indicated that ER stress plays a critical role in many neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease [5,6].

Resveratrol (trans-3,4′,5-trihydroxystilbene), a unique polyphenol phytoalexin widely distributed in the natural plants, possesses strong antioxidant activities to resist oxidative damages [7]. Recently, extensive studies have also shown that resveratrol has anti-aging and anti-inflammatory properties [8-9]. For instance, several studies have demonstrated that resveratrol protects a variety of neuronal cell types against oxidative stress-induced cell death [10-12]. However, whether resveratrol plays a neuroprotective role against TG-induced cell damage is still unknown, and the molecular mechanisms of how resveratrol works has not yet been fully delineated.

Using the mouse neuroblastoma cell line Neuro-2a, the current study demonstrated that resveratrol may have neuroprotective potentials against thapsigargin induced damage.

2. Materials and Methods

2.1 Reagents and chemicals.

Mouse neuroblastoma cell line (Neuro-2a) was purchased from American Type Culture Collection (Manassas,USA). Modified Eagle's medium (MEM), fetal bovine serum (FBS), and penicillin/streptomycin mixture were purchased from Life Technologies (Carlsbad, USA).

Resveratrol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), thapsigargin (TG), and dimethylsulfoxide (DMSO) were from Sigma-Aldrich (St Louis, USA).

2.2 Cell culture.

Neuro-2a cells were cultured in growth medium containing MEM, 10% heat-inactivated FBS, and 100 U/ml penicillin/streptomycin [13]. Cells were subcultured at a ratio of 1: 6 every three days.

2.3 Cell viability.

MTT assay was used to determine the effects of resveratrol on Neuro-2a cell viability. Neuro-2a cells were cultured at a density of 2×10^4/well in 96-well plates. The next day, cells were treated with resveratrol at different concentrations for 4 h. The designed three experimental groups were: (1) control, (2) TG, and (3) TG + resveratrol. In the third group, resveratrol was added to the culture media 1 h before TG and resveratrol were treated. MTT was added to each well for 3 h, which was converted by the viable cells to generate purple formazan dye. Finally, 100 µl DMSO was added to dissolve the formazan, and absorbance at 595 nm was analyzed by a DTX880 multimode detector (Beckman Coulter, USA).

2.4 Statistical analysis.

All experiments were carried out independently at least three times. Data were expressed as the mean±SEM (standard error of the mean). Statistical analysis was performed using one-way ANOVA followed by the Tukey's test. $P < 0.05$ was determined to be statistical significant.

3. Results

3.1 Test of Resveratrol dosage on Neuro-2a cells.

To determine the biologically safe concentrations of resveratrol, Neuro-2a cells were exposed to resveratrol with increasing concentrations from 2.5 to 40 µM for 4 h (Fig. 1). The MTT assay revealed that Neuro-2a cells treated with 40 µM or lower concentrations of resveratrol were ~100% viable comparing to control, which indicated that resveratrol from 2.5 to 40 µM does not influence cell viability.

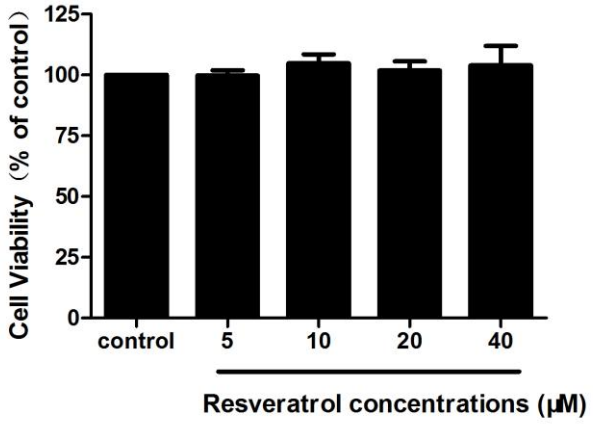

Fig 1. Test of resveratrol concentrations on cell viability. MTT assay showed that resveratrol (5–40 µM) does not affect the viability of Neuro-2a cells. Values were obtained from three independent experiments and expressed as mean ± SEM (% of control).

3.2 Resveratrol inhibited TG-induced cell viability decrease.

We next examined whether resveratrol protects TG-injured Neuro-2a cells. Three biologically safe concentrations of resveratrol (10, 20 and 40 µM) were tested. Our data showed that cell viability decreased prominently after TG injury. However, significant restoration of cell viability was observed when 20 µM resveratrol was treated comparing to the TG only group (Fig. 2).

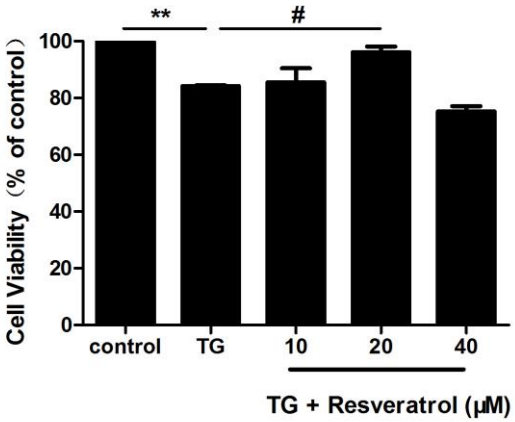

Fig 2. Resveratrol restores normal cell viability after TG treatment. Neuro-2a cells were pretreated with resveratrol (10–40 μM) before exposed to 50 nM TG. 20 μM of resveratrol showed protective effects on cell viability. Values obtained from three independent experiments are expressed as mean ± SEM (% of control).

4. Conclusion

In this study, we found that resveratrol with concentrations from 2.5 to 40 μM exerts no harm to Neuro-2a cells. TG, a specific calcium-ATPase inhibitor that causes ER stress and oxidative stress, induces remarkable damage to Neuro-2a cells. Importantly, 20 μM resveratrol restores the cell viability after TG injury. With detailed molecular mechanisms to be fully elucidated, this study demonstrates that resveratrol plays a critical role in protecting the neuronal damage caused by ER and oxidative stress, thus providing new evidence on the understanding of resveratrol as a beneficial drug for neuroprotection.

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