Resveratrol, a Polyphenol Found in Red Wine, Protects Against Rotenone-induced Apoptosis Through Autophagy Induction

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Objective: To examine the neuroprotective effects of resveratrol on rotenone-induced apoptosis in SH-SY5Y cells. Background: Resveratrol, an antioxidant polyphenol found in red wine, has been reported to increase the expression of mammalian Sirt1 deacetylase (SIRT1). The fact that SIRT1 is an important regulator of autophagy led us to hypothesize that resveratrol may enhance autophagy through SIRT1 and prevent neuronal death related to accumulation of aggregated/misfolded proteins found in several age-related neurodegenerative disorders, including PD. Resveratrol is an inhibitor of mitochondrial complex I and induces neuronal death accompanied by the inhibition of proteasome activity, which, in turn, leads to an increase in aggregated/misfolded proteins associated with the pathogenesis of PD. Here, the neuroprotective effects of resveratrol on rotenone-induced injury were examined in SH-SY5Y cells. Methods: The SH-SY5Y cells were treated with resveratrol at various concentrations for different time periods, or exposed to rotenone with or without resveratrol pretreatment. Protein levels of SIRT1, LC3, a marker of autophagy, or PARP, a marker of apoptosis, were determined by immunoblotting assay. The induction of autophagy was verified by electron microscopy. The cells were then transfected with SIRT1 siRNA or Atg5 siRNA followed by addition of rotenone with or without resveratrol pretreatment. Apoptosis and autophagy were determined by immunoblotting with anti-PARP or anti-Atg5 antibody. The transfection efficiency of 50 % was achieved using the corresponding secondary antibody and signals were detected by Enhanced chemiluminescence or Immunoblotting analysis. The morphology changes and accordingly the changes of live and dead cells were subjected to immunoblotting assay with anti-PARP or anti-Atg5 antibody. The transfection efficiency of 50 % was achieved using the corresponding secondary antibody and signals were detected by Enhanced chemiluminescence or Immunoblotting analysis. In addition to the induction of SIRT1, autophagy or PARP, a marker of apoptosis, were determined by immunoblotting analysis.

Results: Resveratrol protected against rotenone-induced apoptosis and enhanced degradation of aggregated proteins in SH-SY5Y cells. Cells were exposed to rotenone with or without resveratrol pretreatment. The total proteins isolated from the cells were subjected to ELISA assay to determine the percentage of autophagic cells in SH-SY5Y cells (15% versus 2%, P < 0.01; Fig. 1C). The autophagosome and autolysosome, collectively referred to as autophagic vacuoles (AVs), are considered as the characteristic components of autophagy. To verify that the observed changes in LC3 reflected increased double membrane structures of AVs, the cells were studied by transmission electron microscopy. As shown in Fig. 1D, the structures of AVs could be observed largely in resveratrol-treated cells. To correlate the induction of autophagy to SIRT1 expression, the cells were transfected with SIRT1 siRNA followed by addition of rotenone. SIRT1 silencing treatment resulted in a significant decrease of SIRT1 expression (Fig. 2A), and at same time, resveratrol-induced enhancement of autophagy was partially inhibited due to the SIRT1 silencing (Fig. 1B).

Conclusions: Resveratrol prevents against rotenone-induced cell death Exposure of cells to rotenone resulted in an increase of histone-associated DNA fragmentation (Fig. 3A) and an increase of cleaved PARP protein level (Fig. 3B), both of which are characteristics of apoptosis induced by resveratrol pretreatment (Fig. 3A, B). Rotenone caused shrinkage of SH-SY5Y cells, while resveratrol pretreatment inhibited these changes in cell morphology (Fig. 3C). Rotenone treatment resulted in an increase of red fluorescent dye (indicating dead cells) and a decrease of green fluorescent signal (indicating live cells) (Fig. 3C). Rotenone pretreatment salvaged cells from rotenone-toxicity as compared to untreated control cells. The high molecular bands of ubiquitinated proteins in detergent-insoluble fractions were detected by immunoblotting assay with anti-ubiquitin antibody (Fig. 3B). Data were expressed as the means ± SE. ** P < 0.01; a compared to control (a) or rotenone (b). The high molecular bands of ubiquitinated proteins in detergent-insoluble fractions were detected by immunoblotting assay with anti-ubiquitin antibody (Fig. 3B). Data were expressed as the means ± SE. ** P < 0.01; a compared to control (a) or rotenone (b). The high molecular bands of ubiquitinated proteins in detergent-insoluble fractions were detected by immunoblotting assay with anti-ubiquitin antibody (Fig. 3B). Data were expressed as the means ± SE. ** P < 0.01; a compared to control (a) or rotenone (b). The high molecular bands of ubiquitinated proteins in detergent-insoluble fractions were detected by immunoblotting assay with anti-ubiquitin antibody (Fig. 3B). Data were expressed as the means ± SE. ** P < 0.01; a compared to control (a) or rotenone (b). The high molecular bands of ubiquitinated proteins in detergent-insoluble fractions were detected by immunoblotting assay with anti-ubiquitin antibody (Fig. 3B). 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