17-AAG Protects Against Rotenone-Induced Apoptosis in SH-SY5Y Cells via HSP70 Induction

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Abstract

Objectives To examine the protective effects of 17-AAG on rotenone-induced apoptosis in SH-SY5Y cells. Background Heat shock proteins (HSPs), such as HSP70, represent an important cellular protective mechanism against neuronal cell death in various models of neurodegenerative disorders. 17-AAG, 17-deacetyl-17-demethoxygeldanamycin (17-AAG), an HSP70 inducer acting via Hsp90 inhibition, is now in clinical trials for a wide range of cancers. 17-AAG has been intended to be used as a protective agent against non-neurological diseases as neuroprotective agent. Here, we investigated the possible role of 17-AAG in rotenone-induced apoptosis in SH-SY5Y cells, an in vitro model related to PD. Methods Cells were exposed to rotenone with or without 17-AAG pretreatment. The apoptosis was determined by Hoechst 33342 staining and immunoblotting assay. The changes in mitochondrial membrane potential were determined using Mitotracker Red CMXROS staining. The release of cytochrome c was determined by immunoblotting assay. The protein levels of HSP70 and P53, a major protein of apoptotic signal pathway, were determined by immunoblotting assay. To correlate the role of Hsp70 in the neuroprotection of 17-AAG, the induction of Hsp70 was measured by western blot and/or by HSP70 mRNA transfection, followed by addition of rotenone with or without 17-AAG pretreatment. Then the apoptosis was evaluated by measuring the protein levels of cleaved PARP and HSP70. 17-AAG treatment caused a time- and dose-dependent increase of HSP70 and decrease of P53. Pretreatment with 17-AAG alleviated rotenone-induced apoptosis, decreased cytochrome c release, and reduced the release of active caspase-3 caused by rotenone. The protective effect of 17-AAG was related to HSP70 induction and P33 reduction, and it was partially blocked by KNK437, or by HSP70 siRNA transfection, in which the HSP70 gene was suppressed. The attenuated accumulation of high molecular weight ubiquitin levels in HSP70 siRNA transfected cells further support the anti-apoptotic effect of 17-AAG. Conclusions The protective effects of 17-AAG against rotenone-induced apoptosis through HSP70 induction may lead to a novel approach to neurodegenerative disorders.

Methods

Background

Heat shock proteins (HSPs), such as HSP70, represent an important cellular protective mechanism against neuronal cell death in various models of neurodegenerative disorders. 17-AAG, 17-deacetyl-17-demethoxygeldanamycin (17-AAG), an HSP70 inducer acting via Hsp90 inhibition, is now in clinical trials for a wide range of cancers. 17-AAG has been intended to be used as a protective agent against non-neurological diseases as neuroprotective agent. Human neuroblastoma SH-SY5Y cell line, subcloned from SK-N-SH cells, is often used as a model of dopaminergic neurons. Rotenone, a widely used insecticide, has been shown to inhibit mitochondrial complex I and induces apoptosis, selectively destroys dopaminergic neurons and produces impaired motor function, characteristic of Parkinson’s disease (PD) in rats. Here, we used rotenone-induced apoptosis in SH-SY5Y cells as an in vitro model that is related to PD to demonstrate the neuroprotective effects of 17-AAG on rotenone-induced injury of cells and to explore its possible mechanisms.

Cell culture and reagents

SH-SY5Y cells were routinely grown in 10% DMEM and cultured at 37°C under humidified 5% CO2 atmosphere.

Apoptosis assay

For microscopic nuclear DNA analysis, cells were stained with Hoechst 33342. The cells with condensed nuclei were stained with Hoechst 33342. The cells with condensed nuclei were counted and value was expressed as percentage of total cells. Data were expressed as the means ± S.D. (lower). Data were analyzed by one-way analysis of variance (ANOVA). *: p < 0.05 as compared with vehicle treated control (C), Rat group (B), (Con + control = Rot + rotenone). After specific treatment, Mitotracker Red CMXROS was used to stain the mitochondria. The release of cytochrome c was used as an equal loading of proteins.

Results

Conclusions

The protective effects of 17-AAG against rotenone-induced apoptosis through HSP70 induction may lead to a novel approach to neurodegenerative disorders involving mitochondrial dysfunction. Further studies are needed to examine the effect of 17-AAG in PD related animal models in vivo and other mechanisms involved in the neuroprotection will also be further investigated.

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