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Research Report

Salubrinal protects against rotenone-induced SH-SY5Y cell death via ATF4-parkin pathway

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ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder, for which there are no effective disease-modifying therapies. Growing evidence from studies in human PD brain, in addition to genetic and toxicological models, indicates that endoplasmic reticulum (ER) stress is a common feature of the disease and contributes to neurodegeneration. We examine whether salubrinal, a ER stress inhibitor, can protect the rotenone-induced SH-SY5Y cell death and explore the mechanisms underlying this protection. Our results demonstrated that rotenone induced a significant ER stress response and caused cell apoptosis, which was inhibited by salubrinal. Activating transcription factor 4 (ATF4), a member of the ATF/CREB family of basic leucine zipper transcription factors, has been implicated in the pathogenesis of neurodegeneration. We showed that salubrinal increased the up-regulation of ATF4 expression. An ATF4 siRNA significantly increased the rotenone cytotoxicity and decreased the salubrinal's protection. Further, we showed that ATF4 siRNA inhibited the expression of parkin, and parkin knockdown similarly aggravated the rotenone cytotoxicity and reduced the salubrinal's protection. Additionally, the protein level of parkin was declined after treatment with rotenone, whereas this reduction was rescued by salubrinal. These findings indicate ATF4-parkin pathway plays an important role in the salubrinal-mediated neuroprotection of rotenone-induced dopaminergic cell death.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the death of dopaminergic neurons in the substantia nigra (Martin et al., 2011). The mechanisms of neuronal loss remain unclear, and current therapies principally alleviate symptoms instead of targeting the underlying neuronal loss (Levy et al., 2009). Growing evidence

Abbreviations: ATF4, activating transcription factor4; C/EBP, CCAAT-enhancer-binding protein; CHOP, CCAAT-enhancer-binding protein homologous protein; ER, endoplasmic reticulum; PERK, ER membrane proteins double-stranded RNA-activated kinase-like kinase; eIF2α, eukaryotic translation initiation factor 2 α; GRP78, glucose regulated protein 78; IRE1α, inositol requiring 1α enzyme; MTT, 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide; PD, Parkinson's disease

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from research in human PD brain, in addition to genetic and toxicological models, indicates that endoplasmic reticulum (ER) stress is a common feature of PD and contributes to dopaminergic neurodegeneration (Hoozemans et al., 2007; Colla et al., 2012; Chen et al., 2008). Therefore, therapeutic strategies that mitigate ER stress may be beneficial to PD patients.

ER stress is known to activate a series of signals that comprise the unfolded protein response (UPR). The UPR is mediated through three signaling pathways under the control of the ER membrane proteins double-stranded RNA-activated kinase-like kinase (PERK), activating transcription factor (ATF)-6 and inositol requiring-1α (IRE1α) (Ron and Walter, 2007). These signals coordinate the cellular response to unfolded proteins, which includes (1) downregulation of protein translation via activation of PERK and subsequent phosphorylation of the eukaryotic translation initiation factor 2 α (eIF2α) at Ser51, (2) enhanced expression of ER chaperone proteins that promote protein refolding, and (3) activation of proteases involved in the degradation of misfolded proteins (Walter and Ron, 2011). However, prolonged or severe ER stress can lead to the activation of apoptotic cell death pathways. Rotenone is an environmental toxin used to induce experimental Parkinsonism in animals and cell cultures. Many studies reveal that rotenone induces ER stress (Chen et al., 2008; Ryu et al., 2002), and that eIF2α siRNA can decrease rotenone cytotoxicity in SK-N-MC cells (Chen et al., 2008), suggesting that ER stress mediates rotenone-induced cell death.

The effects of ATF4, a member of the ATF/CREB family of basic leucine zipper transcription factors, on neuronal survival or death are complex. Previous studies found that rotenone induces PERK phosphorylation, which results in ATF4 and CHOP (CCAAT-enhancer-binding protein (C/EBP) homologous protein) protein induction in neuronal PC12 cells (Ryu et al., 2002). Indeed, in a previous study, we found that ATF4 may be involved in dopaminergic cell death in the rotenone rat model (Wu et al., 2013). However, many target genes are regulated by ATF4 (Singleton and Harris, 2012), including parkin, which is transcriptionally upregulated by ER stress and can protect cells from ER stress-induced cell death (Sun et al., 2013; Imai et al., 2000). Moreover, a dominant-negative ATF4 mutant prevents the ER stress-induced upregulation of parkin (Bouman et al., 2011). And a recent report showed that ATF4-parkin pathway protects against neuronal death induced by 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-pyridinium (MPP^+) (Sun et al., 2013). However, it is unknown whether ATF4-parkin pathway is beneficial or harmful in the rotenone model.

Salubrinal, a phosphatase inhibitor, selectively inhibits dephosphorylation of the α subunit of eIF2 which is an upstream activator of ATF4. It has been shown that salubrinal can modulate the cellular stress response to protect against ER stress-induced apoptosis (Boyce et al., 2005). In the present

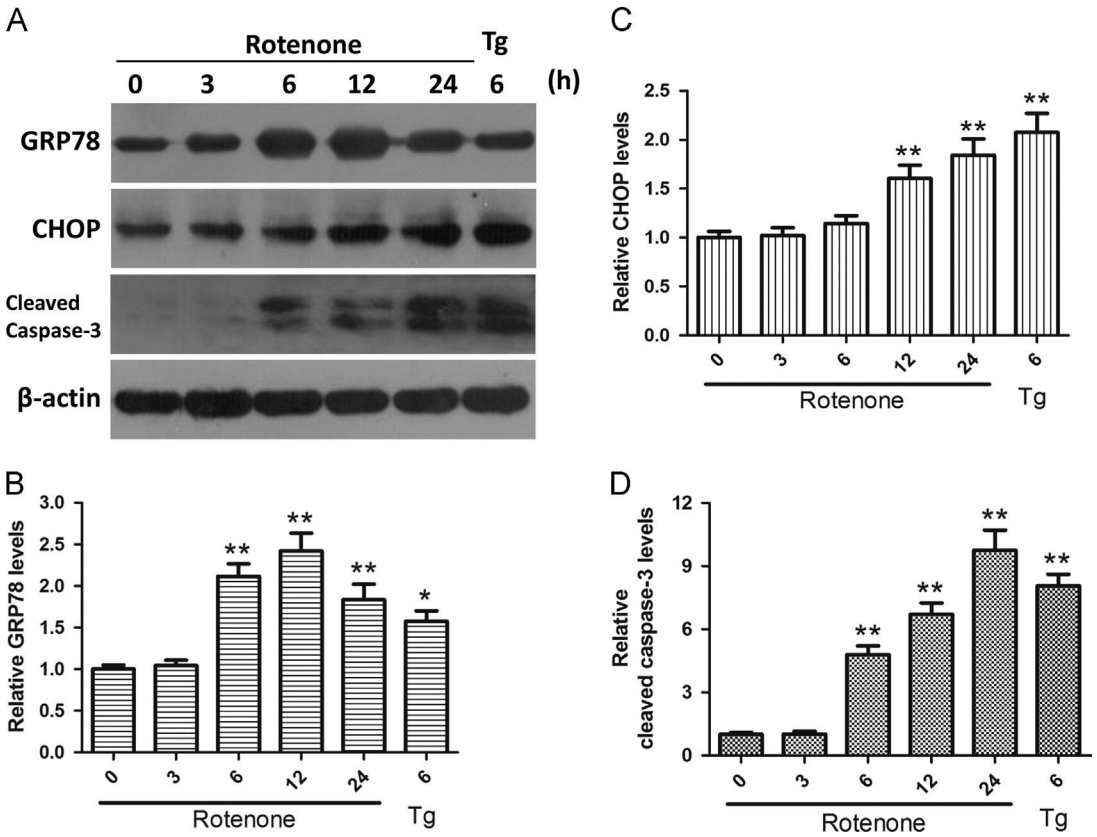

Fig. 1 – Effect of rotenone on ER stress and the death of SH-SY5Y cells. (A) SH-SY5Y cells were treated with rotenone (100 nM) or thapsigargin (Tg, 5 μM) for the specified times. The expression levels of GRP78, CHOP and caspase-3 were determined with immunoblotting. The relative amounts of GRP78 (B), CHOP (C) and cleaved caspase-3 (D) imaged on the films was measured by densitometry and normalized to the expression of β-actin. $^*p<0.05$, $^{**}p<0.01$, vs. control group, $n=3$.