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BRAIN RESEARCH

Research Report

# Rotenone-induced neurotoxicity of THP-1 cells requires production of reactive oxygen species and activation of phosphatidylinositol 3-kinase

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ABSTRACT

Parkinson's disease is characterized by slow and progressive degeneration of dopaminergic neurons. Increasing evidence has suggested an important role for exposure to pesticides such as rotenone in the pathogenesis of Parkinson's disease. Although rotenone can elicit immune responses in microglia, the intracellular signaling events mediating these effects are poorly defined. Here we show that cell-free supernatants of rotenone-treated monocytic THP-1 cells induced cytotoxicity in dopaminergic neuroblastoma SH-SY5Y cells. Exposure of THP-1 cells to rotenone led to transient production of reactive oxygen species (ROS) and phosphorylation of Akt. Akt activation was also induced by exogenous hydrogen peroxide. Pretreatment of THP-1 cells with either a phosphatidylinositol 3-kinase (PI3K) inhibitor or ROS scavengers prevented Akt activation and protected SH-SY5Y cells from the cytotoxic effect of conditioned media from rotenone-treated THP-1 cells. Rotenone treatment of THP-1 cells also led to upregulation of cyclooxygenase-2 and secretion of prostaglandin $E_2$. These results suggest that rotenone-induced activation of ROS/PI3K/Akt pathway in THP-1 cells leads to the release of factors that are toxic to SH-SY5Y cells and have implications for the onset of Parkinson's disease.



## 1. Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, decreased dopamine levels, and consequent extrapyramidal motor dysfunction. Increasing evidence suggests that environmental toxicants and inflammation may contribute to the development of PD (Orr et al., 2002). Rotenone, a naturally occurring lipophilic compound from the roots of certain plants (*Derris* species) is used as the main component of many herbicides. Chronic (Alam and Schmidt, 2002; Betarbet et al., 2000) and acute (Saravanan et

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*Abbreviations:* PD, Parkinson's disease; ROS, reactive oxygen species; PI3K, phosphatidylinositol 3-kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; PKB, protein kinase B; COX-2, cyclooxygenase-2; $PGE_2$, prostaglandin $E_2$; CM, conditioned media; NAC, *N*-acetylcysteine; LPS, lipopolysaccharide; NSAIDs, nonsteroidal anti-inflammatory drugs; TNFα, tumor necrosis factor-α; IL-1β, interleukin-1β; NO, nitric oxide; PTEN, phosphatase and tension homologue deleted on chromosome 10; DCFH-DA, 7′-dichlorodihydrofluorescein diacetate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; DMEM–F12, Dulbecco's modified Eagle's medium–nutrient mixture F12 ham; DMSO, dimethylsulfoxide; RIPA, radioimmunoprecipitation assay

✠ Deceased.

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al., 2005) rotenone infusion in rats reproduces PD features. The rotenone-induced neurotoxicity is generally attributed to its inhibition of the activity of neuronal mitochondrial complex I. However, recent studies demonstrate that microglia play a pivotal role in rotenone-induced degeneration of dopaminergic neurons. The presence of microglia greatly enhances the vulnerability of mesencephalic neurons to rotenone (Gao et al., 2002a). Extensive microglia activation is observed in the striatum and substantia nigra of rotenone-treated animals (Sherer et al., 2003).

Microglial activation is a hallmark of the pathogenesis of a number of neurodegenerative diseases including PD (McGeer et al., 1988). Activated microglia contribute to neurodegeneration via the production of a variety of proinflammatory and neurotoxic factors including tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), or nitric oxide (NO), eicosanoids, and superoxide (Gao et al., 2002a; McGeer and McGeer, 2004). Since dopaminergic neurons are more vulnerable to oxidative stress compared to other neurons (Hirsch et al., 1997), activated microglia-produced reactive oxygen species (ROS) are proposed to mediate the dopaminergic neurodegeneration induced by rotenone via inflammation-mediated oxidative damage to neurons (Gao et al., 2002a). However, ROS also activate critical signaling pathways, including the stress activated kinases, c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK) (Droge, 2002; Martindale and Holbrook, 2002). Exposure of astrocytes and oligodendrocytes to hydrogen peroxide results in activation of Akt/protein kinase B (PKB; hereafter referred to as Akt) (Salsman et al., 2001) and multiple MAPKs (Bhat and Zhang, 1999). Although Akt is well-known to play a role in pro-survival responses to growth factors, it can also be activated in response to heat shock or hydrogen peroxide (Konishi et al., 1997). Phosphatidylinositol 3-kinase (PI3K) activation leads to formation of various 3′-phosphorylated phosphoinositides in the plasma membrane that promote membrane recruitment of Akt via its pleckstrin homology domain and activation by PDK-1 (Vanhaesebroeck and Alessi, 2000). Although the intracellular signaling events underlying rotenone-induced microglia/monocytes activation are still poorly defined, the ROS/PI3K/Akt pathway could be involved.

Recently, we adapted an in vitro inflammatory neurodegeneration model (Klegeris et al., 1999) to study the effects of factors released from rotenone-treated monocytic THP-1 cells on neuron-like SH-SY5Y cells (Zhao et al., 2006), a human neuroblastoma cell line expressing dopaminergic activity (Bollimuntha et al., 2006). The THP-1 cell line has been widely used as a model for human microglia because of functional similarities and the ability to activate similar signaling pathways (Combs et al., 1999; Lee et al., 2005; McDonald et al., 1997). We observed that conditioned media from rotenone-treated monocytic THP-1 cells induced dopaminergic neuroblastoma SH-SY5Y cell degeneration (Zhao et al., 2006). In the current study, we have obtained additional insights into the potential mechanism of rotenone signaling in THP-1 cells. We find that rotenone treatment of THP-1 cells results in ROS production, activation of the PI3K/Akt pathway, cyclooxygenase-2 (COX-2) upregulation, and secretion of prostaglandin $E_2$ ($PGE_2$). Most importantly, inhibitors of ROS production and the PI3K pathway could prevent the cytotoxicity of conditioned media from rotenone-treated THP-1 cells on SH-SY5Y cells.

## 2. Results

### 2.1. *Rotenone-induced cytotoxicity of THP-1 cells to SH-SY5Y cells*

We recently established a model for rotenone-induced neurotoxicity using conditioned media from rotenone-treated THP-1 cells (Zhao et al., 2006). Here we used the optional doses of rotenone (25–100 nM) to stimulate THP-1 cells for 24 h. Cell-free supernatants, or conditioned media (CM), were collected and used to culture human dopaminergic SH-SY5Y cells for 48 h. Subsequently, the viability of SH-SY5Y cells was measured using the MTT assay (Fig. 1). When SH-SY5Y cells

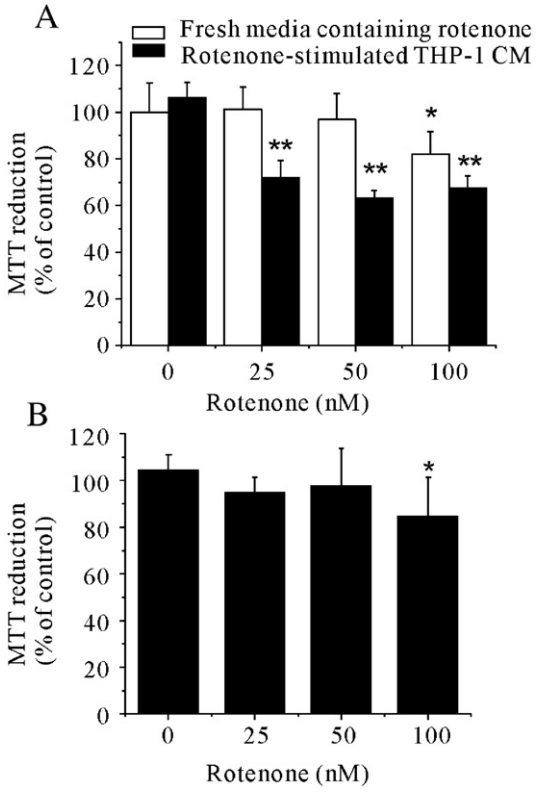


**Fig. 1 – Rotenone-treated THP-1 cells secrete factors that are toxic to SH-SY5Y cells. (A) THP-1 cells were seeded at $4\times10^5$ per well in a 24-well plate and stimulated with rotenone (25–100 nM). After 24 h of incubation the cell-free supernatants or conditioned media (CM) were transferred to the wells containing SH-SY5Y cells. Viability of SH-SY5Y cells was assessed after 48 h by MTT assay. White bars represent SH-SY5Y cells that were treated with fresh media containing rotenone; dark bars represent SH-SY5Y cells that were treated with CM from rotenone-stimulated THP-1 cells. (B) SH-SY5Y cells were treated with media from unstimulated THP-1 cells along with rotenone, rotenone is added to SH-SY5Y cells by the time that media are transferred from THP-1 cells to SH-SY5Y cells. Data are mean ± SD from three independent experiments. Statistical significance was assessed with an ANOVA, followed by Bonferroni's *t* test. *$P<0.05$ compared with control, **$P<0.01$ compared with control.**