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Research Report

NF-κB contributes to 6-hydroxydopamine-induced apoptosis of nigral dopaminergic neurons through p53

Zhong-Qin Liang[a], *Yun-Lin Li*[a], *Xi-Lin Zhao*[a], *Rong Han*[a], *Xiao-Xia Wang*[b], *Yumei Wang*[a], *Thomas N. Chase*[b], *M. Catherine Bennett*[c], *Zheng-Hong Qin*[a,*]

[a]*Department of Pharmacology, Soochow University School of Medicine, Suzhou-Singapore Industrial Park, Suzhou 215123, China*
[b]*Experimental Therapeutic Branch, National Institute of Neurological disorders and Stroke, NIH, Bethesda, MD, USA*
[c]*Blanchette Rockefeller Neuroscience Institute, Rockville, MD, USA*

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ABSTRACT

To evaluate the contribution of NF-κB and the NF-κB target gene *p53* to nigral dopaminergic neuron degeneration in rodent models of Parkinson's disease, time-course of dopaminergic neuron loss as well as changes in the expression of some NF-κB-regulated proapoptotic proteins were assayed after unilateral infusion of 6-hydroxydopamine into rat medial forebrain bundle. Substantial loss of tyrosine hydroxylase immunoreactivity in nigral was observed 24 h after 6-hydroxydopamine treatment. The degenerative processes began 12 h after 6-hydroxydopamine administration as evidenced by a positive silver staining. Apoptotic death of dopaminergic neurons was suggested by the appearance of TUNEL-positive nuclei in substantia nigra and internucleosomal DNA fragmentation as detected by agarose gel electrophoresis. NF-κB activation in dopaminergic neurons as revealed by immunohistochemistry and electrophoresis mobility shift assay, began at 12 h after 6-hydroxydopamine administration. Levels of c-Myc and p53 immunoreactivities increased after 6-hydroxydopamine treatment, mainly in dopaminergic neurons as indicated by co-localization with tyrosine hydroxylase immunoreactivity. Blockade of NF-κB nuclear translocation with recombinant cell-permeable peptide NF-κB SN50 inhibited NF-κB nuclear translocation and p53 induction. SN50 and the p53 antagonist pifithrin-α significantly reduced nigral dopaminergic neuron degeneration. These results suggest that NF-κB activation contributes, at least in part, to oxidative stress-induced degeneration of dopaminergic neurons through a NF-κB-dependent p53-signaling pathway.

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1. Introduction

Parkinson's disease (PD) is a motor function disorder primarily reflecting a reduction in striatal dopaminergic transmission as a result of nigral dopamine (DA) neuron degeneration (Fornai et al., 2003). Recent studies have identified gene mutations linked to several forms of familial PD. These studies indicate that genetic defects play an important pathogenetic role in

* *Corresponding author.* Fax: +86 512 65190599.
E-mail address: zhqin5@hotmail.com (Z.-H. Qin).

Abbreviations: 6-OHDA, 6-hydroxydopamine; MFB, medial forebrain bundle; NF-κB, nuclear factor-kappaB; PBS, phosphate-buffered saline; PFT-α, pifithrin-alpha; SN, substantia nigra; TBS, Tris-buffered saline; TDT, terminal deoxynucleotidyl transferase; TH, tyrosine hydroxylase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling

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these familial cases (Autere et al., 2002; Bennett, 2005; Burke, 2004; Mouradian, 2002). Sporadic PD, however, is the predominant form of the disease, and the cause of DA neuron degeneration in these cases remains poorly understood (Dawson and Dawson, 2003).

Increasing evidence suggests that oxidative stress contributes to DA neuron degeneration (Jackson-Lewis and Smeyne, 2005; Jenner, 2003; Yoo et al., 2003; Yu et al., 2005). Dopamine neurons have relatively heavy burden of endogenous oxidative stress, since both the synthesis and degradation of DA produce free radicals (Gerlach et al., 2003; Metodiewa and Koska, 2002). Mitochondria are a major source of free radicals, including the superoxide anion and hydrogen peroxide (Papa and Skulachev, 1997). The nigrostriatal DA system is exceptionally vulnerable to mitochondrial insufficiency induced by a variety of toxins, including 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) and rotenone (Betarbet et al., 2005; Sherer et al., 2002). Mitochondrial inhibition increases oxidative stress and induces DA neuron degeneration *in vivo* (Betarbet et al., 2000; Zhang et al., 2003).

Dopamine itself is a potential neurotoxin that has been shown to induce cell degeneration at relatively low concentrations *in vitro* and *in vivo* (Lou et al., 1999; Pedrosa and Soares-da-Silva, 2002). The genetic mutations that produce PD can exacerbate DA toxicity (Junn and Mouradian, 2002). The DA neurotoxin 6-hydroxydopamine (6-OHDA), which induces neurotoxicity in large part through oxidative stress (Glinka et al., 1997; Napolitano et al., 1995), can be formed endogenously (Gerlach et al., 2003; Jones et al., 2003; Obata and Yamanaka, 2001; Power et al., 2002).

6-OHDA could induce death of nigral DA neurons partially by apoptotic mechanisms. Apoptosis, i.e., gene-controlled cell self-destruction, is involved in several neurodegenerative disorders including PD (Przedborski, 2005; Tatton et al., 2003). Activation of p53 has been reported in animal models of PD and inhibition of p53 activity prevented MPTP-induced degeneration of DA neurons, suggesting an important role of p53 in apoptotic death of DA neurons (Biswas et al., 2005; Blum et al., 1997; Duan et al., 2002). In previous studies we have found that the transcription factor NF-κB plays a proapoptotic role in excitotoxin-induced apoptotic death of striatal neurons, possibly through up-regulating p53 and c-Myc (Qin et al., 1998; Qin et al., 1999). Similarly, activation of NF-κB is associated with DA- and 6-OHDA-induced neuronal injury in rat striatum (Lou et al., 1999; Panet et al., 2001; Tarabin and Schwaninger, 2004). The NF-κB activator, C2-ceramide induces NF-κB activation and apoptosis in cultured DA neurons through the production of free radicals (France-Lanord et al., 1997; Hunot et al., 1997). Increased NF-κB immunoreactivity has been reported in the substantia nigra of patients with PD, although a causal relationship to pathogenesis has not been established (Hunot et al., 1997; Soos et al., 2004). Collectively, these findings suggest that NF-κB contributes to oxidative stress-induced DA neuron degeneration in PD (Panet et al., 2001; Youdim et al., 1999). In the present studies, we tested the hypothesis that 6-OHDA-induced oxidative damage to the nigrostriatal neurons involves activation of NF-κB and its target gene *p53* in rat brain.

2. Results

2.1. *Degenerative changes of DA neurons in substantia nigra (SN)*

To evaluate the temporal correlation between degenerative changes in DA neurons, the time course of the loss of TH immunoreactivity, the appearance of silver-stained denatured proteins and TUNEL-positive nuclei were observed following 6-OHDA administration. Infusion of 6-OHDA to the MFB caused a rapid and consistent loss of TH immunoreactivity in SN, and to a lesser extent, in the ventral tegmental area. There was no apparent loss of TH immunoreactivity in SN 12 h after 6-OHDA infusion. A substantial loss of TH immunoreactivity in SN was observed starting 24 h after 6-OHDA {Fig. 1A′ (A–F)}. We found no recovery of TH immunoreactivity 8 to 32 days after 6-OHDA treatment (data not shown). A robust loss of TH immunoreactivity was also found in the ipsilateral striatum, the primary target region of the nigral dopaminergic pathway, starting 24 h after 6-OHDA treatment {Fig. 1A′ (G–J)}. Quantitative analysis revealed that about 70% of TH-positive cells disappeared 48 h after 6-OHDA infusion (Fig. 1B′).

Degenerative changes in SN DA neurons after 6-OHDA were indicated by the appearance of positive silver stained neurons, using the FD NeuroSilver Staining kit, which is specialized for detecting denatured proteins. A few positive cells were observed as early as 12 h after 6-OHDA injection and the number of these cells increased thereafter up to 24 h. By 48 h, the number of silver-positive cells decreased (Fig. 2). These degenerative changes were not seen in the contralateral SN of 6-OHDA-injected animals (data not shown) or in the ipsilateral SN of saline-injected animals (Figs. 2I and J).

Evidence for apoptosis in neurons of the SN was revealed by assay of internucleosomal DNA fragmentation, a biochemical hallmark of apoptosis. DNA laddering of multimers of 180–200 bp was observed at both 24 and 48 h after 6-OHDA administration (Fig. 3). In order to identify if apoptosis occurred in SN, TUNEL was performed in brain sections throughout the SN region. In these studies, increasing numbers of TUNEL-positive nuclei were observed from 12 to 48 h after 6-OHDA infusion. The number of TUNEL-positive nuclei was estimated to be about 20, 37 and 46 (average from three animals) in 6-OHDA-injected SN at 12, 24 and 48 h after toxin administration, respectively. While only a few (3, averaged from three animals) TUNEL-positive nuclei were seen in saline-injected SN at 24 h after saline administration (Figs. 4G and H). These TUNEL-positive nuclei were round in shape and slightly shrunken, and fragmented nuclei were occasionally observed (Fig. 4). There were no TUNEL-positive nuclei in the contralateral SN of 6-OHDA-injected animals (data not shown). In combination with loss of TH-positive neurons and positive silver staining in SN, we can attribute that TUNEL labeled degenerative DA neurons.

2.2. *Activation of NF-κB in neurons*

To evaluate activation of NF-κB and AP-1 in SN following 6-OHDA injection, DNA binding activities of transcription

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