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Research Report

Pretreatment with near-infrared light via light-emitting diode provides added benefit against rotenone- and MPP$^+$-induced neurotoxicity

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ABSTRACT

Parkinson's disease (PD) is a movement disorder caused by the loss of dopaminergic neurons in the substantia nigra pars compacta, leading to nigrostriatal degeneration. The inhibition of mitochondrial respiratory chain complex I and oxidative stress-induced damage have been implicated in the pathogenesis of PD. The present study used these specific mitochondrial complex I inhibitors (rotenone and 1-methyl-4-phenylpyridinium or MPP$^+$) on striatal and cortical neurons in culture. The goal was to test our hypothesis that pretreatment with near-infrared light (NIR) via light-emitting diode (LED) had a greater beneficial effect on primary neurons grown in media with rotenone or MPP$^+$ than those with or without LED treatment during exposure to poisons. Striatal and visual cortical neurons from newborn rats were cultured in a media with or without 200 nM of rotenone or 250 μM of MPP$^+$ for 48 h. They were treated with NIR-LED twice a day before, during, and both before and during the exposure to the poison. Results indicate that pretreatment with NIR-LED significantly suppressed rotenone- or MPP$^+$-induced apoptosis in both striatal and cortical neurons ($P<0.001$), and that pretreatment plus LED treatment during neurotoxin exposure was significantly better than LED treatment alone during exposure to neurotoxins. In addition, MPP$^+$ induced a decrease in neuronal ATP levels (to 48% of control level) that was reversed significantly to 70% of control by NIR-LED pretreatment. These data suggest that LED pretreatment is an effective adjunct preventative therapy in rescuing neurons from neurotoxins linked to PD.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by progressive motor dysfunction and variable cognitive impairment (Dauer and Przedborski, 2003; Gandhi and Wood, 2005). The key neuropathological feature of PD is the loss of striatal dopamine content, which leads to bradykinesia, tremors, and postural instability (Gandhi and Wood, 2005). The etiology of PD remains largely unknown, as no study has demonstrated thus far that the majority of PD

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cases are due to a single factor. Present evidence suggests that PD is a multifactorial disorder, probably caused by a combination of age, genetics, and environmental factors (Veldman et al., 1998; Zhang et al., 2000). Epidemiological studies have suggested that environmental factors play an important role in the pathogenesis of PD and other related disorders. These include trace metals, pesticides, herbicides, and industrial chemicals (Gorell et al., 1998; Veldman et al., 1998). In particular, the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and the pesticide rotenone have been found to produce, at least in experimental animals, a Parkinsonian syndrome characterized by highly selective nigrostriatal dopaminergic degeneration just as in idiopathic PD. Biochemical studies have implicated mitochondrial complex I dysfunction in both the pathogenesis of PD as well as in MPTP- or rotenone-induced neurotoxicity. MPTP is highly lipophilic and easily crosses the blood-brain barrier. In the brain, MPTP is converted to MPP^+ in glial cells and then incorporated into neurons via the dopamine transporter (DAT) (Gainetdinov et al., 1997; Cappelletti et al., 2005). MPP^+ is believed to be concentrated in the mitochondria and to inhibit the multi-subunit complex I (Nicklas et al., 1985; Ramsay et al., 1986) of the electron transport chain, leading to the depletion of ATP and the production of reactive oxygen species (ROS) (Dauer and Przedborski, 2003). MPP^+ causes damage to dopaminergic neurons and has been widely used to generate models of Parkinson's disease (Gainetdinov et al., 1997; Cappelletti et al., 2005).

Rotenone has recently been shown to reproduce the same progressive, selective, nigrostriatal dopaminergic degeneration seen in PD (Betarbet et al., 2000). This rotenone model of PD has reinforced the notion that complex I inhibition may be a key factor involved in the death of dopaminergic neurons and the development of Parkinsonism, mainly by increasing oxidative stress.

Near-infrared light (NIR) via light-emitting diode (LED) is a well-accepted therapeutic tool in the treatment of infected, ischemic, and hypoxic wounds and other soft tissue injuries in humans and animals (Whelan et al., 2001, 2003). The mechanism of NIR-LED action is the up-regulation of cytochrome *c* oxidase activity and the production of adenosine triphosphate (ATP), as shown in primary cultures of rat visual cortical neurons functionally inactivated by tetrodotoxin, potassium cyanide (KCN), or sodium azide (N_3Na) (Wong-Riley et al., 2001, 2005). NIR-LED treatment effectively rescues poisoned neurons from apoptotic cell death (Wong-Riley et al., 2005; Liang et al., 2006). Moreover, such treatment has recently been shown to partially rescue neurons from both rotenone- and MPP^+-induced neurotoxicity (Liang et al., 2008). The goal of the present study was to test our hypothesis that pretreatment with NIR-LED enhances this protective effect.

2. Results

2.1. *LED treatment rescued neurons from rotenone-induced apoptotic cell death*

In the normal rat primary neuronal cultures (control), very few striatal neurons or cortical neurons underwent cell death (Figs. 1A, E and 2A, E). However, exposure to 200 nM of rotenone for 48 h induced a 29.81% of striatal neurons to undergo apoptosis ($P<0.001$) (Fig. 1B and E). Similar exposure to rotenone rendered higher numbers of cortical neurons to

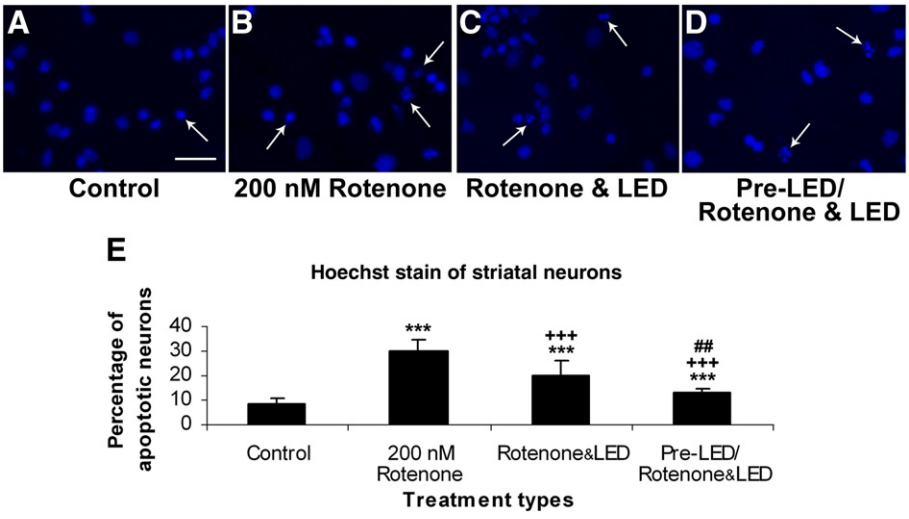

Fig. 1 – Hoechst staining of cultured striatal neurons in control (A), rotenone exposed (B), rotenone plus LED treated (C), and LED pretreatment for 2 days before rotenone exposure plus LED treatment during rotenone exposure (D). The arrows show apoptotic neurons with nuclear condensation or decreased nuclear size, with or without nuclear fragmentation. Quantitative assays of percent apoptotic neurons under various conditions are shown in (E). Rotenone exposure significantly increased the number of apoptotic neurons ($P<0.001$), and LED treatment markedly reduced this percentage ($P<0.001$). However, LED pretreatment further reduced this percentage ($P<0.01$). All "*P" values were compared to controls: $^{**}P<0.01$, $^{***}P<0.001$. All "$^{+}$P" values were compared to rotenone alone: $^{++}P<0.01$, $^{+++}P<0.001$. All "$^{\#}$P" values compared "LED pretreatment plus LED treatment during toxin exposure group" to "LED during toxin exposure only group". Scale bar: 25 μm for all.