BRAIN RESEARCH 1535 (2013) 61–70

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

The impact of near-infrared light on dopaminergic cell survival in a transgenic mouse model of parkinsonism

Sivaraman Purushothuman[a,c], *Charith Nandasena*[a,c], *Daniel M. Johnstone*[a,c,*], *Jonathan Stone*[a,c], *John Mitrofanis*[b,c]

[a]*Discipline of Physiology, University of Sydney, Australia*
[b]*Discipline of Anatomy & Histology, University of Sydney, Australia*
[c]*Bosch Institute, University of Sydney, Australia*

ARTICLE INFO

Article history:
Accepted 24 August 2013
Available online 30 August 2013

Keywords:
Tyrosine hydroxylase
Substantia nigra
Photobiomodulation
K369I mice
Hyperphosphorylated tau
Oxidative stress

ABSTRACT

We have examined whether near-infrared light (NIr) treatment mitigates oxidative stress and increased expression of hyperphosphorylated tau in a tau transgenic mouse strain (K3) that has a progressive degeneration of dopaminergic cells in the substantia nigra pars compacta (SNc). The brains of wild-type (WT), untreated K3 and NIr-treated K3 mice, aged five months (thus after the onset of parkinsonian signs and neuropathology), were labelled immunohistochemically for the oxidative stress markers 4-hydroxynonenal (4-HNE) and 8-hydroxy-2′-deoxyguanosine (8-OHDG), hyperphosphorylated tau (using the AT8 antibody) and tyrosine hydroxylase (TH). The average intensity and area of 4-HNE, 8-OHDG and AT8 immunoreactivity were measured using the MetaMorph software and TH^+ cell number was estimated using stereology. Our results showed immunoreactivity for 4-HNE, 8-OHDG and AT8 within the SNc was increased in K3 mice compared to WT, and that this increase was mitigated by NIr. Results further showed that TH^+ cell number was lower in K3 mice than in WT, and that this loss was mitigated by NIr. In summary, NIr treatment reduced the oxidative stress caused by the tau transgene in the SNc of K3 mice and saved SNc cells from degeneration. Our results, when taken together with those in other models, strengthen the notion that NIr treatment saves dopaminergic cells in the parkinsonian condition.

1. Introduction

The distinct signs associated with Parkinson's disease, namely resting tremor, akinesia and/or lead-pipe rigidity, arise after degeneration of many dopaminergic cells of the substantia nigra pars compacta (SNc) (Bergman and Deuschl, 2002; Blandini et al., 2000). The cause of this cell loss is not entirely clear, but recent evidence indicates that mitochondrial dysfunction and oxidative stress (Jenner, 2003; Muqit et al., 2006), caused by toxic insult (e.g., 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)) (Langston, 1996) and/or genetic mutation (Kruger et al., 1998), play a central role.

*Correspondence to: Discipline of Physiology, University of Sydney, Anderson Stuart Building F13, NSW 2006, Australia. Fax: +61 2 9351 6470.
E-mail address: Daniel.Johnstone@sydney.edu.au (D.M. Johnstone).

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.08.047

Indeed, many recent neuroprotective strategies, for example anti-oxidant treatment (LeWitt, 2006; Ma et al., 2009; Purushothuman et al., 2013), deep brain stimulation (to reduce glutamate excitotoxicity) (Piallat et al., 1996; Wallace et al., 2007) and near infrared light (NIr) treatment (Moro et al., 2013; Peoples et al., 2012; Shaw et al., 2010), have been developed with the goal of reducing oxidative stress and improving mitochondrial function in parkinsonian cases.

Our recent work has focused on neuroprotection of the SNc using NIr treatment, also known as photobiomodulation. We have shown that NIr exposure saves many dopaminergic SNc cells from degeneration in both acute (Moro et al., 2013; Shaw et al., 2010) and chronic (Peoples et al., 2012) MPTP mouse models of SNc degeneration. Further, we have reported that NIr treatment partially corrects abnormal neuronal activity in the subthalamic nucleus and zona incerta of the basal ganglia (Shaw et al., 2012) and improves locomotor activity (Moro et al., 2013) in MPTP-treated mice. These *in vivo* experiments have built on the pioneering *in vitro* findings of improved cell survival and function against Parkinsonian toxins (e.g., rotenone and MPTP) after NIr treatment (Liang et al., 2008; Trimmer et al., 2009; Ying et al., 2008).

In this study, we sought to extend our previous work on the MPTP mouse model to a transgenic model of parkinsonism. We took advantage of the availability of mice that have been genetically modified to overexpress human tau protein carrying the pathogenic K369I (K3) mutation (Ittner et al., 2008; van Eersel et al., 2010). These mice were originally generated as a model for frontotemporal dementia but have since been shown to have a chronic and progressive loss of dopaminergic cells in the SNc (Ittner et al., 2008; van Eersel et al., 2010). In this respect, the K3 mice mimic the pattern of degeneration seen in Parkinson's disease. For the present study, we analysed the levels of oxidative stress markers (4-hydroxynonenal (4-HNE) and 8-hydroxy-2′-deoxyguanosine (8-OHDG)) and hyperphosphorylated tau within the SNc using immunohistochemistry. Previous studies have shown that increases in oxidative stress and hyperphosphorylated tau are associated with the neurodegenerative process (Jenner, 2003; Jomova et al., 2010; Nakabeppu et al., 2007); we wanted to determine if NIr treatment could reduce these toxic species within the SNc. In addition, we examined the impact of NIr treatment on the number of dopaminergic cells in the SNc of K3 mice. As with previous studies (Moro et al., 2013; Peoples et al., 2012; Shaw et al., 2010), we used tyrosine hydroxylase (TH) immunohistochemistry to identify and count (using stereological methods) the number of dopaminergic cells. In general, our aim was to explore whether NIr treatment saved SNc cells from pathology and degeneration in a transgenic mouse model of chronic parkinsonism. Our results provide an important comparison to previous work on the MPTP model and a broader view on the effect of NIr treatment on the parkinsonian condition.

2. Results

2.1. *NIr reduced markers of oxidative stress*

4-HNE is a toxic by-product of oxidative stress and has been shown to accumulate within SNc cells of Parkinsonian cases. Its toxicity modifies proteins and causes mitochondrial dysfunction, leading ultimately to cell death (Butterfield et al., 2011; Hattoria et al., 2009; Jenner, 2003; Roede and Jones, 2010). Fig. 1 shows graphs of the average intensity (Fig. 1A) and area (Fig. 1B) of 4-HNE immunoreactivity within the SNc. Overall, the variations between the groups were significant for both intensity (ANOVA: $F=22.1$; $p<0.0001$; $n=5$ per group) and area (ANOVA: $F=13.2$; $p<0.0001$; $n=5$ per group) of immunolabelling.

In the K3 group, average intensity and area of immunolabelling were significantly higher (Tukey test: $p<0.0001$) than in the WT (~8-fold and ~60-fold, respectively) and the K3–NIr (~2-fold and ~8-fold, respectively) groups. The difference between the WT and the K3–NIr groups reached significance for intensity ($p<0.05$) but not for area ($p>0.05$). Fig. 1 shows immunolabelled sections of the WT (Fig. 1C and C′), K3 (Fig. 1D and D′) and K3–NIr (Fig. 1E and E′) groups; adjacent panels show TH (Fig. 1C–E) and 4-HNE (Fig. 1C′–E′) immunoreactivity of the same SNc region in each group. 4-HNE immunoreactivity was very sparse in the SNc of the WT (arrow; Fig. 1C′) and K3–NIr (arrows; Fig. 1E′) groups, but readily apparent in structures within this nucleus in the K3 group (arrows; Fig. 1D′).

In nuclear and mitochondrial DNA, 8-OHDG is one of the major forms of free radical-induced oxidative lesions and has been used in many previous studies as a marker for oxidative stress (Fu et al., 2012; Nakabeppu et al., 2007; Valavanidis et al., 2009). Fig. 2 shows graphs of the average intensity (Fig. 2A) and area (Fig. 2B) of 8-OHDG immunoreactivity within the SNc. Overall, the variations between the groups were significant for both intensity (ANOVA: $F=37.9$; $p<0.0001$; $n=5$ per group) and area (ANOVA: $F=40.6$; $p<0.0001$; $n=5$ per group) of immunolabelling.

In the K3 group, average intensity and area of 8-OHDG immunolabelling were significantly greater ($p<0.0001$) than in the WT control (~14-fold and 40-fold, respectively) and the K3–NIr (~4-fold) groups. Values of average intensity and area did not differ significantly between the WT and K3–NIr groups ($p>0.05$). The immunolabelled sections in Fig. 2 (organised the same way as in Fig. 1) show that 8-OHDG immunoreactivity was very sparse in the SNc of the WT (Fig. 2C′) and K3–NIr (arrow; Fig. 2E′) groups, but readily apparent in structures within this nucleus in the K3 group (arrows; Fig. 2D′).

2.2. *NIr reduced the overexpression of hyperphosphorylated tau*

Tau is a phosphoprotein that functions to stabilise microtubules in cells. Tau is minimally phosphorylated in normal brains, but becomes hyperphosphorylated in diseased brains. It is the main component in paired helical filaments and hence neurofibrillary tangles, the hallmark of degenerating cells in neurodegenerations such as Alzheimer's disease (Augustinack et al., 2002; Crowther et al., 1989). Immunohistochemistry using AT8, an antibody recognising hyperphosphorylated tau, is an established method for identifying hyperphosphorylated tau protein and neurofibrillary tangles (Augustinack et al., 2002; Braak and Del Tredici, 2011; Ward et al., 2012).