Brain Research 1648 (2016) 19–26

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

# Effects of a higher dose of near-infrared light on clinical signs and neuroprotection in a monkey model of Parkinson's disease

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ARTICLE INFO

*Article history:*
Received 15 June 2016
Received in revised form
5 July 2016
Accepted 6 July 2016
Available online 7 July 2016

*Keywords:*
Tyrosine hydroxylase
Substantia nigra
MPTP
Macaque monkeys
Behaviour
670 nm

ABSTRACT

We have reported previously that intracranial application of near-infrared light (NIr) – when delivered at the lower doses of 25 J and 35 J – reduces clinical signs and offers neuroprotection in a subacute MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) monkey model of Parkinson's disease. In this study, we explored whether a higher NIr dose (125 J) generated beneficial effects in the same MPTP monkey model (n=15). We implanted an NIr (670 nm) optical fibre device within a midline region of the midbrain in macaque monkeys, close to the substantia nigra of both sides. MPTP injections (1.8–2.1 mg/kg) were made over a five day period, during which time the NIr device was turned on and left on continuously throughout the ensuing three week survival period. Monkeys were evaluated clinically and their brains processed for immunohistochemistry and stereology. Our results showed that the higher NIr dose did not have any toxic impact on cells at the midbrain implant site. Further, this NIr dose resulted in a higher number of nigral tyrosine hydroxylase immunoreactive cells when compared to the MPTP group. However, the higher NIr dose monkeys showed little evidence for an increase in mean clinical score, number of nigral Nissl-stained cells and density of striatal tyrosine hydroxylase terminations. In summary, the higher NIr dose of 125 J was not as beneficial to MPTP-treated monkeys as compared to the lower doses of 25 J and 35 J, boding well for strategies of NIr dose delivery and device energy consumption in a future clinical trial.



## 1. Introduction

The discovery of a disease-modifying or neuroprotective therapy for neurodegenerative diseases, for example Alzheimer's and Parkinson's disease, remains frustratingly elusive. Notwithstanding much endeavour by many, there is no current treatment option available that slows or stops the pathology and the neurodegeneration progresses relentlessly (Olanow et al., 2008; Jankovic and Poewe, 2012; Schapira et al., 2014).

In this context, and following on from many promising previous reports in cell culture (Liang et al., 2008; Ying et al., 2008) and in various insect (Vos et al., 2013) and rodent (Whelan et al., 2008; Shaw et al., 2010; Peoples et al., 2012; Purushothuman et al., 2013; Moro et al., 2014; Reinhart et al., 2015; Oueslati et al., 2015; El Massri et al., 2016) models, we have shown recently that near-infrared light (NIr) therapy (⌊=670 nm) – when delivered intracranially – reduces the clinical signs and offers neuroprotection in a non-human primate model (MPTP; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) of Parkinson's disease (Darlot et al., 2016). These beneficial effects were achieved using a lower NIr dose regime of 25 J and 35 J, applied during the time of MPTP insult. Further, at these doses, there was no evidence of NIr causing adverse behavioural effects nor being toxic to cells at the midbrain implant site.

The major aim of the present study was to explore the potential clinical and neuroprotective of a much higher dose of NIr (125 J) delivered intracranially to the midbrain in the same monkey MPTP model (Darlot et al., 2016). In addition, we explored whether the higher NIr dose had any toxicity at the midbrain implant site. Our

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http://dx.doi.org/10.1016/j.brainres.2016.07.005
0006-8993/

results have significance when considering strategies of NIr dose delivery and intracranial device energy consumption in a future clinical trial.

## 2. Results

### 2.1. General

We found no major adverse effects in the monkeys that underwent surgery for the optical fibre device. They resumed normal activity – eating and grooming - within a few hours after surgery (Moro et al., 2014; Reinhart et al., 2015; Darlot et al., 2016). The NIr-treated monkeys had to have the battery of their implantable device recharged twice during the survival period – for a period of six to seven hours – and this required these animals to be anaesthetised (see Section 4). In each case, recovery from anaesthetic was uneventful.

### 2.2. Clinical evaluations

From our previous study (Darlot et al., 2016), there were two groups of NIr-treated MPTP monkeys; (1) MPTP-NIr1: these had MPTP injections together with optical fibre implants delivering NIr at lower doses (25 J or 35 J). These monkeys developed virtually no clinical signs and had an average mean clinical score of 2.8 (by comparison, control monkeys had a clinical score of 0; see Section 4 for details of our method of clinical evaluation); (2) MPTP-NIr2: these had MPTP injections together with implants delivering the same lower NIr doses. These monkeys developed more moderate clinical signs and had an average mean clinical score of 12.3. From the NIr-treated MPTP monkeys examined in this present study – those with MPTP injections together with optical fibre implants delivering NIr at a higher dose (125 J) – there appeared a further distinctive group. These monkeys, which we will refer to as the MPTP-NIr3 group, developed quite severe clinical signs (Fig. 1). Their mean clinical scores increased dramatically soon after the last MPTP injection, during the first few days of the survival period (Fig. 2A). They then stabilised, remaining consistently high up until the last day of testing. This time-line and development of clinical impairment mirrored closely that of the MPTP group, although mean clinical scores of the MPTP-NIr3 group were always slightly lower (Fig. 2A). The average mean clinical score of the MPTP-NIr3 group was 16.2 ($n=7$), ~30% lower than the MPTP group (*t* test, $p=0.3$), which had an average mean clinical score of 22 ($n=5$).

All of the monkeys of the MPTP-NIr3 group – except for one – received L-dopa or apomorphine treatment immediately after behavioural testing, in order for some autonomous activity and for eating and drinking. This was in a similar pattern to the MPTP group, where all of the monkeys required dopamine replacement drug treatment. By contrast, none of the monkeys of the MPTP-NIr1 group – those that developed very few clinical signs – required this supplementary drug treatment (Darlot et al., 2016).

### 2.3. Implant sites

All of the implant sites in the Control and MPTP-NIr3 groups were found close to the midline in the midbrain, traversing the IIIrd ventricle and often encompassing the ventral tegmental area and red nucleus (Fig. 3A); the tip of the optical fibre was usually 1–2 mm to the left of the midline, with the focal point of NIr intensity being very near the midline itself, equidistant from the SNc of both sides (see Section 4).

The graph in Fig. 3B shows that the area of the implant sites in the Control ($n=3$) and MPTP-NIr3 ($n=7$) groups were similar (*t* test, $p=0.3$). In the MPTP-NIr3 group, we found no large zones of necrosis or gliosis surrounding the optical fibre implant site, particularly in the midline regions where the NIr was most intense. In fact, from Nissl-stained (labelling all cells; Fig. 3D,G), GFAP-immunostained (labelling astrocytes; Fig. 3E,H) and IBA1-immunostained (labelling microglia; Fig. 3F,I) sections, there were no major differences in the cellular organisation surrounding the implant sites of the two groups. Further to this point, Fig. 3C plots all the $GFAP^+$ astrocytes in the regions surrounding the distal end of the optical fibre in a MPTP-NIr3 and a Control case; the patterns of gliosis were comparable, notwithstanding the MPTP-NIr3 case having a continuous delivery of NIr for the entire experimental period. The only cellular damage we found at the implant site appeared mechanical, being caused after tissue displacement by the fibre and/or tip itself; in both MPTP-NIr3 and Control groups, there were similar patterns of gliosis along the course of the optical fibre tract through the forebrain and midbrain as there were near implant site where, in the MPTP-NIr3 group, the NIr intensity was focussed.

Fig. 1B and C shows MRI images of the same brain regions of a monkey of the MPTP-NIr3 group, before (T1 weighted; Fig. 1B) and after surgery (T2 weighted; Fig. 1C). There is a distinct region depicting the optical fibre within the midbrain, near IIIrd ventricle (arrow Fig. 1C), but there were no signs of a major inflammatory response surrounding the optical fibre.

### 2.4. Cell analysis

Fig. 2 shows the mean number of nigral Nissl-stained and $TH^+$ cells (Fig. 2B) and the mean density of striatal $TH^+$ terminals (Fig. 2C), in the Control, MPTP and MPTP-NIr3 groups. Fig. 2 also shows photomicrographs of the nigral Nissl-stained (Fig. 2D,G) and $TH^+$ (Fig. 2E,H) cells, together with the patterns of TH immunoreactivity in the striatum (Fig. 2F,I) evident in the MPTP (Fig. 2D,E,F) and MPTP-NIr3 (Fig. 2G,H,I) groups.

Overall, the differences in the number of nigral Nissl-stained (ANOVA: $F=17$; $p<0.0001$; $n=15$) and $TH^+$ (ANOVA: $F=39$; $p<0.0001$; $n=15$) cells and density of striatal $TH^+$ terminations (ANOVA: $F=116$; $p<0.0001$; $n=15$) between the Control ($n=3$), MPTP ($n=5$), MPTP-NIr3 ($n=7$) groups were significant. For the MPTP group, there were major reductions in the total number of nigral Nissl-stained (Fig. 2A; Tukey-Kramer test, $p<0.001$) and $TH^+$ (Fig. 2A; Tukey-Kramer test, $p<0.0001$) cells and, in particular, the density of striatal terminations (Fig. 2B; Tukey-Kramer test, $p<0.0001$) compared to the Control group. For the MPTP-NIr3 group, the total number nigral Nissl-stained (Fig. 2B) and the density of striatal terminations (Fig. 2C) were very similar to the MPTP group (Tukey-Kramer test, $p>0.05$) indicating little evidence for neuroprotection using these measures (see Fig. 2D,G,F,I). By contrast, the number of nigral $TH^+$ cells in the MPTP-NIr3 was considerably higher (~40%) than the MPTP group (Tukey-Kramer test, $p<0.001$), indicating some preservation, a neuroprotection of TH expression in nigral cells for the MPTP-NIr3 group (see Fig. 2E,H). When compared to the MPTP-NIr1 group of our previous study (Darlot et al., 2016), the neuroprotection in the MPTP-NIr3 group was much less extensive; the MPTP-NIr1 group had a greater preservation of, not only nigral TH+ cells, but also nigral Nissl-stained cells and striatal $TH^+$ terminations.

## 3. Discussion

Our results showed that, when delivered intracranially at a higher dose (125 J), NIr had (i) no toxic effects on brain parenchyma and (ii) less therapeutic benefit, in terms of a reduction in clinical signs and offering neuroprotection, than when delivered at lower doses (25 J and 35 J) (Darlot et al., 2016). These issues will

Please cite this article as: Moro, C., et al., Effects of a higher dose of near-infrared light on clinical signs and neuroprotection in a monkey model of Parkinson's disease. Brain Research (2016), http://dx.doi.org/10.1016/j.brainres.2016.07.005