Therapeutic effect of near infrared (NIR) light on Parkinson's disease models

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## 1. ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder that affects large numbers of people, particularly those of a more advanced age. Mitochondrial dysfunction plays a central role in PD, especially in the electron transport chain. This mitochondrial role allows the use of inhibitors of complex I and IV in PD models, and enhancers of complex IV activity, such as NIR light, to be used as possible therapy. PD models fall into two main categories; cell cultures and animal models. In cell cultures, primary neurons, mutant neuroblastoma cells, and cell cybrids have been studied in conjunction with NIR light. Primary neurons show protection or recovery of function and morphology by NIR light after toxic insult. Neuroblastoma cells, with a gene for mutant alpha-synuclein, show similar results. Cell cybrids, containing mtDNA from PD patients, show restoration of mitochondrial transport and complex I and IV assembly. Animal models include toxin-insulted mice, and alpha-synuclein transgenic mice. Functional recovery of the animals, chemical and histological evidence, and delayed disease progression show the potential of NIR light in treating Parkinson's disease.

## 2. INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease that affects 1-2% of the population over 50 years of age, with 1.5 million in the United States alone (1-3). Characteristics include progressive motor dysfunction and variable cognitive impairment (4,5). The key neuropathological change is a loss of striatal dopamine content and degeneration of dopaminergic neurons (5,6). Age, genetics, and environmental factors all play a role in the pathogenesis of Parkinson's disease (7,8). Various toxins such as trace metals, industrial chemicals, pesticides, and herbicides may be involved (7,9). Genetic factors that have been identified include two mutations in the genes that code for alpha-synuclein (1). This protein is a major component of Lewy bodies, a defining feature in both inherited and sporadic Parkinson's disease. Other mutations linked to inherited PD include mutations in the genes for parkin, DJ-1, PINK1, and LRRK2 (10).

## 3. MITOCHONDRIAL DYSFUNCTION

Mitochondrial dysfunction appears to play a central role in the pathogenesis of Parkinson's disease.

Complexes I through IV are a set of membrane bound protein assemblies whose role is to shuttle electrons, generated during oxidative phosphorylation, to the ultimate acceptor, molecular oxygen. In the process, the energy afforded is used to pump protons across the mitochondrial inner membrane. The resultant proton gradient is used by complex V, ATP synthase, to produce ATP, which is made available elsewhere as needs indicate.

This electron transport chain allows us two points of access regarding models relevant to Parkinson's disease. Inhibition of complex I results in display of PD type symptoms and decreases function of the electron transport chain (11). Complex IV, which is also known as cytochrome c oxidase and is the site of the terminal electron transfer, may allow an opportunity to rectify this decreased electron transport chain function, and restore energy production. A therapeutic process that restores electron transport chain activity may counter the tendency toward dopaminergic cell death seen in Parkinson's disease.

## 4. NIR LIGHT THERAPY

The therapeutic potential and associated mechanisms of near infrared (NIR) therapy have been studied for nearly 40 years, and NIR light has been found to affect various biological processes both *in vivo* and *in vitro* (12,13). NIR light can be produced both by low intensity laser and light emitting diode, and typically falls in the range of 630-1000 nm. Cytochrome c oxidase is postulated to be the primary NIR photoacceptor in the cell, with effects including improved mitochondrial energy production, reduced free radical damage, increased cell proliferation, and decreased apoptosis (12,13). Cytochrome c oxidase contains a number of metal centers that may act as photoacceptors. These include a binuclear copper center and two heme prosthetic groups, one of which exists as a member of a binuclear metal center in combination with an additional copper. Clinical applications of NIR light therapy have included areas as diverse as oral mucositis, wound healing, and retinal toxicity (14-17).

## 5. COMPLEX I INHIBITORS

Of particular interest, as they can be used to create useful models of Parkinson's disease, are the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and the pesticide rotenone. MPTP is converted in glial cells into 1-methyl-4-phenylpyridinium (MPP$^+$) and incorporated into neurons via the dopamine transporter (18). MPP$^+$ is an inhibitor of complex I in the electron transport chain, and can result in damage to dopaminergic neurons and the display of a Parkinson's disease-like syndrome (Parkinsonism) in humans (11,18). Rotenone is also a complex I inhibitor, and can lead to nigrostriatal dopaminergic degeneration (19). These complex I inhibitors play a key role in several of the PD models used in conjunction with NIR light therapy.

## 6. CELL CULTURE MODELS

### 6.1. Primary neurons

Wong-Riley, Whelan, and co-workers have explored the effects of NIR light on a primary neuron cell culture model of Parkinson's in a series of studies spanning the past ten years (20-24). As Parkinson's is connected to both mitochondrial bioenergetics and apoptosis, experiments focused on the ability of NIR light to protect these neuronal cells against the effects of toxins relating to Parkinsonism.

Initial studies focused on tetrodotoxin (TTX), a neurotoxin that blocks the voltage–dependent sodium channels in neurons (20). Although not a Parkinsonism inducing toxin, TTX poisoning affords a useful method in which to study functionally silenced neurons. TTX at a 0.4 micromolar concentration decreased cytochrome c oxidase activity in cultured rat visual cortex primary neurons. Application of 670 nm NIR light restored cytochrome c oxidase activity in TTX poisoned cells, and even increased cytochrome c oxidase activity above baseline in normal cells.

From here, work was expanded to include cyanide poisoning, using KCN, an irreversible inhibitor of cytochrome c oxidase (21). NIR light at 670 nm partially restored cytochrome c oxidase activity that had been reduced by micromolar levels of KCN. Neuronal cell death, which was nearly complete at 300 micromolar KCN (84%), was reduced significantly (to 44%) by NIR light application. Millimolar levels of KCN resulted in cell death of a magnitude beyond the reach of NIR light therapeutic effects. Cellular ATP content was also improved in KCN treated cells. At 10 micromolar KCN, NIR light significantly restored neuronal ATP levels. Higher KCN, however, resulted in ATP reductions insensitive to NIR light application.

The question of optimum wavelength was addressed by measuring cytochrome c oxidase activity and ATP content with NIR light applied at several wavelengths in the range of 670-880 nm. The most effective wavelengths, 670 & 830 nm, correspond to maxima in the absorption spectra of cytochrome c oxidase, while the least effective, 728 nm, falls into a spectral minimum. This correspondence of the ATP and cytochrome c oxidase activity spectra with the cytochrome c oxidase absorption spectrum lends support to the hypothesis that the intracellular target for NIR light therapy may be the terminal electron transport chain enzyme cytochrome c oxidase.

The actual cell death pathway, whether apoptosis or necrosis, induced by KCN and protected against by NIR light, was the next issue addressed (22). With 100 & 300 micromolar KCN, the apoptotic pathway was confirmed by electron microscopy, Hoechst staining, single-stranded DNA, and the pro-apoptotic proteins Bax and activated caspase-3. Cell death was reduced by NIR light, in the presence of caspase inhibitor I, by 33% to 50%. NIR light pretreatment of KCN treated cells also decreased

expression of caspase-3, decreased Bax, and increased the anti-apoptotic protein Bcl-2. Reactive oxygen species (ROS) were also decreased.

These results indicate that light-emitting diode pretreatment partially protects neurons against cyanide-induced caspase-mediated apoptosis, most likely by decreasing reactive oxygen species production, down-regulating pro-apoptotic proteins and activating anti-apoptotic proteins.

In order to determine the optimum dosage patterns for NIR light, both striatal and cortical neurons were insulted with 300 micromolar KCN (23). Using a combination of histology and ATP and cytochrome c oxidase assays, the optimal frequency of NIR light treatment was determined to be twice a day. This was extended to MPP$^+$ and rotenone, both Parkinsonism inducing inhibitors of complex I in the electron transport chain.

Twice a day NIR light treatments significantly increased cellular ATP, decreased the number of neurons undergoing cell death, and significantly reduced the expressions of reactive oxygen species and reactive nitrogen species in rotenone- or MPP$^+$-exposed neurons as compared with untreated ones. These results strongly suggest that LED treatment may be therapeutic to neurons damaged by neurotoxins linked to PD by energizing the cells and increasing their viability.

Since NIR light appears to be therapeutic both by decreasing apoptosis and by improving mitochondrial bioenergetics, there may be an added benefit to a pretreatment strategy, in addition to concurrent or post-insult treatment. Pre- and concurrent NIR light treatments, alone and combined, were applied to striatal and cortical neurons cultured in 200 nM rotenone or 250 micromolar MPP$^+$ (24). The pretreatment alone significantly suppressed rotenone- or MPP$^+$-induced apoptosis. Pretreatment plus concurrent NIR light was significantly better than concurrent treatment alone. 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 light pretreatment. These results suggest that NIR light pretreatment is an effective adjunct preventative therapy in rescuing neurons from neurotoxins linked to Parkinson's disease.

### 6.2. Mutant neuroblastoma cells

A human dopaminergic neuroblastoma cell line (SH-SY5Y) has been engineered to stably over-express the human A30P alpha-synuclein mutation, which is linked to an early-onset form of Parkinson's disease (25,26). Previous studies utilizing this cell line have demonstrated that cell viability is not affected by over-expression of mutant alpha-synuclein, despite an increase in basal expression of reactive oxidative species (ROS). These cells do, however, show a receptiveness to NIR light therapy. Application of 670 nm LED light at 4 J/cm$^2$ resulted in a 50-125% increase in incorporation of tritiated thymidine compared to control, implying an NIR light related increase

in cell proliferation (Whelan, data not shown). Most effective was a single treatment per day, but results were significant with up to four treatments per day.

Although SH-SY5Y A30P cells do not show decreased cell viability due to over-expression of mutant alpha-synuclein, they do exhibit an enhanced susceptibility to additional oxidative stress (25,26). Exposure of these cells to the complex I inhibitor, MPP$^+$, disrupts mitochondrial function and further increases oxidative stress. The combination of the A30P alpha-synuclein mutation with exposure to this mitochondrial toxin recapitulates many of the features associated with the pathogenesis of Parkinson's disease. Specifically, this dopaminergic cell model of PD combines genetic susceptibility with an environmental insult.

This cellular model of PD was used by Whelan, *et al* to examine the potential of NIR light treatment in attenuating MPP$^+$-induced cytotoxicity. SH-SY5Y A30P cell cultures were exposed to increasing concentrations of the dopaminergic toxin MPP$^+$ for 14 hours (27). Cell proliferation, mitochondrial function (mitochondrial dehydrogenase activity), oxidative stress (hydrogen peroxide production), and cell viability (LDH release) were assessed 12 hours later. Exposure to MPP$^+$ produced a concentration-dependent decrease in these parameters accompanied by a concentration-dependent increase in the production of reactive oxygen species. Three 670 nm light (8 J/cm$^2$) treatments significantly attenuated the cytotoxic actions of MPP$^+$.

In summary, these studies demonstrate that NIR light treatment promotes cell survival in SH-SY5Y A30P cells exposed to MPP$^+$ by mechanisms that include increased cellular proliferation, profoundly enhanced mitochondrial function, reduced oxidative stress, and increased viability.

### 6.3. Cybrid cells

A novel model of sporadic PD has been developed that avoids many of the problems associated with genetic and neurotoxin models (28). Cybrid cells are created by fusing mitochondrial DNA (mtDNA)-free SH-SY5Y neuroblastoma or NT2 teratocarcinoma cells with platelets containing mtDNA from either control or PD volunteers. Multiple replications results in cell lines with a uniform nuclear genetic and environmental background that differ from each other only in the source of their mitochondrial genes. Deletions in mtDNA increase with age, and are more abundant in PD brain tissue. These mitochondrial genes code for important complex I and IV proteins, and may be related to mitochondrial dysfunction. As these mutations are expressed against a uniform cellular background, differences in cell lines are minimized, while functional differences in mitochondrial genomes are isolated.

Studies with cybrids show that PD mitochondrial genes harm cell survival in a manner that correlates with what is seen in PD brain tissue. These changes include, among others, decreased complex I activity, increased

oxidative stress, decreased cytochrome c oxidase activity, more LDH release, less ATP, and increased alpha-synuclein oligomerization. Morphologically abnormal and swollen mitochondria were observed, and Lewy bodies were formed.

Trimmer *et al* have put forth the hypothesis that reduced axonal transport of mitochondria contributes to degeneration of neuronal processes in Parkinson's disease (29). Mitochondria supply the ATP necessary to power this axonal transport, in addition to many other functions necessary for cell survival. They have developed a method to label mitochondria, follow their movement with time lapse microscopy, and calculate average velocities. The mitochondrial velocity and total distance traveled in PD cybrids was significantly reduced compared to controls. Average distance and time spent moving was also reduced, although not significantly.

The cybrids were given NIR light treatment at 810 nm for 40 seconds, with a total dose of 2 $J/cm^2$. For two hours after NIR light treatment, mitochondrial movement was restored to levels comparable to controls, while the controls themselves were unaffected. PD cybrid neuronal cells lines showed a decreased proportion of correctly assembled complexes in the electron transport chain, while control cybrids had correctly assembled complexes. Those PD cybrid lines that showed the greatest response to NIR light also had a larger proportion of intact assemblies. The cybrids with the most dysfunctional oxygen utilization profiles also were the least responsive to NIR light.

As reduced neuronal transport could underlie the loss of dopaminergic cells in Parkinson's disease, through degeneration of the axonal terminals, the increased mitochondrial velocity seen with NIR light therapy could possibly have a positive effect in preventing, or decreasing, neuronal degeneration. This suggests that NIR light therapy could be developed into a means to improve neuronal function in PD patients.

## 7. ANIMAL MODELS

### 7.1. MPTP mice

NIR light treatment can also augment mitochondrial function and stimulate antioxidant protective pathways in specific neurons that offer protection against degeneration in a mouse model of Parkinson's disease. Mammals treated with MPTP, a metabolic precursor of $MPP^+$, develop many of the cardinal features of Parkinson's disease, manifested predominately as a marked reduction in locomotor activity hours after administration of the toxin. The rapid onset of the Parkinsonian syndrome following acute MPTP intoxication thus provides an excellent paradigm for initial assessment of the therapeutic potential of NIR light therapy. MPTP has the added advantage that it poisons the very process thought to account for the beneficial actions of NIR light, namely, mitochondrial energy production.

To investigate the ability of NIR light to ameliorate the acute toxicity of the MPTP, mice were either pretreated with 670 nm NIR light or were treated after MPTP exposure.[27] Each animal was tested for locomotor activity from 0–12, 23–24, 47–48, and 71–72 hours post-injection. Administration of MPTP alone caused a profound depression of all of the locomotor parameters measured, which was sustained for at least 48 hours. A single 670 nm light treatment (10 min, 60 $J/cm^2$) administered following MPTP did not alter the locomotor behavior brought about by MPTP. Thus, 670 nm light treatment was not able to reverse the effects of MPTP when applied after the toxin. Conversely, 670 nm NIR light pretreatment for 5 min (30 $J/cm^2$) twice a day over 3 days attenuated the deficits in locomotor behavior (length of time spent moving, the number of movements made, distance moved, and velocity) induced by a single injection of MPTP. Furthermore, by 48 hours the behaviors were essentially restored to control levels.

Mitrofanis and co-workers have also explored the effects of NIR light therapy of MPTP treated mice (30). Mice were subjected to two levels of MPTP (50 or 100 mg/kg) and treated immediately with NIR light at 670 nm and 40 $mW/cm^2$ for a total dose of 14.4 $J/cm^2$ over a thirty hour period. Measurements using an intracranial sensor showed that the energy intensity of the light that actually penetrated through the skull was 5.3 $mW/cm^2$, giving the actual dosage to the brain as 1.9 $J/cm^2$.

The mice were sacrificed after six days, and the brains were processed for tyrosine hydroxylase immunochemistry. Dopaminergic cells from two regions were studied, the substantia nigra pars compacta (SNc) and the zona incerta-hypothalamus (ZI-Hyp). The SNc is the region showing the main loss of dopaminergic cells seen in Parkinson's disease. The major finding was the presence of significantly more dopaminergic cells in the MPTP NIR light-treated animals as compared to MPTP alone animals. In contrast, the ZI-Hyp samples showed no significant difference between these groups. In addition, cell survival was MPTP dose dependent. After the higher MPTP dose, the magnitude of cell loss was similar in the two regions, while cell loss was greater in the SNc than the ZI-Hyp after the lower dose. This neuroprotection of dopaminergic cells by NIR light therapy, more evident in the PD relevant SNc, has clear clinical applications in the treatment of Parkinson's disease.

### 7.2. Transgenic mice

The study of alpha-synuclein point mutations has been expanded from the neuroblastoma cell model to a transgenic mouse model by investigating the protective effects of NIR light therapy in transgenic mice expressing the A53T human alpha-synuclein point mutation (31). A53T transgenic mice received NIR light treatment at 670 nm and 7.5 $J/cm^2$ three times per week beginning at eight weeks of age. At phenotype onset mice received NIR light once per day until they exhibited signs of extreme distress. Onset and severity of disease phenotype were analyzed.

NIR light therapy delayed disease progression and attenuated the severity of the disease phenotype. The median age of disease phenotype onset was 455 days for non-NIR light treated mice and 535 days for NIR light-treated mice. At 500 days, significantly fewer NIR light treated mice developed the disease phenotype. Also, of those mice that developed the disease phenotype, NIR light treated mice demonstrated delayed progression of disease measured from time of phenotype onset to sacrifice. Utilizing a grading scale developed to score disease phenotype, the median scores of NIR light treated mice was 1, whereas non-NIR light treated A53T mice had a median score of 2. These findings support the neuroprotective actions of NIR light therapy in an established animal model of Parkinson's disease.

## 8. PERSPECTIVE

The PD models detailed in this review allow us a way to test a promising new therapy for neurodegenerative diseases using near infrared radiation. As the cellular mechanisms and processes involved when using NIR light therapy are unraveled, against a background of established PD models, we can work out the details of the necessary light exposure and the changes we expect to see. Work has been pushed forward to include rodent models and actual behavioral measures, which brings us closer to our goal of an effective therapy relevant to the needs of actual human Parkinson's sufferers. As current Parkinson's treatments revolve around counteracting the effect of neuronal loss, we are still in the stage of trying to make the surviving neurons "work harder". A more elegant, and possibly more effective, goal would be to prevent the loss of dopaminergic neurons in the first place. Although this would not reverse any degeneration that may have occurred already, we may be able, for the first time, to actually arrest, or at least slow down, further neuronal degeneration and perhaps halt disease progress. As Parkinson's patients do not normally present until the disease stage is well advanced, this is perhaps the best course we can offer.

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