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Harnessing the cell's own ability to repair and prevent neurodegenerative disease

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Near-IR light treatment modifies cellular function, promotes cell survival, and improves outcomes in laboratory and mouse models of Parkinson's disease.

The technique of photobiomodulation is based on the phenomenon that exposure to low-level laser light can alter cellular function. The observed physiologic effects of such treatment include increased rates of tissue regeneration as well as inflammation and pain relief. The exact cellular mechanisms underlying photobiomodulation, however, are not entirely understood and are actively being investigated.

Evidence has indicated that near-IR light treatment can prevent cell death (apoptosis) in cultured neuronal cells. As seen in Figure 1, control cultures had relatively few dead cells (4.75% of total cell population). On the other hand, cultures subjected to 300μM KCN for 28 hours to induce apoptosis showed a profusion of neurons with highly condensed DNA (83.6%), indicative of cell death. Similar cultures that underwent 10 minutes of near-IR light treatment demonstrated substantially reduced numbers of neurons exhibiting cell death (43.5%),[1] supporting the role of photobiomodulation in promoting cell survival.

Recent reports have ascribed the ability of specific wavelengths of light to promote cellular proliferation to the activation of mitochondria, the energy-producing organelles within the cell.[2, 3, 4] The growing body of evidence suggests that one specific mitochondrial protein, cytochrome oxidase, is a key photoacceptor of light in the far-red to near-IR spectral range.[2, 3, 4] Cytochrome oxidase is a membrane-associated protein that contains four redox active metal centers and is a critical component of the energy-producing machinery (see Figure 2). This protein demonstrated a strong absorbance in the far-red to near-IR spectral range, detectable within the cell by near-IR spectroscopy.[5]

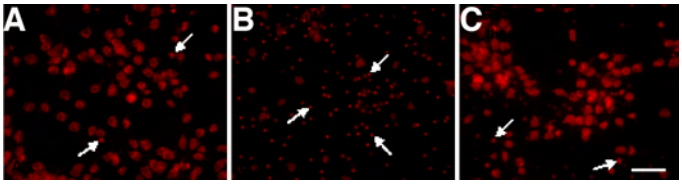

Figure 1. *670nm near-IR light treatment attenuates potassium cyanide (KCN)-induced apoptosis (i.e., programed cell death) in primary cultured neurons. Primary visual cortical neurons subdivided into 3 groups: (A) control, (B) exposed to 300μM KCN for 28 h, and (C) pretreatment with 670nm light for 10 min before exposure to 300μM KCN for 28 h. Cultures were then stained with propidium iodide for neurons undergoing cell death (arrows).*

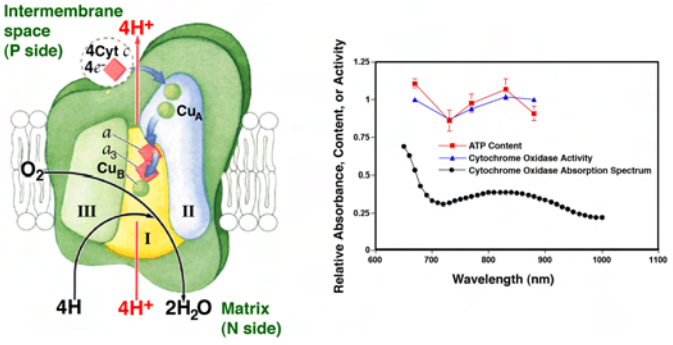

Figure 2. *Cytochrome oxidase and the correlation between the near-IR absorption spectrum of cytochrome oxidase, ATP content, and cytochrome oxidase activity in cultured primary neuronal cells subjected to metabolic inhibition and near-IR light treatment.*

Studies in our laboratory have demonstrated that the action spectrum for the stimulation of cytochrome oxidase activity and

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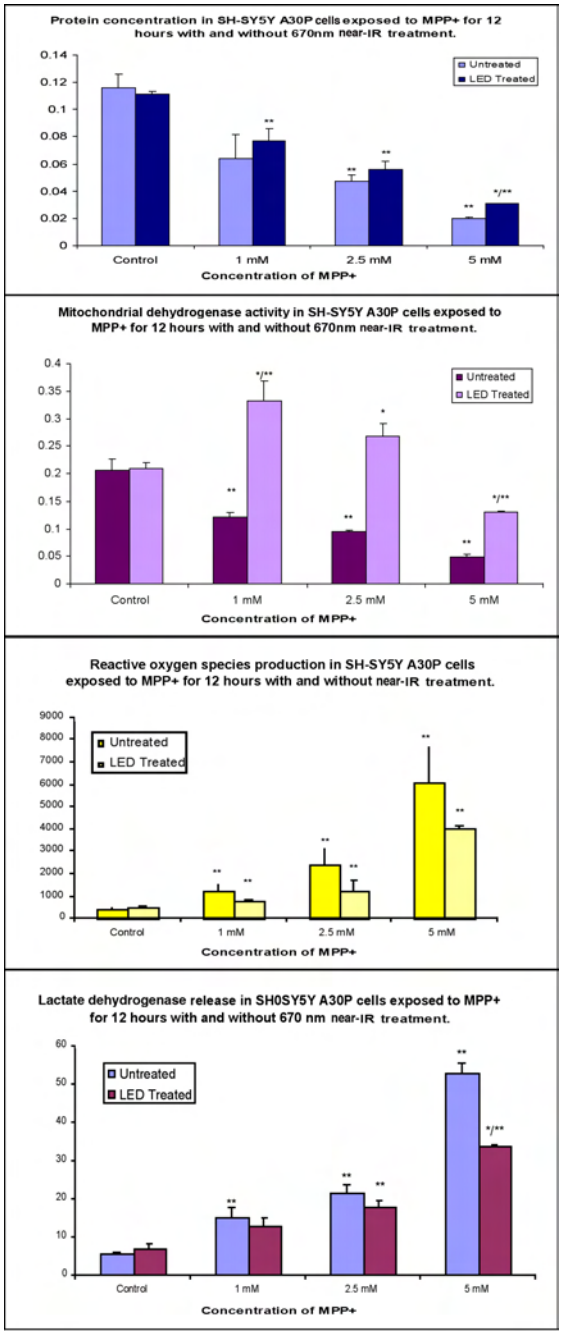

Figure 3. *670nm light treatment attenuates MPP^+ cytotoxicity in human dopaminergic cells expressing A30P mutant α-synuclein. Results expressed as mean values ± SE of replicated culture wells within an experiment. Differences between groups determined by one-way ANOVA. ** $p < 0.05$.*

cellular energy stores (i.e., ATP content) parallels the near-IR absorption spectrum of cytochrome oxidase (see Figure 2). Moreover, irradiation at 660–680nm has been shown to increase the activity of purified cytochrome oxidase,[3] increase the energy production rates of isolated mitochondria,[3] and upregulate cytochrome oxidase activity in cultured neuronal cells.[4] Together, these data indicated that cytochrome oxidase, and thus mitochondria energy production, are cellular targets influenced by near-IR light treatment.

The evidence indicating that near-IR treatment can augment mitochondrial function and stimulate antioxidant protective pathways comes from photobiomodulation experiments conducted in a laboratory model of Parkinson's disease (PD). Cultures of human dopaminergic neuronal cells engineered to stably overexpress the PD mutant form of α-synuclein were exposed to increasing concentrations of the dopaminergic toxin MPP^+ for 14 hours (Figure 3). Cell proliferation (protein concentration), mitochondrial function (mitochondrial dehydrogenase activity), oxidative stress (H_2O_2 production), and cell viability (lactate dehydrogenase release) were assessed 12 hours later. Exposure to MPP^+ produced a concentration-dependent decrease in cell proliferation, mitochondrial function and cell viability accompanied by a concentration-dependent increase in reactive oxygen species production. Three 670-nm light (8 J/cm^2) treatments significantly attenuated the cytotoxic actions of MPP^+, resulting in increased cellular proliferation, profoundly enhanced mitochondrial function, reduced oxidative stress, and increased viability.

Near-IR treatment can also augment mitochondrial function and stimulate antioxidant protective pathways in specific neurons that offer protection against neuronal degeneration in a mouse model of PD. Mammals treated with MPTP, a metabolic precursor of MPP^+, develop many of the cardinal features of PD, 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 the initial assessment of the therapeutic potential of near-IR photon therapy. MPTP has the added advantage in that it poisons the very process thought to account for the beneficial actions of near-IR light—namely, mitochondrial energy production.

To investigate the ability of near-IR light to ameliorate the acute toxicity of the neurotoxin MPTP, mice were either pretreated with 670nm photon irradiation or were treated after MPTP exposure. Each animal was tested for locomotor activity from 0–12, 23–24, 47–48, and 71–72 hours post injection.

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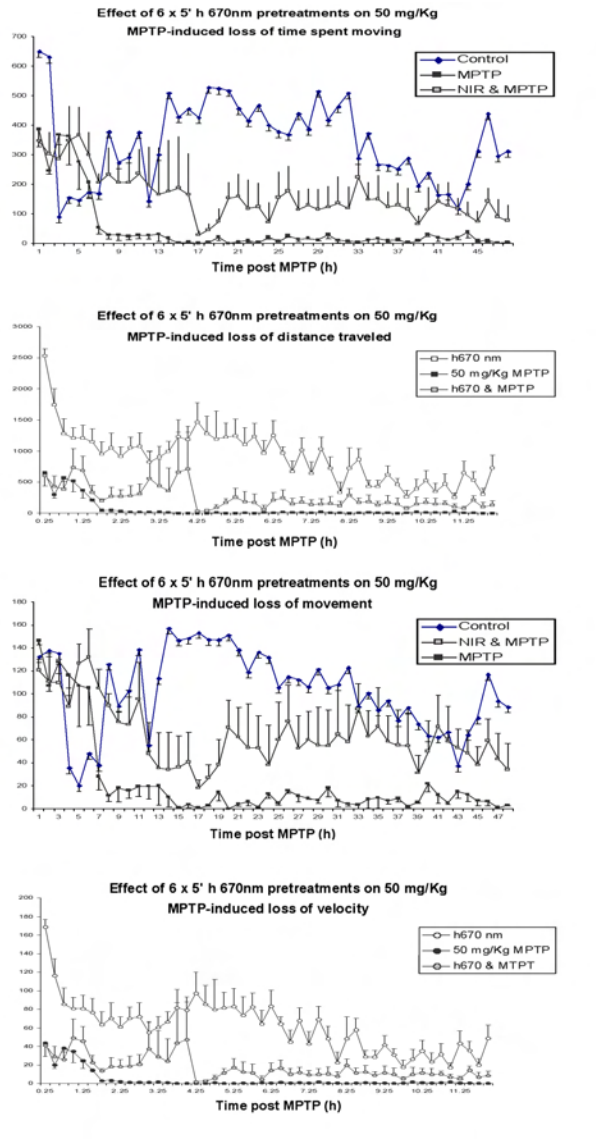

Figure 4. *670nm light pretreatment ameliorates the toxicity of the Parkinsonian drug MPTP*

Administration of MPTP alone caused a profound depression of all of the locomotor parameters measured, and this depression was sustained for at least 48 hours (see Figure 4). A single 670nm light treatment (10 min, 60 J/cm^2) administered following MPTP did not alter the locomotor behavior brought about by MPTP (data not shown). Thus, 670nm light treatment was not able to reverse the effects of MPTP when applied after the toxin. Conversely, 670nm light pretreatment for 5 minutes (30 J/cm^2) twice a day over 3 days attenuated the deficits in locomotor behavior induced by a single injection of MPTP. Near-IR light pretreatment attenuated the effects of MPTP on the length of time spent moving, the number of movements made, distance moved, and velocity (see Figure 4). Moreover, 670nm light pretreatments essentially restored these behaviors to control levels by 48 hours.

Taken together, these observations demonstrate the potential clinical applications of near-IR photobiomodulation. The identification of the key mitochondrial component involved in this process, cytochrome oxidase, suggests a plausible mechanism of action whereby near-IR light treatment upregulates energy production, thereby promoting cell survival. Furthermore, the ability of near-IR light treatment to attenuate the cytotoxity and dopaminergic cell death in a laboratory model of PD and the clear therapeutic benefit against the acute toxicity of MPTP indicates the potential benefit of this therapy for patients suffering with PD and possibly other neurodegenerative diseases.

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References

1. M.T. Wong-Riley, H.L. Liang, J.T. Eells, B. Chance, M.M. Henry, E. Buchmann, M. Kane, and H.T. Whelan, *Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome oxidase*, **J Biol Chem 280** (6), pp. 4761–4771, 2005.

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2. T. Karu, *Primary and secondary mechanisms of action of visible to near-IR radiation on cells*, **J Photochem Photobio 49** (1), pp. 1–17, 1999.
3. T. Karu, *Low power laser therapy*, in T. Vo-Dinh ed., **Biomedical Photonics Handbook**, CRC Press LLC, Bica Raton, FL, 2003.
4. M.T. Wong-Riley, X. Bai, E. Buchmann, and H.T. Whelan, *Light-emitting diode treatment reverses the effect of TTX on cytochrome oxidase in neurons*, **NeuroReport 12** (14), pp. 3033–7, 2001.
5. B. Beauvoit, S. M. Evans, T. W. Jenkins, E. E. Miller, and B. Chance, *Correlation between the light scattering and the mitochondrial content of normal tissues and transplantable rodent tumors*, **Analytical Biochemistry 226** (1), pp. 167–174, 1995.