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Mitochondrial signal transduction in accelerated wound and retinal healing by near-infrared light therapy

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Abstract

Photobiomodulation by light in the red to near infrared range (630–1000 nm) using low energy lasers or light-emitting diode (LED) arrays has been shown to accelerate wound healing, improve recovery from ischemic injury in the heart and attenuate degeneration in the injured optic nerve. Recent evidence indicates that the therapeutic effects of red to near infrared light result, in part, from intracellular signaling mechanisms triggered by the interaction of NIR light with the mitochondrial photoacceptor molecule cytochrome *c* oxidase. We have demonstrated that NIR-LED photo-irradiation increases the production of cytochrome oxidase in cultured primary neurons and reverses the reduction of cytochrome oxidase activity produced by metabolic inhibitors. We have also shown that NIR-LED treatment prevents the development of oral mucositis in pediatric bone marrow transplant patients. Photobiomodulation improves wound healing in genetically diabetic mice by upregulating genes important in the promotion of wound healing. More recent studies have provided evidence for the therapeutic benefit of NIR-LED treatment in the survival and functional recovery of the retina and optic nerve in vivo after acute injury by the mitochondrial toxin, formic acid generated in the course of methanol intoxication. Gene discovery studies conducted using microarray technology documented a significant upregulation of gene expression in pathways involved in mitochondrial energy production and antioxidant cellular protection. These findings provide a link between the actions of red to near infrared light on mitochondrial oxidative metabolism in vitro and cell injury in vivo. Based on these findings and the strong evidence that

Abbreviations: NIR, near infrared; LED, light-emitting diode; ERG, electroretinogram; LRRI, log relative retinal illumination.

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mitochondrial dysfunction is involved in the pathogenesis of numerous diseases processes, we propose that NIR-LED photobiomodulation represents an innovative and non-invasive therapeutic approach for the treatment of tissue injury and disease processes in which mitochondrial dysfunction is postulated to play a role including diabetic retinopathy, age-related macular degeneration, Leber's hereditary optic neuropathy and Parkinson's disease.

1. Introduction

Low energy photon irradiation by light in the far red to near infrared spectral range (630–1000 nm) using low energy lasers or light emitting diode arrays has been found to modulate various biological processes in cell culture and animal models (Karu, 1999, 2003). At the cellular level, photo-irradiation at low fluences can generate significant biological effects including cellular proliferation, collagen synthesis and the release of growth factors from cells (Sommer et al., 2001). This phenomenon of photobiomodulation has been shown to accelerate wound healing, improve recovery from ischemic injury in the heart and attenuate degeneration in the injured optic nerve (Whelan et al., 2001; Oron et al., 2001, Assa et al., 1989). Moreover, photobiomodulation has been applied clinically in the treatment soft tissue injuries and to accelerate wound healing for more than 30 years (Karu, 1999, 2003).

Investigations into low-energy stimulation of tissues by lasers have shown increased cellular activity during wound healing, including increased collagen production and angiogenesis (Mester et al., 1998; Sommer et al., 2001) The data suggest that monochromatic, near-infrared laser biostimulation produces its primary effect during the cell proliferation phase. Lasers, however, have some inherent characteristics that make their use in a clinical setting problematic, including limitations in wavelength capabilities and beam width. The combined wavelengths of the light for optimal wound healing cannot be efficiently produced, the size of wounds which may be treated is limited (due to laser production of a beam of light; a fact inconsistent with treating large areas), heat production from the laser light itself can actually damage tissue, and the pin-point beam of laser light can damage the eye. NASA developed LEDs offer an effective alternative to lasers (Fig. 1). These diodes can be configured to produce multiple wavelengths, can be arranged in large, flat arrays (allowing treatment of large wounds), and produce no heat. It is also of importance to note that LED light therapy has been deemed a nonsignificant risk by the FDA and FDA approval for the use of LEDs in humans for light therapy has been obtained. Optimal light wavelengths [proven in prior studies of laser and LED light (Karu, 1989; Lubart et al., 1992; Beauvoit et al., 1994, 1995; Whelan, 1999, 2000, 2001; Sommer et al., 2001)] to speed wound healing include range from 630–900 nm. The depth of near-infrared light penetration into human tissue has been measured spectroscopically (Chance et al., 1988; Beauvoit et al., 1994, 1995). Spectra taken from the wrist flexor muscles in the forearm and muscles in the calf of the leg demonstrate that most of the photons at wavelengths between 630–800 nm travel approximately 23 cm through the skin surface (light input) and muscle, exiting at the photon detector.

2. Cytochrome oxidase as a primary photoacceptor molecule

The mechanism of photobiomodulation by red to near infrared light at the cellular level has been ascribed to the activation of mitochondrial respiratory chain components resulting in initiation of a signaling cascade which promotes cellular proliferation and cytoprotecton (Karu, 1999, Grossman et al. 1998, Wong-Riley et al., 2001). A growing body of evidence suggests that cytochrome oxidase is a key photoacceptor of light in the far red to near infrared spectral range (Karu, 1999, Wong-Riley et al., 2001). A comparison of the action spectrum for cellular proliferation following photoirradiation with the absorption spectrum of potential photoacceptors first lead Karu and colleagues to suggest that cytochrome oxidase is a primary photoreceptor of light in the red to near infrared region of the spectrum (Karu 1999, 2003). Cytochrome oxidase (Fig. 2) is an integral membrane protein which contains four redox active

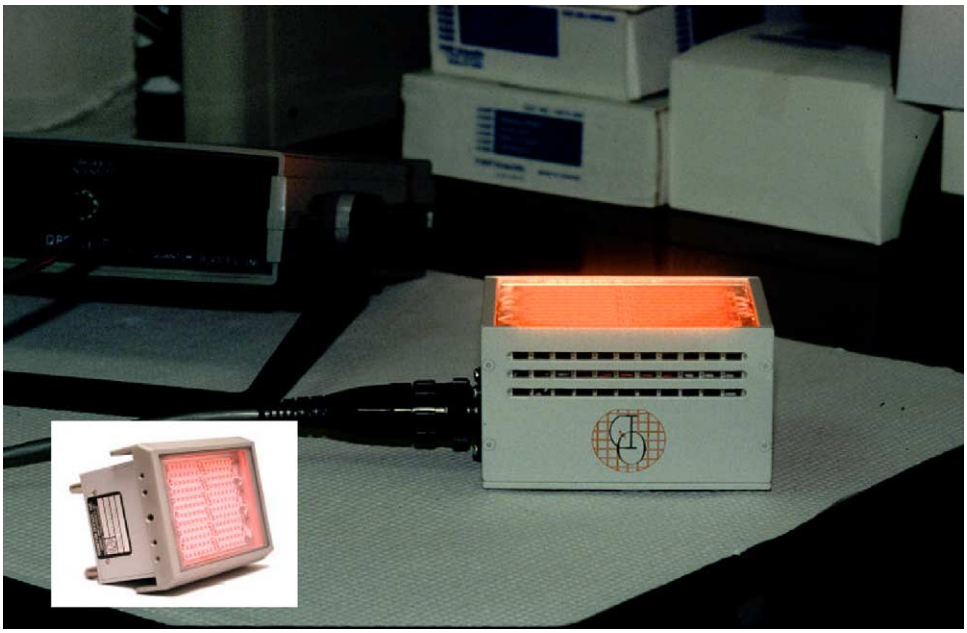

Fig. 1. Quantum devices 670 nm light-emitting diode array.

metal centers and has a strong absorbance in the far red to near-infrared spectral range detectable in vivo by near-infrared spectroscopy (Beauvoit et al., 1994). Moreover, 660–680 nm irradiation has been shown to increase electron transfer in purified cytochrome oxidase (Pastore et al., 2000) to increase mitochondrial respiration and ATP synthesis in isolated mitochondria (Pasarell et al., 1984) and to upregulate cytochrome oxidase activity in cultured neuronal cells (Wong-Riley et al., 2001).

3. NIR-LED for the prevention of oral mucositis in pediatric bone marrow transplant patients

As a final life-saving effort, leukemia patients are given healthy bone marrow from an HLA-matched donor. Prior to the bone marrow transplant (BMT), the patient is given a lethal dose of chemotherapy in order to destroy his/her own, cancerous bone marrow. Because many chemotherapeutic drugs, as well as radiation therapy, kill all rapidly dividing cells

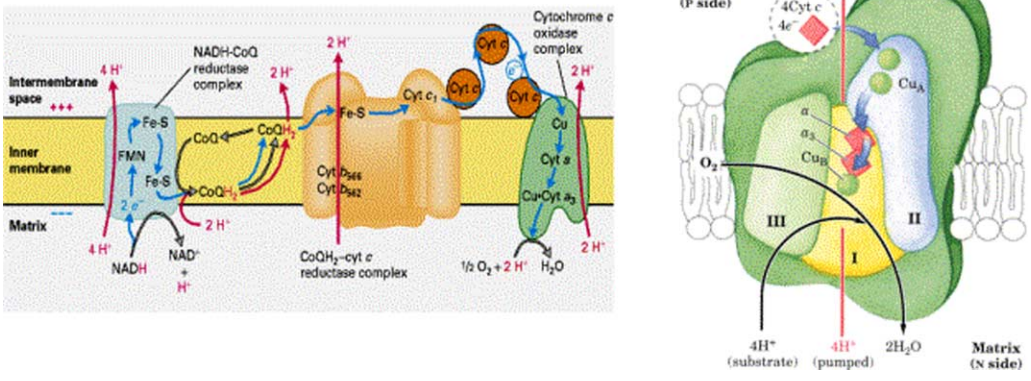

Fig. 2. Cytochrome oxidase is a photoacceptor moleculae for NIR light.

indiscriminately, the mucosal linings of the gastrointestinal tract are often damaged during the treatment. As a result of these GI effects, a majority of patients treated with cytotoxic drugs develop ulcers in their mouths (oral mucositis) and/or suffer from nausea and diarrhea. Oral mucositis, which causes severe pain, bleeding, an increased risk for infection, and compromised ability to chew and swallow, is a significant risk for this population. Current treatment for mucositis addresses pain management and infection prevention. The use of oral agents to promote cleansing, debridement, and comfort are recommended, and prophylactic oral antiviral and antifungal agents have been used to minimize infections. Laser light treatment has been shown to speed healing of oral mucositis (Barasch et al., 1995; Cowen et al., 1997) and we have recently obtained evidence that NIR-LED therapy protects against the development of these oral lesions. (Whelan et al., 2002).

Studies were performed in 32 pediatric patients undergoing myeloablative therapy in preparation for BMT. Patients were examined by two of three pediatric dentists trained in assessing the Schubert oral mucositis index (OMI) for left and right buccal and lateral tongue mucosal surfaces, while the patients were asked to rate their current left and right mouth pain, left and right xerostomia, and throat pain. LED therapy consisted of daily treatment at an fluence of 4 J/cm^2 using a 670 nm LED array held to the left extra-oral epithelium starting on the day of transplant, with a concurrent sham treatment on the right. Patients were assessed before BMT and every two to three days through post-transplant day 14. Outcomes included the percentage of patients with ulcerative oral mucositis (UOM) compared to historical epidemiological controls, the comparison of left and right buccal pain to throat pain, and the comparison between sides of the buccal and lateral tongue OMI and buccal pain. As shown in Fig. 3, the incidence of UOM was 53%, compared to an expected rate of 70–90%. There was also a 48 and 39% reduction of treated left and right buccal pain respectively compared to untreated throat pain at about post-transplant day seven ($P < 0.05$). We are currently engaged in a multi-center trial of NIR-photobiomodulation for the prevention of oral mucositis in BMT patients and are confident that this non invasive treatment regimen may soon become the standard of care.

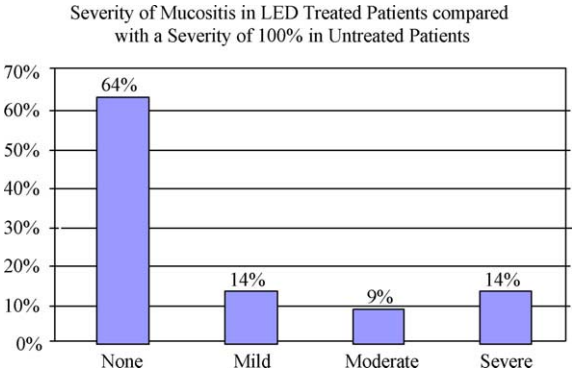

Fig. 3. 670 nm LED treatment reduces the incidence of ulcerative mucositis in pediatric bone marrow transplant patients.

4. Molecular basis for NIR-LED-induced acceleration of wound healing

Wound healing has three phases: first a substrate is laid down, then cells proliferate, and finally there is remodeling of tissue. The data published so far suggests that laser biostimulation produces its primary effect during the cell proliferation phase of the wound healing process. It has been demonstrated that mitochondria are receptive to monochromatic near-infrared light and that laser light likely increases respiratory metabolism of certain cells (Beauvoit et al., 1994, 1995; Cooper and Springett, 1997) Processes such as fibroblast proliferation, attachment and synthesis of collagen and procollagen, growth factor production [including keratinocyte growth factor (KGF), transforming growth factor (TGF) and platelet-derived growth factor (PDGF)], macrophage stimulation, lymphocyte stimulation (Mester et al., 1998) and greater rate of extracellular matrix production have been reported with laser light treatment (Lubart et al., 1992; Miller and Truhe, 1993; Yu et al., 1994; Whelan, 1999, 2000, 2001; Sommer et al., 2001). Animal studies on the enhanced wound healing effect of laser light of low power density have been performed in toads, mice, rats, guinea pigs, and swine (Bibikova and Oron, 1995). Human studies with laser light have demonstrated greater amounts of epithelialization for wound closure and stimulation of skin graft healing (Miller and Truhe, 1993; Conlan et al., 1996). An excellent review of recent human experience with near-infrared light

therapy for wound healing was published by Conlan, et al. in 1996.

We have recently investigated the explored the molecular basis of the promotion of wound healing by near-infrared light in a genetically diabetic mouse model of impaired wound healing (BKS.Cg-*m*+/+ *Lepr*^{db} from Jackson Laboratory,Bar Harbor, ME.) (Whelan et al., 2003). Polyvinyl acetal (PVA) sponges were subcutaneously implanted in the dorsum of BKS.Cg-*m*+/+*Lepr*^{db} mice. LED treatments were given once daily, and at the sacrifice day, the sponges, incision line and skin over the sponges were harvested and used for RNA extraction. Using cDNA gene array technology we observed a variety of gene families such as basement membrane components to be upregulated by NIR-LED treatment compared to the untreated controls (Fig. 4). Expression of basement membrane components occurs during sequential phases of wound healing and angiogenesis. Nidogen is one such protein along with gap junction proteins, actin that were upregulated by LED treatment. Laminin and nidogen transcripts are greatest during the early proliferative-migratory phase of angiogenesis but decrease significantly in later phases, when vessel maturation and tube formation predominate. There are reports that suggest that wound-induced epithelial cell migration is a finely tuned process that is dependent upon the regulated function and localization of specific laminins and their integrin receptors (Lotz et al., 1997).

Integrin alpha 7 beta 1 is a specific cellular receptor for the basement membrane protein laminin-1, as well as for the laminin isoforms −2 and −4. The alpha 7 subunit is expressed mainly in skeletal and cardiac muscle and has been suggested to be involved in differentiation and migration processes during myogenesis. Both integrins and laminins were among the many upregulated genes upon LED treatment when compared to the untreated controls. Principal stages of epidermal wound healing in human skin implies a linkage between BM assembly, integrin distribution and the compartment of proliferation competent cells, which in turn determines the onset of differentiation. Thus, apart from the balance of diffusible growth regulators, there is positional control of keratinocytes, largely accomplished by integrin-matrix interactions, which seems to be prerequisite to establishment and maintenance of tissue homeostasis (Breitkreutz et al., 1997).

We have identified semaphorins/collapsins to be markedly increased upon exposure to LED that may in turn decrease pain. Mouse semaphorin H functions as a chemorepellent to guide or block sensory peripheral nerve ingrowth, most likely via neuropilin as a receptor (Miyazaki et al., 1999). With the increase of semaphoring D at the site of the wound, nerve growth would likely be directed to occur around, rather than through the wound area. Numerous studies have shown that pain slows the healing process probably due to CNS-directed recruitment of inflammatory cells to the site of injury and their ubsequent release of cytokines/eicosanoids and other mediators.

Using gene discovery techniques one can begin to understand the biochemical mechanisms that are triggered by LED and may be playing a role in ultimately enhancing the healing process. LED photobiomodulation alters the expression of genes

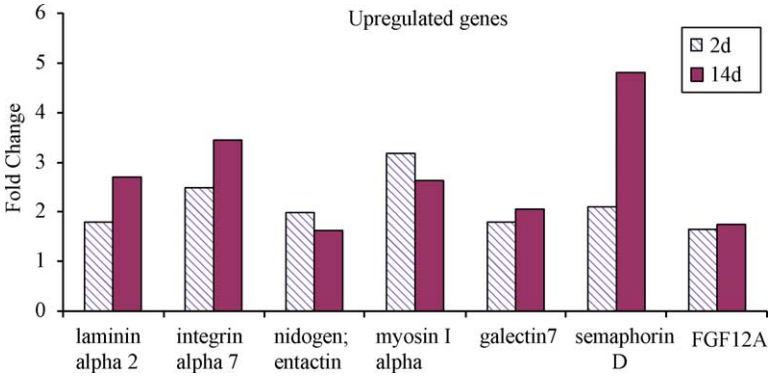

Fig. 4. Genes associated with wound healing are upregulated in a mouse model of diabetic wound healing treated with 670 nm LED.

involved in wound healing and possibly pain modulation thus enhancing the healing process.

5. NIR-LED photobiomodulation in the treatment of retinal toxicity

Decrements in mitochondrial function have been postulated to be involved in the pathogenesis of numerous retinal and optic nerve diseases including age-related macular degeneration, diabetic retinopathy and Leber's hereditary optic neuropathy (Carelli et al., 2002). Decrements in mitochondrial function have also been postulated to be involved in the pathogenesis in methanol intoxication (Eells et al., 2000). Methanol intoxication produces toxic injury to the retina and optic nerve frequently resulting in blindness. The toxic metabolite in methanol intoxication is formic acid, a mitochondrial toxin known to inhibit the essential mitochondrial enzyme, cytochrome oxidase. Studies were undertaken to test the hypothesis that exposure to monochromatic red radiation from light-emitting diode (LED) arrays would protect the retina against the toxic actions of methanol-derived formic acid in a rodent model of methanol toxicity (Eells et al., 2003). Using the electroretinogram as a sensitive indicator of retinal function, we demonstrated that three brief (2 min. 24 s.) 670 nm LED treatments (4 J/cm^2), delivered at 5, 25 and 50 h of methanol intoxication, significantly attenuated the retinotoxic effects of methanol-derived formate during intoxication and profoundly improved the recovery of retinal function following intoxication (Fig. 5). We further show that LED treatment protected the retina from the histopathologic changes induced by methanol-derived formate (Fig. 6). These findings provide a link between the actions of monochromatic red to near infrared light on mitochondrial oxidative metabolism in vitro and retinoprotection in vivo.

The prolonged effect of 3 brief LED treatments in mediating the retinoprotective actions in methanol intoxication suggests that 670 nm LED photostimulation induces a cascade of signaling events initiated by the initial absorption of light by cytochrome oxidase. These signaling events may include the activation of immediate early genes, transcription factors, cytochrome oxidase subunit gene expression and a host of other enzymes and pathways related to increased oxidative metabolism. In addition to increased oxidative metabolism, red to near infrared light stimulation of mitochondrial electron transfer is also known to increase the generation of reactive oxygen species (Karu, 1999 Karu, 2003). These

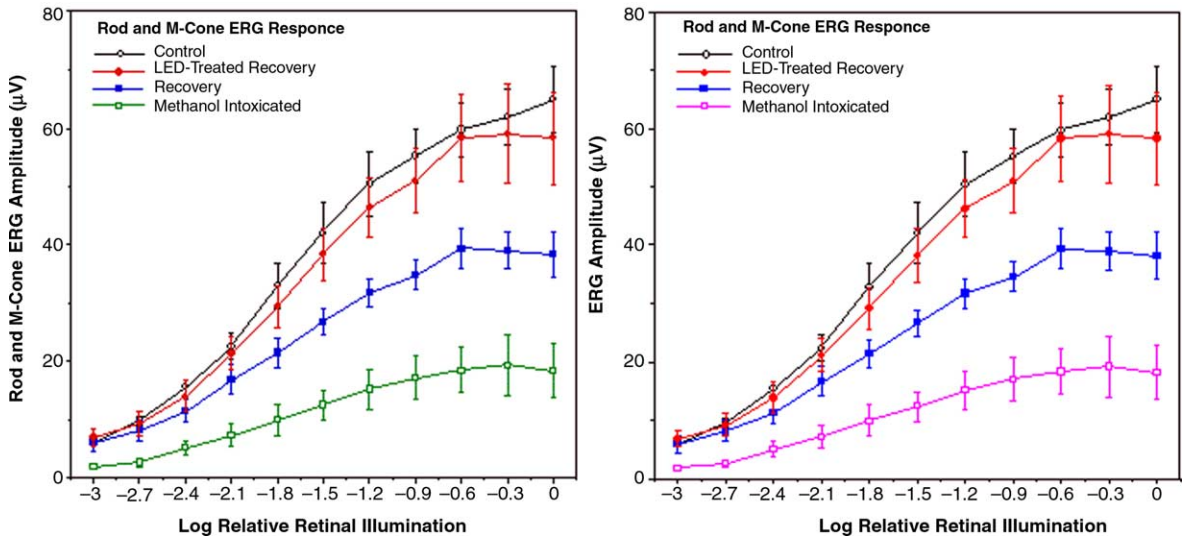

Fig. 5. 670 nm LED treatment significantly attenuated the retinotoxic effects of methanol-derived formate during intoxication (Left panel) and profoundly improved the recovery of retinal function following intoxication (Right panel).

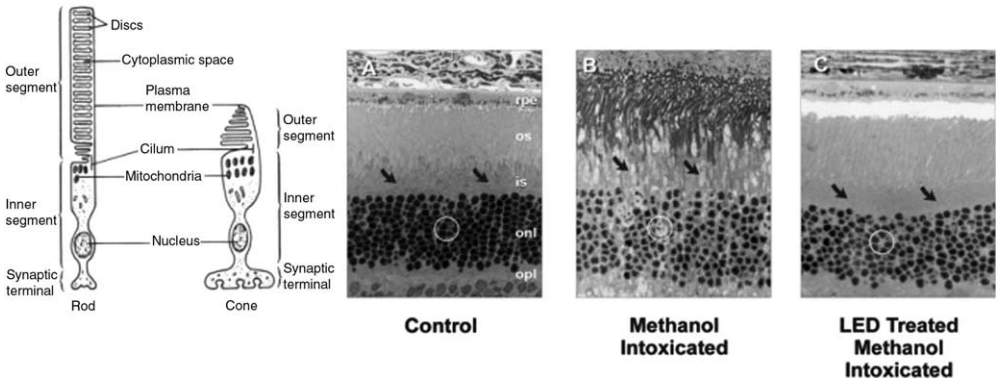

Fig. 6. 670 nm LED treatment protects against methanol-induced retinal damage.

mitochondrially generated reactive oxygen species may function as signaling molecules to provide communication between mitochondria and the cytosol and nucleus and thus play an important signaling role in the activation of retinoprotective processes following LED treatment.

We have compared gene expression profiles in the neural retina of untreated rats with those from the neural retina of methanol-intoxicated rats and LED-treated methanol-intoxicated rats. Results from these studies indicate that methanol intoxication and LED treatment altered the retinal expression of nearly 80 genes. At least 26 of these genes that were up-regulated in the retinas of methanol intoxicated rats were correspondingly down-regulated by in the retinas of LED treated methanol intoxicated rats and vise-versa**.** Several functional subcategories of genes regulated by NIR-LED were identified in retinal samples, including those encoding DNA repair proteins, antioxidant defense enzymes, molecular chaperones, protein biosynthesis enzymes, and trafficking and degradation proteins. Striking differences were observed in genes from cytochrome oxidase family, peroxiredoxin family and genes involved in cell growth and maintenance (Fig. 7). Differential expression of selected genes was confirmed at

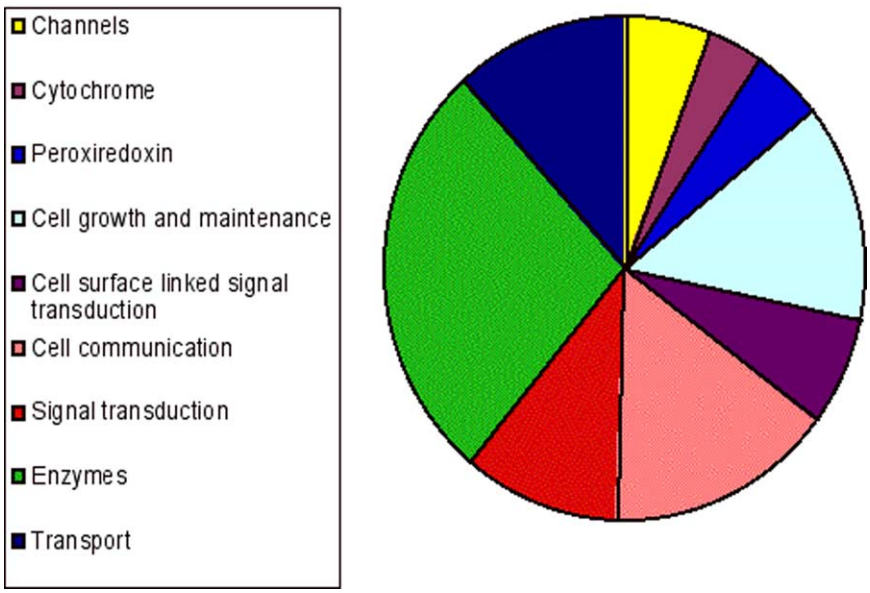

Fig. 7. Gene families regulated by 670 nm LED treatment in the retinas of in methanol intoxicated rats.

the level of RNA. We intend to further substantiate these findings using real time PCR, Northern and Western analysis and to investigate the roles of several of these genes in cellular energy production and cellular survival.

In summary, we have reviewed the evidence that photobiomodulation with red to near infrared light augments recovery pathways promoting cellular viability and restoring cellular function following injury. These findings provide a link between the actions of red to near infrared light on mitochondrial oxidative metabolism in vitro and cell injury in vivo. Based on mounting evidence that mitochondrial dysfunction is involved in the pathogenesis of numerous diseases processes, we propose that NIR-LED photobiomodulation represents an innovative and non-invasive therapeutic approach for the treatment of tissue injury and disease processes in which mitochondrial dysfunction is postulated to play a role including diabetic retinopathy, age-related macular degeneration, Leber's hereditary optic neuropathy and Parkinson's disease.

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