Clinical results from low-level laser therapy in patients with autosomal dominant cone-rod dystrophy

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Abstract. The objective of this study is to examine long-term effects of low-level laser therapy (LLLT) in patients with autosomal dominant cone-rod dystrophy (CRDs). A He-Ne Laser with continuous emission at 633 nm (01 mW/cm²) was used on five patients with autosomal dominant pedigree of Romani origin with non-syndromic CRDs. The laser radiation was applied transpupillarily to the macula six times for three minutes every other day. The experiment was conducted for a period of three years. The clinical evaluation included best corrected visual acuity determination, funduscopy, Humphrey perimetry, Farnsworth Hue-28 color testing, fluorescein angiography, and full-field electroretinogram (ERG).

All affected individuals presented reduced visual acuity (0.01 – 0.4) and photophobia. The funduscopic examination and fluorescein angiography revealed advanced changes including bone spicule-like pigment deposits in the midperiphery and the macular area, along with retinal atrophy, narrowing of the vessels, and waxy optic discs. The visual fields demonstrated central scotoma. The electrophysiologic examination of the patients detected an abnormal cone-rod ERG (20 – 30 μV) with photopic amplitudes more markedly reduced than the scotopic. Flicker responses were missing and Farnsworth Hue-28 test found protanopia. There was a statistically significant increase in the visual acuity (p<0.001, end of study versus baseline) for CRDs patients for the period of three years after the treatment with LLLT. Following the LLLT, the central absolute scotoma in CRDs was reduced, as was the prevalence of metamorphopsia in CRDs.

This study shows that LLLT may prove to be a novel long-lasting therapeutic option for both forms of CRDs. It is a highly effective treatment resulting in a long-term improvement of the visual acuity.

1. Introduction
Cone rod dystrophies (CRDs) is characterized by a loss of cone cells, the photoreceptors responsible for both central and color vision [1]. The cones and rods transform light into electric nerve messages that are transferred to our brain via our optic nerve [2]. CRDs (prevalence 1/40,000) are inherited retinal dystrophies that belong to the group of pigmentary retinopathies [3]. There are more than 30 types of cone-rod dystrophy, which are distinguished by their genetic cause and their pattern of

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inheritance: autosomal recessive, autosomal dominant, and X-linked. CRDs are characterized by retinal pigment deposits, predominantly localized in the macular region [4, 5]. There is no one effective treatment for the different types of CRD. However, there may be treatment options that may help slow down the degenerative process, such as light avoidance and the use of low-vision aids. Dry AMD is characterized by drusen, retinal pigment epithelial (RPE) cell atrophy and subjacent photoreceptor degeneration [6]. Factors involved in causing RPE cell injury and dysfunction have been shown to include mitochondrial dysfunction, oxidative stress, inflammation and genetic disposition [7].

Photobiomodulation (PBM) was discovered almost 50 years ago by Endre Mester in Hungary [8]. For most of this time PBM was known as “low-level laser therapy” as ruby laser (694nm) and HeNe lasers (633nm) were the first devices used. Low-level laser therapy (LLLT) produces significant bioeffects [9-12] These effects are manifested in biochemical, physiological and proliferative phenomena in various enzymes, cells, tissues, organs and organisms [13, 14]. Published studies demonstrate that mitochondrial cytochrome C oxidase (CCO) is a key photoacceptor of light at these wavelengths and improves blood flow and ATP formation, enhances O2 binding and reduces oxidative stress and inflammation [15, 16].

Although early studies identified mitochondrial CCO as an endogenous photoacceptor for PBM, the cellular and molecular mechanisms underlying PBM are better understood [17]. Recent findings provide important new insight. First, nitric oxide has been implicated. Second, CCO, an enzyme known to reduce oxygen to water at the end of the mitochondrial respiratory chain, has been shown to have a new enzymatic activity – the reduction of nitrite to nitric oxide [18]. This nitrite reductase activity is elevated under hypoxic conditions but also occurs under normoxia. And third, low-intensity light regulates nitric oxide synthesis by CCO without altering its ability to reduce oxygen.

The first signs and symptoms of cone-rod dystrophy are usually decreased sharpness of visual acuity and photophobia [2]. These features are typically followed by impaired color vision (dyschromatopsia), scotomas in the center of the visual field, and peripheral vision loss [3]. Photobiomodulation (PBM) utilizes very low energy levels causing no tissue damage. Infrared (NIR, 770–1200 nm) where rhodopsin absorbs, has shown positive results [9, 12].

PBM, also known as low-level laser therapy, uses red and near-infrared light to stimulate healing, relieve pain, and reduce inflammation [14, 22, 23]. The primary chromophores identified were cytochrome c oxidase in mitochondria, and calcium ion channels (possibly mediated by light absorption by opsins). Secondary effects of photon absorption include increases in ATP, a brief burst of reactive oxygen species, an increase in nitric oxide, and modulation of calcium levels. Tertiary effects include activation of a wide range of transcription factors leading to improved cell survival, increased proliferation and migration, and new protein synthesis. Many wavelengths in the red (600–700 nm) and near-infrared (NIR, 770–1200 nm) spectral regions have shown positive results; however there is a region in between (700–770 nm) where, broadly speaking, the results are likely to be disappointing [24]. Some studies have looked at animal models of neuropathic pain such as the “spared nerve injury” [25]. This involves ligating two out of three branches of the sciatic nerve in rats and causes long lasting (>6 months) mechanical allodynia [27].

Some researchers [26] have used a transgenic mouse strain (FVB/N-Tg(iNOS-luc)) that had been engineered to express luciferase under control of the inducible nitric oxide synthase promoter, to allow bioluminescence imaging of PBM of the zymosal-induced arthritis model in mice knees [26]. They
compared the same fluence at 635 nm, 660 nm, 690 nm, and 905 nm (in CW mode), and 905 nm (short pulse). Animals younger than 15 weeks showed mostly reduction of iNOS expression, while older animals showed increased iNOS expression. Pulsed 905 nm also increased iNOS expression. In recent years, the use of PBM as a treatment for traumatic brain injury, and other brain disorders including stroke, neurodegenerative diseases and even psychiatric disorders, has increased remarkably [25, 27-29].

Photoneuromodulation of cytochrome oxidase activity is the most important primary mechanism of action of LLLT [12]. Cytochrome oxidase is the primary photoacceptor of light in the red to near-infrared region of the electromagnetic spectrum. It is also a key mitochondrial enzyme for cellular bioenergetics, especially for nerve cells in the retina and the brain. Evidence shows that LLLT can secondarily enhance neural metabolism by regulating mitochondrial function, intraneuronal signaling systems, and redox states.

The aim of this study was to examine long-term effects of low-level laser therapy (LLLT) in patients with cone-rod dystrophy.

2. Methods
For LLLT, a He-Ne Laser with continuous emission at 633 nm (0.1 mW/cm2) was used in five patients with autosomal dominant pedigree of Romani origin with non-syndromic CRDs. A new ophthalmologic system for bio-stimulation and LLLT of eye diseases based on a He-Ne laser (Mediray 04, Optella Ltd., Sofia, Bulgaria, developed by K. Koev, V. Tanev, L. Avramov) with emission wavelength of 632 nm was used for the investigation. The system has the ability to regulate the size of the laser spot and the laser power density from 0.05 mW/cm2 to 0.4 mW/cm2. The system is compact, portable and with minimal optical losses and high reliability. The device is convenient for exploitation, both for the patients and the treating personnel.

Laser radiation was applied transpupillary to the macula for six times for three minutes every other day. The experiment lasted for a period of three years. The clinical evaluation included best corrected visual acuity determination, funduscopy, Humphrey perimetry, Farnsworth Hue-28 color testing, fluorescein angiography, and full-field electroretinogram (ERG).

3. Results
All individuals affected with CRDs presented reduced visual acuity (0.01-0.4) and slightly variable photophobia. Funduscopy examination and fluorescein angiography revealed advanced changes, including bone spicule-like pigment deposits in the midperiphery and macular area along with retinal atrophy, narrowing of the vessels, and waxy optic discs. Using Humphrey perimetry, central scotoma of the visual fields was established. The electrophysiologic examination (ERG) of the patients detected an abnormal cone-rod ERG (20-30 μV) with photopic amplitudes more markedly reduced than the scotopic. Flicker responses were missing, and the Farnsworth Hue-28 test found protanopia. After the LLLT treatment, we found a statistically significant increase in the visual acuity (p<0.001, end of study versus baseline) for CRDs patients for the period of three years. Following the LLLT, the visual acuity in a larger proportion of patients improved.

Visual acuity improved optotypes in 9/10 eyes (90%; p<0.001)
Eyes: by one row of optotypes in 3/10 (30%),
by two rows in 4/10 (40%),
by three rows in 2/10 (20%),
Visual acuity remained unchanged in 1/10 eyes (1.0%).

The central absolute scotoma in CRDs was reduced after LLLT, as established with Humphrey perimetry and fluorescein angiography. The prevalence of metamorphopsia in CRDs was also reduced.

4. Discussion
We report studies on applying LLLT to members of a family of Gypsy origin affected by autosomal
dominant cone-rod dystrophy. To the best of our knowledge, this is the first publication of clinical results from the use of LLLT in patients with autosomal dominant CRDs.

The LLLT resulted in an improvement in the visual acuity in most patients (90%) with CRDs. An increase of two to three rows of optotypes was observed in 6/10 (60%) eyes with CRDs.

We also found a reduction in the central absolute scotoma and metamorphopsia in CRDs.

In contrast to laser treatment, PBM utilizes very low energy levels causing no tissue damage [14]. Photobiomodulation stimulates cellular processes, thus providing an approach to targeting the underlying degenerative pathology with disease-modifying potential. A recent review paper of PBM in retinal diseases has reported that the low-cost and non-invasive nature of PBM coupled with the first promising clinical reports and the numerous preclinical studies in animal models make PBM well poised to become an important player in the treatment of retinal disorders [28]. The good clinical results that we have found prove that LLLT can be successfully used in CRDs.

5. Conclusions
This study shows that LLLT may become a novel long-term therapeutic option for both forms of CRDs, as we demonstrated its effectiveness in improving the patients’ visual acuity.

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