Neuroscience & Medicine, 2015, 6, 35-41
Published Online March 2015 in SciRes. http://www.scirp.org/journal/nm
http://dx.doi.org/10.4236/nm.2015.61006

Pulsed Led's Light at 650 nm Promote and at 470 nm Suppress Melatonin's Secretion

F. Ferro Milone[1*], A. Bolner[2], G. P. Nordera[2], S. Z. Scalinci[3]

[1]L. D. Università, Verona & Prosenectute, Vicenza, Italy
[2]Casa di Cura Villa Margherita, Arcugnano, Vicenza, Italy
[3]Centro Studio e Ricerca Ipovisione e Glaucoma, Università, Bologna, Italy
Email: *fferromilone@gmail.com

Received 28 February 2015; accepted 16 March 2015; published 20 March 2015

Abstract

In a previous research we have studied the effect of the stimulation of the retina, by pulsed LED's light of different wavelength, on the spectral density of the alpha rhythms of the electroencephalogram [1] [2]. In conformity to our results and the recent discovery of a nonvisual pathway of light from the retina to the brain, we are induced to search for the effect of stimulation of the retina, with different wavelength, on the melatonin's secretion. We have, therefore, stimulated the retina with blue LED's light 470 nm and red LED's light 650 nm, and measured the melatonin's secretion in saliva by means of High Pressure Liquid Chromatography (HPLC). The results show that melatonin values are higher with long wavelength stimulation (red, 650 nm) to be confronted with short wavelength stimulation where the values are lower (blue, 470 nm), the difference being significant (***). Action spectrum of short wavelength, producing melatonin suppression, was already evidenced *in vivo*; it was also demonstrated that blue LED's light differentially modulated cell's survival and growth, inducing mitochondrial suppression *in vitro*. We speculate, therefore, that long wavelength light (red) produces photobiomodulation effect at the level of the retina and that this effect is the opposite of the effect produced by the short wavelength (blue). The molecular mechanism underlying both effects may be, we suppose, the activation (red) or depression (blue) of the mitochondrial cytochrome c oxidase activity at the level of the pool of the retina's ganglion cells.

Keywords

Wavelength, Nonvisual Pathway, Cytochrome C Oxidase, Phtobiomodulation

1. Introduction

Since 1998, the discovery of a new photopigment, melanopsin, in a small fraction of retinal ganglion cells in-

*Corresponding author.

How to cite this paper: Ferro Milone, F., Bolner, A., Nordera, G.P. and Scalinci, S.Z. (2015) Pulsed Led's Light at 650 nm Promote and at 470 nm Suppress Melatonin's Secretion. *Neuroscience & Medicine*, **6**, 35-41.
http://dx.doi.org/10.4236/nm.2015.61006

trinsecally photosensitive (ipRGCs), has induced to the discovery of a nonvisual pathway of light, which sets the pupillary reflex and synchronizes the circadian rhythms, with the solar light. Steps of this pathway are the suprachiasmatic nucleus (SCN) and the pineal gland (PG). Collectively these structures, with the paraventricular nucleus (PVN), the intermedio-lateral column of the spinal cord (IMC), and the superior cervical ganglion (SCG), set the photoneuroendocrine system, which provides coordination of circadian timing in mammals [3]-[5].

This highly specific and sophisticated regulatory system is governed by the activation of the photopigment melanopsin, exclusively present in pituitary adenilate cyclase-activating polypeptide-containing retinal ganglion cells of the retinohypothalmic tract [6].

Melatonin, a key hormone in the photoneuroendocrine system, is a highly conserved molecule that exists in animals as well as in bacteria, unicellular organism and in plants. Since melatonin is an antioxidant, it is speculated to be present in plants and animals to protect from environmental oxidative stress [7].

In previous studies we have shown that pulsed LED's light 650 nm provokes increase of the spectral density of the alpha rhythms in the electroencephalogram, which is more evident than blue LED's light 470 nm, the difference between the spectral density of photostimulated and non photostimulated records, being significant (***). The difference was postulated to be due to photobiomodulation effect of the red light on the retinal tissue [2].

Recently red and near infrared light (red-NIR light 630 to 800 and over nm) produced by Light Emitting Diodes (LED) is widely used in human and veterinarian medicine, although the molecular mechanisms involved are not clearly understood [8] [9]. Red-NIR light seems to have better penetrative capacity in tissue without thermal increase and optimal response in activating the chromophore's cytochrome c oxidase activity at the level of the mitochondrial electron transport chain, which produces ATP [10]-[12]. Moreover, recent findings provide importantly new insight in photobiomodulation, in which nitric oxide (NO) synthesized also by cytochrome c oxidase has been implicated [13].

Collectively those researches show that different wavelengths may make their effects through both visual and nonvisual pathways. In this last case, the photoneuroendocrine system activation is at the core of the timekeeper mechanism in many different tissues throughout the body. With the aim to clarify the response of the photoneuroendocrine system to different wavelength, we carried out a research on the melatonin's secretion, evaluated through dosage of melatonin in the saliva.

2. Method

Informed consent was subscribed by 20 voluntary subjects male (10) and female (10) aged 30 - 60 years + 1 subject (Case 21, 55 y), sufferers of insomnia and cured with melatonin caps 5 mg.

Photostimulation was performed 30 - 40 minutes before the first collection of saliva (hs 08-20), duration 10 - 20 minutes, by means of Kingbright's Ltd LED's lamp: HYPER RED (InGaAIP), peak wavelength 650 nm, intensity 11 μW/cm², and BLUE (InGaN), peak wavelength 470 nm, intensity 29 μW/cm², assembled in a device and pulsed at 10 Hz, duty cycle 50%.

Saliva (5 - 10 ml) was collected during the day at hours 08-12-16 and during the night at hours 20-24-04, and stocked at −20˚C. Melatonin's level in saliva was calculated as the mean of the value during the day (08-12-16) and during the night (20-24-04). The mean of three values during day and night was considered of interest, due to the variability of melatonin's level (activity day living, food intake, wine, stress and others).

Melatonin evaluation was performed by means of High Pressure Liquid Chromatography (HPLC) with electrochemical and fluorescence detection. This method was preferred due to its high degree of specificity and sensitivity [14].

The saliva samples were purified by a solid-liquid extraction procedure; 100 uL of the extracts were injected into the HPLC system. The mobile phase consisted of a solution of formic acid 0.1 mol /L and acetonitrile at concentration increasing from 23% to 70% with flow rate 2 mL/min. The stationary phase was a reversed-phase C18 column, 5 mm, X-BRIDGE 250 × 4.6 mm. Detection was performed by a FP920 fluorometer (Jasco) with a pair of wavelengths 286/352 nm. The chromatography was completed in 15 minutes with a retention time for melatonin of 5.5 minutes (modified from [15]).

2.1. Chemicals and Solution Preparation

All the reagents were of analytical and HPLC grade and purchased from Farmitalia (Milan, Italy); melatonin powder was purchased from Sigma-Aldrich (Milan, Italy), and cartridges for solid phase extraction (MF C18 Isolute 50 mg/1mL, cod. 240-0005-A) from StepBio (Bologna, Italy). Stock solution (2 mg/mL) of melatonin in

water was prepared from powder and kept at −80˚C. At the time of the analysis, one aliquot of stock solutions was adequately diluted in a saliva matrix (pooled from donor samples) in order to obtain working standard solutions at concentrations of 0, 50 and 100 pg/mL.

2.2. Collection and Samples Preparation

Saliva samples were collected in 10 mL empty tubes within 30 minutes, immediately centrifuged at 3500 rpm, for 10 min, at +4˚C and subsequently frozen at −80˚C until analysis. Both the standards and the unknown samples were processed according to the extractive procedure summarised below. The saliva samples were purified by a solid-liquid extraction procedure. The extraction column (SPE C18 MF 50 mg/1mL-ISOLUTE) was conditioned using 1 mL of ethanol and 1 mL of water: 5 mL of saliva were passed slowly (by gravity) on the SPE column. After a first washing step with 1 mL of water and a further 1 mL of water: ethanol 90:10 (v/v), the elution was performed with 0.5 mL of a solution of water: ethanol 50:50 (v/v). 100 uL of the extract were injected into the HPLC system.

2.3. Apparatus and Chromatographic Conditions

The HPLC system consisted of two model 307 pumps (A and B) and a model 234 autosampler with a 100 mL loop, all from Gilson, Villiers-le-Bel, France. The separation was performed on a reversed phase C18 stainless-steel column (XBridge Shield 250 mm × 4.6 mm i.d.) packed with 5 ml particles (Waters, Duren, Germany), with a 10 mm × 4.6 mm i.d. precolumn, packed with the same material. The mobile phase flushed by pump A was an acqueous mixture of formic acid 0.1 mol/L, citric acid 1.0 mmol/L, EDTA 0.5 mmol/L, sodium azide 500 mg/L, acetonitrile 23% (v/v) and diethylamine 2.5 mL/L, pH 3 (not modified); the mobile phase flushed by pump B was an almost identical solution containing 70% acetonitrile (v/v). Both mobile phases were flushed at 2 ml/min according to the following gradient: from 0 to 3 minutes pump B 0%, from 3 to 15 minutes pump B from 0 to 10%. At room temperature, the pressures were between 200 and 250 kg/cm^2 and melatonin capacity factors (k') was of 4.5. The analytical detection was performed by a fluorometer Jasco FP920 with excitation and emission lambda fixed at 286 and 352 nm respectively (other settings: normal mode, standard response, gain 1000 and attenuation 1).

(Details of the laboratory procedure may be obtained from bolner.andrea@gmail.com).

3. Results

Some case report may clarify the method for calculation of melatonin's baseline values and the effect of red-blue LED's light photostimulation (30 - 40 minutes before the saliva's collection at h 08 and 20). Note: day light means "natural" day light; night light means that "low artificial" ambient light was present.

Case 1 (31 y) basal evaluation during day light (h. 8-12-16) mean = 0.4 pg/ml; night light (h. 20-24-04) mean = 9.1 pg/ml; stimulated red LED 30 - 40 minutes before h. 08 and h. 20 saliva collection, day light (08-12-16) mean = 39.7 pg/ml; night light (h. 20-24-04) mean = 16.8 pg/ml; stimulated blue LED day light (h. 8-12-16) mean = 2.2 pg /ml; night light (h. 20-24-04) mean = 1.9 pg/ml.

Case 2 (65 y): basal day light (h. 08-12-16) mean = 5.3 pg/ml ; night light (h. 20-24-04) mean = 11.7 pg/ml; double stimulation red LED 30 - 40 minutes before hs 08 and 20, day light (h. 8-12-16) mean = 15.5 pg/ml; night light (h. 20-24-04) mean 161.4 pg/ml; with a peak at midnight (h. 24, 364.9 pg/ml); stimulated blue LED day light (h. 8-12-16) mean = 6.5 pg/ml; night light (h. 20-24-04) mean = 1.2 pg/ml.

Case 21 (55y): suffers chronic insomnia, assumes melatonin's caps 5 mg, regularly in the last 10 years: Melatonin's values, basal day light (h. 08-12-16) mean = 18.3 pg/ml; night light (h. 20-24-04) mean = 14.7 pg/ml; stimulation red LED day light (h. 8-12-16) mean 9.8 pg/ml ; night light (h. 29-24-04) mean 19.3 pg/ml; 5 mg melatonin's caps h. 20 provokes, within 1 h. the appearance of 379.7 pg/ml melatonin in the saliva ; 2 hours later was 14.7 pg/ml; stimulation blue LED day light (h. 08-12-16) mean = 3.8 pg/ml; night light (h. 20-24-04) means = 6.9 pg/ml.

Statistics

Mean and standard deviation of the values (20 subjects, values of melatonin are in pg/ml) are summarized in the tables: **Figure 1** shows melatonin's level in natural condition; **Figure 2** shows melatonin's level under stimulation with red and blue LIED's light.

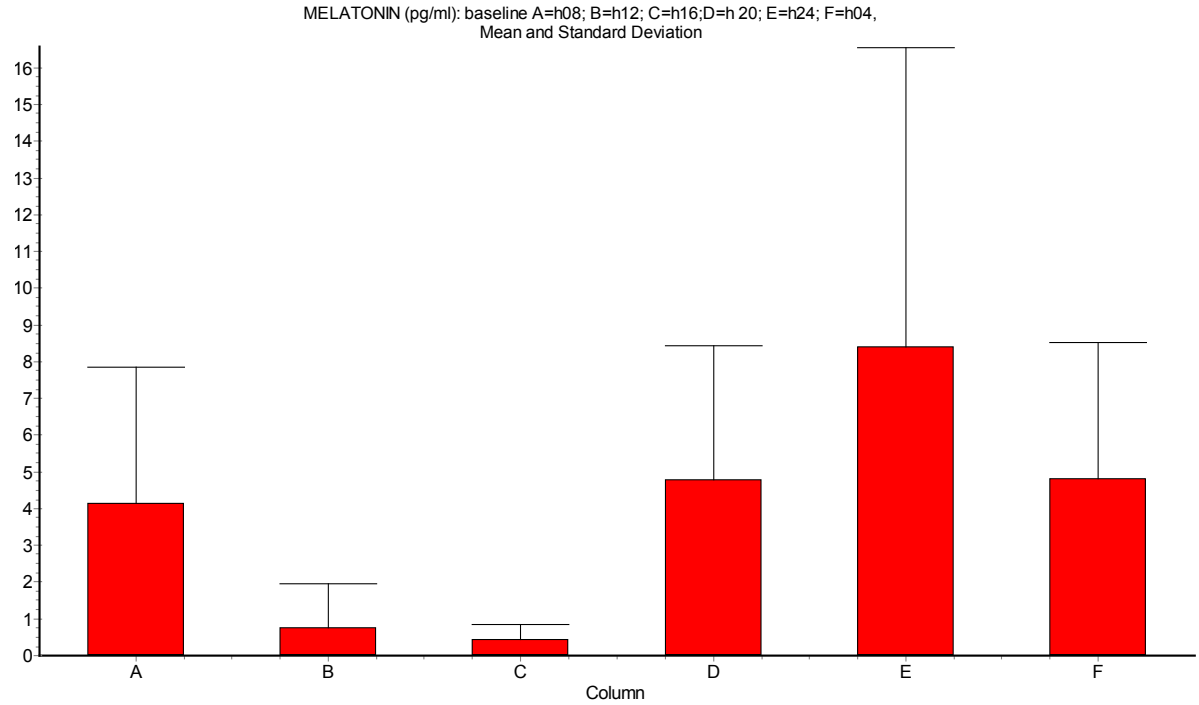

Figure 1. MELATONIN baseline values (pg/ml): A = h08; B = h12; C = h16; D = h20; E = h24; F = h04, Kruskal-Wallis Statistic (Nonparametric ANOVA); Dunn's Multiple Comparisons Test: Summary: A-C, B-D, B-F = $^{*}(p < 0.05)$; B-E, C-D, C-E, C-F = $^{**}(p < 0.01)$.

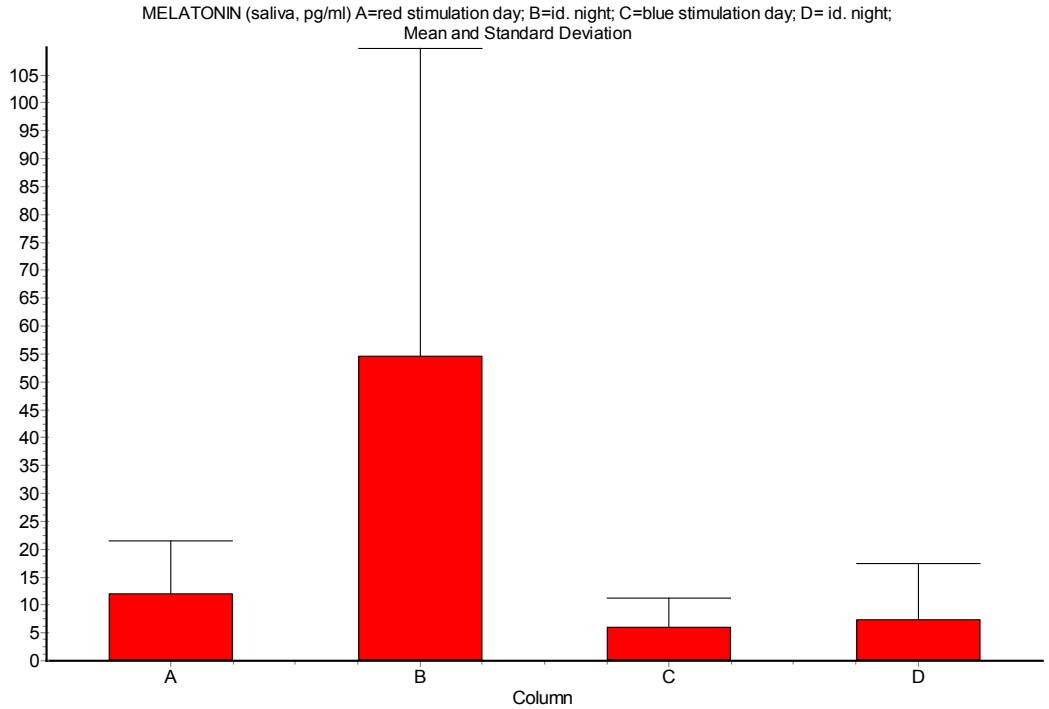

Figure 2. MELATONIN in saliva (pg/ml), A-B = stimulated red (650 nm); C-D = stimulated blue (470 nm); A (mean of hs 08-12-16) = stimulation red LED's light (h 07,30), intensity 11 µW/cm^2, pulsed 10 Hz; B mean (hs 20-24-04) = stimulation (hs 19,30) as in A; C mean (hs 08-12-16) = stimulation blue LED's light (h 07,30), intensity 29 µW/cm², pulsed 10 Hz; D mean (hs 20-24-04) = stimulation (h 19,30) as in C; Statistics: One-way Analysis of Variance (ANOVA); Tukey-Kramer Multiple Comparisons Test; Summary: A-B, B-C, B-D = $^{***}(p < 0.001)$.

Collectively these results show that in basal condition, diurnal melatonin has a mean value of 1.5 pg/ml (SD 2.6) being highest value at h 08 a.m. = 11.1 pg/mL, and lowest at 04 p.m. = 0.3 pg/mL; during the night the mean values are 5.0 pg/mL (SD 5.4) with the highest value at midnight = 19.5 pg/mL.

Photostimulation shows: red LED 650 nm, provokes low increase of melatonin during the day (mean 12.4 SD 9.4), and marked increase during the night (mean 54.5; SD 55.6), the difference being significant (***). Blue LED's light 470 nm stimulation, on the contrary, shows that the difference between basal level of melatonin and stimulated with blue, is not significant. This relative suppression is not comparable to the suppression found in the literature [16] [17]. The difference may be due to differences in the tissue examined (plasma, urine, saliva), the target (melatonin, or its metabolite 6-sulphatoxymelatonin) the method of melatonin assay (radioimmunoassay, HPLC), intensity of stimulation (dose-response effect).

Case 21 (out of the statistic) shows that the response to red stimulation in this subject, cured with daily assumption of melatonin 5 mg, is suppressed also at night due to probable blocking of secretion at the level of the pineal gland. The value of melatonin in saliva 1 h after the assumption of 5 mg melatonin caps is similar to the value of melatonin after the double stimulation with red LED in Case 2.

4. Discussion

In a previous paper, we have shown that photostimulation with pulsed red LED's light is more efficacious than blue light in provoking the increase of the spectral density in the alpha rhythms of the electroencephalogram. This results, we hypothesized, may be due to photobiomodulation effect at the level of the cone and rod cells (visual pathway) that will be summed to the effect of the light on the ganglion cells (nonvisual pathway) at the level of the inner retinal tissue. Therefore the signal in the final common pathway, the optic nerve, results enhanced [2].

The possible selective activation of the nonvisual pathway (originated in the ganglion cell intrinsecally photosensitive), at present, is deduced from the literature about short wavelength light sensitivity of circadian pupillary and visual awareness in human lacking the outer retina [18]. Moreover, melatonin suppression was also demonstrated with increasing irradiance, in non-rod, non-cone, photoacceptor system [19].

Recently red and near-infrared light (red-NIR light 650 - 1000 nm), produced by light emitting diodes (LED), named photobiomodulation, is widely used in human and veterinary medicine, although the molecular mechanisms involved are not clearly understood. But widespread practice and numerous experimental and certified data show the dominant role of the activation of mitochondrial cytochrome c oxidase activity [9] [16].

The red LED's light 650 nm seems have the better penetrative capacity in tissue without thermal increase and optimal response in activating chromophore's cytochrome c oxidase in the mitochondrial electron transport chain that produce adenosintriphosphate (ATP) [11] [12].

The mitochondrial decay, on the other side, due to oxidant by products, is the principal underlying contributor to aging and neurodegenerative diseases. The energy of the basic metabolism's processes comes from mitochondria, and their decay impairs cellular metabolism and leads to cellular decline. The progress in delaying the mitochondrial decline may be considered a basic procedure to preventing the disease bound to aging and neurodegeneration [17] [20].

The photobiological action mechanism via activation of the respiratory chain is a universal mechanism. Primary photoacceptors are terminal oxidases (cytochrome c oxidase) in eukariotic cells. All biological effects induced by light depend on the parameters of irradiation: wavelenght, dose, intensity, irradiation time, and pulsed mode [21] [22].

Our experimental data show that red LED's light stimulate the secretion of melatonin whereas blue LED's light reduce or suppress it; this results may support the hypothesis that both effects belong to a common molecular mechanism, enclosed in the mitochondrial cytochrome c oxidase activity and its derivative the adenosintriphosphate (ATP).

Previous studies have reported that blue light (400 - 500 nm) inhibits mitochondrial activity in vivo as well as in cultured cells [23] [24]. Red LED's light on the contrary promotes the activity of the mitochondrial cytochromo c oxidase [12]. Mitochondrial respiratory chain activity may be considered, therefore, the common target of the action of different wavelength light, but in opposite direction: red light increases activity of mitochondrial cytochrome c oxidase instead of blue light depress or suppress it.

5. Conclusions

In the field of the photobiology, the irradiance of the retina's tissue with red LED's light has distinctive properties that induce both local effects, *i.e*. activation of the chromohore's cytochrome c oxidase (photobiomodulation) at the level of photosensitive ganglion cells, and generalized effects at the level of the living organism, through activation of the photoneuroendocrine system, which upregulates melatonin. Blue LED's light, at appropriate parameters of irradiation, makes opposite effects locally at the level of retina, consistent in mitochondrial suppression (blue light hazard), and at the level of the living organism, downregulation of melatonin's secrtion.

It is postulated that the local (retinal) photobiomodulation by red light may be relevant for a non invasive, therapeutic intervention for treatment of some eyes diseases linked to mitochondrial disfunction and/or neurotoxin's damage, including age-related macular degeneration and optic neuropathy [8] [9] [25]. Whereas the effects on the photoneuro-endocrine system, due to the increase of melatonin's pool, may promote beneficial effects in some general disfunction of the living organism, due to circadian disrhythmicity, like insomnia and jet lag, but also in some diseases related to age and neurodegeneration [26]-[28].

References

[1] Ferro Milone, F., Minelli, T., Porro, A. and Binda, F. (2009) Memoria comportamentale (test di Rivermead): studio di una possibile interdipendenza in soggetti normali e con deficit cognitivo lieve. *Psicogeriatria*, **2**, 59-65.

[2] Ferro Milone, F., Minelli, A.T. and Cian, R. (2013) Alpha Rhythms Response to 10 Hz Flicker Is Wavelength Dependent. *Neuroscience & Medicine*, **4**, 4-10.

[3] Berson, D.M. (2003) Strange Vision: Ganglion Cells as Circadian Photoreceptors. *Trends in Neurosciences*, **25**, 314-320. http://dx.doi.org/10.1016/S0166-2236(03)00130-9

[4] Korf, W., Schomerus, C. and Stehele, J.H. (1998) The Pineal Organ, Its Hormone Melatonin, and the Photoneuro-Endocrine System (Advances in Anatomy, Embriology and Cell Biology). Vol. 146, Springer Verlag, Berlin, 1-8.

[5] Reppert, S. and Weaver, D.R. (2002) Coordination of Circadian Timing in Mammals. *Nature*, **418**, 935-841.

[6] Hannibal, J., Hindersson, P., Knudesen, S.M., Georg, B. and Fahrenkrug, J. (2002) The Photopigment Melanoopsin Is Exclusively Present in Pituitary Adenilate Cyclase-Activating Polypeptide-Containing Retinal Ganglion Cells of the Retinohypothalamic Tract. *The Journal of Neuroscience*, **22**, 1-7.

[7] Chen, G., Huo, Y., Tan, D.X., Liang, Z., Zhang, W. and Zhang, Y. (2003) Melatonin in Chinese Medicinal Herbs. *Life Sciences*, **73**, 19-26. http://dx.doi.org/10.1016/S0024-3205(03)00252-2

[8] Karu, T. and Kolyakov, S.F. (2005) Exact Action Spectra for Cellular Responses Relevant to Phototherapy. *Photomedicine and Laser Surgery*, **23**, 355-361. http://dx.doi.org/10.1089/pho.2005.23.355

[9] Wong Riley, M.T., Liang, H.L., Eelles, J.T., Chance, B., Henry, M.M., Buchmann, E., Kane, M. and Whelan, H.T. (2005) Photobiomodulation Directly Benefits Primary Neuron Functionally Inactivated by Toxins: Role of Cytochrome c Oxidase. *Journal of Biological Chemistry*, **280**, 4761-4771.

[10] Karu, T. (2010) Mitochondrial Mechanisms of Photobiomodulation in Context of New Data about Multiple Roles of ATP. *Photomedicine and Laser Surgery*, **28**, 159-160. http://dx.doi.org/10.1089/pho.2010.2789

[11] Timon, C.L., Jan-Ling, J., Xiao-Yang, X., Xiao-Guang, L., Shu-Xun, D. and Song-Hao, L. (2005) Photobio-Modulation: Phenomenology and Its Mechanism. The Smithsonian/NASA Astrophysics Data System, Vol. 5630, 185-191.

[12] Amat, A., Rigau, J., Waymant, R.W., Ilev, I.K., Thomas, J. and Anders, J.J. (2005) Modification of Intrinsic Fluorescence and the Biochemical Behavior of ATP after Irradiation with Visible and near Infrared Laser Light. *Journal of Photochemistry and Photobiology B*: *Biology*, **81**, 26-32. http://dx.doi.org/10.1016/j.jphotobiol.2005.05.012

[13] Poyton, R.O. and Ball, K.A. (2011) Therapeutic Photobiomodulation: Nitric Oxide and a Novel Function of Mitochondrial Cytochrome c Oxidase. *Discovery Medicine*, **11**,154-159.

[14] Alvesde Almeida, E., Mascio, P., Harumi, T., Spence, W., Moscovitch, A., Hardeland, R., Cardinali, D.P., Brown, G.M. and Pandi-Perunal, S.R. (2011) Measurement of Melatonin in Body Fluids: Standards, Protocols and Procedures. *Child's Nervous System*, **27**, 879-891. http://dx.doi.org/10.1007/s00381-010-1278-8

[15] Lagana, A., Pardo-Martinez, B., Marino, A., Fago, G. and Bizzarri, M. (1995) Determination of Serum Total Lipid and Free Nacetylneuraminic Acid in Genitourinary Malignancies by Fluorimetric High Performance Liquid Chromatography. Relevance of Free N-Acetylneuraminic Acid as Tumour Marker. *Clinica Chimica Acta*, **243**, 165-179. http://dx.doi.org/10.1016/0009-8981(95)06165-7

[16] Desmet, K.D., David, A.P., Corry, J.J., Eelles, J.T., Wong-Riley, M., *et al*. (2006) Clinical and Experimental Application of NIR-LED Photobiomodulation. *Photomedicine and Laser Surgery*, **24**, 121-126.

http://dx.doi.org/10.1089/pho.2006.24.121

[17] Ames, B.N. (2004) Mitochondrial Decay, a Major Cause of Aging, Can Be Delayed. In Special Issue: Oxidative Stress in Aging and Neurodegenerative Diseases: From Biology to Therapy. *Journal of Alzheimer's Disease*, **6**, 117-121.

[18] Zaldi, F., Hull, J.T., Peirson, S., Wulff, K., Aeschbach, D., Gooley, J.J., Brainard, G., Gregory-Evans, K., Rizzo Ill, J.F., Czeisler, C.A., Foster, R.G., Moseley, M.J. and Lockley, S.W. (2007) Short-Wavelength Sensitivity of Circadian, Pupillary and Visual Awareness in Humans Lacking an Outer Retina. *Current Biology*, **17**, 2122-2128. http://dx.doi.org/10.1016/j.cub.2007.11.034

[19] Thapan, K., Arendt, J. and Skene, D.J. (2001) An Action Spectrum for Melatonin Suppression: Evidence for a Novel Non-Rod, Non-Cone Photoreceptor System in Human. *The Journal of Physiology*, **535**, 261-267. http://dx.doi.org/10.1111/j.1469-7793.2001.t01-1-00261.x

[20] Pizzo, P., Brini, M., Leo, S., Fottino, C., Pinton, P. and Rizzuto, R. (2009) Mitochondria, Calcium and Cell Death: A Deadly Triad in Neurodegeneration. *Biochimica et Biophysica Acta* (*BBA*)—*Bioenergetics*, **1787**, 335-344. http://dx.doi.org/10.1016/j.bbabio.2009.02.021

[21] Huang, Y.Y., Chen, A.C.H., Carroll, J.D. and Hamblin, M.R. (2009) Biphasic Dose Response in Low Level Laser Therapy. *Dose-Response*, **7**, 358-383.

[22] West, K.E., Jabonski, M., Warfield, B., Cecil, K.S., James, M., Ayers, M.A., Maida, J., Bowen, C., Sliney, D.H., Rollag, M.D., Hanifin, J.P. and Brainard, G.C. (2011) Blue Light from Light-Emitting Diodes Elicits a Dose-Dependent Suppression of Melatonin in Human. *Journal of Applied Physiology*, **110**, 619-626. http://dx.doi.org/10.1152/japplphysiol.01413.2009

[23] Rolenberg, S., Lewis, J.B., Lockwood, P.E., Tseng, W.Y., Messer, R.L., Hsu, S.D., Omata, Y. and Wataha, J.C. (2006) Extracellular Environment as One Mediator of Blue Light-Induced Mitochondrial Suppression. *Dental Materials*, **22**, 759-764. http://dx.doi.org/10.1016/j.dental.2005.11.003

[24] Wataha, J.C., Lewis, J.B., Lckwood, P.E., Hsu, S., Messer, B.L., Rueggeberg, F.A. and Bouillaget, S. (2004) Blue Light Differentially Modulates Cell Survival and Growth. *Journal of Dental Research*, **83**, 104-108.

[25] Rodriguez-Santanal, E., Reyes, H., Santana-Rodriguez, K.E. and Santana-Bank, L. (2008) Photo-Infrared Puled Biomodulation in Age-Related Macular Degeneration Associated to Neurologial Disease: One Interventional Case Report and Mini-Review. *Journal of Chinese Clinical Medicine*, **3**, 35-43.

[26] Mecocci, P. (2004) Oxidative Stress in Mild Cognitive Impairment and Alzheimer's Disease: A Continuum. Special Issue: Oxidative Stress in Aging and Neurodegenerative Diseases: From Biology to Therapy. *Journal of Alzheimer's Disease*, **6**, 69-74.

[27] Reiter, R.J., Tan, D.X., Mayo, J.C., Sainz, R.M., Leon, J. and Czarnocki, Z. (2003) Melatonin as an Antioxidant: Biochemical Mechanisms and Pathophysiological Implications in Humans. *Acta Biochimica Polonica*, **50**, 1129-1146.

[28] Reiter, R.J., Manchester, L.C. and Tan, D.X. (2010) Neurotoxins, Free Radical Mechanism, and Melatonin Protection. *Current Opinion in Pharmacology*, **8**, 194-210.