Chapter 10

COPY OF THE PAPER PUBLISHED/ACCEPTED
Protective effect of light emitting diode phototherapy on fluorescent light induced retinal damage in Wistar strain albino rats

A. Ahamed Basha a,+, D.C. Mathangi a, R. Shyamala a, K. Ramesh Rao b
a Department of Physiology, Chettinad Hospital and Research Institute, Rajiv Gandhi Salai, Kelambakkam, Chennai 603103, India
b Department of Pathology, Chettinad Hospital and Research Institute, Rajiv Gandhi Salai, Kelambakkam, Chennai 603103, India

ARTICLE INFO
Article history:
Received 14 February 2014
Received in revised form 15 April 2014
Accepted 16 April 2014
Available online xxx

Keywords:
Fluorescent light
LED phototherapy
Outer nuclear layer
Retina

ABSTRACT
Background: Artificial light at night alters retinal physiology. Several studies have shown that light emitting diode phototherapy protects the retina from the damaging effects of acute light exposure.
Objective: The aim of this study has been to elucidate the protective effects of 670 nm LED light on retinal damage induced by chronic fluorescent light in Wistar rats.
Methods: Male Wistar albino rats were divided into four groups: group 1 were control (CL), group 2, 3 and 4 were exposed to fluorescent light (FL), LED preexposure + fluorescent light exposure (LL) and only LED light exposure (OL) respectively. All animals were maintained in their specific exposure regime for 30 days. Fluorescent light of 1800 lx was exposed between 8 pm to 8 am. Rats were exposed to therapeutic LED light of 670 nm of 9 J/cm² at 25 mW/cm² for 6 min duration. Histopathological changes in the retina were studied.
Results: Animals of the FL group showed a significant reduction in the outer nuclear layer thickness and cell count in addition to the total thickness of the retina. LL group which were exposed to 670 nm LED prior to exposure to fluorescent light showed a significant decrease in the degree of damage.
Conclusions: 670 nm LED light preexposure is protective to retinal cells against fluorescent light-induced damage.

© 2014 Elsevier GmbH. All rights reserved.

1. Introduction

Circadian rhythms of hormonal secretion and the sleep wake cycle play a major role in maintaining homeostasis and well being. This is influenced by external cues called Zeitgebers, the most important of which is light. Among the artificial light sources, exposure to fluorescent light is the most common in office buildings, shopping markets and homes. Fluorescent light exposure leads to negative effects such as an increase in incidence of eye diseases (Walls et al., 2011), alterations in secretion of hormones like melatonin and cortisol (Miyachi et al., 1990; Rahman et al., 2008), disturbed circadian rhythms and sleep patterns (Anisimov et al., 2012). It also causes migraine (Noseda et al., 2010), fatigue and depression (Santhi et al., 2008). Exposure to fluorescent light at night causes a greater amount of damage when compared to day-time light exposure (Organisciak et al., 2000). Even a dim light of 5 lx at night decreases immune response (Bedrosian et al., 2011).

Night shift workers, especially health care professionals, software engineers, call center employees, telephone operators or street sweepers are continuously exposed to artificial light during their night shifts. Life threatening diseases such as cancer (Stevens et al., 2013), coronary artery disease (Brown et al., 2009) and diabetes (McMullan et al., 2013) have been linked to light exposure at night. Reports using animal models show that fluorescent light exposure affects circadian rhythms (Dauchy et al., 2010), retinal antioxidants and photoreceptors (Penn et al., 1987; Wiegand et al., 1983). Fluorescent light exposure also causes oxidative stress and spontaneous carcinogenesis (Anisimov et al., 2004). Studies show that the effects of light exposure at night in animal models mimic similar effects found in humans (Organisciak and Vaughan, 2010; Radetsky et al., 2013). Hence, this study was initiated in a rat model to determine the effects of exposure to fluorescent light at night.

Therapies which have been tested to overcome light induced damages includes, vitamins (Organisciak et al., 1985), melatonin supplements (Cajochen et al., 1998), tempro derivatives (Tanito et al., 2007) and non-erythropoietin derivatives (Colella et al., 2011). These therapies are expensive, invasive and must be consumed for a long period. Light emitting diode light (LED) is known to enhance the activity of antioxidant systems (Lim et al., 2009). It
not only hastens wound healing (Erdle et al., 2008) but also protects the retina (Qu et al., 2010), attenuating degeneration in the injured optic nerve (Eeils et al., 2010) and is used as a therapeutic option for non-center-involving diabetic macular edema in type 2 diabetic patients (Tang et al., 2014). Hence, the current study was initiated to assess protective effect of 670 nm LED photo therapy in Wistar rats exposed to fluorescent light at night.

2. Materials and methods

Albino rats have been identified as the most suitable model for study of retinal degeneration (Tanito et al., 2007) and several studies have been done using albino rats to study the effect of light on retinal function (Li et al., 2003; Penn et al., 1987; Specht et al., 1999; Wiegand et al., 1983), hence Wistar strain albino rats were chosen for the study. The study protocol was adherent to ARVO (Association for Research in Vision and Ophthalmology) guidelines for the use of animals in ophthalmic and vision research. The study was initiated after obtaining ethical clearance from the Institutional ethics committee.

2.1. Fluorescent light exposure

Light exposure was fixed at 1800 lx from fluorescent tubes (18 W, four tubes, 6500 K). 6500 K is a widely preferred color temperature for light source as it appears “most natural” to human eyes (David, 1955). The intensity of 1800 lx is the minimum light intensity required for regular tasks according to the Illuminating Engineering Society of North America (IES). The tube lights were kept at a height of 50 cm distance from the cage level (Dilly and Rajala, 2008). Fluorescent light exposure at 1800 lx was measured randomly at 10 different points from the cage level for uniformity of exposure to all the animals (Yamamoto et al., 1999). The animals were exposed to fluorescent light for 12 h between 8pm and 8am (Organisciak et al., 2000). The temperature of the cage was maintained at 24 ± 1 °C to avoid thermal effects.

2.2. LED light exposure

The LED lighting device (15 cm × 9 cm dimension) consisted of 32 LED bulbs arranged in 7 rows (with 5 bulb and 4 bulbs alternating in each row). This lighting devise was closely fit on to a plastic mesh box without gap to avoid dissipation of light. The plastic mesh box was further covered with white cardboard on all sides to facilitate reflection of LED light on to the animal. LED was placed approximately 2.5 cm from the rat to enable exposure to whole body (Qu et al., 2010). This light exposure set up was further placed in normal sized mice cage to avoid the dissipation of LED light to the external environment (Fig. 1(a and b)). Each animal was placed individually in this plastic mesh box and exposed to 670 nm LED light of 25 mW/cm² for a period of 6 min in order to obtain an exposure of 9J/cm². LED light of 9J/cm² was chosen because it is a known energy density to offer better protection in retina and liver during LED light exposure (Albarracin et al., 2011; Lim et al., 2009). LED light of 670 nm (red) was used because it is the optimal intensity for absorption of light by our body cells and offers maximum protective effect in mitochondrial energy metabolism (Lim et al., 2009). Animals in the LED exposed groups were exposed to LED prior to the exposure to fluorescent light every day.

2.3. Grouping

Inbred male Wistar rats weighing between 150 and 160 g were grouped based on their exposure to fluorescent light/LED light as follows with 6 animals in each group.

1 Group 1 – control animals (CL) maintained under standard animal care facility of the institution with ambient light (12L: 12D) and temperature.
2 Group 2 – fluorescent light exposure (FL).
3 Group 3 – LED preexposure + fluorescent light exposure (LL)
4 Group 4 – only LED light exposure (OL)

All of the animals were maintained in their specific exposure regime for a period of 30 days. After 30 days, animals were maintained for a period of 24 h in standard animal facility lighting conditions and euthanized the following day with sodium pentothal anesthesia. A jugular venous blood sample (1 ml) was collected using a heparinized syringe. Plasma was separated by cooling centrifugation and used for the estimation of corticosterone. Plasma corticosterone was measured by a quantitative and sensitive enzyme-labeled immunosorbent assay (ELISA) kit purchased from IBL International, Hamburg, Germany. Manufacturer instructions were adhered to for the assay protocol.
2.4. Morphologic evaluation of outer nuclear layer thickness and cell count by quantitative histology

Eyes were enucleated, 5 mm calotte was taken along the horizontal meridian in the equatorial plane, fixed in buffered formalin, embedded in paraffin and 5 μm sections obtained were stained by routine histological stain combination of Harris hematoxylin and eosin. In each section, the outer nuclear layer (ONL) thickness and total retinal thickness were measured at nine defined points (Cao et al., 2001). ONL and total thickness were analyzed using Image J software with length expressed in μm. The total numbers of cells in the outer nuclear layer were counted for each section by the deconvolution, segmentation and multi thresholding technique with the same software.

2.5. Statistical analysis

All statistical analysis was performed using an SPSS statistical package (Version 17). One-way ANOVA and Tukey’s multiple comparison tests were used to compare the groups for plasma corticosterone levels, Kruskal–Wallis test was used to test the efficacy of LED light on the retinal histopathology related parameters. p < 0.05 was considered to be statistically significant.

3. Results

Plasma corticosterone was significantly lower in group 2 and group 3 and higher in group 4 in comparison with the control group (Table 1).

Morphologic evaluation of total thickness, outer nuclear thickness and cell count were performed in retinal sections of all groups by quantitative histology (Fig. 2(a–d)). In group 2, exposure to fluorescent light significantly reduced the ONL thickness (p = 0.02, Kruskal–Wallis test) and total retinal thickness (p = 0.02, Kruskal–Wallis test) when compared to control animals (group 1). In group 3, there was a significant reduction in ONL thickness when compared to control (group 1) (p = 0.04, Kruskal–Wallis test). When group 3 was compared with group 2, a decrease in ONL thickness was observed which was not statistically significant. However, a significant increase in the number of cells in the ONL (p = 0.05, Kruskal–Wallis test) as well as the total retinal thickness (p = 0.02, Kruskal–Wallis test) was observed in group 3 when compared with group 2. In the animals exposed only to LED (group 4) a significant increase in the ONL cell count was observed (p = 0.05, Kruskal–Wallis test) however this did not significantly affect the thickness of the ONL or the total retinal thickness.

4. Discussion

The current study showed that continuous exposure to fluorescent light at night results in retinal damage mainly in the outer nuclear layer and LED light preexposure ameliorates the retinal damage as evidenced by increase in the cell count in the outer nuclear layer.

The retina is the most sensitive region to light exposure. A decrease in the outer nuclear layer thickness and ganglion cell count (Organisciak et al., 2000) are indicative of significant retinal damage. The mechanism of fluorescent light damage might be due to the production of reactive oxygen free radicals (Wu et al., 2006). The other possible mechanism could be due to retinal DNA strand breaks (Specht et al., 1999), alteration in rhodopsin as the trigger for photoreceptor cell damage (Ranchon et al., 1999), overexpression of cFos and cjun gene (Grimm et al., 2000), modification of proteins (Tanioto et al., 2005) or alteration in mitochondrial functions (Danan et al., 2001). Studies done by Qu (Qu et al., 2010) showed a decrease in retinal thickness following fluorescent light exposure of 900 lx, 1800 lx and 2700 lx for a duration of 3 h, which is in agreement with the current study. However, the duration of exposure to 1800 lx in our study has been for a period of 30 days.

We have used a 670 nm LED light source to study its protective effect on fluorescent light induced retinal damage. Most of the previous studies used 670 nm LED light and there is substantial evidence that LED treatment at 670 nm is beneficial for its stimulating effect on cellular metabolism (Eells et al., 2010; Fitzgerald et al., 2010; Qu et al., 2010). The wavelength of 670 nm of LED has been proven to be non toxic and offer significant retinal protection (Eells et al., 2010; Qu et al., 2010). LED light exposure exhibits hormetic effects in retinal tissues with energy densities less than 10 J/cm² exhibiting a photo stimulatory effect and greater than 10 J/cm² resulting in a photo inhibitory effect. In addition, a maximum protective effect of LED on the retina has been observed between 600 and 1000 nm range (Rojas and Lima, 2011).

In this study, preexposure with LED light of 670 nm for a period of 6 min at intensity of 91 J/cm², once every day for a period of 30 days, prevented retinal damage caused by fluorescent light exposure. This was seen in group 3 (increase in ONL cell count and total thickness) and group 4 (increase in ONL cell count) following LED exposure. A similar study with 24 h of exposure to fluorescent light of 1000 lx on rats showed a significant protective effect of pre, concomitant and postexposure to LED light (Albarracin et al., 2011). However, they have shown that maximum protective effect of LED light on the retina was observed only with pre and concomitant exposures. Exposure to LED with increased frequency (50 mW/cm²), duration (30 min) and intensity (90 J/cm²) have also shown similar results (Qu et al., 2010).

The cellular mechanism of LED protection could be due to either improved mitochondrial energy metabolism or efficient antioxidant defence mechanisms resulting in cell survival (Eells et al., 2008). Other possible mechanisms might be influence of LED on reduction/oxidation (redox) signaling (Tafur and Mills, 2008) or by alteration in glial fibrillary acidic protein in Muller cells (Albarracin et al., 2011) or by influencing melatonin, a principal component in red light therapy (Yeager et al., 2007). According to Qu et al. (2010)
cytochrome c oxidase absorbs the LED and oxidizes the substrate cytochrome c and reduces the free radical oxidation to water. This produces substantial deoxygenized substance and ATP for the activity of Na/K ATPase and thereby retaining the physiological ionic distribution. The net beneficial effect is the maintenance of intracellular homeostasis and prevention of cellular damage and apoptosis. The significant increase in cell count observed in group 4, even in the absence of stress, could be attributed to above mentioned effects of LED light. This is also substantiated by the clinical use of low level light therapy from laser source as a treatment regime in retinal ailments like retinitis pigmentosa amplyopia (Ivandic and Ivandic, 2014) and visual acuity (Ivandic and Ivandic, 2012).

Decrease in plasma corticosterone following 30 days exposure to fluorescent light is indicative of adaptive process in the HPA axis (Dijkstra et al., 2012; Ishida et al., 2005). The other probable cause for the decrease in the cortisol level might be due to the polysynaptic neural pathways between retina and suprachiasmatic nucleus to adrenal glands which bypass the Hypothalamic pituitary axis (Buijs et al., 1999). Hence, alterations in retinal layer architecture and morphology are suitable markers to quantify the damaging effects of light irrespective of adaptation (Organisciak and Vaughan, 2010). A significant increase in plasma corticosterone in the group 4 as observed in this study is in agreement with an earlier report (Figueiro and Rea, 2010). The probable mechanism suggested is that a separate non visual pathway could increase cortisol secretion following exposure to red and blue light.

5. Conclusion

Exposure to fluorescent light of 1800 lx at night for a period of 30 days resulted in retinal damage as evidenced in the outer nuclear layer of the retina. Preexposure to red LED of 670 nm offers significant protection to the retina from chronic fluorescent light exposure. Though the Wistar rat is suitable for such studies, further in depth studies are required to ascertain this claim and also elucidate the molecular mechanism(s) involved in this protective action.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aanat.2014.04.004.

References


