CYTOTOXICITY AND GENOTOXICITY OF LIGHT EMITTED BY INCANDESCENT, HALOGEN AND LED BULBS ON RETINAL PIGMENT EPITHELIUM CELLS

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ABSTRACT

LED technology has the extraordinary ability to reduce energy consumption, constituting an economic and ecological advantage, so it is planned to replace incandescent, halogen and other inefficient bulbs for public and domestic lighting with LEDs. LEDs present specific spectral and energetic characteristics compared with that of other domestic light sources, so the potential risks for human health of these bulbs need to be explored.

The aim of this study was to assess cytotoxicity and genotoxicity of light emitted by different commercial light bulbs: incandescent, halogen and two LED bulbs with different Correlated Colour Temperatures. The evaluation was done on the ARPE-19 as a specific cell model for eye toxicity and on BEAS-2B as a good cell model for toxicology tests.

Light induced mainly cytotoxic effects on ARPE-19 and DNA damage on BEAS-2B, so different cell line showed different biological response. Moreover, our findings indicates that, among the four bulbs, cold LED caused the major cytotoxic effect on ARPE-19 and the major genotoxic and oxidative effect on BEAS-2B. Cold LED probably is able to cause more cellular damage because contains more high-energy radiations (blue). These results suggests that LED technology could be a safe alternative to older technologies but the use of warm LED should be preferred to cold LED, which can potentially cause adverse effects on retinal cells.

Keywords: ARPE-19, WST-1 assay, Comet assay, light-emitting diodes, halogen bulb.

1. INTRODUCTION

In the past century conventional incandescent bulb was almost the only source of electric light used in households. Due to energy saving policy (Commission regulation 244/2009), conventional incandescent bulbs (and other inefficient lighting methods) had to be phased out until September 2012. Incandescent bulbs have to be replaced with energy efficient light sources such as halogen bulbs, compact fluorescent bulbs (CFLs) or light-emitting diode bulbs (LED). All these light sources are extensively used for public and domestic lighting, but for the future it is planned to replace halogen bulbs and CFLs with LEDs (Necz and Bakos 2014).

LED technology has the extraordinary ability to reduce energy consumption, constituting an economic and ecological advantage. The importance of this technology has been recognized by giving the 2014 Nobel Prize in Physics to Isamu Akasaki, Hiroshi Amano and Shuji Nakamura "for the invention of efficient blue light emitting diodes (LEDs) which has enabled bright and energy-saving white light sources" (Haim and Zubidat 2015).

LEDs are also incorporated in all the screens of electronical devices, such as computers and mobile phones. The development of handheld computer-based technology has provided the opportunity for long-term viewing of illuminated screens. It is recognized that many people are using laptop or tablet computers, or mobile phone technology, for many hours per day (O'Hagan, Khazova and Price 2016).

The LED technology is currently being viewed as a huge step in cost-efficient solution for lighting systems and these light sources are extensively used, so it is important assess the potential risks to the environment and human health linked to this new technology.

Optical radiation includes ultraviolet light (UV) (100 – 380 nm), visible light (380 – 780 nm) and infrared radiation (IR) (780 – 10 000 nm). Visible light can be divided into blue (short-wavelength radiation), green (medium-wavelength radiation) and red light (long-wavelength radiation) (Youn et al. 2009).

Overall, our household light bulbs emit mainly optical radiation but not only in the visible spectrum. There are some other ranges of non-ionizing radiation that are emitted by bulbs and that are possibly hazardous for human health, such as: UV and IR. Also visible light, especially blue light, can impair eyesight (Necz and Bakos 2014). Ultraviolet light and the shorter wavelengths of the visible light pose a potential hazard because they contain more energy (Youn et al. 2009). In particular, the blue light (400 – 500 nm) is likely to be important since it has a relatively high energy (Godley et al. 2005).

LEDs present specific spectral and energetic characteristics compared with that of other domestic light sources, so the potential risks of these new light sources need to be explored to answer whether they could be eventually harmful for people (Chamorro et al. 2013).

Most white LEDs consist of a short-wavelength emitting diode (blue light mostly) and phosphor emitting at a larger wavelength (mixed white light generation), so they emit many blue radiations (Shen et al. 2016). Blue light, emitted by LED, has been demonstrated to be the most effective frequency for melatonin suppression compared with conventional lighting technologies (Falchi et al. 2011; West et al. 2011). Melatonin strongly regulates numerous vital functions including antioxidant, antiaging and most relevant anti-oncogenic properties (Srinivasan et al. 2011). Reduced levels of melatonin in women exposed to artificial light-at-night during night work and sleep deprivation are associated with an increase in breast cancer risk (Davis, Mirick and Stevens 2001; Schernhammer et al. 2001; Viswanathan, Hankinson, and Schernhammer 2007; Haim and Zubidat 2015).

The eye is constantly exposed to radiations. Light in excess (high energy or long-time exposure) may cause eye injury when focused onto retina. Although the eye has developed very precise mechanism of light adaptation and has several protective mechanism against light exposure, prolonged or intense exposure may affect the human vision (Contin et al. 2016).

European Standard EN 62471:2008 (European Standard 2008) gives guidance for evaluating the photobiological safety of broad band lighting sources (including LED) and systems, it specifies the spectral blue-light hazard function B(λ), and states the limiting values, measuring quantities useful to evaluate the potential photobiological hazard of light exposure.

Constant exposure to light in excess can produce retinal degeneration as a consequence of photoreceptor or retinal pigment epithelium (RPE) cells death (Contin et al. 2016). Moreover, light in excess may damage the human vision promoting retinal degeneration or accelerating some genetic diseases, such as retinitis pigmentosa or age-related macular degeneration (Contin et al. 2016).

Visible light affects mitochondrial respiration and decreases mitochondrial homeostasis (Osborne et al. 2010; Li, Fan, and Ma 2011) and it can also directly cause nuclear DNA damage in retinal ganglion cells (Li, Fan, and Ma 2011).

It has been hypothesized that in particular blue light can damage the retina causing photoretinitis (Necz and Bakos 2014) and the development of age-related macular degeneration (Youn et al. 2009). Studies *in vivo* show that retinal exposure at elevated levels of blue light leads to photochemical damage on the photoreceptors and retinal pigment epithelial cells (Youn et al. 2009). It has been reported that blue light induced retinal damage is mainly caused by the production of reactive oxygen species (ROS) (Moon et al. 2017). Excessive oxidative stress can cause dysfunction in retinal cells by the oxidation of proteins, lipids and DNA and eventually results in cell death by apoptosis (Moon et al. 2017). Studies *in vitro* have shown that irradiation of mammalian cells (human primary epithelial cells) with blue light induces both mitochondrial and DNA damage via reactive oxygen species (ROS) (Godley et al. 2005). Also low intensity of blue light can induce ROS production and apoptosis in RPE cells (A2E-loaded ARPE-19) (Moon et al. 2017). Moreover, the study of Nakanishi-Ueda and collaborators (2013), showed that blue light emitted by LED causes an increase of ROS, lipid peroxidation and subsequent cellular injuries in cultured bovine RPE cells. Others authors (Kuse et al. 2014) demonstrated that also the cone photoreceptor-derived cells (661 W) can be damaged via ROS by blue light emitted by LED. The harmful blue light effect was also confirmed *in vivo* (Wu et al. 1999; Narimatsu et al. 2015; Ham, Mueller and Sliney 1976; Gorgels and Norren 1995; Moon et al. 2017). Blue light induced retinal damage in rats, whereas green light did not (Wu et al. 1999). The retinal damage was mediated by apoptosis, and the damage in the rat retina increased with the use of shorter wavelength of blue light (Gorgels and Norren 1995). Moreover similar results were confirmed in a previous study using a rhesus monkey (Ham, Mueller and Sliney 1976). Recently, it was demonstrated that blue light exacerbated the increase in the ROS level and inflammatory cytokine expression as well as macrophage recruitment in the RPE-choroid of mice exposed to light (Narimatsu et al. 2015).

The mechanisms by which light can cause damage to the retina have not been completely understood and properties of light that induce this damage have not been precisely related to simple photometric characteristics like peak wavelength and Correlated Colour Temperature (CCT). Few studies evaluated genotoxicity induced by light and little is known about the biological effects induced by different types of LED bulbs.

The aim of this study was to evaluate cytotoxicity and genotoxicity of light emitted by different commercial light bulbs that have the same amount of luminous flux emitted. In particular, the tested bulbs were a halogen lamp bulb, two LED bulbs with different Correlated Colour Temperatures (CCT) (warm white and cold white) and, in comparison, an old incandescent bulb, which is currently no commercially available because it does not comply with energy requirements.

The human RPE cells (ARPE-19) were used as a specific cell model for eye toxicity and the human bronchial epithelial cells (BEAS-2B) were used as a good cell model for *in vitro* toxicology tests.

2. METHODS AND MATERIALS

2.1 Cell culture

The spontaneously immortal human RPE cells (ARPE-19) and the virus transformed human bronchial epithelial cells (BEAS-2B) were obtained from the American Type Culture Collection.

ARPE-19 were grown as a monolayer, maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum, 2% L-glutamine 200 mM, 1% sodium pyruvate 100 mM and 1% penicillin 10 000 U/ml - streptomycin 10 000 µg/ml, at 37°C in an humidified atmosphere containing 5% CO_2.

BEAS-2B were grown as a monolayer, maintained in RPMI 1640 supplemented with 10% (v/v) FCS, 2% L-glutamine 200 mM and 1% penicillin 10 000 U/ml - streptomycin 10 000 µg/ml, at 37°C in an humidified atmosphere containing 5% CO_2.

2.2 Lamps and exposure

Experimental illuminating system included commercial warm LED bulb (provided by KADELED-light line S.r.l.), commercial cold LED bulb (provided by SI S.r.l.) and halogen bulb (provided by GREENPLUX S.r.l.). In comparison, an old incandescent bulb was tested, this type of lamp is currently no commercially available because it does not comply with the energy requirements. The characteristics of the four bulbs are reported in Table 1: bulbs differ for power, Correlated Colour Temperature and energy efficiency class but produce an equivalent luminous flux (lumen). The spectral intensity distribution of all sources was measured with a Minolta CL500A illuminance spectrophotometer in three different conditions: in air in a dark room with the sensitive area of the meter toward the lamp (Cond.

A) and in two different positions inside the cell culture incubator, one with the meter on the bottom of the incubator (Cond. B) to evaluate the changes induced by selective wavelengths reflections of the incubator walls, and one to assess the spectral distribution of the incident light on cells with the meter inside the incubator with the sensitive area toward the lamp and a plate between the sensitive area and the lamp (Cond. C). The results are shown in Fig. 1.

The experimental illuminating system was installed into the cell culture incubator, which maintained a temperature of 37°C. In order to reduce the interference of medium, each illuminating system irradiated the basal surface of culture plates (Shen et al. 2016), which were positioned 14 cm above the light sources directly. The distance of the light form the cell cultures was based on the distance used by other recent studies (Shen et al. 2016; Xie et al. 2014). In addition, during light exposure, the culture medium was changed to DMEM or RPMI 1640 without phenol red containing 2% HEPES buffer and without fetal calf serum to reduce the chromophores present in the culture medium (Xie et al. 2014).

The cultured cells were irradiated for 1h and 4h. The spectral irradiance and the illuminance on the cells cultures on plates were measured inside the incubator with the CL500A in measurement condition (Cond C). From the measured values of spectral irradiance, the total blue-light weighted exposure, calculated as the integral of irradiance weighted against the spectral blue-light hazard function B(λ) for the exposure time (European Standard 2008), was calculated for the two exposure times (1h and 4h). Illuminance and total blue-light weighted exposure are shown in Table 2 (the measurement uncertainty is 5%), while the blue-light weighted exposure is shown in Figure 2.

The discrepancies in the illuminance and total blue-light weighted exposure values among the lamps, are due to the different luminous spatial intensity distributions of the lamps: incandescent lamp and cold LED have a strong light emission in the vertical direction (i.e.

toward the cell plates), while warm LED has a horizontal emission, due to the geometrical arrangement of the emitting surface.

Cells kept in the dark, incubated in the same incubator of the exposed cells, are considered as control group. The exposure times were chosen in order to evaluate short-time effects induced by light. The exposure times are comparable with previous studies (Nankanishi-Ueda et al. 2013; Godley et al. 2005; Roechelecke et al. 2009; Youn et al. 2009).

To ensure that assays were not influenced by an eventually system temperature increase caused by bulbs, during the incubation the temperature was measured at 10, 15, 20, 30, 60, 90, 120, 150, 180, 210, 240 minutes after incubation using RS Digital Thermometer 1319, K-Type. Figure 3 shows the temperature variation induced by different types of light inside the irradiated and control wells. After inserting the plates into the incubator, the temperature inside wells rises, is stabilized during 20 minutes and remains almost unchanged for the whole incubation time. Comparing the temperature among wells exposed to different light bulbs, the incandescent bulb and the halogen bulb determined the highest temperature values, probably because these bulbs (less energetically efficient) dissipate part of the energy as heat. The slight difference in temperature variation (1° C between the minimum and maximum recorded temperature) reasonably did not influence the results of biological assays performed on the cells.

2.3 WST-1

The cell viability was evaluated using the Cell Proliferation Reagent WST-1 (Roche). This assay is based on the cleavage of tetrazolium salts to soluble formazan dye by mitochondrial succinate-tetrazolium reductase which exists in the mitochondrial respiratory chain and is active only in viable cells. The quantity of formazan dye in the medium is directly proportional to the number of viable metabolically active cells.

Briefly, cells were seeded in 24-well plates at a density of 5×10^4 cells/well and, after exposure, 50 µl of Cell Proliferation Reagent WST-1 (Roche) were added to each cell culture well and incubated for 3 h at 37°C, protecting the plate from the light.

To avoid any interference in light absorption owing to the cells and cell debris, at the end of incubation, contents of each well were transferred in an optically clear 96-well flat bottom plate. Formazan dye formed by metabolically active cells was quantified by measuring its absorbance (440nm) using a microtiter plate reader (Tecan Infinite Reader M200 Pro).

Negative control were obtained by absorbance measurement of culture cell medium of control cells. Data from exposed cells were expressed as a percent of viable cells. All experiments were performed in quadruplicate and the data were represented as the mean ± standard deviation.

2.4 Comet assay

DNA damage has been evaluated by alkaline single cell gel electrophoresis (Comet assay), according to the recommended procedure (Tice et al. 2000). Cells were cultured for 12 h in 6-well plates at a density of 3×10^5 cells/well before exposure to light. The proportion of living cells was determined by trypan blue staining. Cells were treated with different light bulbs for 1h or 4h. After exposure, cell viability was checked again. Cells (3×10^5) were mixed with 140 µl of 0.7% low melting point agarose (LMA) and 20 µl were placed on the slides coated with 1% of normal melting agarose (NMA), with LMA added as the top layer. Cells were lysed at 4°C in the dark overnight (8 mM Tris–HCl, 2.5 M NaCl, 100 mM EDTA disodium salt dihydrate, 1% TRITON X-100 and 10% DMSO, pH 10). DNA was allowed to unwind for 20 min in alkaline electrophoresis buffer (1 mM EDTA tetrasodium salt dihydrate, 300 mM NaOH, 10% DMSO, pH >13) and subjected to electrophoresis in the same buffer for 20 min (1 V/cm and 300 mA). The slides were then soaked with neutralization buffer (0.4 M Tris-HCl, pH 7.5, 4 °C, 3 min), fixed with ethanol 70% (-20 °C, 5 min) and air dried.

All steps for slide preparation were performed under yellow light to prevent additional DNA damage.

DNA was stained with ethidium bromide (20 µg/ml) and analyzed using a fluorescence microscope (Axioskop HBO 50, Zeiss). A hundred randomly selected cells per sample (2 spot) were analyzed using an image analysis system (Comet Assay IV) (Perceptive Instruments Ltd, Stone, Staffordshire, UK). The % tail DNA was selected as the parameter to estimate DNA damage (Tice et al. 2000; Collins 2004).

2.5 Fpg-Comet

The formamidopyrimidine glycosylase (Fpg)-modified Comet assay was used to evaluate oxidative DNA damage. The test was carried out as described above with the exception that, after lysis, the slides were washed three times for 5 min with Fpg Buffer (40 mM Hepes, 0.1M KCl, 0.5 mM EDTA disodium salt dihydrate, 0.2 mg/ml bovine serum albumin, pH 8). Then, the slides were incubated with 0.5 unit of Fpg enzyme (Escherichia coli Fpg Enzyme and Buffer- TREVIGEN) at 37°C for 30 min. Control slides were incubated with buffer only. A hundred randomly selected cells per sample (2 spot) were analyzed using an image analysis system (Comet Assay IV) (Perceptive Instruments Ltd, Stone, Staffordshire, UK). For each experimental point, the mean % tail DNA from enzyme untreated cells (direct DNA damage) and mean % tail DNA for Fpg-enzyme treated cells (direct and indirect DNA damage) were calculated.

2.6 Statistical analyses

Statistical analyses were performed using IBM SPSS software (ver. 24.0). The results of WST-1 and Comet assay are presented as the mean of quadruplicate (WST-1) and duplicate (Comet assay) ± standard deviation. Differences between exposed and control cells for each time of exposure (1h and 4h) were tested by T-test Student. Differences of cytotoxicity and genotoxicity induced by different bulbs after the same time of exposure were tested by one-way analysis of variance (ANOVA) followed by Tukey's test procedure. Significance was accepted at $p<0.05$.

3. RESULTS

3.1 Cytotoxicity – WST-1

The results of the effects of different light on cell viability (WST-1 assay) on ARPE-19 are presented in figure 4. The incandescent bulb induced a decrease in viability after only 4h ($p<0.05$) (fig. 4a), while halogen bulb caused a significant cytotoxic effect both after 1h and 4h ($p<0.001$) and the effect increased with the increase of exposure time (fig. 4b). Considering effects induced by LEDs, warm LED induced a decrease in viability after 4h that was not significant (fig. 4c), on the contrary cold LED, similar to halogen bulb, showed a significant cytotoxic effect both after 1h and 4h ($p<0.001$) and the effect increased with the increase of exposure time (fig. 4d).The ANOVA analysis, performed assuming cytotoxicity induced after exposure (1h) as dependent variables and the different bulbs as independent variables, showed the general significance of the model ($F= 28.422$, $p<0.001$). Post hoc Tukey's test emphasised the cytotoxicity induced by cold LED that was the highest compared to other light bulbs (cold LED *vs* incandescent bulb $p<0.001$, cold LED *vs* halogen bulb $p<0.05$, cold LED *vs* warm LED $p<0.001$).

The results of the effects of different light on cell viability (WST-1 assay) on BEAS-2B are presented in figure 5. As reported on ARPE-19, the incandescent bulb induced slight decrease in viability that was significant after only 4h ($p<0.001$) (fig. 5a). Differently from ARPE-19, halogen bulb was not cytotoxic on BEAS-2B (fig. 5b). Considering effects induced by LEDs, contrary to ARPE-19 a low cytotoxic effect was observed for warm LED (1h and 4h, $p<0.001$) (fig. 5c), while no cytotoxicity was observed for cold LED (fig. 5d). The ANOVA analysis, performed assuming cytotoxicity induced after exposure (4h) as dependent variables and the different bulbs as independent variables, showed the general significance of the model (F= 116.753, $p<0.001$). Post hoc Tukey's test confirmed the major cytotoxic effect induced by incandescent bulb and warm LED than halogen bulb and cold LED (incandescent bulb *vs* halogen bulb $p<0.001$, incandescent bulb *vs* cold LED $p<0.001$, warm LED *vs* halogen bulb $p<0.001$, warm LED *vs* cold LED $p<0.001$).

3.2 Genotoxicity – Comet and Fpg-Comet assays

The alkaline version of the Comet assay (sensitive to DNA strand breaks, direct oxidative DNA lesions and alkali-labile sites) was used to evaluate the genotoxic effects of light, while the Fpg- modified Comet assay was used to assess the oxidative (direct and indirect) DNA damage.

The results of genotoxic effect induced by different lamp bulbs on ARPE-19 are presented in figure 6. Considering the exposure with incandescent light, no genotoxic effect was showed in enzyme untreated cells (direct DNA damage) (fig. 6a). On the contrary, halogen lamp exposure caused a significant DNA damage after 4 hours ($p<0.05$) (fig. 6b). Similar to halogen lamp, also LEDs (warm and cold) induced genotoxicity after 4 hours exposure ($p<0.05$) (fig. 6c and 6d). The ANOVA analysis showed no significance of the model, so the comparison of genotoxicity induced after 4h by different bulbs on ARPE-19 was not statistically significant.

On the ARPE-19 cells, it was not possible to perform the Fpg-Comet assay because the oxidized sites were high in the control cells (results not shown).

The results of genotoxic effect induced by different lamp bulbs on BEAS-2B are presented in figure 7. On BEAS-2B, incandescent light caused a significant DNA damage after 4 hours ($p<0.05$) (fig. 7a). As reported on ARPE-19, halogen lamp, warm LED and cold LED were genotoxic after 4 hours on BEAS-2B ($p<0.05$) (fig. 7b, 7c, 7d). The damage induced by cold LED was higher than damage caused by exposure to incandescent, halogen and warm LED lamps (fig. 7d). The ANOVA analysis, performed assuming genotoxicity induced after exposure (4h) as dependent variables and the different bulbs as independent variables, showed the general significance of the model ($F= 27.730$, $p<0.05$). Post hoc Tukey's test emphasised major genotoxicity induced by cold LED than the others bulbs (cold LED *vs* incandescent bulb, halogen bulb and warm LED; $p<0.05$). The genotoxic effect induced on BEAS-2B was higher than the effect induced on ARPE-19, although the observed genotoxicity was overall low.

On the BEAS-2B cells, the Fpg modified Comet assay was performed successfully. The results of genotoxic effect (direct and indirect DNA damage) induced by different lamp bulbs on BEAS-2B are reported in figure 8. Incandescent, halogen and warm LED bulbs induced a significant DNA damage with respect to the control cells in enzyme treated cells ($p<0.05$) (fig. 8a, 8b and 8c respectively). However, there were no differences between the DNA damage in enzyme treated cells and the DNA damage induced in enzyme untreated cells, resulting in no oxidative damage induced by these bulbs. On the contrary, a major statistically significant increase of DNA damage was observed in enzyme treated cells (direct and indirect DNA damage) with respect to the control cells after 4 hours exposure with cold LED ($p<0.05$) (fig. 8d). Considering that, the subtraction of the mean % tail DNA in enzyme treated cells from the relative mean % tail DNA in enzyme untreated cells, compared with unexposed cells at each experimental point, provides the intensity of the oxidative damage, a significant oxidative damage was observed for cold LED ($p<0.05$). The ANOVA analysis, performed assuming genotoxic effect (direct and indirect DNA damage) induced after exposure (4h) as dependent variables and the different bulbs as independent variables, showed the general significance of the model (F= 126.643, p<0.001). Post hoc Tukey's test emphasised major genotoxicity induced by cold LED than the others bulbs (cold LED *vs* incandescent bulb, halogen bulb and warm LED; p<0.001).

4. DISCUSSION

In vitro assays provide rapid and effective means of screening and ranking chemicals and physical agents for a number of toxicological endpoints. They allow targeted investigations on issues that can not be adequately addressed by other methods, such as analysis of mechanisms of toxicity at both the molecular and cellular level (Eisenbrand et al. 2002). Toxicity testing can be refined by considering the target organ of the test compound *in vivo* and selecting a cell system that is appropriate on the basis of metabolic competence and of organ/tissue specific toxicity (Eisenbrand et al. 2002). However, it should be kept in mind that some tissues/cell lines may be more susceptible to cytotoxicity or other biological effects than others (Vinken and Blaauboer 2017), so it is useful testing chemical or physical agent on different cell types.

This study investigated the cytotoxicity and genotoxicity of different commercially available light bulbs: incandescent bulb, halogen bulb, warm LED and cold LED. The effect was evaluated *in vitro* on two different human cell lines deriving from the RPE (ARPE-19) and bronchial epithelium (BEAS-2B). These cell lines were chosen as a specific cell model for eye toxicity (ARPE-19) and a good cell model for *in vitro* toxicology tests (BEAS-2B) in order to evaluate possible different biological response. To support comparison, we chose cell lines derived from epithelium and non-tumoral, because cell lines originates from cancer might perform aberrant functionality (Vinken and Blaauboer 2017).

As a specific cell model for eye, we chose ARPE-19 because the RPE cells have vital support functions for retina (e.g. maintain ionic composition, filter nutrients and provide photoprotection) and are important for the physiology and pathology of the retina. It has been documented that RPE cell cultures and also immortalized cell lines may adopt a variety of morphological and biochemical phenotype, more or less resembling the equivalent RPE tissue (Pfeffer and Philp 2014).

In comparison, we chose to use a human bronchial cell line, which is extensively used to study the impact of toxicants on lung, the BEAS-2B cells. According to a recent study on human lung cell model (Courcot et al. 2012), BEAS-2B exhibited the highest similarities with primary cells and the lowest number of dysregulated genes compared with non-tumoral lung tissues, so they are a good model for toxicology studies.

The WST-1 assay was performed to assess cytotoxicity on the two cell lines. On ARPE-19 different bulbs induced different cytotoxic effect: halogen bulb and cold LED caused the major cytotoxic effect. Comparing the characteristics of the two tested LEDs, they differ in CCT and spectrum (Fig. 1 and Table 1). The CCT is a characteristic that identifies the perceived tonality of light distribution of the radiation in the spectral band of the visible. If the dominant colour of the light tends to red, the light emitted will have a warm perceived tone (low CCT values); if the dominant colour of the light tends to blue, the light emitted will have a more cold perceived tone (high CCT values). Therefore, the CCT reflects the optical and spectral characteristics of a specific white LED light to some extent (Xie et al. 2014). Our LED, warm and cold, have respectively low and high CCT values, so they emit light composed by radiations that tends respectively to red and blue colours. We hypothesize that our cold LED was more cytotoxic than warm LED because it emits more blue radiations and in the region were $B(\lambda)$ (European Standard 2008) is close to its maximum value (Fig. 1(b)). According with our results, other studies demonstrated that the blue component of the visible spectrum (400-500 nm) is associated with retinal damage and the development of age-related macular degeneration (Necz and Bakos 2014; Nakanishi-Ueda et al. 2013; Kuse et al. 2014; Shaban and Richter 2002). Negative effect of blue light exposure on ARPE-19 cells was previous demonstrated by others. Youn and collaborators (2009), testing lights with different wavelengths (400, 420 and 435.8 nm), found that only the 400 nm light can cause significant dose-dependent decreases in ARPE-19 cell viability. Moon et al. (2017) recently obtained similar results testing the blue light effect on ARPE-19 containing A2E (a fluorophore): shorter wavelength blue light resulted in an increased production of ROS and induced reduction in viability and activation of caspase-3/7. Also King and collaborators (2004) and Roehlecke and collaborators (2009), after exposure to blue light, demonstrated increase of ROS production, induction of cytotoxicity through mitochondrial-dependent mechanism and mitochondrial damage on ARPE-19. Therefore, as shown by other studies, we can hypothesized that the CCT is an important parameter that could induce different biological effects: with the increase of CCT there is a major cytotoxic effect. This is a direct implication of the physical principle that shorter Electromagnetic wavelengths (like blue light wavelengths) have higher energy, but it is expressed through a more simple parameter of easy understanding.

Considering our results on ARPE-19, not only cold LED but also halogen bulb was highly cytotoxic, although the tested halogen light has a low CCT. This result is in agreement with the study of Yoshida and collaborators (2013). They found that blue light irradiation by quartz tungsten halogen lamp and LED decreased cell proliferation of human gingival fibroblasts (HGF) in a time-dependent manner and caused morphological changes especially in the mitochondria. Moreover, according to our results they found that cytotoxicity was significant higher after LED irradiation than after quartz tungsten halogen irradiation.

Therefore our study and the study of Yoshida and collaborators (2013) suggests that also halogen bulbs with a low CCT can induce cytotoxic effect.

Our study showed that light induced more cytotoxicity on ARPE-19 than on BEAS-2B cells. ARPE-19 are retinal cells and the most common mechanism by which light is thought to cause damage to retina is the photochemical. Considering our results, we hypothesized that ARPE-19 cells were particularly susceptible to blue radiations because, as RPE cells, have many mitochondria, so they have many cytochromes. The cytochromes are chromophores, so they interact with wavelengths in the high-energy portion of the visible spectrum and cause the generation of free radicals (King et al. 2004; Youssef, Sheibani and Albert 2011). This hypothesis is confirmed by an experimental study attesting that mitochondria are an important source of toxic oxygen radicals in the short wavelength light-exposed RPE cells (King et al. 2004; Youn et al. 2009). Moreover RPE cells are especially susceptible to oxidative stress, induced by light, because of their high membrane lipid levels (e.g. polyunsaturated fatty acids) (Youn et al. 2009).

The Comet assay was used to assess the genotoxicity induced by light on ARPE-19 and BEAS-2B cells. Considering results on ARPE-19, halogen, warm LED and cold LED bulbs caused a significant slight genotoxic damage after 4h exposure. During Comet assay, after 4h exposure, the wells treated with light presented cells detached from the bottom, confirming the major cytotoxicity observed on ARPE-19 than on BEAS-2B. The detached cells probably was lost after washing with PBS increasing the proportion of living cells detected by trypan blue staining, which was not lower than 70% (incandescent: 87.5%, halogen: 96.7%, warm LED: 86.2%, cold LED: 84.7%). Moreover, during Comet assay scoring, many hedgehogs (comets with almost all DNA in the tail) were scored after treatment with light, especially after 4h exposure. In agreement with the major cytotoxicity observed in our study on ARPE-19 than on BEAS-2B, in literature, it was largely suggested that these comets come from heavily damaged cells and represent cells engaging in apoptosis. However, some authors do not agree with this interpretation claiming that hedgehogs can correspond to one level on a continuum of genotoxic damage and are not diagnostic of apoptosis (Lorenzo et al. 2013). We hypothesized that the high cytotoxicity detected on ARPE-19, probably concealed the detection of a high genotoxic effect.

On the ARPE-19 cells, it was not possible to perform the Fpg modified Comet assay because the oxidized sites were high in the control. The same evidence was found by Sparrow, Zhou, and Cai (2003). They evaluated DNA damage induced by blue light (430 nm) on ARPE-19 cells loaded with A2E and the ability of cells to repair DNA. They found high oxidized site in the control and hypothesized the presence of pre-existing base changes. According to our results, the same study demonstrated that the light caused a time-dependent DNA damage. The DNA damage induced by light exposure on ARPE-19 was studied also by Youn and collaborators (2009), using confocal laser scanner microscopy. Their results showed that only radiations with lower wavelength caused the increased degradation of DNA/RNA (especially RNA) in comparison with the control cells. Previously also Hafezi and collaborators (1997) and Seko and collaborators (2001) showed that light induced apoptosis in the retinal cells, especially revealing DNA fragmentation and nucleic chromatin alteration (Youn et al. 2009). The results of Youn and collaborators, Hafezi and collaborators and Seko and collaborators correspond to our results on cytotoxicity and to the presence of hedgehog during the scoring of Comet assay.

Considering our genotoxicity results on BEAS-2B cells, the Comet assay performed without Fpg showed that all type of bulbs caused a significant direct DNA damage after 4h exposure. Cold LED caused the major genotoxic effect. Similar results were obtained by Chamorro and collaborators (2013). They investigated the effects of LED radiations (blue-468nm, green-525nm, red-616nm and white light) on human RPE cells (HREpiC). They found that all types of light induced a significant DNA damage and the greatest damage was observed for the blue LED light (468 nm). A major genotoxic effect induced by cold light was found also by Xie and collaborators (2014) using Comet assay on lens epithelial cells (hLECs).

Our study investigated the oxidative DNA damage using Fpg modified Comet assay. Contrary to ARPE-19, Fpg modified Comet assay was performed successfully on BEAS-2B cells. Incandescent, halogen and warm LED bulbs induced no oxidative DNA damage. On the contrary, a statistically significant increase of oxidative DNA damage was observed after 4 hours exposure with cold LED. Our results using Fpg enzyme confirmed the major biological effects of cold LED and suggested that blue light could induced reactive oxygen species and oxidative stress leading to oxidative DNA damage, as supposed above and confirmed by other studies (Sparrow, Zhou, and Cai 2003; King et al. 2004; Roehlecke et al. 2009; Moon et al. 2017).

5. CONCLUSION

Our results indicates that light induced mainly cytotoxic effects on ARPE-19 and DNA damage on BEAS-2B, so different cell line models showed different biological response. The difference is probably due to a different susceptibility between the two cell lines. In particular, ARPE-19 cells seemed to be more susceptible to light exposure. On ARPE-19 cells light induced a cytotoxic effect which probably concealed the detection of a high genotoxic effect. The use of different cell models was important because only on BEAS-2B cells (which are more resistant) it was possible the detection of oxidative DNA damage induced by blue light. This kind of damage leaded to the hypothesis that light induced effects are mediated by oxidative stress, confirming hypothesis made before by other authors. Moreover, in our experimental conditions, among the four (incandescent, halogen, warm LED, cold LED) commercial bulbs, cold LED caused the major cytotoxic effect on ARPE-19 and the major genotoxic and oxidative effect on BEAS-2B. Commercial cold LED is able to cause more cellular damage probably because contains more high-energy radiations (blue) than the other bulbs. While further evaluations are be needed to assess biological effects of light emitted by different sources for the same amount of total exposure, the different Total Blue-light Exposure among sources gives also relevance to the findings on cytotoxic effects of halogen bulb and highlights the need of more investigations on the topics. Ultimately our results indicates that LED technology could be a safe alternative to older technologies but the use of warm LED should be preferred because the light emitted by cold LED can potentially cause adverse effects on retinal cells.

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TABLES

Table 1. Characteristics of the four tested bulbs.

	Incandescent bulb	Halogen bulb	Warm LED	Cold LED
Power	25 W – 230 V	18 W – 220/240 V	3 W	3.5 W – 230 V
Declared luminous flux	≈ 200 Lumen	210 Lumen	250 Lumen	300 Lumen
Measured Correlated Colour Temperature	warm white 2589 ± 5 K	warm white 2652 ± 5 K	warm white 2700 ± 5 K	cold white 6500 ± 14 K
Energy efficiency class	≈E	C	A+	A+

Table 2. Illuminance and Total Exposure of the four tested bulbs measured and calculated for Cond. C

	Incandescent bulb	**Halogen bulb**	Warm **LED**	Cold **LED**
Measured Illuminance, Cond.C	602 ± 27 lx	215 ± 9 lx	434 ± 20 lx	1126 ± 50 lx
Calculated Total blue-light weighted exposure 1 h Cond.C.	0,144 ± 0,007 W/m^2 h	0,049 ± 0,002 W/m^2 h	0,091 ± 0,004 W/m^2 h	0,878 ± 0,044 W/m^2 h
Calculated Total blue-light weighted exposure 4 h Cond.C.	0,577 ± 0,028 W/m^2 h	0,196 ± 0,009 W/m^2 h	0,364 ± 0,018 W/m^2 h	3,512 ± 0,018 W/m^2 h

LEGEND TO FIGURES

Table 1. Characteristics of the four tested bulbs.

Table 2. Illuminance and Total Exposure of the four tested bulbs measured and calculated for Cond. C.

Figure 1. Normalized Spectral intensity distribution of the four lamps as measured in Cond. A, Cond. B, Cond.C (a) and Normalized Spectral intensity distribution of the four lamps in

Cond. A, and Cond.C with Blue-light hazard weighting function $B(\lambda)$ (b), the purple lines identifies the Blue-light range.

Figure 2. Blue light weighted spectral exposure measured in Cond C.

Figure 3. Temperature variations induced by the four different (incandescent, halogen, warm LED, cold LED) bulbs inside irradiated and control wells.

Figure 4. Cytotoxicity of ARPE-19 cells exposed for 1h or 4h to light emitted by the different bulbs: incandescent (a), halogen (b), warm LED (c), cold LED (d). Bars represent the mean % cell viability (quadruplicate), error bars represent standard deviation of mean. Asterisks indicate statistically significant differences vs control cells (C- cell viability 100%) *$p< 0.05$; **$p<0.001$ (T- test Student).

Figure 5. Cytotoxicity of BEAS-2B cells exposed for 1h or 4h to light emitted by the different bulbs: incandescent bulb (a), halogen bulb (b), warm LED (c), cold LED (d). Bars represent the mean % cell viability (quadruplicate), error bars represent standard deviation of mean. Asterisks indicate statistically significant differences vs control cells (C- cell viability 100%) *$p< 0.05$; **$p<0.001$ (T- test Student).

Figure 6. Genotoxic effect, evaluated by the Comet assay, of ARPE-19 cells exposure (1h or 4h) to light emitted by the different bulbs: incandescent bulb (a), halogen bulb (b), warm LED (c), cold LED (d). Bars represent the mean % tail intensity value from two spots, error bars represent standard deviation of mean. Asterisks indicate statistically significant differences vs control cells (C-) *$p< 0.05$ (T- test Student).

Figure 7. Genotoxic effect, evaluated by the Comet assay, of BEAS-2B cells exposure (1h or 4h) to light emitted by the different bulbs: incandescent bulb (a), halogen bulb (b), warm

LED (c), cold LED (d). Bars represent the mean % tail intensity value from two spots, error bars represent standard deviation of mean. Asterisks indicate statistically significant differences vs control cells (C-) *$p < 0.05$ (T- test Student).

Figure 8. Genotoxic effect, evaluated by the Fpg-Comet assay, of BEAS-2B cells exposure (1h or 4h) to light emitted by the different bulbs: incandescent bulb (a), halogen bulb (b), warm LED (c), cold LED (d). Bars represent the mean % tail intensity value from two spots, error bars represent standard deviation of mean. Asterisks indicate statistically significant differences vs control cells (C-) *$p < 0.05$ (T- test Student).