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TOP 2019 PANORAMA LE AZIENDE DOVE SI LAVORA MEGLIO IN ITALIA
IN COLLABORAZIONE CON statista

EFFECTS OF BLUE LED LIGHT ON CELL CULTURES OF HUMAN RETINAL PIGMENTED EPITHELIUM

Final report

Recent studies suggest that the blue component of the light emitted by LEDs and other light sources could be harmful to the retina (1-3). In fact, some works have shown that prolonged exposure of retinal pigment epithelium cells (RPE) to blue light causes suffering and an increased propensity to die from apoptosis (1,4,5). These results are certainly indicative that blue light is potentially harmful for the retina, but do not show that aggression is followed by a real alteration of the retinal function (if the magnitude of the damage induced by blue light were not be particularly marked, it could to be eventually compensated for by intrinsic retinal defense mechanisms).

In this regard, a recently published study that suggests for the first time that the retinal pigment epithelium exposed to blue light may begin to secrete VEGF (vascular endothelial growth factor, a pro-angiogenic factor) in a significantly increased quantity appears to be very interesting (6).

If confirmed, the increased production of VEGF by the retinal pigment epithelium exposed to blue light could in fact directly link the toxic effect of blue light to extremely serious retinal pathologies such as age-related retinal macular degeneration (AMD) and diabetic macular edema (DME) (7,8). In both of these pathologies a local increase in VEGF produced in excess by retinal neurons causes a choroidal neovascularization (due to VEGF-dependent proliferation of endothelial cells) with consequent subretinal edema and deformation of the macula which, if not treated with anti-VEGF drugs, rapidly leads to a drastic reduction in visual acuity (7,8).

The recent development of the LUUM LED™, characterized by a low emission of blue light component (similar to the one detectable in the solar spectrum) may allow to clarify the issue of the "toxicity" of the blue light component.

To this aim we set up a research project based on two major targets:

1) To verify whether, by exposing human RPE to a high, supraphysiologic dose of luminous intensity emitted by a conventional LED), it is possible to induce dysfunctions in viability, VEGF synthesis and CAIX (a marker of cellular hypoxia) synthesis. The results of this experiment were compared to the ones obtained by exposing RPE to a LUUM LED set up to generate the same luminous intensity. A RPE cell culture, used as control, was kept in the dark.

2) To verify whether, by exposing human RPE to a normal, physiologic dose of luminous intensity emitted by a conventional LED), it is possible to induce dysfunctions in viability, VEGF synthesis and CAIX (a marker of cellular hypoxia) synthesis. The results of this experiment were compared to the ones obtained by exposing RPE to a LUUM LED set up to generate the same luminous intensity. A RPE cell culture, used as control, was kept in the dark.

Research protocol
Clonetics™ Human Retinal Pigment Epithelial Cell Systems by Lonza® were grown in culture according to the manifacturer instructions and finally seeded (50.000 cells/well) with completed RtEGM® (growth medium) BulletKit®. At 70–80% confluency, adherent cells were detached using versene-EDTA (Lonza), centrifuged at 1000 × g for 3 minutes, and versene-EDTA was aspirated carefully without disturbing the cell pellet followed by addition of regular cell culture media at a cell density of 1 × 106 cells/mL. Cells were then cultured on 35 mm glass bottom dishes (In Vitro Scientific) with 1 × 105/mL cell density.
To culture the cells under LED light, two dome-shaped structures were used. In each one the LED light was placed underneath. The experiments were conducted in blind.

Project 1: High luminous intensity. For every experiment 3 identical H-RPE cell cultures were used. One was kept in dark condition and was used as a control, one was exposed to conventional blue LED light (set at 25000 lux, x 20 normal illuminance in a working area), and the third one was exposed to Luum LED (set at 25000 lux, x 20 normal illuminance in a working area).
To test at which condition (in term of duration of exposure) the H-RPE cells started to suffer, the same experiment was repeated changing the incubation period. Test at 13, 24, 48 and 72 hours were conducted. Once the determined time of incubation was completed, cell cultures were washed 1 time with a physiologic solution and fixed with formalin (pH 7.4) for 10 min at room temperature. Cell suffering was determined by immunofluorescence looking for the synthesis of two specific markers: VEGF, the factor secreted by stressed RPE that has been involved in the pathogenesis of AMD and DME (using an Anti VEGF rabbit polyclonal Ab, Abcam®) and CAIX, an established marker of cellular hypoxia (Anti CAIX- mouse monoclonal Ab, Santa Cruz Biotechnology®).
Cell viability at the end of the experiment was verified by means of the LIVE/DEAD® Viability/Cytotoxicity Assay Kit by Molecular Probes. The kit is based on the simultaneous determination of live and dead cells with two probes that measure recognized parameters of cell viability: intracellular esterase activity and plasma membrane integrity.
To verify the cause of death of RPE, the apoptosis TUNEL assay was performed (Boehringer–Mannheim, Indianapolis, USA).

Project 2: Normal luminous intensity. For every experiment 3 identical H-RPE cell cultures were used. One was kept in dark condition and was used as a control, one was exposed to conventional LED light (set at 250 lux, the normal illuminance in a working area), and the third one was exposed to Luum LED (set at 250 lux, the normal illuminance in a working area).
Cell cultures were exposed to LED lights or kept in the dark for 35 days and then studied as described for Project 1.

Results
During the study the human RPE did not have any problem in growing and there was no infection of the cell cultures.

Project 1: High luminous intensity.
As shown in Figure 1, after 24 hours of exposure of RPE to high luminous intensity there were no signs of cell stress and the expression of the two stress markers considered, i.e. VEGF and CAIX was similar in cells grown in the dark and under blue LED and Luum LED.

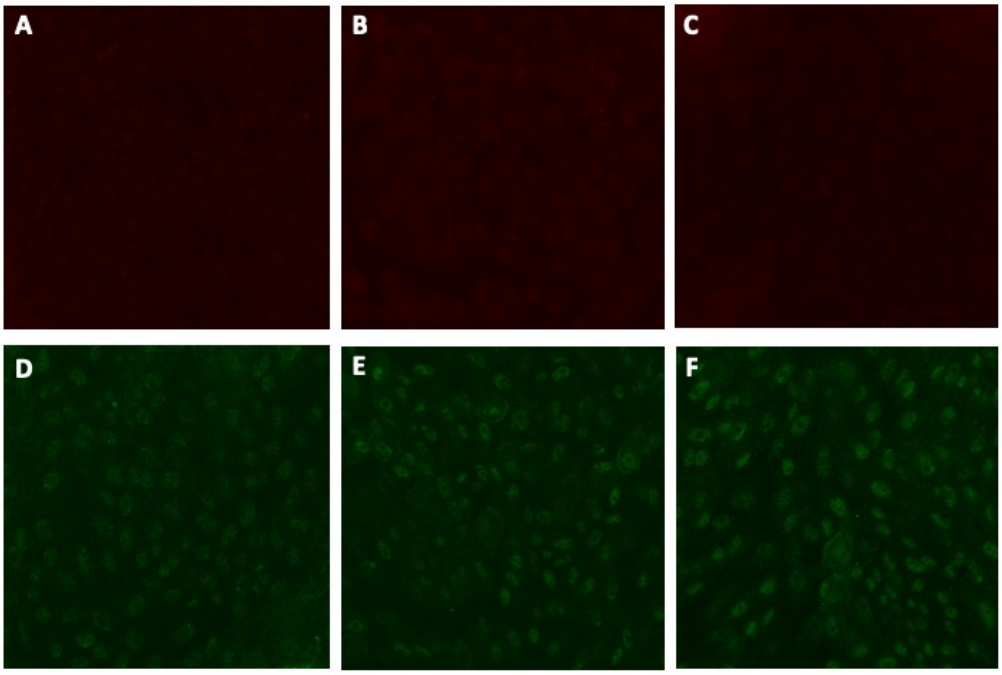

Figure 1. *Effect of LED light (24 hours exposure) on human pigmented epithelial cells in culture* ***Panel A:*** *VEGF expression in control cells (dark).* ***Panel B****: VEGF expression in cells exposed to blue LED.* ***Panel C****: VEGF expression in cells exposed to Luum LED.* ***Panel D:*** *CAIX expression in control cells (dark).* ***Panel E****: CAIX expression in cells exposed to blue LED.* ***Panel F:*** *CAIX expression in cells exposed to Luum LED.*

Forty-eight hours of exposure of RPE to high luminous intensity, as shown in Figure 2 gave rise to a significant increase of both VEGF and CAIX expression in cells exposed to blue LED when compared to both Luum LED and the dark. A result suggesting a strong suffering of the cells specifically induced by the blue component of light present in the conventional LED

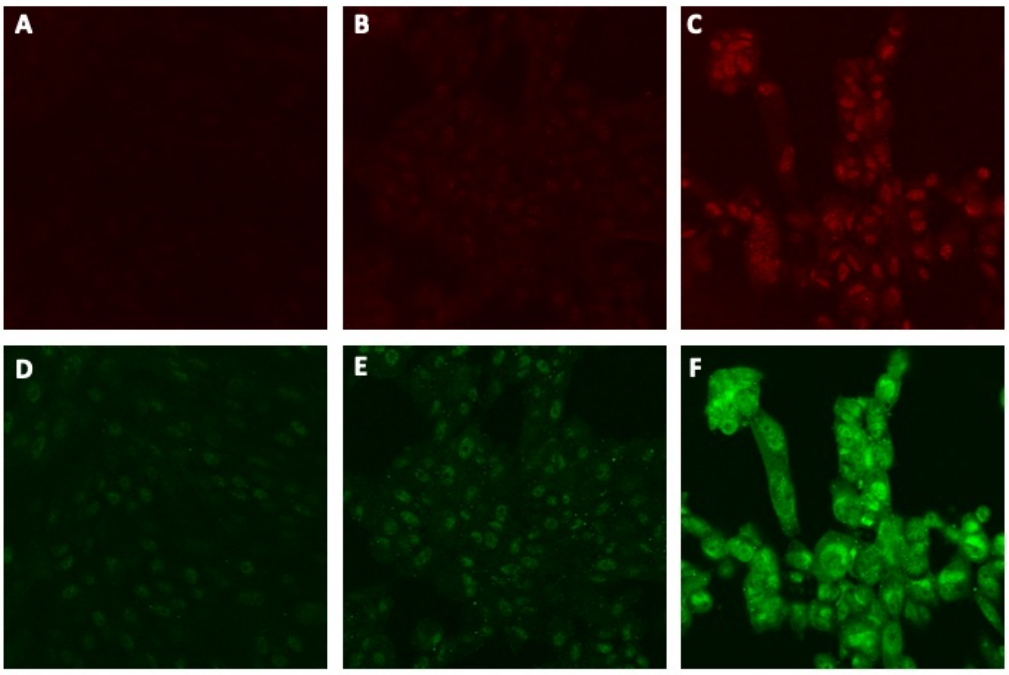

Figure 2. *Effect LED light (48 hours exposure) on human pigmented epithelial cells in culture.* ***Panel A****: VEGF expression in control cells (dark).* ***Panel B:*** *VEGF expression in cells exposed to blue LED.* ***Panel C:*** *VEGF*

expression in cells exposed to Luum LED. ***Panel D****: CAIX expression in control cells (dark).* ***Panel E:*** *CAIX expression in cells exposed to blue LED.* ***Panel F****: CAIX expression in cells exposed to Luum LED.*

This finding was confirmed by the evidence that the number of dead RPE cells was significantly increased among the cells exposed to blue LED when compared to both Luum LED and the dark (Figure 3)

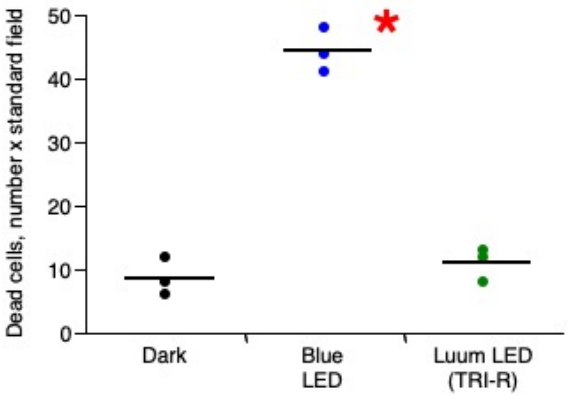

Figure 3. *The number of dead epithelial pigmented cells after 48 hours of culture under blue LED was significantly increased when compared to cells grown for 48 hours in the dark (*p<0.0001) and to cells grown for 48 hours under Luum LED (*p>0.0001).*

Of interest, the Tunel assay confirmed the increased mortality in case of exposure to the blue LED, the protection induced by the Luum LED and demonstrated that the death of RPE was the consequence of apoptosis.

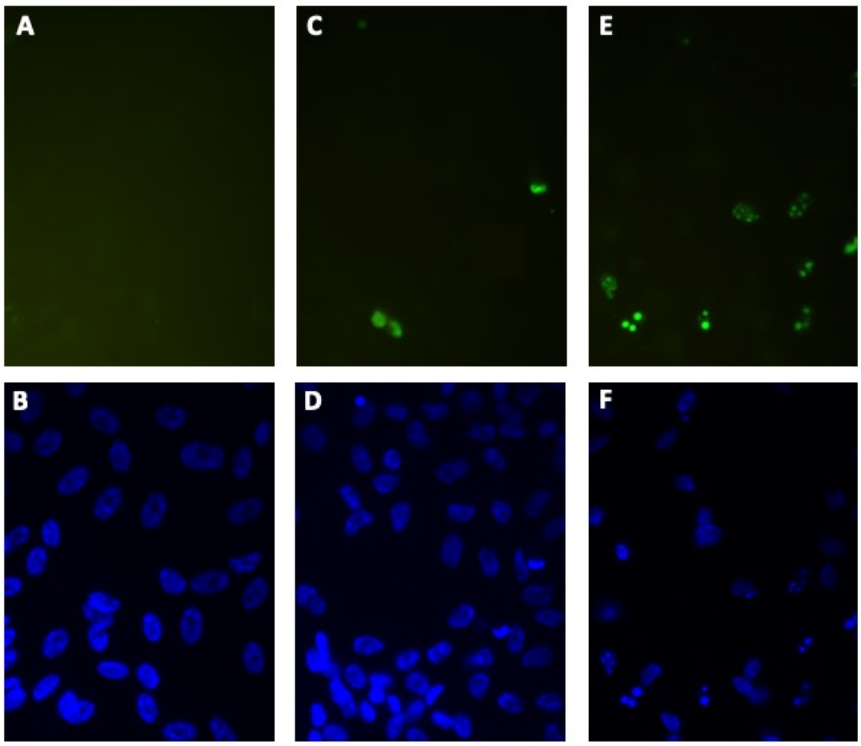

Figure 4. *Effect LED light (48 hours exposure) on the apoptosis of human pigmented epithelial cells in culture as evaluated by Tunel assay.* ***Panel A:*** *Apoptosis among control cells (dark).* ***Panel B:*** *Distribution of nuclei of control cells as seen by DAPI staining.* ***Panel C:*** *Apoptosis among cells exposed to Luum LED.* ***Panel D:***

Distribution of nuclei of cells exposed to Luum LED as seen by DAPI staining. ***Panel E:*** *Apoptosis among cells exposed to blue LED.* ***Panel F:*** *Distribution of nuclei of cells exposed to blue LED as seen by DAPI staining.*

Project 2: Normal luminous intensity.

After exposure of RPE to normal luminous intensity for 35 days, no change in cell mortality could be demonstrated in cells exposed to blue LED, to Luum LED or to the dark, as shown in Figure 5.

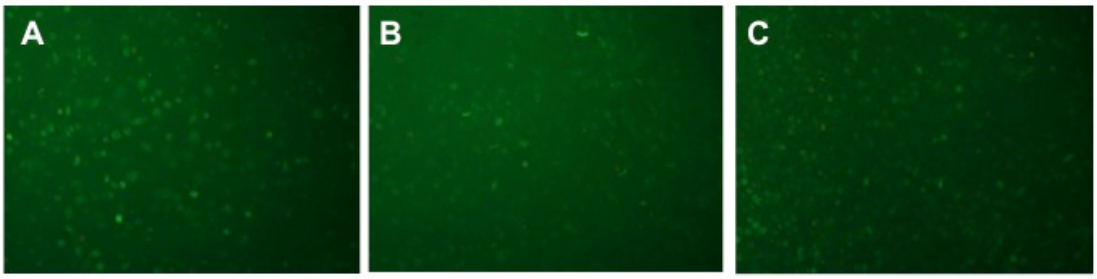

Figure 5. *Viability of RPE under the different experimental conditions.* ***Panel A:*** *Substantial absence of dead cells in RPE culture grown in the dark.* ***Panel B:*** *Substantial absence of dead cells in RPE culture grown under the blue LED.* ***Panel C:*** *Substantial absence of dead cells in RPE culture grown under the Luum LED.*

Despite the evidence that the mortality of RPE does not differ in the different experimental conditions, the synthesis of VEGF, a known stimulus of angiogenesis in case of both AMD and DME was significantly increased in RPE exposed for 35 days to blue LED when compared to dark and to Luum LED as shown in Figure 6

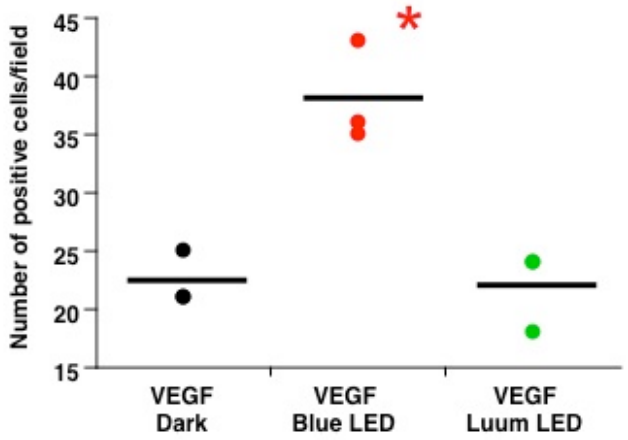

Figure 6. *The number of epithelial pigmented cells expressing VEGF after 35 days of culture under blue LED was significantly increased when compared to cells grown for 35 days in the dark (*p<0.0001) and to cells grown for 35 days under Luum LED (*p>0.0001).*

Similarly, also in case of the hypoxia marker CAIX, the synthesis was significantly increased in RPE exposed for 35 days to blue LED when compared to dark and to Luum LED as shown in Figure 7

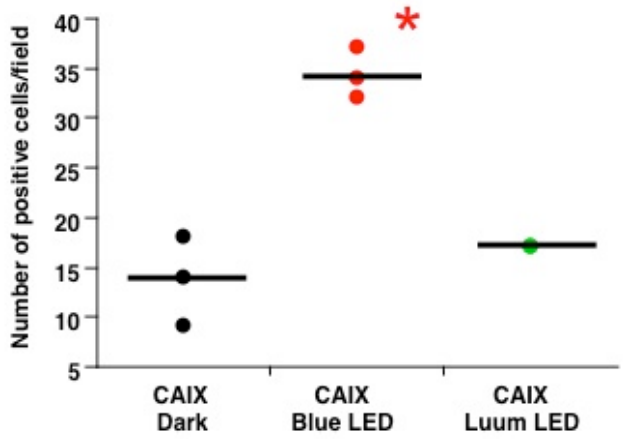

Figure 7. *The number of epithelial pigmented cells expressing CAIX after 35 days of culture under blue LED was significantly increased when compared to cells grown for 35 days in the dark (*p<0.0001) and to cells grown for 35 days under Luum LED (*p>0.0001).*

Conclusions

RPE are the cells directly involved in the activation of angiogenesis in case of AMD and DME. Hypoxia is considered as the major cause of RPE stress with consequent overproduction of VEGF and development of retinal neovascularization.

The results of this study demonstrates for the first time that exposure of human RPE to high intensity of the conventional blue LED results directly in the increased death of these cells and in the activation of the VEGF and CAIX pathways.

When the intensity of the blue LED is decreased to normal levels, the death rate is normalized but the signs of stress, in particular the increased synthesis of VEGF and CAIX, are maintained.

Of interest, the use of the Luum LED characterized by a blue light component similar to the one present in the solar spectrum, not only prevent death in case of high luminous intensity, but also prevent the increased synthesis of VEGF and CAIX in case of normal luminous intensity.

Taken together the results of this study demonstrate that, when compared to the conventional blue LED, the Luum LED protects from the acute and chronic effect of the blue light component on RPE in culture and could, at least in principle, exert the same beneficial effect also on RPE and retinal neurons present in the human eye.

References

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