IMPACT OF BLUE LIGHT EXPOSURE ON PREMATURE SKIN AGEING

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Abstract

Blue light, thanks to its relatively high energy and long wavelength, can penetrate deeper into the skin layers and reach the dermis. Studies have shown that blue light induces reactive oxygen species, reduces the intracellular antioxidative defences and impairs the proliferative capacity of cells.

In the present study, we assessed, *in vitro*, the effect of blue light exposure (415nm) on normal human skin fibroblasts. Reactive oxygen species (ROS) were quantified with dichlorofluorescin diacetate (DCFH-DA) assay. Expression of genes involved in antioxidant enzymes and extracellular matrix (ECM) synthesis were studied through RT-PCR. Finally, cell migration was assessed on monolayer fibroblasts through wound-healing assay using silicone inserts with a defined cell-free gap.

The results showed that blue light exposure induced the production of intracellular ROS and down-regulated the expression of main antioxidant enzymes. Other keys components of extracellular matrix were also regulated, as a clear under expression of several types of collagens. In contrast, the expression of MMP1, MMP3 and MMP12 were highly up-regulated. Blue light exposure also induced a clear slowdown of fibroblasts migration compared to non-exposed cells.

Based on these results, we highlighted that blue light reduced the antioxidative defenses of fibroblasts. It also decreased the synthesis of keys components of the extracellular matrix and disturbed the normal wound-healing process by fibroblasts. Thus, we can conclude that blue light exposure impairs the homeostasis of the dermis which could contribute to premature skin ageing.

Keywords : Blue light ; Fibroblasts ; Cell migration; Premature skin ageing

Introduction

The human skin is constantly exposed to sunlight. Solar radiation reaching the earth surface includes ultraviolet radiation (290-400nm), visible light (400-760nm) and infrared radiation (760nm to 1mm). Visible light constitutes a very important part of the sun light spectrum, about 50% of the solar radiation reaching the earth surface, where blue light is emitted in wavelengths between 400 and 500nm. From this high energy, blue light can penetrate deeper into the skin layers and reach the dermis.

Studies have especially shown that blue light induces reactive oxygen species, reduces the intracellular antioxidative defences and impairs the proliferative capacity of cells [1, 2, 3]. More recently, it has been shown that blue wavelengths create genotoxic lesions, both oxidative and cyclobutane-pyrimidine-dimer DNA lesions are generated [4].

In this study, we assessed, *in vitro*, the effect of blue light exposure (415nm) on normal human fibroblasts. Reactive oxygen species (ROS) were quantified with dichlorofluorescin diacetate (DCFH-DA) assay. Expression of genes involved in antioxidant enzymes and extracellular matrix (ECM) synthesis were studied through RT-PCR. Finally, cell migration capacity was measured by a monolayer wound-healing assay.

Materials and Methods

Blue light irradiation

Blue Light irradiation was performed with a BioLambda Blue Light Irradiator (BioLambda) emitting a wavelength to 415nm, with an irradiance of 8mW/cm².

Intracellular ROS measurements

Normal human skin fibroblasts were incubated with dichlorofluorescin diacetate (DCFH-DA) for 30 min and then exposed to several doses of irradiation. The emitted fluorescence was recorded with a fluorescence plate reader equipped with the excitation filter at 485nm and the emission filter at 520nm. The results were expressed as the dichlorofluorescein fluorescence increase and compared to the non-irradiated cells. Results were compared using a Student's t test, a p value < 0.05 was considered statistically significant.

Gene expression

Normal human skin fibroblasts were exposed to blue light irradiation. After 24 hours of incubation, total RNA were extracted with NucleoMag RNA kit (Macherey-Nagel)

and quantified with a spectrophotometer at 260nm. First strand cDNA were then synthesized by using High Capacity cDNA Reverse Transcription kit. Real-Time RT-PCR reactions were carried out with the Quantstudio 7 Flex Real-Time PCR System by using array cards containing TaqMan primers and probes (Applied Biosystems) specific to each gene. Relative changes in gene expression (RQ) were calculated according to the 2-ΔΔCT method, utilizing multiple housekeeping genes. Results were compared using a Student's t-test, a p value < 0.05 was considered statistically significant.

Wound healing assay

Normal human skin fibroblasts were seeded in culture-inserts containing a defined cell-free gap and incubated at 37°C with CO_2 5%. The inserts are removed from the petri dish creating a cell-free gap. Cells were then exposed to blue light and incubated during 72hours. Cell culture images were regularly recorded by using a cell culture real-time monitoring system (Cytonote) connected to Horus software (iPrasense). The migration of the cells inside the wound is quantified by image analysis and the migration rate is calculated.

Results

Intracellular ROS production

Normal human skin fibroblasts were exposed to increasing blue light irradiation from 4.8 to 28.8 J/cm² and intracellular ROS were then quantified. Whatever the blue light dose, a significant ROS production was observed, the highest level being reached with the highest exposure (fig.1).

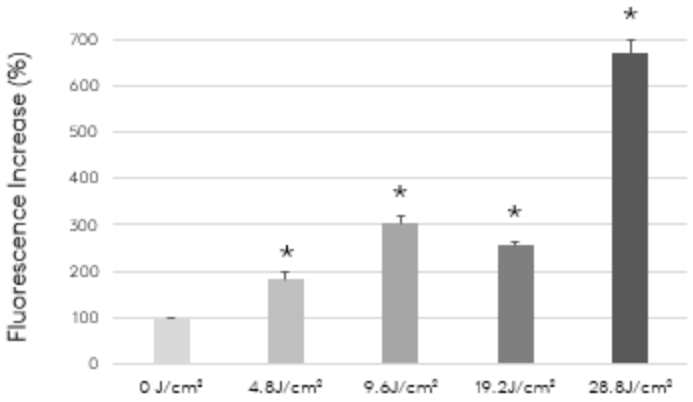


Figure 1: Intracellular reactive oxygen species generated in normal human skin fibroblasts. Results were expressed as the fluorescence increase compared to non irradiated cells (mean ± sem, n=12) * significantly different to non irradiated cells ($p<0,05$).

Gene expression of antioxidant enzymes

Normal human skin fibroblasts were exposed to increasing doses of blue light from 4.8 to 28.8 J/cm² and the expression of some antioxidant enzymes were studied. Under the experimental conditions used, catalase and glutathione peroxidase (GPX1) were significantly down-regulated with the two highest blue light irradiations (fig.2).

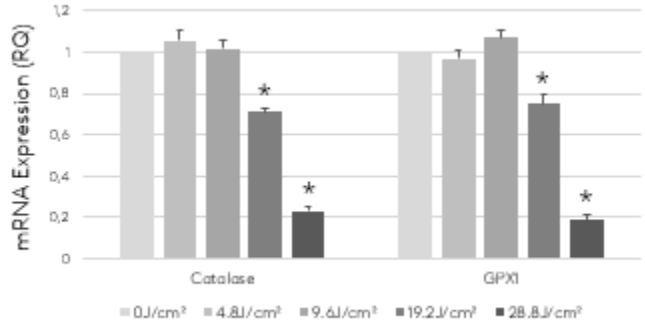


Figure 2: Gene expression of antioxidant enzymes on normal human skin fibroblasts (mean ± sem, n=5). * significantly different to non irradiated cells ($p<0,05$).

Expression of genes involved in extracellular matrix components synthesis

Normal human skin fibroblasts were exposed to blue light irradiations and various genes related to the extracellular matrix were study 24 hours later. Results showed that the expression of some keys genes involved in the collagen network were impaired.

Collagen type 5 was significantly down-regulated from the 9.6J/cm² dose. Collagen type I and collagen type 6 were also down-regulated with the two highest blue light irradiations, whereas the collagen type 3 mRNA expression was impaired whatever the dose. In these experimental conditions, the highest blue light dose, 28.8J/cm², induced a gene expression decrease about 80% of the studied collagens (fig.3).

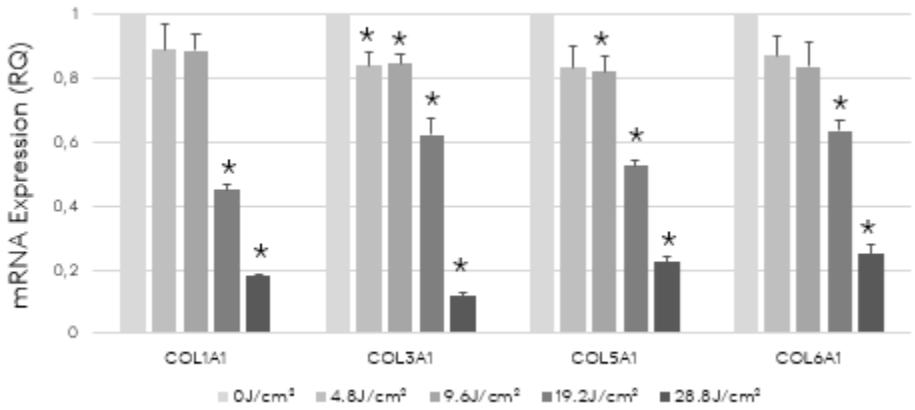


Figure 3: mRNA expression of extracellular matrix genes 24h after blue light irradiation on normal human fibroblasts (mean ± sem, n=5). * significantly different to non irradiated cells ($p<0,05$).

Moreover, the gene expression of some matrix metalloproteinases was regulated. MMP1 and MMP3 were highly up-regulated in a dose-dependent response. MMP12 was significantly increased with the two highest doses (fig.4).

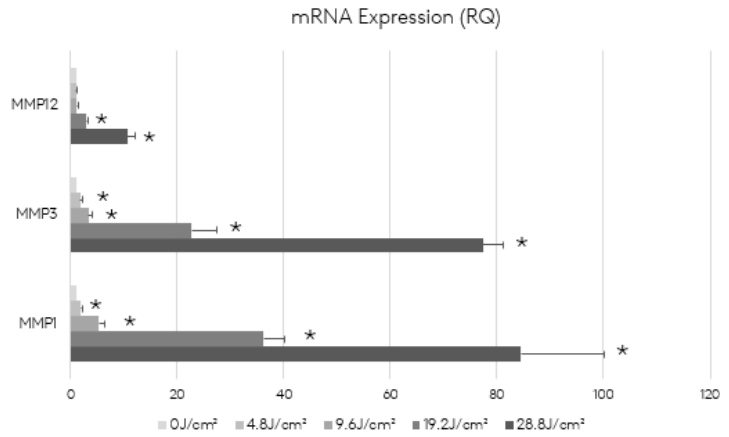
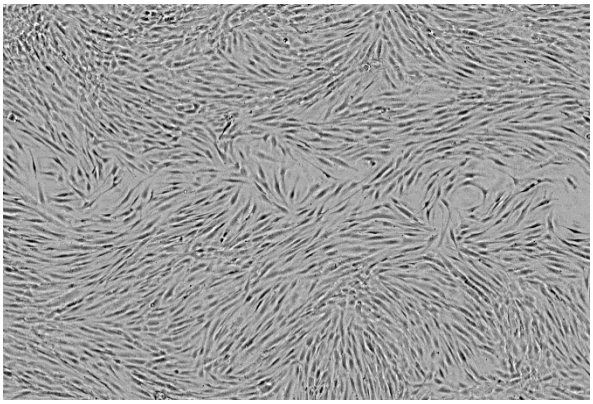


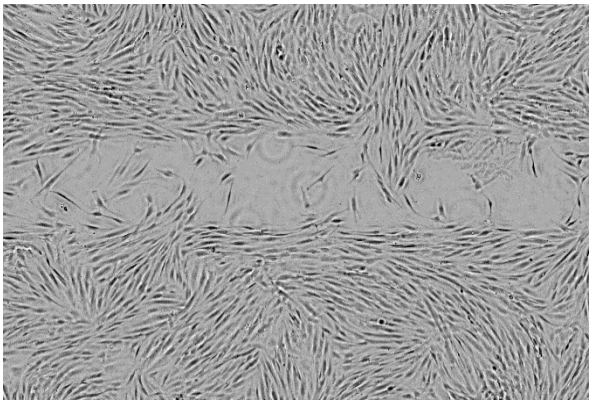
Figure 4: mRNA expression of matrix metalloproteinases on normal human skin fibroblasts (mean ± sem, n=5). * significantly different to non irradiated cells ($p<0,05$).

Cell migration

Normal human skin fibroblasts were irradiated with blue light and cell migration was recorded during 72 hours. Irradiation with blue light resulted in a clear decrease of the cell migration speed, about 2.5 lower compared to non irradiated cell (fig.5).



Non-irradiated cells



Blue light irradiated cells

Figure 5: Effect of blue light exposure on normal human skin fibroblast migration

Discussion

The objective of this study was to investigate the effect of blue light irradiation on dermal fibroblasts. Results showed that blue light exposure induced the production of intracellular ROS which are detrimental for cells. On another side, genes expression of relevant antioxidant enzymes, catalase and glutathione peroxidase, involved in the enzymatic cascade of free radicals detoxification were altered. The synthesis of the extracellular matrix was impaired, a down-regulation of fibrillar collagen type 1 and collagen type 3 was observed. Collagen type 5 and collagen type 6 expression were also reduced while they interact with several components of ECM and are essential for optimal dermal quality. In contrast, some of matrix metalloproteinases which are proven to degrade collagen were induced. Consequently, this imbalance in the dermal extracellular matrix predicting a possible impairment of the skin collagen network. Interestingly, blue light irradiation reduced the fibroblasts migration, impacting the wound healing and tissue repair.

Conclusion

The present study showed that a single blue light irradiation induced the production of intracellular ROS and reduced the antioxidative defenses of fibroblasts. The homeostasis of the extracellular matrix of the skin was altered and the wound-healing process was also disturbed. Thus, we concluded that, beside the UV induced-photo-damage, the blue light may also contribute to premature skin ageing.

Conflict of Interest Statement: NONE

References

Opländer C, Hidding S, Werners FB, Born M, Pallua N, Suschek CV (2011) Effects of blue light irradiation on human dermal fibroblasts. J Photochem Photobiol B Biol 103:118–125.

Nakashima Y, Ohta S, Wolf AM (2017) Blue light-induced oxidative stress in live skin. Free Radic Biol Med 108:300-310.

Vandersee S, Beyer M, Lademann J, Darvin ME (2015) Blue-Violet light irradiation dose dependently decreases carotenoids in human skin, which indicates the generation of free radicals. Oxid Med Cell Longev 5965:1-7.

Chamayou-Robert C, DiGiorgio C, Brack O, Doucet O (2021) Blue light induces DNA damage in normal human skin keratinocytes. Photodermatol Photoimmunol Photomed 38:69-75.