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Impact of Blue Light Exposure On Premature Skin Ageing

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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. Cell migration capacity was measured by a monolayer wound-healing assay.

Materials & 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 CO2 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 & Discussion:

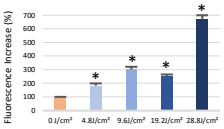

Fig 1: Intracellular ROS production. Blue light irradiation induced a significant generation of ROS.

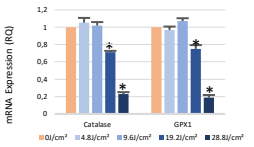

Fig 2: Gene expression of antioxidant enzymes. Catalase and glutathione peroxidase (GPX1) were significantly down-regulated with the two highest blue light irradiations.

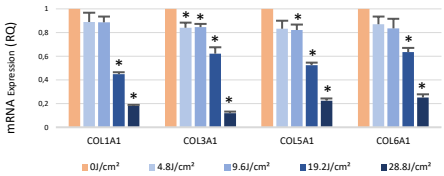

Fig 3: Genes expression of ECM components. Collagen type 5 was significantly down-regulated from the 9.6J/cm² dose. Collagen type I and collagen type 6 were also down-regulated with the two highest blue light irradiations, whereas the collagen type 3 mRNA expression was impaired whatever the dose. In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

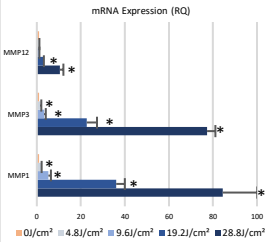

Fig 4: MMP genes expression. MMP1 and MMP3 were highly up-regulated in a dose-dependent response. MMP12 was significantly increased with the two highest doses.

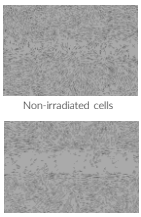

Fig 5: Cell migration. Irradiation with blue light resulted in a clear decrease of the cell migration speed, about 2.5 lower compared to non irradiated cell.

Conclusions:

The present study showed that a single blue light irradiation induced the production of intracellular ROS and reduced the antioxidative defenses of fibroblasts.
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 process and tissue repair.
Thus, we concluded that, beside the UV induced-photo-damage, the blue light may also contribute to premature skin ageing.

References:

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