THE EFFECTS OF BLUE LIGHT ON THE SKIN: CHALLENGING PREVIOUS FINDINGS?

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INTRODUCTION

The effects of blue light (BL) from sunlight or artificial sources (LED lights, televisions, laptops, digital screens, and cell phones) on the skin remain highly inconsistent in scientific literature. Studies on skin aging often associate BL with negative outcomes, such as skin damage and accelerated aging. Conversely, research focusing on photobiomodulation therapies frequently highlights positive effects.

One probable reason for this inconsistency is the significant variation in experimental designs across studies, including differences in skin models, fluences, and irradiances of BL sources. To address this, our study aimed to compare the effects of BL of different fluences (doses) and irradiances, simulating artificial light and sunlight, on selected markers and pathways associated with skin aging. The experiments were conducted using different skin models, including cell cultures of keratinocytes, fibroblasts, and melanocytes, porcine skin explants, and human skin in vivo. We selected wavelengths between 425–475 nm, with a peak at approximately 450–455 nm, corresponding to most common artificial light sources.

MATERIALS AND METHODS

Cell cultures: HaCaT keratinocytes, 3T3 Swiss albino mouse embryonic fibroblasts, and B16-F10 mouse melanoma cells.

Porcine skin explants: Full-thickness skin specimens were excised from the inner side of the porcine cadaver ears obtained as by-products from meat production. No experiments were performed on living animals.

BL irradiation of cell cultures and skin explants: A LED tape (444-464 nm, peak 455 nm) was attached to the steel lid of an in-house developed steel box. The irradiance was adjustable between 0-16.6 mW/cm^2 and was measured before each experiment.

MTT cell viability assay: HaCaT, 3T3, and B16-F10 cells were seeded in 96-well plates at an appropriate density, irradiated with BL as described above, and cultivated for 24 h. After cultivation, standard MTT cell viability assay was performed. For the evaluation of viability of porcine epidermis, the skin specimens prepared as described above were placed into vertical Franz diffusion cells with the acceptor chamber filled with 350 µL of cell culture medium. Skin explants were irradiated with BL and incubated for 18 h at 37 °C in a humidified atmosphere. After incubation, the skin samples were washed with PBS and the exposed epidermis was separated from the dermis by heat shock (90 s in 60 °C water) and transferred to a 24-well

plate. The epidermis was incubated with 1 mg/mL MTT in PBS for 3 h at 37 °C and then with the solubilizing solution for 2 h at RT with shaking. The resulting solution was transferred to a 96-well plate for the absorbance (570 and 690 nm) measurement.

Evaluation of late apoptotic and necrotic cells: HaCaT cells seeded in 24-well plates were irradiated with BL and cultivated for 24 h. After incubation, Hoechst 33342 and propidium iodide (PI) were added to the cell culture medium to final concentrations of 20 μM and 10 μM, respectively. The cells were then examined using a fluorescent microscope.

Determination of ROS: HaCaT cells seeded in a 24-well plate were treated with 10 μM DCFH-DA in PBS for 30 min at 37 °C in the dark. After incubation, the cells were washed twice with PBS, which was then replaced with cell culture medium for BL irradiation. The medium was replaced with PBS and the cells were observed under a fluorescent microscope.

Quantitative real-time reverse transcription PCR (qRT-PCR): HaCaT, 3T3 and B16-F10 cells were seeded at an appropriate density into 6-well plates, irradiated with BL and cultivated in fresh culture medium for 24 h. The cells were washed with PBS and the total RNA was isolated by the acid guanidine thiocyanate-phenol extraction method. Then, the standard reverse transcription reaction was performed with 1 μg of the isolated RNA followed by qPCR with specific TaqMan gene expression assays. The data were analyzed using the $2^{-\Delta\Delta Ct}$ method. RPL13A was used as a reference gene.

Determination of melanin level: For evaluation of the effect of BL on melanogenesis, B16-F10 cells were seeded in 6-well plates, irradiated with BL and cultivated in fresh culture medium for 48 h. Then, cell viability was assessed using a resazurin dye as described earlier [1]. Next, melanin content in the cells was determined colorimetrically as described previously [2]. The level of melanin was normalized to the cell viability. For the evaluation of the effect of BL on immediate pigment darkening (IPD), B16-F10 cells seeded in 6-well plates were treated with α-MSH in the cell culture medium for 48 h to stimulate melanogenesis. Then, the cells were irradiated with BL, incubated for 2 h and the resazurin cell viability assay together with melanin colorimetric assessment were carried out as described above.

In vivo study on human volunteers: The study was conducted in accordance with the WMA Declaration of Helsinki, approved by Contipro´s ethical committee, and informed consent was obtained from all participants. We enrolled 8 Caucasian subjects (6 women and 2 men) with Fitzpatrick skin type II, aged 28-44 years (average 35.8 years). The sun-exposed (outer) and sun-protected (volar) areas (Ø 4 cm) of the forearms were irradiated with BL using a Xenon arc lamp combined with a bandpass filter 450 ± 25 nm providing an irradiance of 15 mW/cm^2 simulating sunlight. A high final fluence of 120 J/cm^2 corresponding to an exposure time of 133 min. Measurements of the skin parameters: melanin and erythema indices (mexameter), TEWL (tewameter), elasticity (R2, cutometer) and photo documentation (VisiaCR) were performed before irradiation and 1, 2, 3, 4 and 7 days post-irradiation, always after 30 min of

acclimation in a room with controlled conditions. Suction-blister technique was performed to collect epidermal samples from the irradiated and non-irradiated (control) areas of the volar forearms 24 h after irradiation as described previously [3]. Samples were homogenized and processed for qRT-PCR as for porcine epidermis. Isolated RNA was used for microarray analysis (Agilent, Cy3-labeled cRNA), followed by data processing in R (quantile normalization, differential expression with limma, clustering, PCA, and GO/KEGG/UniProt enrichment via DAVID). Significance was set at Benjamini-adjusted p value < 0.05.

Statistical analysis: If not stated otherwise, the data represents mean±SEM from at least 3 independent experiments. T test was used for statistical analysis. Asterisks indicate statistically significant differences at the following levels: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

RESULTS AND DISCUSSION

Generation of ROS by BL

The generation of ROS in response to BL exposure has been reported in numerous studies [4]. ROS production has been shown to be wavelength-dependent, increasing with shorter BL wavelengths [5]. Our findings are consistent with these reports, confirming a fluence-dependent increase in ROS production in both cell cultures and porcine epidermis, with ROS generation detectable even at the lowest BL fluences tested (**Fig 1A, 1B**).

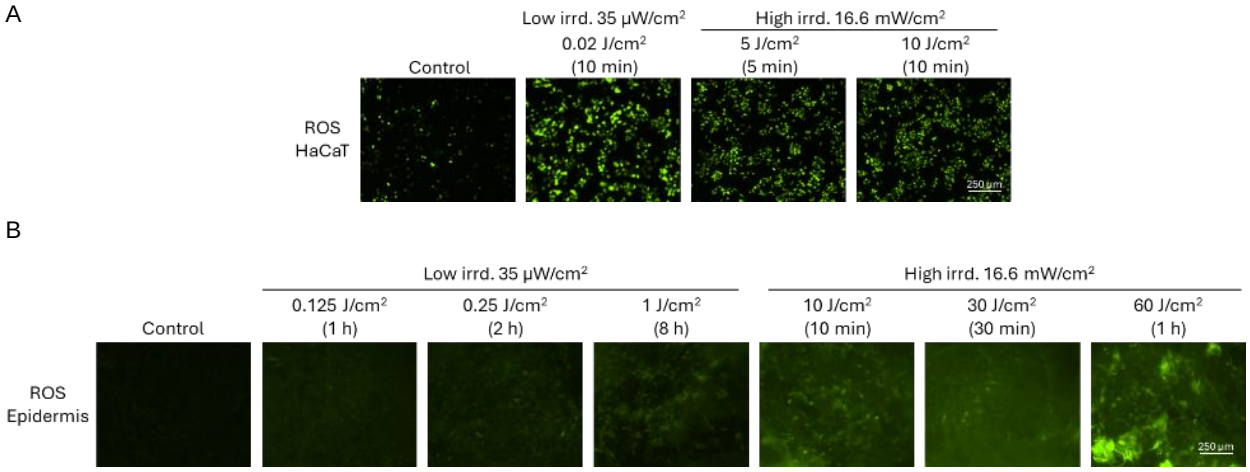

Fig 1 *Generation of ROS in (A) HaCaT keratinocytes and (B) porcine epidermis (view of the skin surface) after irradiation with BL at low irradiance (35 μW/cm²) and high irradiance (16.6 mW/cm²). ROS level was determined by DCFH-DA using fluorescence microscopy.*

The effect of BL on cell viability

Consistent with some prior studies [7], [8], [9], BL irradiation led to a fluence-dependent reduction in cell viability across HaCaT, 3T3, and B16-F10 cells (Chyba! Nenalezen zdroj odkazů.**2A**). When comparing a dose 1 J/cm² achieved either by 8h-exposure to low-intensity BL or short exposure to high-intensity BL, only slight differences were observed with low-intensity BL having more negative impact (Chyba! Nenalezen zdroj odkazů.**2B**). Fluorescence microscopy images showed no significant increase in PI-positive cells in HaCaT cells after

irradiation, suggesting that the observed decrease in viability was not primarily due to apoptosis or necrosis (Chyba! Nenalezen zdroj odkazů.**3**). No significant changes were detected in porcine epidermis, even at the highest fluences tested (**Fig 4**).

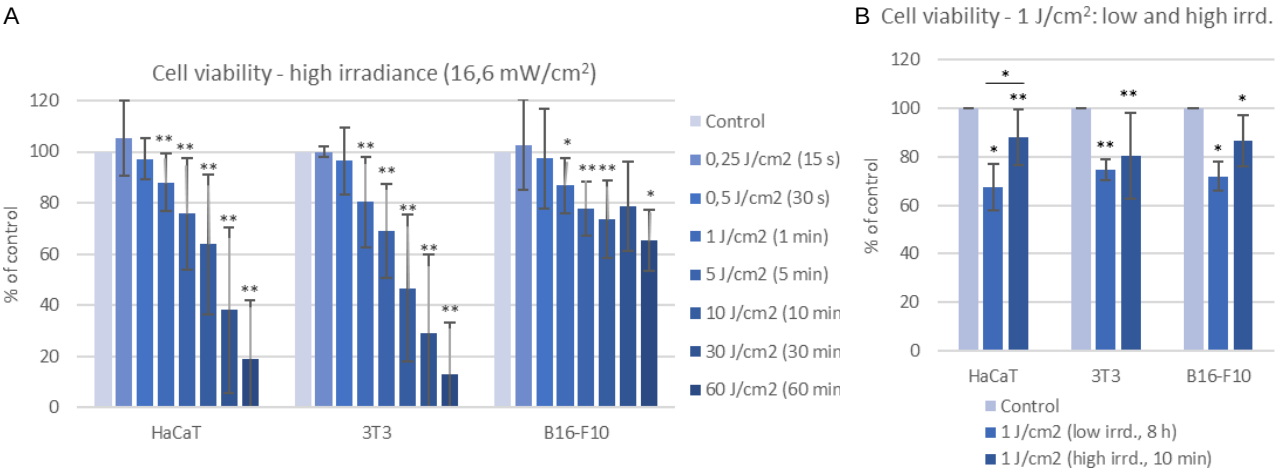

Fig 2 *Cell viability of HaCaT, 3T3 and B16-F10 cells irradiated with BL and incubated for 24 h. Cell viability was determined by MTT assay. (A) Irradiation with BL at high irradiance (16.6 mW/cm²). (B) Comparison of irradiation with BL of the same fluence 1 J/cm² BL but at a high (16,6 mW/cm²) or low (35 µW/cm²) irradiance, and a respective short (1 min) or long (8 h) exposure time.*

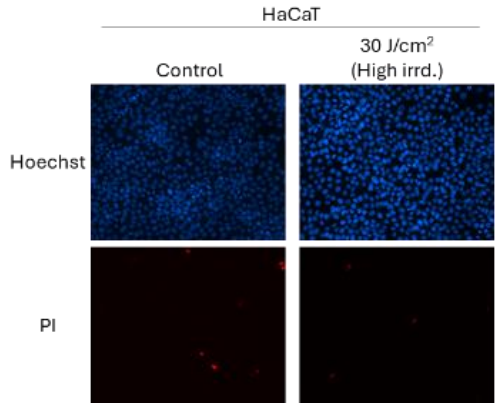

Fig 3 *Evaluation of late apoptosis and necrosis in HaCaT cells irradiated with 30 J/cm² of BL at high irradiance (16.6 mW/cm²) and incubated for 24 h. Cell nuclei are stained with Hoechst (blue); cells with increased membrane permeability are stained by propidium iodide (PI, red).*

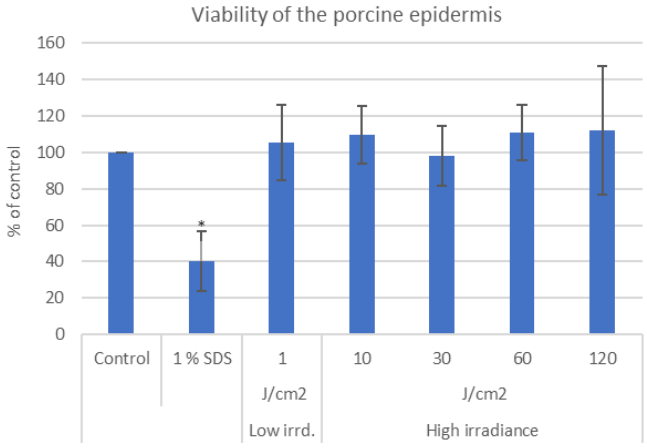

Fig 4 *Cell viability of the porcine epidermis irradiated with BL at low (35 µW/cm²) and high irradiances (16.6 mW/cm²). 1 % SDS was used as a cytotoxic control. Cell viability was determined 24 h after BL irradiation or SDS treatment by MTT assay.*

Gene expression analysis of selected genes

For gene expression analysis in HaCaT keratinocytes, 3T3 fibroblasts and B16-F10 melanocytes, we used a non-cytotoxic BL fluence of 1 J/cm² delivered either via prolonged (8 h) exposure to low-irradiance BL (35 µW/cm²) or a short (1 min) exposure to high-irradiance BL (16.6 mW/cm²) mimicking artificial BL sources and sunlight, respectively. The results are shown in **Fig 5**. Generally, prolonged expression to low-intensity BL had a more pronounced impact on the cells increasing markers of oxidative stress (antioxidant enzyme *SOD2*), pro-inflammatory response (cytokines *IL6*, *IL8* and *TNF*), cell differentiation (filaggrin *FLG*, occludin

OCLN), activation of the aryl hydrocarbon receptor (cytochrome P450, *CYP1A1*), collagen degradation (collagenase *MMP1*) and melanogenesis (tyrosinase, *TYR*). On the other hand, short exposure to high-intensity BL had only limited effect causing upregulation of *SOD2* and a slight decrease in differentiation markers and *VEGFA*, a marker of angiogenesis. No effect on collagen (*COL1A1*) was observed.

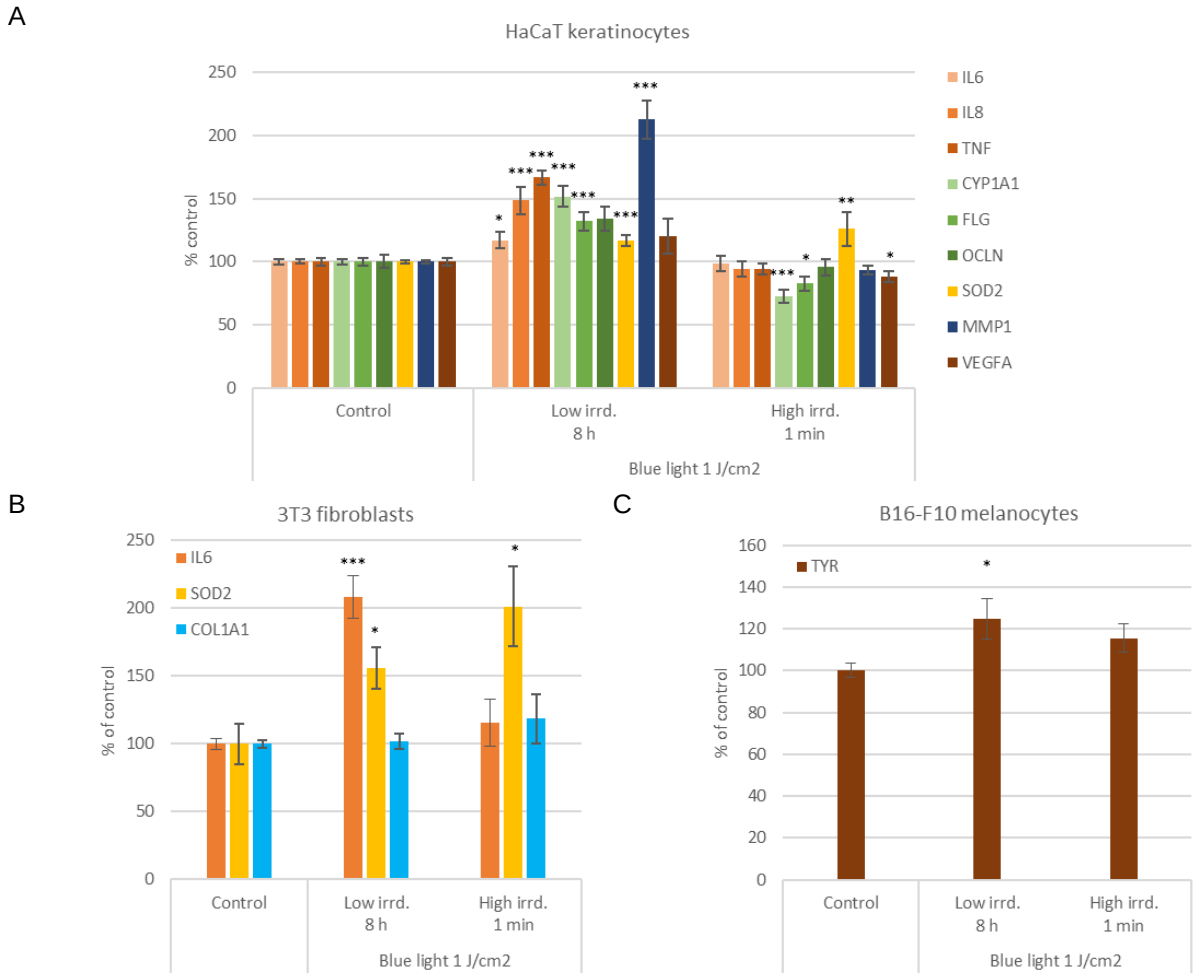

Fig 5. *Gene expression analysis in HaCaT keratinocytes (A), 3T3 fibroblasts (B) and B16-F10 melanocytes (C) irradiated with 1 J/cm² BL at low irradiance (35 µW/cm²) and prolonged (8 h) exposure time, or at high irradiance (16.6 mW/cm²) and shorter exposure time (1 min), and post-incubated for 24 h. Gene expression was determined by qRT-PCR.*

In porcine epidermis, skin samples were irradiated with 1 J/cm² at low irradiance for 8 h, and with 60 J/cm² or 120 J/cm² at high irradiance for 1 h or 2 h, respectively, and the same genes as in HaCaT keratinocytes were analyzed (**Fig 6**). A non-significant upregulation of *SOD2* confirmed ROS production and oxidative stress. Pro-inflammatory cytokines showed divergent responses: *IL6* was downregulated under all conditions, while *TNF* was reduced only after exposure to 120 J/cm². In contrast, high-intensity blue light induced *IL8* upregulation. Differentiation markers showed no consistent pattern: low-intensity irradiation downregulated *CYP1A1* and *FLG2*, with no effect on *OCLN*. High-intensity exposure upregulated *CYP1A1* and *OCLN*, while *FLG2* was downregulated. *MMP1*, associated with collagen degradation,

was significantly upregulated under all conditions. Additionally, low-intensity irradiation increased *VEGFA* expression, whereas high-intensity exposure had no effect.

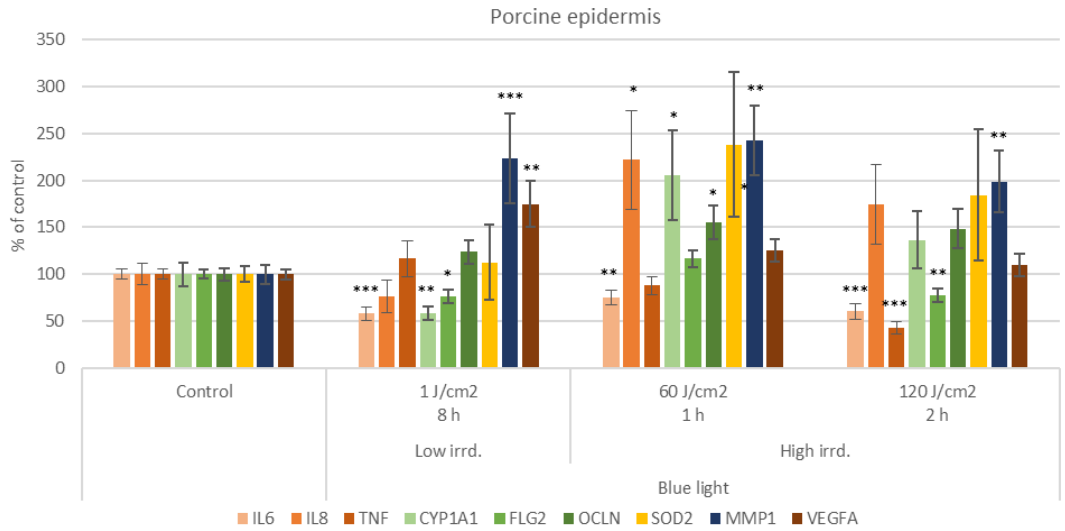

Fig 6 *Gene expression analysis in porcine epidermis irradiated with 1 J/cm2 BL at low irradiance (35 µW/cm2) and prolonged (8 h) exposure time, or with 60 J/cm2 and 120 J/cm2 at high irradiance (16.6 mW/cm2) for 60 min or 120 min, respectively. Gene expression was determined by qRT-PCR.*

Melanin production

The stimulation of melanogenesis was evaluated in B16-F10 melanocytes 48 h after irradiation with BL at both low (35 µW/cm^2) and high (16.6 mW/cm^2) irradiances (**Fig 7A**). A decreasing trend was observed under all BL conditions though the changes were not statistically significant. We also showed immediate pigment darkening (IPD), as shown by a significantly higher level of melanin in the α-MSH stimulated cells irradiated with BL compared to non-irradiated α-MSH controls (**Fig 7B**). Given the previously observed inhibitory effect of BL on melanogenesis, we propose that the increase in melanin levels following the 1-h BL irradiation reflects the oxidation of melanin precursors (IPD) rather than enhanced melanin synthesis.

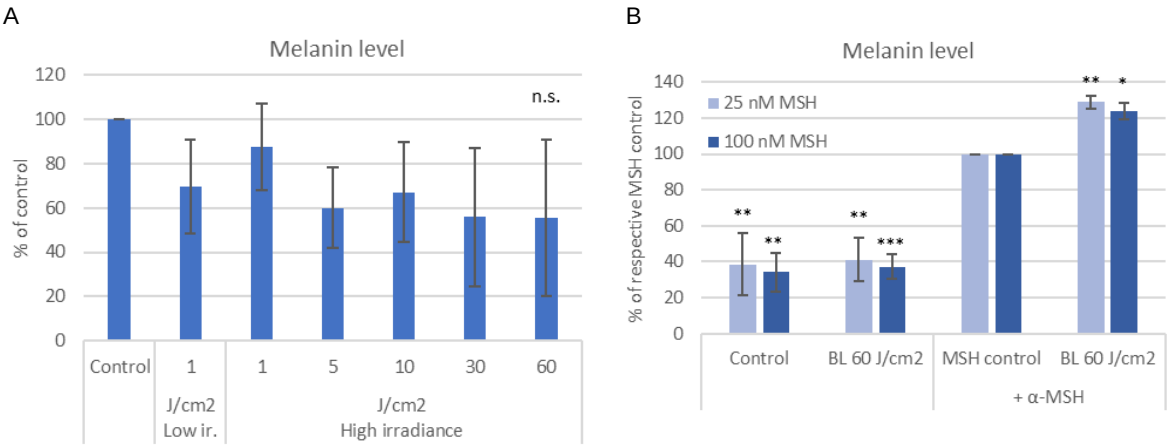

Fig 7 *Melanin level in B16-F10 melanocytes. (A) B16-F10 melanocytes were first irradiated with BL at low irradiance (35 µW/cm^2), and high irradiance (16.6 mW/cm^2) and then incubated for 48 h to evaluate the effect of BL on melanogenesis. (B) B16-F10 melanocytes were first treated with α-MSH (25 nM and 100 nM) for 48 h to stimulate melanin production, then they were irradiated with 60 J/cm^2 BL at high irradiance (16.6 mW/cm^2) and post-incubated for 2 h to evaluate immediate pigment darkening. Melanin level was determined colorimetrically and normalized to the cell viability measured by resazurin.*

In vivo study on human volunteers

Visual assessment of both sun-protected and sun-exposed forearms revealed no or only very faint skin darkening after BL irradiation (**Fig 8**). However, the melanin index, measured using a mexameter, showed a slight but significant increase up to 4 days post-irradiation, which then decreased close to baseline levels by day 7 (**Fig 9A**). No visible signs of erythema were observed on either sun-protected or sun-exposed skin upon visual evaluation (**Fig 8**). However, the erythema index, determined by a mexameter, showed a slight but significant increase in the sun-protected area at all measured timepoints post-irradiation (**Fig 9B**). On the sun-exposed area, the increase in erythema index was transient, being detected only 24 h after BL irradiation. This finding suggests a possible protective role of melanin in tanned, sun-exposed skin. A non-significant increasing trend in transepidermal water loss (TEWL) was noted in the sun-protected area (**Fig 9C**) suggesting a potential, though mild, attenuation of the skin barrier function upon BL irradiation. Interestingly, an increase in skin elasticity was observed in the sun-exposed forearm skin following BL irradiation (**Fig 9D**). No significant changes in skin hydration, epidermal thickness or collagen level were observed in either sun-protected or sun-exposed areas following BL irradiation (data not shown).

Gene expression analysis showed upregulation, though non-significant, of pro-inflammatory cytokines, CYP1A1 and MMP1 suggesting a subacute inflammatory response (**Fig 9E**). No significant changes in markers of proliferation (Ki-67) and DNA damage (γ-H2AX) were observed in histological preparations (data not shown).

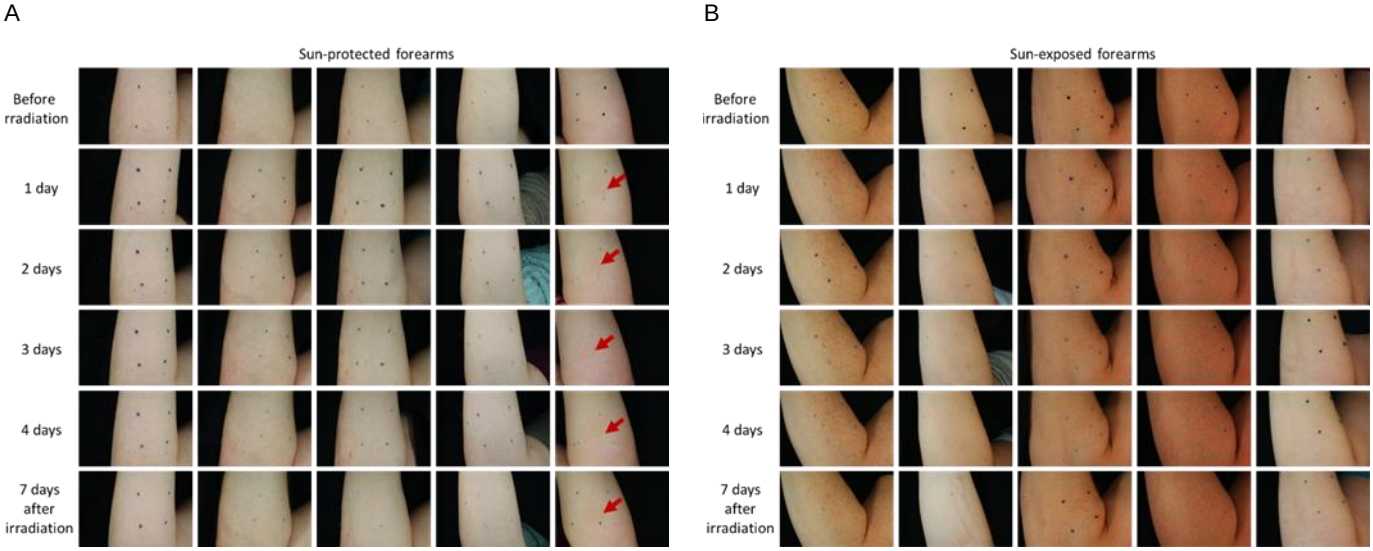

Fig 8 *Representative images of the sun-protected (A) and sun-exposed (B) forearm skin of human volunteers after irradiation with 120 J/cm2 BL. Faint skin darkening is marked with red arrows.*

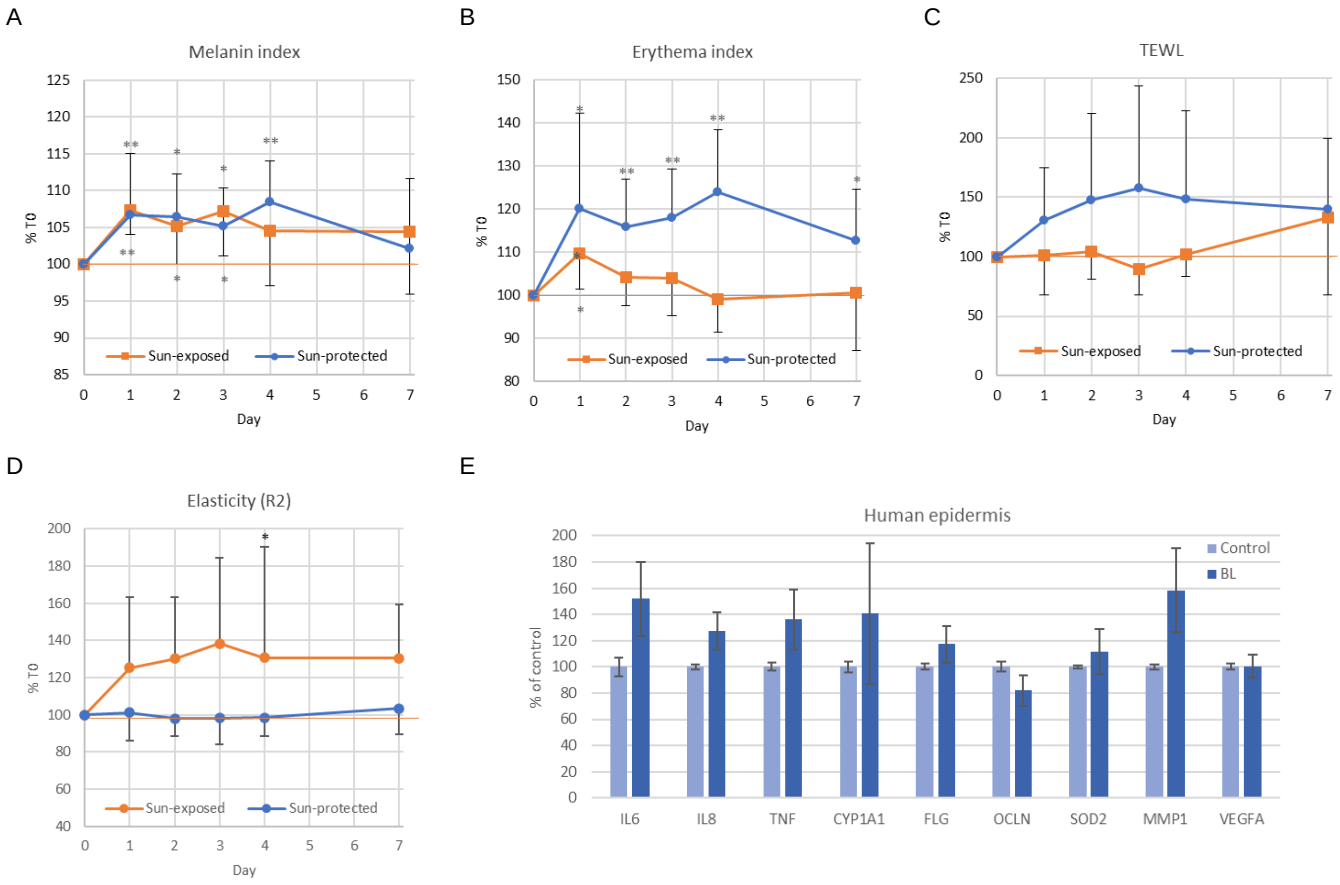

Fig 9 *Selected skin parameters after irradiation of the sun-protected and sun-exposed forearm skin with 120 J/cm² BL. (A) Melanin index, (B) erythema index, (C) TEWL, (D) skin elasticity (parameter R2). (E) Gene expression determined in the epidermal samples obtained by suction-blistering 24h after BL irradiation by qRT-PCR.*

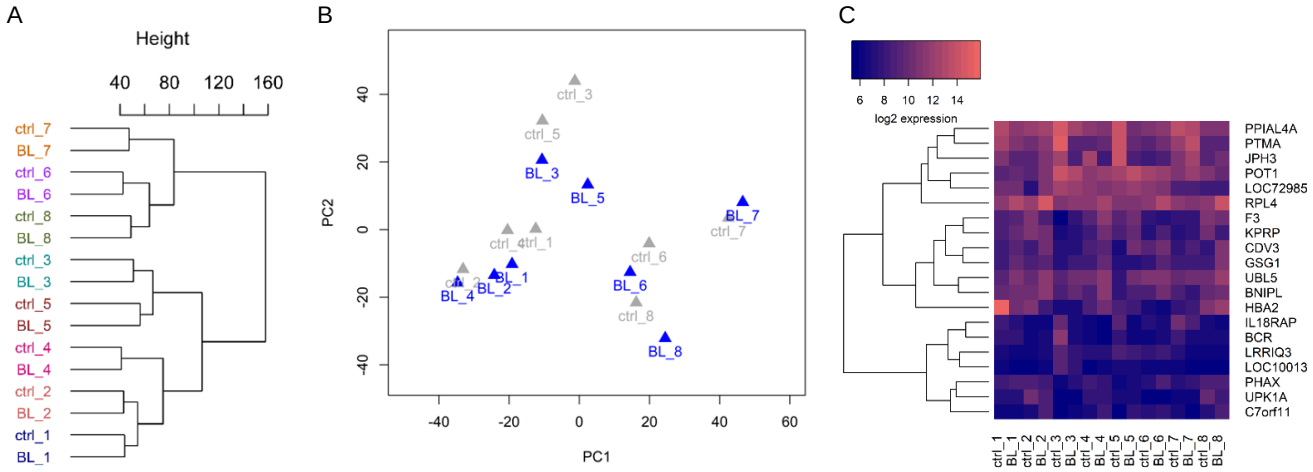

Fig 10 *Microarray analysis in the human epidermis 24 h after 120 J/cm² BL irradiation. (A) Hierarchical clustering analysis, (B) PCA plot of the first and second principal components (PC1, PC2) of gene expression profiles, (C) Heatmap of the 20 genes most influential in PC2*

To gain an overview of the trends in gene expression data, hierarchical clustering was performed (**Fig 10A**). The clustering revealed that samples grouped within each individual pair (irradiated and non-irradiated), indicating that BL treatment did not result in substantial changes to overall gene expression and that inter-subject variability remained predominant.

Reduction of dimensionality via principal component analysis (PCA) further confirmed that gene expression profiles were similar within each individual pair (**Fig 10B**). While no clear trend was observed along the first principal component (PC1), BL irradiation appeared to reduce the second principal component (PC2) consistently within each pair. Analysis of the 20 most influential genes contributing to PC2 revealed that their expression changed in most subjects, though often in opposite directions between individuals (**Fig 10C**). These findings underscore the high degree of individuality in response to BL and suggest that BL irradiation may induce variable effects among subjects.

To further explore the effects of BL, pathway and gene ontology (GO) analyses were performed on 103 most upregulated genes. We observed enrichment in terms: Ribonucleoprotein, RNA binding, Methylation, Acetylation, Citrullination, Phosphoprotein, Nucleosomal DNA binding, Protein binding, Extracellular exosome, Cytoplasm, and Cadherin binding. In the case of 104 most downregulated genes we revealed significant suppression of pathways associated with Oxygen transport, Hemoglobin complex, and Heme binding, Hydrogen peroxide catabolism, and Peroxidase activity. No significant changes were observed in pathways involved in inflammation, melanogenesis, xenobiotics metabolism, steroid hormone synthesis, and collagen synthesis or degradation observed in previous studies [8], [12], [13], [14].

CONCLUSION

In summary, BL induces relatively mild effects strongly depending on the experimental model, fluence, irradiance, and exposure duration. In this study, we confirmed ROS production even at the lowest doses accompanied by upregulation of defensive antioxidant mechanisms (*SOD2*). Cell-based models were generally more sensitive than ex vivo or in vivo skin; viability decreased in a dose-dependent manner due to reduced proliferation, without signs of apoptosis or necrosis. Notably, low-intensity, long-term exposure had a more pronounced effect than short-term, high-intensity exposure, inducing pro-inflammatory cytokines, MMP1, and differentiation markers.

In ex vivo and in vivo skin, no significant changes in viability or proliferation were observed. Instead, only mild immunomodulatory effects emerged, with the long-term consequences remaining unclear. However, in vivo exposure to a high fluence of intense BL simulating sunlight elicited a weak pro-inflammatory response, including cytokine and MMP1 upregulation, accompanied by a slight erythema index increase – potentially contributing to skin damage or premature aging. The inflammatory effects of BL remain highly controversial, with studies demonstrating both pro-inflammatory cytokines upregulation [9], [15], [16], [17] and anti-inflammatory responses [10], [18].

None of the tested doses induced melanogenesis in vitro despite slight tyrosinase upregulation, but IPD was observed. No visible pigmentation occurred in vivo, although a minor

melanin index increase was recorded, likely attributable to IPD. Our study focused on phototype II, other studies suggest that darker skin types may exhibit visible pigmentation upon BL exposure [19].

The effects of BL on collagen synthesis also remain poorly understood as both upregulation [9], [10] and downregulation [8], [9] of its synthesis has been observed together with MMP1 increased [8], [20] or unchanged levels [8].

The effects of BL on angiogenesis remain controversial including our study, with studies reporting pro-angiogenic responses [10], [21], [22], while others observed opposite results [23].

Microarray analysis showed only modest overall effects on the gene expression and high inter-subject variability even with the highest tested fluence with high-intensity BL. Further studies are needed to determine whether these molecular alterations have long-term physiological impacts or are part of a transient stress response.

Given the current state of knowledge, individual skin responses to BL cannot yet be reliably predicted. Therefore, we recommend a cautious approach and suggest preventive protection against BL exposure. A deliberate exposure to BL in the context of photobiomodulation therapies may not be advisable.

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