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Prospects for using laser fluorescence spectroscopy and optical oximetry for an objective assessment of the minimal erythema dose

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ABSTRACT

Minimal erythema dose (MED) is the amount of ultraviolet (UV) radiation needed to induce a mild skin erythema reaction after 24 hours following exposure. Determination of MED is based on the assessment of UV-erythema and traditionally performed visually by naked eye, which is subjective and connected with errors due to high intrarater and interrater variability. The application of non-invasive and quantitative techniques such as optical methods could improve MED calculation, allowing us to detect and quantify alterations in epidermis and dermis induced by UV irradiation. In the current study the analysis of microcirculation parameters by non-invasive optical methods revealed the relationship between the oxygen consumption and a dose of UV radiation. Results also showed the correlation of oxygen consumption of UV-exposed tissues normalized to intact skin with a dose of UV. Moreover, we described tendencies in dynamics of porphyrin fluorescence intensity at different time points after UV-exposure. Optical methods have some prospects in non-invasive and predictive evaluation of UV erythema and MED and more research should be conducted in this field.

Keywords: minimal erythema dose, laser fluorescence spectroscopy, optical tissue oximetry, melanin index, non-invasive, ultraviolet erythema, ultraviolet irradiation

1. INTRODUCTION

Nowadays, minimal erythema dose (MED) is widely used to assess the sensitivity of individual's skin to ultraviolet (UV) exposure. MED is an amount of UV radiation leading to the development of minor, barely perceptible redness on untanned skin of human or animal within 24 hours after irradiation[1]. MED is widely used in the evaluation of photosensitivity and diagnostics of photodermatoses, to determine the UVA and UVB starting doses in the phototherapy[1] and in a cosmetic industry – to calculate sun protection factor (SPF)[2] and to determine photoprotective properties of different substances[3].

Even in modern clinics MED is still determined visually, which is subjective and connected with errors due to high intrarater and interrater variability. Schalka and Reis in their study showed that assessing the MED was one of the most serious challenges for participants as traditional MED determination lacks quantification, reproducibility and accuracy[4].

The inaccurate MED calculation could cause excessive UV exposure and some undesirable effects. A short-term excessive UV irradiation can induce burns, xerosis, hyperpigmentation, stinging, infections[5]. These adverse effects can lead to interruption in the phototherapy course, thus leading to the additional economic costs due to the increased duration of the hospitalization or number of outpatient visits and patients' rehabilitation. Moreover, the connection between erythema and molecular changes in the deoxyribonucleic acid (DNA) was shown in several studies, indicating the pathogenic correlation between sunburn erythema (due to incorrect MED reading) and the risk of premature photoaging and skin malignancies in the long-term period[6]. Based on abovementioned problems we can conclude that development, research and standardization of non-invasive objective quantitative methods for the MED assessment as well as their implication in clinical practice are currently needed. In this area, optical diagnostic methods seem to be the most applicable choice.

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These approaches do not require consumables/reagents, they are non-invasive, aseptic and allow to obtain ready-made quantitative data characterizing the pathological process for a short period of time. We found several studies dedicated to the use of the following optical methods for assessing UV-erythema characteristics and MED: diffuse reflectance spectroscopy[7], high resolution laser Doppler imaging[8], colorimetric determination of erythema using a chromameter[9], skin water content calculation[10], confocal microscopy[11] and optical coherence tomography[12].

However, described methods have several drawbacks: some of them are difficult to master and expensive, they were rarely used in combination with each other and the majority of measurements were performed on developed UV-erythema 24 hours after the irradiation not allow to predict erythema formation and MED. Laser fluorescence spectroscopy (LFS) and optical tissue oximetry (OTO) have potential to overcome disadvantages of mentioned optical methods. The aim of this study was to investigate prospects for using complex optical technologies including LFS and OTO in the evaluation of ultraviolet-induced skin damage and MED at different time points after UV exposure.

2. MATERIALS AND METHODS

The study was carried out on a group of young healthy volunteers (n = 7: 5 male and 2 female) aged 25.1 ± 2.2 years with Fitzpatrick skin phototypes II and III. In all participants traditional MED phototesting described by Heckman et al.[1] was utilized. MED testing was performed on the skin of the upper back or on the skin of left abdomen. UVB irradiation was induced by Dr. Honle Dermalight 500-1 series (manufactured by Dr. Henle Medical Technology GmbH, Germany), equipped with Phillips UV-B Narrowband PL lamps with a wavelength of 311 nm. The Daavlin DosePatch with six square windows (a square size of 1x1 cm^2) was attached to the skin of the back or abdomen. The distance to the UV source was 30 cm and irradiation duration was between 1.5 and 11.5 minutes. The UV intensity was measured using a Waldmann Variocontrol spectroradiometer (UV meter). The dose of UV radiation increased stepwise from window to window depending on the skin phototype of the participant according to reference tables[13]. The skin in the windows was cumulatively exposed to UV radiation in the range from 100 to 770 mJ/cm^2. 24 hours after UV-B exposure, the participant's erythema was visually assessed by 2 trained observers. The erythema reaction was graded using a visual rating scale[14]. Based on the results of the visual examination, the site corresponding to the MED (barely noticeable erythema) was determined and the dose of UVB was calculated. Detailed characteristics of MED and volunteers' phototype are presented in Table 1.

Before UVB irradiation and 0.5, 3, 6, 24 hours after it, on the skin in each of 6 square windows and on the contralateral area of intact skin the endogenous fluorescence of porphyrins was evaluated by LFS and local blood flow characteristics were measured by optical tissue oximetry (OTO) implemented in the LAKK-M system (SPE 'LAZMA' Ltd, Russia), as described in the study by Chursinova et al.[15]

The choice of the abovementioned time points was based on the literature data on the pathogenesis of an acute UV damage[6]. The measurements of optical parameters are presented in the Figure 1.

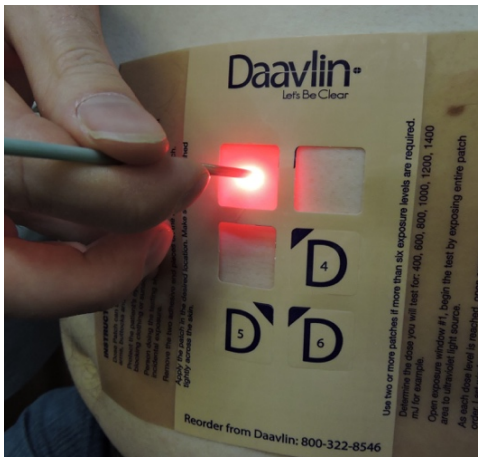

Figure 1. Optical measurements of the abdomen skin in square windows after ultraviolet-B radiation.

Porphyrin is characterized by a two-hump fluorescence spectrum with maxima at wavelengths of 630 and 710 nm[16]. In the wavelength range of 650 - 750 nm porphyrins make the main contribution to the endogenous fluorescence of biological tissue, but at a wavelength of 630 nm fluorescence of porphyrins is more pronounced. Although, other fluorophores (e.g., lipofuscin) may also fluoresce at this wavelength. Therefore, for the porphyrin assessment in biological tissue, spectra of secondary radiation (diffusely reflected and fluorescence) were recorded from each skin area after its excitation by low-power laser sources with wavelengths λ_e = 635 nm and λ_e = 535 nm. The fluorescence intensities I_f of porphyrins were estimated at wavelengths λ_f = 710 and 630 nm, respectively.

To exclude the variability of the initial endogenous fluorescence of volunteers' skin, the fluorescence intensity was normalized to the intact region $\mu(\lambda_f)$:

$$\mu(\lambda_f) = I(\lambda_f)/ I_0(\lambda_f) \quad (1)$$

where $I(\lambda_f)$ is the fluorescence intensity from the irradiated area, $I_0(\lambda_f)$ is the fluorescence intensity from the intact area.

To evaluate the parameters of local blood flow, the relative blood volume V_b (total hemoglobin content) and tissue oxyhemoglobin saturation (StO_2) were recorded for each region of interest for 20 seconds. Then, according to the time-averaged data the specific oxygen consumption of the tissues U characterized by the oxygen intake per tissue blood flow volume unit was calculated with the use of the following formula[17]

$$U= (SpO_2 - StO_2)/ V_b \quad (2)$$

In this formula SpO_2 is the functional pulse saturation of the oxyhemoglobin fraction in the arterial peripheral blood. It was assumed equal to 98%.

In the intact skin area, a melanin index (MI) was measured for each participant using a spectrophotometric instrument «Spectrotest» (SPE 'Cyclone-Test' Ltd, Russia)[18]. Results of measurements are showed in Table 1.

Statistical analysis was performed in Microsoft Excel 2016 and Statistica 12 (Statsoft inc., USA). The analysis of dynamic changes in the optical parameters described above was carried out using the Wilcoxon test. The relationship of the obtained optical data with the dose of UV radiation was evaluated using the Spearman rank correlation coefficient (r). The probability of an error of the first kind was considered statistically significant to be less than 5% ($p < 0.05$)

Table 1. Characteristics of volunteers enrolled in the study.

Volunteer N	Phototype (Fitzpatrick scale)	Melanin Index (MI)	MED, mJ/cm^2	Site of MED assessment
1	3	0.0524	280	abdomen
2	3	0.0566	400	upper back
3	2	0.0445	280	upper back
4	2	0.0501	200	abdomen
5	3	-	470	abdomen
6	2	0.0501	280	upper back
7	3	-	380	abdomen

3. RESULTS AND DISCUSSION

Examples of measured fluorescence spectrum of the intact (non-irradiated) and irradiated skin sites at excitation wavelength λ_e = 535 nm and λ_e = 635 3 and 24 hours after the UV-irradiation are shown in the Figures 2 and 3.

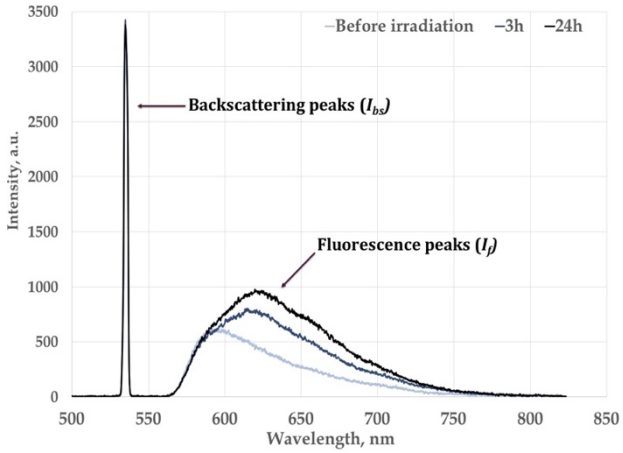

Figure 2. The example of the fluorescence spectra of skin before exposure and 3 and 24 hours after UVB exposure; λ_e 535 nm.

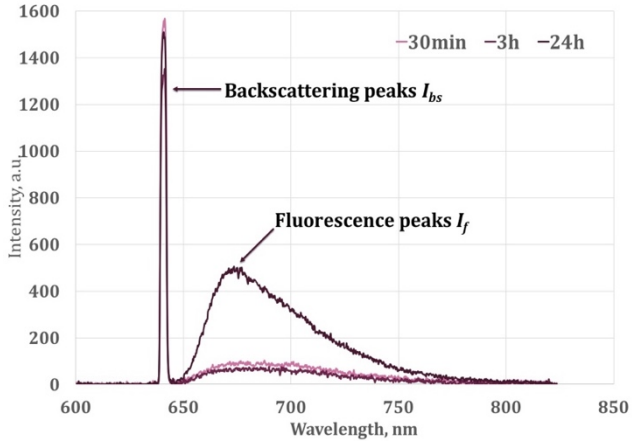

Figure 3. The example of the fluorescence spectra in irradiated skin 0.5, 3 and 24 hours after UVB exposure; λ_e = 635 nm

It was found that the values of *μ(670)* in all irradiated skin segments regularly changed stepwise over time. The most pronounced increase in this parameter was noted in all 6 square zones after 24 hours compared with 0.5 hours after UV exposure. No corresponding increase in *μ(670)* connected with dose of UV radiation was detected (Figure 4). The wide deviations in *μ(670)* presented on the graph could be associated with a number of research flaws: 1 - volunteers were not divided by the anatomical zone where the phototest was carried out; 2 - the diet and constitution of the volunteers were not taken into account; 3 - special skin characteristics (oily, seborrheic. acne-prone skin) of the participants were not considered; a preliminary cleaning of the skin with antiseptic was not carried out - a number of studies showed that the presence of enlarged pores and comedones is associated with increased synthesis of porphyrins by *Cutibacterium acnes* and increased fluorescence intensity of porphyrins[19].

It is important to note that recording the intensity of porphyrins in the red waveband has advantages: the contribution of other fluorophores to its signal is practically undetectable and can be neglected.

We assumed that the pull of porphyrins in the skin changes under the influence of UV and this phenomenon has a certain pattern. Porphyrins are orange-red fluorescent compounds consisting of 4 pyrrole rings that are formed during heme biosynthesis. A number of authors in their studies revealed that porphyrins accumulate in tissues susceptible to ischemia, hypoxia, and inflammation[20]. In the experiments performed by Schneckenburger and colleagues using the LFS method demonstrated the accumulation of porphyrins in the area of artificially induced skin inflammation in Wistar rats[21].

It is also shown that porphyrins contribute to the activation of compliment components, a change in the metabolism of eicosanoids, recruitment of inflammatory cells in the lesion area, and mast cell activation with the release of vasoactive mediators[22]. In acute UV damage, a persistent inflammatory response in the skin is known to form 24 hours after the

initial exposure. It is characterized by damage to the cells of the epidermis and vascular endothelium, a change in the population of Langerhans cells, mast cell degranulation, vasodilatation and the release of vasoactive cytokines and chemokines[23]. Probably, the porphyrin molecules are involved in the implementation of inflammatory UV-induced reactions and the attraction of immune cells with the subsequent development and preservation of persistent inflammatory infiltrate[24], which are clinically manifested in the form of UV erythema. In addition, a marked increase in the fluorescence of porphyrins in all windows (or at all irradiated skin zones with increasing doses) by 24 hours can be associated with the pronounced vasodilation observed in UV erythema[23].

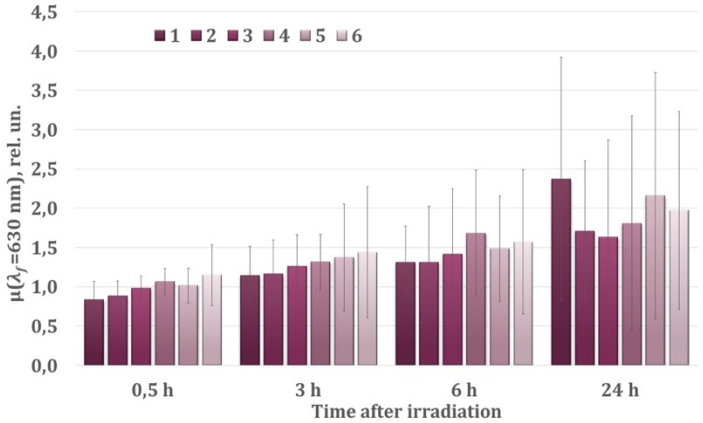

Figure 4: The dynamics of the porphyrin's intensity normalized to intact tissue in skin sites irradiated with stepwise increasing doses of UVB (6 – lowest dose, 1 – highest dose) in 4 time points after UV-exposure; λ_e = 630 nm

Using Spearman's rank correlation coefficient, correlation relationships were found between the UV dose in the square window and the tissue specific oxygen consumption index (U) normalized to intact skin after 3 hours ($r = -0.418$; $p = 0.006$) and 24 hours ($r = -0.422$; $p = 0.005$) after irradiation. These results may indicate changes in metabolic activity of cells of the UV-damaged area of the skin at the indicated time points. The results obtained are consistent with the literature: it has been shown that during the indicated time periods the most active skin infiltration by inflammatory cells, the appearance of apoptotic keratinocytes "sunburn cells" and the thickening of the epidermis were observed. After exposure to 3 UVB MED on human skin, sunburn cells appeared in the skin after 30 minutes. At first, they were found in the lower half of the epidermis, but 24 hours after UV exposure they were present in the upper layers of the epidermis. In humans, mast cells have been shown to degranulate and release histamine 4 hours after exposure to ultraviolet light[6].

Meanwhile, we did not find any significant correlation between the dose of UV and the intensity of fluorescence of porphyrins.

Additionally, using Spearman's rank correlation coefficient, it was found that the MED of volunteers correlated with Fitzpatrick's skin phototype ($r = 0.749$; $p = 0.02$), the melanin index (MI) showed correlation with the skin phototype ($r = 0.889$; $p = 0.02$). The results obtained are not surprising and correspond to published data.

4. CONCLUSION

The results of this pilot study showed that the integrated application of the LFS and OTO for objective non-invasive assessment of erythema has prospects for further research in larger studies. To obtain more accurate and qualitative results, it is advisable to analyze the differences in UV-induced changes of optical parameters of the skin of different anatomical zones (for example, back and abdomen) in a larger population of young volunteers. In addition, it is necessary to consider the factors of the diet (the presence of food rich in porphyrins) and participant's skin type (the presence of comedones, enlarged pores, acne elements). Considering abovementioned remarks, in the future these investigations may help to develop diagnostic algorithm for quantitative and predictive assessment of MED.

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