Optical Technology for Ultraviolet Erythema Assessment and Minimal Erythema Dose Determination in Healthy Volunteers

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Abstract: Currently, in clinical practice, the assessment of ultraviolet (UV) -induced erythema and the determination of the minimal erythema dose (MED) is done visually, which is subjective, inaccurate and associated with high variability of the results. To solve this problem, the application of optical methods seems promising, allowing us to evaluate changes in epidermis and dermis induced by UV exposure. In this study the analysis of endogenous fluorescence and microcirculation characteristics by non-invasive optical methods revealed the relationship between the intensity of endogenous fluorescence of porphyrins and oxygen consumption with a dose of UV radiation. The correlation of the intensity of endogenous fluorescence of the irradiated region normalized to intact tissue with a dose of UV was demonstrated. Therefore, optical diagnostic methods can be a promising tool for non-invasive and quantitative assessment of UV erythema and MED.

1 INTRODUCTION

The assessment of skin reaction on different doses of ultraviolet radiation (UVR) with different wavelengths is one of the challenging issues of modern photobiology and medicine. Currently, the traditional method of assessing the degree of exposure to UV radiation in humans and animals is based on the calculation of the minimal erythema dose. Minimal erythema dose (MED) is an amount of UV exposure leading to the development of minimally perceptible erythema on untanned skin within 24 hours after irradiation (Heckman et al., 2013). Thus, determination of MED is based on assessment the charactheristics of the pathophysiologic phenomenon - UV-erthema (Makmatov-Rys et al., 2019). MED is usually measured in mJ/cm^2. MED is widely used in clinical practice and experiments in the evaluation of photosensitivity. It is applied to determine the UVA and UVB starting doses in the phototherapy of skin diseases (Krutmann et al., 2008). Moreover, MED assessment is one of the diagnostic methods for some photodermatoses (Hönigsmann, 2008).

MED is traditionally determined visually by naked eye, which is a subjective and inaccurate method (Lock-Andersen et al., 1996). For instance, Falk M. and Ilias M. showed that the agreement between observers on the characteristics of UV erythema, was excellent for skin redness with a sharp border, but for reactions with a diffuse or indistinct border there was a substantial inter-observer variability. Mistakes can occur during the visual assessment of the Fitzpatrick skin type especially in tanned patients or in dark skin when the evaluation is made by untrained doctor (Falk et al., 2008).

Incorrect determination of MED can lead to an overestimation of the dose of UV radiation in the starting point of phototherapy course and to such complications as burns, hyperpigmentation, dry skin, herpes simplex reactivation, and in some cases to aggravation of the underlying skin disease. In addition, the occurrence of such complications leads to an interruption in the phototherapy course, an

increase in the numer of clinic visits and the associated economic costs. In the long term, the risk of malignant skin neoplasms and photoaging increases.

To avoid the limitations of conventional methods for MED assessment additional instrumental methods, in particular optical, are used.

There are data in the literature on the use of the following optical methods for assessing erythema intensity and MED: reflective spectroscopy (Bodekær et al., 2013), colorimetry (Jeon et al., 2014), laser doppler flowmetry (Falk et al. 2008), laser doppler visualization (Wilhelm et al., 2001), and confocal microscopy (Yamashita et al., 2007).

Despite the progress in the field of the objective assessment of UV erythema and MED, the abovementioned methods have some limitations. Current studies are trying to find a correlation between objective optical metrics and subjective visual characteristics of erythema, such as color, distinctness of borders, contours. Unfortunately, the examination of pathophysiological mechanisms of severe UV-induced tissue damage is beyond the scope of these studies.

Meanwhile, laser fluorescence spectroscopy (LFS) has potential applications in this field. Fluorophores responsible for inflammation and hypoxia which play a role in UV-induced skin damage, could be detected by LFS in red and green spectrum range (Franco et al., 2016). The literature describes the use of laser fluorescence spectroscopy in vivo in experimental models for assessment local inflammation (Petritskaya et al., 2015), radiation skin damage (Raznitsyna et al., 2018) and the skin fibrosis (Chursinova et al., 2019). There are studies on the use of fluorescence spectroscopy to assess structural skin changes during chronic UV damage (Tian et al., 2001). Papazoglou E. et al. (2010) compared the data of LFS, skin morphology and expression of matrix metalloproteinase 13 (MMP-13) to assess changes caused by prolonged exposure to UVB radiation on the skin of hairless mice. The authors found that LFS can be used to estimate epidermal thickness and fluorescence parameters correlates with tryptophan expression and cell proliferation and may indicate the presence of “burn cells” in the epidermis (Papazoglou et al., 2010)

The aim of this study was to assess the applicability of complex optical technologies including LFS and optical tissue oximetry in the assessment of ultraviolet-induced skin damage and MED at different time periods after UV irradiation

2 MATERIALS AND METHODS

The study was conducted on a group of healthy volunteers (n = 14, 8 male and 6 female) aged 26 ± 3 years with Fitzpatrick skin phototypes II and III. In all participants traditional MED assessment method (described by Heckman et al. (2013)) was perfomed on the skin of the upper back or on the skin of the abdomen. UVB irradiation was performed using a Dr. Honle Dermalight 500-1 series (manufactured by Dr. Honle Medical Technology GmbH, Germany), equipped with Phillips UV-B Narrowband PL lamps with a wavelength of 311 nm. The Daavlin DosePatch hypoallergenic plate 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. The UV intensity was measured using a Waldmann Variocontrol spectroradiometer (UV meter). The dose of UV radiation from cell to cell increased stepwise depending on the phototype of the skin of the subject according to reference tables (Palmer et al., 2005). 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 participants in the experiment were conducted a subjective visual assessment of erythema by 2 observers. The erythema reaction was graded using a visual rating scale (Faurschou, et al., 2009). 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 phototype participants are presented in table 1.

Before UVB irradiation and after 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 (1 cm2), the endogenous fluorescence of porphyrins was evaluated by LFS and local blood flow characteristics was measured by optical tissue oximetry (OTO) implemented in the LAKK-M system (SPE 'LAZMA' Ltd, Russia), as described in (Chursinova et al., 2019).

The diagnostic system scheme is shown in Figure 2. The choice of the abovementioned time points was based on an analysis of the literature on the pathogenesis of the of acute UV damage (Hruza et al., 1993). The process of measuring of optical parameters is presented in Figure 1.

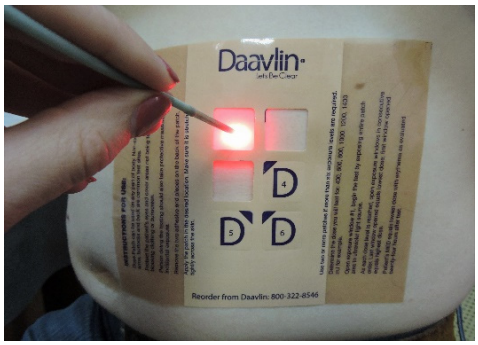

Figure 1: Process of optical measurements of the abdomen skin after irradiation with ultraviolet B.

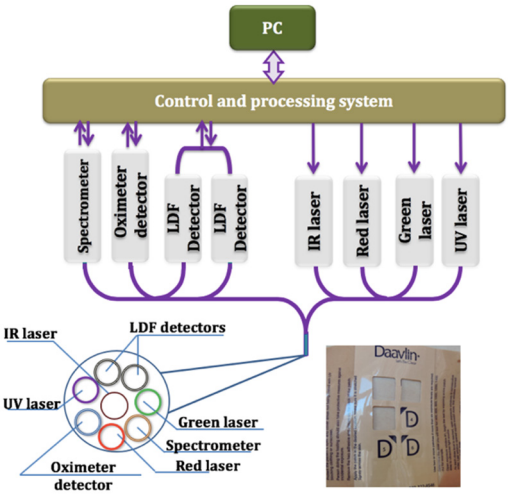

Figure 2: Scheme of the diagnostic system.

As a rule, the maximum absorption of most endogenous fluorophores is observed in the UV wavelength range. However, the radiation of this range has a low penetrating power (less then 0.1 mm (Mustafa et al., 2013) as distinct from the visible part of the spectrum. Therefore, the spectra of secondary radiation (backscattered and fluorescence) were recorded from each region of interest after its irradiation by low-power laser sources with wavelengths λ_e = 635 nm and λ_e = 535 nm.

Porphyrin is characterized by a two-hump fluorescence spectrum with maxima at wavelengths of 625–630 and 700-710 nm (Croce et al., 2014). 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 625–630 nm fluorescence of porphyrins is more pronounced.

The fluorescence intensities I_f of porphyrins were estimated at wavelengths λ_f = 710 to verify theirs presence and at λ_f = 630 nm to quantitative assessment, respectively. Despite the fact that other fluorophores (for example, lipofuscin) can also fluoresce in the range of 625–630 nm, their contribution to the total intensity insignificant.

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 iradiated area, $I_0(\lambda_f)$ is the fluorescence intensity from the intact area.

To evaluate the parameters of local blood flow, blood filling volume (V_b) and tissue oxyhemoglobin saturation (S_tO_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 (Rogatkin et al., 2013):

$$U= (S_pO_2 - S_tO_2)/ V_b \quad (2)$$

In this formula S_pO_2 is the functional pulse saturation of the oxyhaemoglobin 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) (Afanasyev et al., 2007). Results of measurements are showed in Table 1.

Table 1: Characteristics of volunteers enrolled in the study.

N	Phototype	MI	MED, mJ/cm2	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	0.0560	750	upper back
8	2	0.0544	560	upper back
9	3	0.0574	380	upper back
10	3	0.0693	750	upper back
11	3	0.0693	770	upper back
12	2	-	280	upper back
13	3	-	380	upper back
14	3	-	380	abdomen

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 between the obtained optical data and the dose of UV radiation was evaluated using the Spearman rank correlation coefficient. The probability of an error of the first kind was considered statistically significant to be less than 5% ($p < 0.05$).

3 RESULTS AND DISCUSSION

Examples of measured fluorescence spectra from the intact (non-irradiated) and irradiated skin sites at λ_e = 630 nm after 24 hours after the UV-irradiation is shown in the Figure 3.

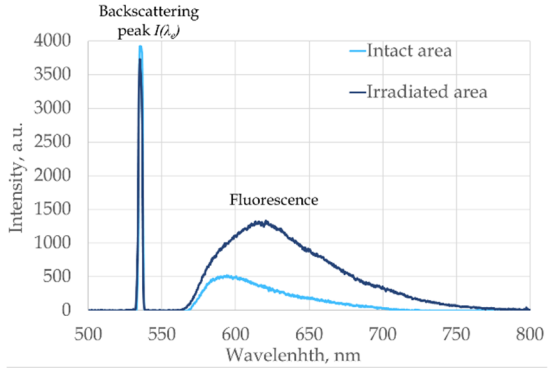

Figure 3: The example of the fluorescence spectra in intact and irradiated skin after 24 hours after UVB exposure; λe = 630 nm.

Using Spearman's rank correlation coefficient, positive correlation relationships were revealed between the cumulative dose of UV and the specific oxygen consumption of the tissues (U) normalized to intact skin after 3 hours (correlation coefficient [r] = 0.297; p = 0.01) and 24 hours (r = 0.307; p = 0.0004) after the irradiation. In addition, a positive correlation was found between the total UV dose and the fluorescence intensity of porphyrins λ_e = 630 nm 6 hours after UV irradiation normalized to intact skin (r = 0.249, p = 0.01).

The findings may reflect the course of acute UV-induced skin damage. Thus, the U index reflects an increase in the metabolic activity of skin cells susceptible to acute UV damage. Under the influence of UV, mast cell degranulation, vascular endothelial damage, vasoditalation are observed, vasoactive substances are released - histamine, nitric oxide, arachidonic acid derivatives, which also contribute to the formation of infiltrate from immune cells in the affected area (Clydesdale et al., 2001). Logan and colleagues showed that one consequence of UV exposure of the skin is damage to epidermal cells which becomes evident as early as 2 hours after UV irradiation (Logan & Wilhelm, 1963). One study showed that peak infiltration of leucocytes after UVB irradiation occurs at 4-6 hours and the response concludes after 48 hours (Logan & Wilhelm, 1966). It has also been shown that with increasing intensity and dose of UV radiation, skin damage becomes more pronounced (Hruza, 1993)

In addition, according to the results of the study, it was found that normalized fluorescence intensity and tissue content index in all irradiated skin sites regularly changed stepwise over time. The most significant increase in the intensity of fluorescence of porphyrins in green light (λ_e = 630 nm), normalized to intact skin in all 6 cells was observed 24 hours compared with 0,5 hours after UV exposure (Figure 4).

These results allow us to hypothesize that UV exposure affects the metabolism and accumulation of porphyrins in the skin.

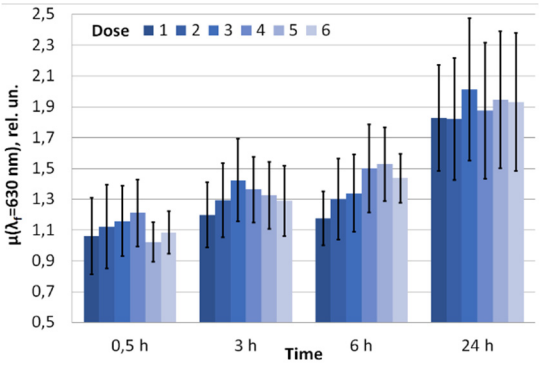

Figure 4: The dynamics of the porphyrin's intensity normalized to intact tissue in skin sites irradiated with stepwise increasing doses of UVB (6 – lowest dose, 1 – highest dose) in 4 time points after UV-exposure; λe = 630 nm.

Additionally, using Spearman's rank correlation coefficient, it was found that the MED of volunteers correlated with Fitzpatrick's skin phototype (r = 0.56; p= 0.036), the melanin index (MI) showed correlation with the skin phototype (r = 0.79; p= 0.007). This results corresponds with previously to published data: for instance, D.L. Damian and colleges in their study showed good correlation between MI, Fitzpatrick phototype and MED in 60 healthy volunteers (Damian et al 1997).

It is important to mention that our work have some limitations. We didn't include subjects with darker skin phototypes (IV-VI) in study population. It is known that melanin content is significantly higher in the skin of subjects with darker skin type (Lu et al., 1996).

Melanin is known to absorb the radiation of the visible spectrum, which reduces the registered signal significantly. In these cases increasing the power of the laser radiation may increase the signal-to-noise ratio and solve the problem. But in further studies it is important to estimate the minimum laser power for skin phototypes IV-VI at which peaks of endogenous fluorophores can be distinguished.

Also, since this study involved young patients of approximately the same age, it is also necessary to conduct the similar studies with subjects of different age groups. Lipofuscin age pigments accumulate in cells with age, also has fluorescent properties (Brunk et al., 2002). Therefore, it is necessary to evaluate its contribution to the total skin spectrum and the possibility of reliable identification of porphyrins in older people.

4 CONCLUSIONS

The results of this pilot study showed that the integrated application of the LFS and OTO methods for objective non-invasive assessment of erythema has prospects for further investigation in larger studies. This techniques give us opportunity to access pathophisiological alternations (e.g. inflmmation and vasodilatation) taking place in the skin after acute UV damage. To gain more precise data, it is worth to analyze optical parameters of of the skin of different anatomical zones irradiated with UV (for example, back and abdomen) in a larger groups of young volunteers darker skin phototypes.

In the future, these developments may become the basis for the development of diagnostic systems for quantitative predictive assessment of MED.

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