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Evaluation of skin parameters in C57BL/6J mice exposed to chemical and environmental factors using non-invasive methods

Dorina Gheorgheosu (Coricovac)[1], Florin Borcan[1*], Nemeth Istfan Balasz[2], Codruta Soica[1], Georgeta Simu[1], Lajos Kemeny[2], Cristina A. Dehelean[1]

[1]*University of Medicine and Pharmacy Victor Babes Timisoara, Faculy of Pharmacy, Eftimie Murgu Square no.2, RO-300041*
[2]*University of Szeged, Department of Dermatology, Szeged, Hungary.*

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Abstract

UVB is an important external exposure factor that created important damages on skin level. PAH-s (ex. DMBA) are also important skin cancer promoters. Non-invasive methods could be applied to evaluate the skin evolution during UVB and chemical agresogen exposures. This external and carcinogen stimulating factors influenced the skin parameters on C57BL/6J mice. The evaluation of these parameters could be applied in experimental studies for therapeutic appreciation and skin carcinoma events pre-evaluation.

Keywords: UVB, C57BL/6J mice, chemical agresogen

1. Introduction

Ultraviolet B radiation (UVB) is considered the environmental factor that plays a key role in skin disorders. UVB (295—315 nm) is the component of solar light, with a terrestrial content of only 5-10% [1], and is responsible for multiple skin alterations such as wrinkle formation, epidermal thickening, degradation of matrix macromolecules, and local and systemic immunosuppression [2]. Moreover, it was shown that UVB induces cutaneous angiogenesis by increasing VEGF mRNA expression in normal mouse skin [2]. A high percent (90%) of UVB radiation is absorbed by the epidermis, more specifically by the aromatic heterocyclic bases of DNA determinating the formation of cyclobutane-pyrimidine dimers and pyrimidine-(6-4)-pyrimidone photoproducts [1]. UVB radiation is also implicated in the production of reactive oxygen and nitrogen species (RONS) [1].

DMBA (7,12-dimethylbenz(a)anthracene) is a chemical agent with carcinogenic potential, frecvently used in experimental research, especially in skin disease models [3]. It was demonstrated that topical application of DMBA led to inflammation and oxidative DNA damage in the skin, lesions with important role in promotion and progression of carcinogenesis [4].

Several studies showed that association of UVB radiation and DMBA application not only determined a carcinogenic effect but also induced specific suppression within the cutaneous immune system [5-7].

The aim of the present study was to evaluate the effects of different aggressive factors such as UVB and 7,12-dimethylbenz(a)anthracene (DMBA) on C57BL/6J mice skin parameters using non-invasive methods.

Corresponding author: *e-mail:* fborcan@yahoo.com

2. Materials and Methods

Animals. Male C57BL/6J (20-22 g, 12-15 weeks) mice were purchased from Charles River (Germany). All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press) on the protection of animals used for scientific purposes. Animals were kept on a 12 h/12 h light/dark cycle, at a normal (24 °C) animal house temperature, humidity above 55%, fed *ad libitum* and had free access to water. The experiments were approved by Bioethical Committee of "*Victor Babes*" University of Medicine and Pharmacy Timisoara.

Design of experimental model. C57BL/6J mice were divided in 3 groups (5 mice/group): group 1 – control group (no intervention), group 2 – mice were exposed to UVB and 7,12-dimethylbenz(a)anthracene (DMBA) (390nmol/0.1 ml acetone) was topically applied on the back skin (a single application in the first week of experiment) before irradiation [8] and group 3 – mice were exposed to UVB. Before UVB exposure C57BL/6J mice were shaved on the back. For UVB exposure, cages were placed in an automatically time-switched irradiation setup. In the experiment, VL-6.M/6W (312 nm wavelength and 680 μW/cm2 intensity at 15 cm) tubes (Vilber Lourmat, France) were used. Under the lamps the minimal erythema dose (MED) of hairless SKH-1 mice, was ≈300 J/m^2 [9]. The exposure protocol was the following: irradiation 3 times/week for 4 weeks and the mice were exposed to a total dose arround 200 J/m^2 UVB radiation. During exposure the mice were maintained in a plastic cage and the distance between the lamp and the back of the mice was 15 cm [10].

Non-invasive skin measurements. All the non-invasive measurements on mice skin were carried out with a Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany. The measurements of melanin and erythema were obtained by means of the MPA5 Mexameter® MX 18 probe, as quantitative results regarding melanin and erythema (haemoglobin) subjected to modifications by tumoral evolution.

The units for melanin and erythema were determined by a spectrophotometer evaluation.

The melanin values were measured using 2 wavelengths: 660 and 880 nm and haemoglobin for erytema 560 and 660 nm [9, 11, 12]. Skin hydration and skin transepidermal water loss TEWL were also determined using a corneometer and a Tewameter®, incorporated in Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany. We used their general units obtained by Mexameter soft evaluation and not the index as value. The measurements were executed every 3 days after exposure to irradiation on the back area. The applied area was 5 mm diameter.

Statistical analysis. Data were analyzed using paired Student's *t* tests or One-way Anova followed by Bonferroni's post-tests were used to determine the statistical difference between experimental and control groups; *,** and *** indicate $p<0.05$, $p<0.01$ and $p<0.001$.

3.Results

Macroscopic aspect of the UVB-induced lesions. UVB irradiation and topical administration of a single subchronic dose of DMBA in the first week of experiment determined significant macroscopic changes of skin aspect. The degree of injury was higher in the group that received DMBA and UVB (group 2) as compared to the group that received only UVB (group 3) (figure 1), indicating that the two aggressive factors had an additive effect.

*Non-invasive skin measurements***.** The skin parameters evaluated in the present study using non-invasive methods were melanin content, erythema, transepidermal water loss (TEWL), skin pH and skin hydration. The results are expressed as differences (delta Δ) between the values measured in the exposed area (exposed to UVB or to UVB and DMBA) and the values measured in a non-exposed area in the same group.

Melanin values. Our results indicated statistically significant changes of melanin content after day 20 of experiment within the groups of study. It was observed that group 2 (exposed to UVB and DMBA) in the first week of experiment when DMBA was applied, showed an important increase in melanin content as compared to both group 1 (control group) and group 3 (exposed to UVB) (figure 2).

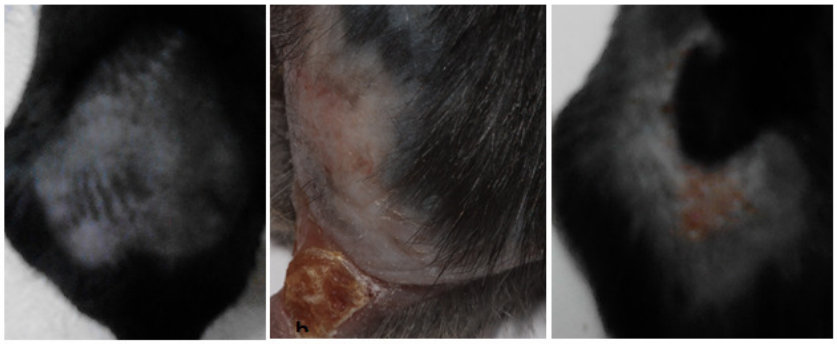

Figure 1. Macroscopic images of C57BL/6J mice: a. Group 1 (no intervention), b. Group 2 (exposed to UVB and DMBA topically applied before exposure) and c. Group 3 (exposed to UVB).

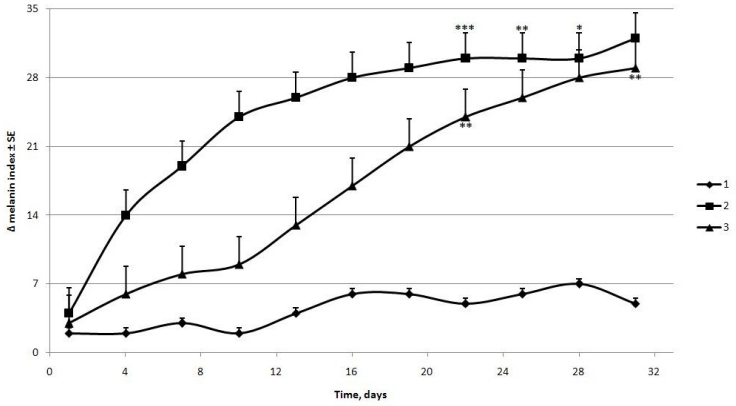

Figure 2. The melanin skin content evaluation for C57BL6/J mice: Group 1 (no intervention), Group 2 (exposed to UVB and DMBA topically applied before exposure) and c. Group 3 (exposed to UVB) (data are expressed as Δ ± SEM).

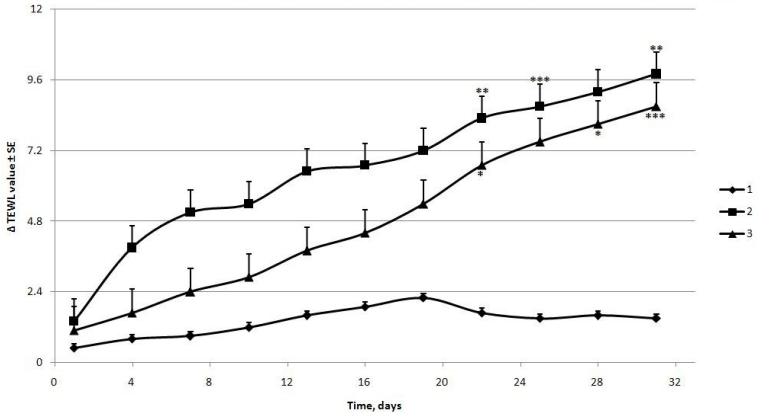

Figure 3. TEWL evaluation for C57BL6/J mice: Group 1 (no intervention) as compared to Group 2 (exposed to UVB and DMBA topically applied before exposure) and Group 3 (exposed to UVB) (data are expressed as Δ ± SEM).

TEWL measuremets. Skin transepidermal water loss (TEWL) was also affected by the action of the two factors UVB and DMBA. Groups 2 and 3 showed a marked increase of TEWL after day 20 as compared to group 1 (control group). The mice exposed to both UVB and DMBA presented a higher degree of TEWL than the ones that were exposed only to UVB (figure 3).

Erythema values. Measurements of erythema indicated a significant increase of this parameter starting with the first week of experiment, especially for group 2 (exposed to UVB and DMBA) and the increase was maintained until the end of the experiment, for groups 2 and 3 in comparison with the control group (group 1) (figure 4).

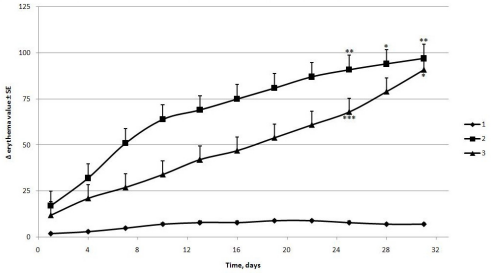

Figure 4. Erythema values for C57BL/6J mice: Group 1 (no intervention) as compared to Group 2 (exposed to UVB and DMBA topically applied before exposure) and Group 3 (exposed to UVB) (data are expressed as Δ ± SEM).

pH values. The pH values measured were chaotic for all three groups of study and it couldn't be established a pattern for their variation, but it was observed that groups 2 and 3 presented higher values of pH (statistically significant) as compared to control group (figure 5).

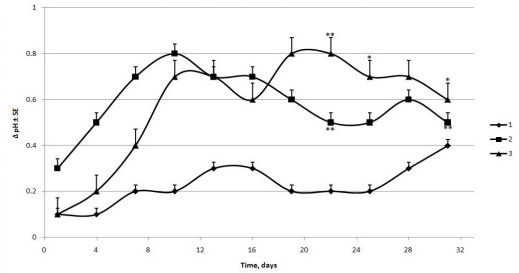

Figure 5. pH values for C57BL/6J mice: Group 1 (no intervention) as compared to Group 2 (exposed to UVB and DMBA topically applied before exposure) and Group 3 (exposed to UVB) (data are expressed as Δ ± SEM).

Skin hydration. Our results indicated an important decrease of skin hydration for groups 2 and 3 *vs.* group 1. In Figure 6 the data are presented as differences (delta) between the values measured in an exposed area and the values measured in a non-exposed area, and it was observed that the differences were significantly higher for groups 2 and 3 in comparison to control group (figure 6).

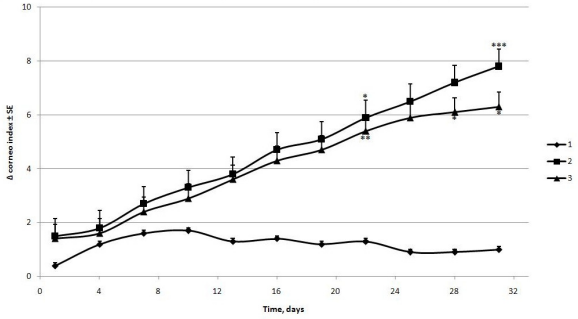

Figure 6. Skin hydration evaluation for C57BL/6J mice: Group 1 (no intervention) as compared to Group 2 (exposed to UVB and DMBA topically applied before exposure) and Group 3 (exposed to UVB) (data are expressed as Δ ± SEM).

4.Discussion

The present study was developed on C57BL/6J mice exposed to two potent carcinogenic agents, UVB radiation and DMBA, and showed that these agents affected skin integrity by inducing lesions observed macroscopically and, also perturbance of different skin parameters including melanin content, erythema, transepidermal water loss (TEWL), skin pH and hydration.

UVB radiation is known to induce DNA damage which can be considered the source of a variety of crucial events in biological systems [7]. The DNA damage induced by UV irradiation can be explained by the presence of dipyrimidine lesions in DNA including the formation of cyclobutane pyrimidine dimers (CPD) and pyrimidine–pyrimidone 6-4 photoproducts [7,13]. UVB radiation is considered an important source of oxygen reactive species (ROS) in the cells and skin, and it represents the cause for inflammation (sunburn) and cancer. It is also known that UVB acts as an initiator and promoter of carcinogenesis in mouse skin [8]. Our results indicated that the group of mice exposed to UVB radiation developed skin lesions and significant

changes in skin parameters values (melanin content, erythema, TEWL and skin hydration) as compared to control group. Similar results were observed in a study developed on hairless mice [8].

A significant number of studies described the role of DMBA as an inducer of two-stage skin carcinogenesis model (initiation with a single subchronic dose of DMBA followed by repeated promotion by a tumor promoter like croton oil or TPA - 12-O-tetradecanoylphorbol-13-acetate) [9, 12,14-16]. It was reported that DMBA administration produced DNA-carcinogen adducts which may induce G - A transitions or A - T transversions, mutations that were frequently observed at the Ki-ras gene in DMBA-induced carcinomas [4]. The mutagenic and carcinogenic potential of DMBA was attributed to the reactive oxygen species (ROS) obtained during its metabolism or secondarily during tumor formation, ROS that might diffuse from the site of generation to other targets within the cells or even propagate the injury to other intact cells [17].

In a study developed on SKH-1 hairless mice it was shown that long time exposure (20 weeks) to UVB radiation after DMBA topical application as initiator of carcinogenesis, induced tumor incidence and the physiological skin parameters were also affected [8].

The present study evaluated the short-term effects (4 weeks of exposure) of DMBA and UVB radiation, and it was observed significant higher skin damage in the group of mice exposed to both carcinogens in comparison with the group that received only UVB radiation. Moreover, no papillomas were detected until the end of the study, results that are in accordance with the data described by Yamada et al., which mentioned that papillomas appeared after 7 weeks of exposure to both agents.

Most of the studies regarding the photobiological research *in vivo* were executed using albino mice which are lacking in epidermal melanocytes and melanin [7,18]. Colored mice presented melanin in the dermis and in the hair follicle, but not in the interfollicular epidermis, with the exception of ears, footpads, and tail [7]. Yamazaki et colab., demonstrated in a study realized using genetically modified albino mice XPA (-/-), which were defective in nucleotide excision repair, and could easily develop UVB and DMBA-induced skin tumors, *versus* hyperpigmented XPA (-/-) SCF transgenic mice, that epidermal melanin was able to protect DNA-induced damage in keratinocytes by UVB radiation, and thereby, prevent UV-induced inflammation, immunosuppression, and carcinogenesis in *in vivo* experiments. No protective effects were observed in the case of DMBA application [7]. The colored mice C57BL/6J that we used in our study presented a lesser degree of injury when were exposed to UVB radiation as compared to those mice that received DMBA treatment, too. We could speculate that these results were influenced by the effect of epidermal melanin as mentioned in Yamazaki's data.

The evaluation of the physiological skin parameters (melanin content, transepidermal water loss – TEWL, erythema, skin pH and skin hydration) was made using a non-invasive measuring method – the Multiprobe Adapter System (MPA5). For the determination of melanin content and erythema it was used MPA5 Mexameter® MX 18 probe, which measured the quantitative changes of skin color. Melanin content and erythema are important parameters of skin pathology and surveillance, and significant changes of these parameters indicate the presence of a skin disease (e.g. melanoma, skin carcinoma) [12,19]. Our results regarding these two parameters showed an increase which confirmed the presence of lesions at skin level induced by both DMBA and UVB radiation.

TEWL is an important physiological skin parameter that indicates the functionality and integrity of skin barrier [20]. An increased value for TEWL compared to normal values can indicate skin damage caused by an injury or infection [19, 21]. In our experiment this parameter was affected by the activity of both UVB radiation and DMBA application, showing a marked increase as compared to control group.

The damage induced by UVB radiation and DMBA topical application at skin level was also confirmed by the significant decrease of skin hydration.

6.Conclusion

Environmental factors that are procarcinorgens influenced the main skin parameters including skin physiology and skin quality. They produce in first

phases of exposure general skin damages. The evaluation of these parameters could be applied in experimental studies for therapeutic surveillance or skin carcinoma events pre-evaluation.

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Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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