Evaluation of Skin Physiological Parameters in SKH1 Mice Experimental Model after Exposure to Aggressive Factors like UVB using Non-invasive Methods

The most incriminated environmental factor for its implication in skin diseases is sun light and ultraviolet (UV)-B radiation is responsible for the most intense negative reports. It is well known that solar UV radiation comprises 3 types: UVA (320-400 nm), UVB (290-320 nm) and UVC (200-280 nm) [1]. From this group UVB radiation is considered the component of solar light with the most energetic, mutagenic and carcinogenic properties due to its direct activity at DNA level, leading to dimeric photoproducts known also as adjacent pyrimidine bases, cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidine photoproducts [2]. On the contrary, UVA seems to have antiphotocarcinogenic properties and a protective effect if it is coupled with UVB exposure. UV radiation determines apparition of epidermal apoptotic sunburn cells with an irreparable DNA damage [3]. Furthermore, UV radiation determines inflammation and erythema (eg. sunburn), photosensitivity and DNA damages. Chronic exposure is related to photoaging and skin carcinoma. At cellular and gene levels, the radiation induces abnormal secretion of cytokines by keratinocytes, also decreases Langerhans cells and increases mast cells effects on cytokine secretion. Skin is the main and the most affected barrier on the body against UV exposure [4].

An increased number of studies reported that ultraviolet radiation acts as a pro-carcinogenic agent [5] and it is important to evaluate the damages. Hairless mice represent a useful tool in studying the UV impact and treatment, albeit mouse skin is different comparing to humans [6].

The aims of the present study were: (i) to evaluate the evolution of the main physiological skin parameters after UVB exposure in a SKH1 mouse model and (ii) to observe the intensity of skin changes induced by UVB on normal and dried skin for correlation with its impact on humans.

**Experimental part**

**Materials and methods**

**Animals**

Male SKH1 (18 – 20 g, 10-12 weeks) mice were purchased from Charles River (Sulzfeld, 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) regarding 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 they had free access to water. The experiments were approved by the Bioethical Committee of “Victor Babes” University of Medicine and Pharmacy Timisoara and also respected the international regulations.

**Design of experimental model**

Mice were divided in 3 groups (5 mice/group): group 1 – control group (no intervention was applied), group 2 – SKH1 mice exposed to UVB, group 3 – SKH1 mice exposed to UVB and sodium lauryl sulphate (SLS) 5% topically applied before irradiation. Application of sodium lauryl sulphate solution mimics a “dry skin syndrome” [7]. 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) used on hairless SKH1 mice, was ≈ 300 J/m² [1]. The exposure protocol was the following: irradiation 3 times/week/3 min for 10 weeks and the mice were exposed to a total dose around 200 J/m² UVB radiation. During exposure the mice were maintained in a specific cage and the distance between the lamp and the back of the mice was 15 cm [8].

**Keywords:** UVB radiation, SLS, TWL, melanin, erythema
Non-invasive skin measurements

All the measurements on the mice skin were carried out with a Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany. In the experiment were evaluated physiological cutaneous parameters, including: melanin and erythema, transepidermal waterloss (TWL), skin pH, the degree of sebum and skin hydration. The measurements of melanin and erythema were obtained by the means of MPA5 Mexameter® MX 18 probe, as quantitative results regarding melanin and erythema (haemoglobin) subject to modifications by tumoral evolution. The units for melanin and erythema were determined by a spectrophotometer evaluation. The applied area was 5 mm diameter.

The melanin values were measured using 2 wavelengths: 660 and 880 nm and haemoglobin for erytema 560 and 660 nm [1]. We used their general units obtained by Mexameter soft evaluation and medium indexes as value. The devices used basic units for different types of skin included in the experimental database.

For the determination of each parameter mentioned above it was used a different instrument, components of MPA5 system: Tewameter® TM300 – for TWL measurement, Skin-pHmeter® PH905 for skin pH, a sebumeter and a corneometer for sebum and hydration values.

Statistical analysis

Data were analyzed using paired Student’s t tests or One-way ANOVA followed by Bonferroni’s post-tests in order to determine the statistical difference between experimental and control groups; *, ** and *** indicate p<0.05, p<0.01 and p<0.001.

Results and discussions

Macroscopic aspect of the UVB and SLS-induced lesions

SKH1 mice included in the present study were exposed for 10 weeks to UVB radiation or/and SLS (sodium lauryl sulfate), two agents with toxic impact at skin level. According to our results, the mice exposed to the action of the two agents developed cutaneous lesions, these lesions being more severe in the group that received both UVB and SLS (group 3) as compared to group 2 (exposed only to UVB) and control group (fig. 1).

As mentioned in the introduction part, it is well known, that UVB radiation is pointed to be the major risk factor for sunburns and most skin cancers [9]. It is reported in the literature that UVB radiation causes direct DNA damage by forming a thymine-thymine cyclobutane dimer [10]. However, also UVA radiation have been incriminated for the induction of different types of skin cancer involving free radicals/ reactive oxygen species (ROS) [11]. On the other hand sodium lauryl sulfate (SLS) also known under the name of sodium dodecyl sulfate (SDS), an surfactant intensively used in cleaning area and personal care products is an irritating organic compound for the skin. It was noticed that the intensity of irritation produced by SLS is directly proportional to the time of contact with the skin and inverse proportional with the thickness of the skin [12]. SLS is commonly used in research as a standard ingredient for irritating the skin [13].

Non-invasive skin measurements

TWL measurements

The skin constantly loses water in form of vapours, loss also known as transepidermal water loss (TWL). It is known that minor lesions of skin result in an increased TWL. TWL can be directly measured with a non-invasive Tewameter probe which is applied for a few seconds on the skin. It consists of two pairs of sensors which measure the humidity and temperature gradients in two different areas. Based on the resulting humidity and temperature gradients, the TWL is automatically calculated and shown. A photo detector analyses the diffuse reflection from the skin [14].

The initial absolute values of TWL were between 1.6-1.8 g/cm²/h. Our data regarding the values of TWL showed that UVB radiation and SLS topically applied increased TWL values. The highest values were determined for group 3 (UVB+SLS) (fig. 2), results that can be confirmed by the severe lesions present in this group (figure 1) and indicate the toxic action of these agents on the barrier function of skin.
Transepidermal water loss (TWL) is a very important parameter that indicates the functionality of barrier of the skin. An increased value for TWL compared to normal values can indicate skin damage due to an injury or infection [1, 15]. TWL is commonly known under the definition of the amount of water that passes from inside a body through the epidermal layer [16]. In this experiment we have found, as revealed by non-invasive skin measurements an increased value for TWL compared to control in both UVB and UVB+SLS exposed groups. Our results are in agreement with the data from the literature since both UVB and SLS have been previously reported for damaging the skin barrier properties and consequently to increase the normal values corresponding to TWL [17, 18].

Skin-pH evaluation
An adequate pH value is essential for a healthy skin. It can be measured with the Skin-pH-Meter probe. Recommended measuring areas of human bodies are the back of the hand, the forearm, the front and the cheeks; however readings are also possible on any other body part.

The absolute values of skin-pH before the addition of the two agents (UVB radiation and SLS) were between 6.1-6.4 units. The results obtained in this experiment showed that skin-pH was maximum changed in the case of Group 3 (0.7 units), but compared with Group 1 - control group used as reference, the increase was not an important one (fig. 3).

Sebum measurements
The measurement of the sebum content consists of a rather basic, direct reading of the sebum secretion on skin, hair and scalp. The measuring is generally taken with a parchment like foil which becomes transparent after contact with lipid substances. The foil is pressed on the skin for a defined period. The change in transparency is then measured with the help of a source of light (photometric method). This method is insensitive to humidity. It is recommended to take sebum readings before any product application. Values of sebum between 8-12 arbitrary units were obtained before the beginning of the experiment. In this study, there were recorded decreases of sebum values after UV exposure and SLS topically applications as compared to control group (fig. 4).

Mexametry - melanin and erythema evaluations
The measuring principle for the melanin and erythema readings is based on a source of light with three specific wavelengths whose radiation is absorbed by the skin and diffusely reflected. A photo detector analyses the diffuse reflection from the skin. If the skin is well supplied with blood also the hemoglobin value is increased. Consequently, it is possible to evaluate the stimulation of the microcirculation before and after applications of different agents by measuring the hemoglobin value. The same measuring probe is used to quantify the skin redness (erythema) and determine the degree of skin tanning (melanin).

The initial absolute values of melanin were between 90-105 arbitrary units. UVB radiation used for exposure of Group 2 and Group 3 leads to a mild skin pigmentation (around 35 melanin units) which are normal values for this mice strain (the scale of Courage-Khazaka instrument is between 0-999 melanin units) (fig. 5). During the period of the experiment, the melanin amount determined by a non-invasive method employing the MPA5 Mexameter® MX 18 was increased for the groups exposed to UVB, respectively UVB and SLS. Hallyday and co-workers showed that SKH1 mice exposed to UVB radiation and retinoic acid did not presented an augmentation of melanin index after 10 weeks of experiment, but at later times the
increase was detected [19]. But, as a different pattern of behaviour, in our experiment higher values were recorded for the arbitrary units corresponding to melanin amount for the SLS-UVB group. It is very well known that skin pigmentation it tightly correlated with the amount of melanin that provides protection against ultraviolet (UV) radiation [15, 20]. Detection of melanin amount by Mexameter MX18 is a non-invasive method currently and intensively used in skin research [15, 21].

We were also interested in the evolution of erythema values under the action of UVB radiation and SLS. Erythema was also detected by the means of non-invasive MPA5 Mexameter® MX 18 method. The absolute values of erythema measured before the experiments were between 32-41 arbitrary units. Our results indicated significant increases of erythema values mainly for Group 2 and Group 3 as a consequence to exposure to UVB radiation and SLS topic applications (fig. 6).

Erythema, caused by rise of blood flow, due to the engorgement of vessels with oxygenated blood, is one of the four typical signs of inflammation [15]. It is very well known that UVB radiation induces erythema [1]. Furthermore, SLS was reported to induce the release of inflammatory mediators like tumor necrosis factor-α, interleukin (IL)-1α, IL-6, and IL-8 [22]. SLS can also cause an increase in skin thickness and an intense immunity activity in the skin [23]. Association of these agents (UVB and SLS) is a noxious problem for the skin even if is a short time exposure. Dry skin seems to be the most affected to UVB exposure.

Hydration of stratum corneum
An adequate moisture content of the stratum corneum is essential for a well-functioning skin barrier. The moisture content is one of the most important parameter for establishing a skin diagnosis and it was measured with a Corneometer probe. The analysis of the moisture retention capacity of the skin is easy to perform, is based on the dielectric constant of the water and measured in the
superficial layers of the stratum corneum as deep as 10-20 μm to ensure that the measurement is not influenced by capillary blood vessels [24]. Hydration of stratum corneum was also analyzed in the present experiment. The initial absolute values of hydration of stratum corneum were between 8.3-11.1 arbitrary units. It was observed that exposure to UVB radiation and SLS topical application induced a significant decrease of skin hydration as compared to control group (fig. 7).

All the physiological skin parameters evaluated in this study suffered important changes as a result of the aggressive effects of the two agents, UVB radiation and SLS. The highest degree of injury and the most relevant induced-changes in parameters quality were observed in group 3 (mice that were exposed to UVB and received SLS topically). It was detected an increase of TWL, melanin content, erythema and pH and a decrease of sebum and hydration of stratum corneum. This situation indicates the intense and fast degradation of skin in these aggressive conditions. The correlation with human skin can be considered relevant even though there are differences between the composition and skin parameters [6, 25] because the quantity and quality of damages is relevant. Also SKH1 mouse model is well related on studies regarding skin evaluation correlated to humans [26].

Conclusions
Aggressive external agents like UVB and SLS can cause intense damages on skin level. They induce fast changes in all skin main parameters including lipid/hydrophil content. Erythema is one of the fast detectable and easy observable increased parameter. All these aspects are more relevant if is included in discussion a dry skin or a more relevant if is included in discussion a dry skin or a observable increased parameter. All these aspects impose a monitoring on skin parameters if there is a susceptibility of UVB exposure and a fast protection.

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References
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