Skin cancer is a multistep process characterized by three main phases: initiation, promotion and progression. The development of mouse models that reproduce these conditions are considered useful tools for the understanding of molecular mechanisms involved in skin carcinoma initiation. The aim of our study was to evaluate the noxious effects at skin level induced by concomitant exposure to 3 pro-carcinogens agents: UVB radiation, DMBA and TPA. Our results indicated that application of these toxic compounds led to the development of skin papilloma and to significant changes in skin physiological parameters (skin pH, transepidermal water loss, erythema and melanin).

Keywords: UVB, skin carcinoma, parameters

Skin cancer has become the most common cancer in various parts of the world including United States [1]. The most commonly diagnosed cancers all around the world are non-melanoma skin cancers including cutaneous basal cell carcinoma and cutaneous squamous cell carcinoma [2]. For example, according World Health Organization, between 2 and 3 million of new non-melanoma skin cancers were globally diagnosed in each year and their rate continues to increase, most of them being caused by ultraviolet (UV) light exposure [3].

Melanoma skin cancer represents only 2% of all skin cancer cases, but it is responsible for the most skin cancer death. In this regard, UV radiation especially UVB (λ, 290–320 nm) is considered to be the major risk factor for both melanoma and non-melanoma skin cancer [4].

UV radiation, including sunburn, is responsible for various physiopathologic cutaneous alterations including inflammation, immunosuppression, premature skin aging and cell death through the activation of death receptors, DNA damage and through decreasing of the anti-oxidant defenses [5].

Furthermore, chronic UV exposure has showed a destructive effect on the connective skin tissue, increasing the amount of the elastic fibers and changing the dermal collagen structure [6].

In this regard, skin cancers animal models are very useful not only for analyzing the mechanisms of induction of skin cancer by UV radiation but also for studying the anti-proliferative effects of different drugs that may be used in skin cancer, in the near future.

Experimental part

Materials and methods

In order to elaborate this study, we used SKH-1 hairless male mice [(20-22) weeks old] purchased from Charles River, Budapest. The experimental protocols used are in agreement with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The animals were fed ad libitum and kept in standard conditions: constant temperature of 22.5 ± 2°C, humidity of 55 ± 5% and a 12 h (light) – 12 h (dark) cycle.

The two-stage skin carcinogenesis mouse model was obtained according to the protocols described in the literature [7, 8], but with several modifications: the first step – double tumor initiation consisted of exposure to ultraviolet radiation (UVB) for 5 min, followed by topical application of 7, 12-dimethyl benzanthracene (DMBA) acetone solution (0.025 - 200 µL/application) at 30 min post-UVB exposure once a week for one week; the second step – tumor promotion: exposure to UVB radiation for 5 min followed by topical application of a pro-inflammatory phorbol ester, 12-O-tetradecanoylphorbol 13-acetate (TPA) acetone solution (15 nM– 200 µL/application) – the procedures in this phase were done twice a week until the end of the experiment – 21 weeks.

The animals were divided in 3 groups of work (n=5 mice/group): control group (group 1) – healthy mice (no interventions were applied); group 2 – mice exposed to UVB – radiation and topically administration of acetone (the solvent of DMBA and TPA – 200 µL/application) and group 3 – mice with skin cancer (exposure to UVB – radiation and topical application of DMBA and TPA solutions).

In this study were measured different skin physiological parameters, including: melanin, erythema, skin-pH and transepidermal water loss (TEWL) by the means of a non-invasive technique using a Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany. The measurements of melanin and erythema were carried out by means of the MPA5 Mxameter® MX 18 probe, a Skin-pH-meter® PH 905 probe for skin pH determination, and a Tewameter®, incorporated in Multiprobe Adapter System (MPA 5). The measurements were conducted twice a week starting from the first week of experiment before the exposure to UVB radiation.

Results and discussions

The aim of this study was to evaluate the noxious effects at skin level induced by concomitant and repeated (21 weeks) exposure to 3 toxic agents (environmental and chemical): UVB radiation (tumor initiator and promoter), DMBA (tumor initiator) and TPA (tumor promoter).

According to the literature, hairless mice represents an important tool to study the effects of different agents at skin level, SKH-1 mice being a strain used very frequently in skin cancer animal models [9,10].
The exposure to UVB radiation 30 min before DMBA and TPA topical applications represented a change in the classical protocol for two-stage skin carcinoma described in the literature. The mice included in the study were monitored and macroscopically analyzed every second day during the experiment for counting and recording of the incidence and numbers of papilloma.

According to our results, the apparition of the first papilloma was observed starting with the 11th week of experiment in the group exposed to UVB radiation and TPA. As it can be seen in figure 1, after 10 weeks of experiment no papilloma was detected in group 3 (exposed to UVB radiation, DMBA and TPA) whereas the skin presented lesions such as redness and dryness both in group 3 and in group 2 (UVB radiation + acetone). At week 16 of experiment, the papillomas were visible and palpable in group 3 and the number of papillomas increased beginning with the 19th week of experiment as compared to group 2 which haven’t developed (week 21 - fig.1). After 21 weeks of experiment the mice were sacrificed and organ samples were analyzed.

UVB-radiation used as a tumor promoter in our experiment is related to noxious cellular, biochemical and molecular events, including: generation of oxygen free radicals and other type of free radicals, depletion of antioxidant systems and acute inflammation expressed as skin edema [11]. Application of DMBA in the initiation phase followed by TPA application (promotion) leads usually to a hyperproliferative cutaneous response like as hyperplasia and promotion of cells that express proliferative markers in the dermis, and also to development of squamous papilloma [12]. A possible toxic mechanism of action of TPA consists of its ability to induce excessive production of ROS and an impairment of antioxidant systems, such as SOD (superoxide dismutase), leading to oxidative stress, one of the factors involved in the development of mouse skin carcinogenesis [12].

No important change of transepidermal water loss was obtained in the case of blank and acetone groups: TEWL values indicate a very slight increase which is specific for any non-toxic compounds topically applied. In the case of mice from group 3, the increase of transepidermal water loss was more pronounced and these two agents may be
considered as agents with harmful effect on skin (fig. 2). Transepidermal water loss is a physiological parameter used to verify the skin water barrier function and integrity, this barrier being disturbed in skin pathologies [13, 14].

Skin-pH values presented very slight and non-linear fluctuations (fig. 2), between 0.2 and 0.6 units. There was a slight increase in the values recorded for those mice which were exposed to UVB, DMBA and TPA.

Values of melanin presented an almost constant trend in the case of mice from groups 1 and 2, and an important upward trend in the case of mice from group 3 (exposed to UVB, DMBA and TPA); this upward trend is a normal one because it is well-known that UVB exposure leads to an increase of melanin content in the skin (fig. 3).

There were significant differences between group 1 and group 2 on the one hand, and group 3 on the other hand in the case of erythema measurements. The values were modified with 12 arbitrary units (group 1) and 18 arbitrary units (group 2), while an important change (43 arbitrary units) was obtained for group 3 after 21 weeks of exposures-evaluations.

Conclusions

Thus, it can be concluded that the exposure to UVB, DMBA and TPA leads to deteriorations of barrier function of skin by increasing the levels of transepidermal water loss and melanin. Furthermore, the values of skin pH and erythema were elevated in the mice that were exposed to UVB, DMBA and TPA what indicates skin damage associated with the apparition of papillomas. Consequently, this model of photochemical-induced skin carcinoma in hairless SKH-1 mice using UVB as a tumor promoter proved to be a viable, reproducible model of skin carcinoma that can be used for testing the anti-proliferative effects of different natural and synthetic drugs.

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