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Correlation between the Energy Density of Ultraviolet Radiation and Skin Thickening In an Animal Mouse Model: Induction of Trichoepithelioma in a Mouse Model

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ABSTRACT

Introduction: Exposure to ultraviolet (UV) radiation causes oxidative damage and cancer in the epidermis. The thickening of the skin layer seems to be correlated with carcinogenicity. The present study aimed to induce trichoepithelioma, a rare benign skin lesion, in an animal model and investigate the relationship between the radiation dose of UV waves and the thickening of skin layers resulting from high-frequency ultrasound images.

Material and Methods: To investigate skin damage process, 25 C57BL6 mice were irradiated with Ultraviolet B-rays (UVB) (5 times a week for 9 weeks) with an energy density of 135, 270, 405, 540, 675, 810, 945, 1080, and 1215 J/m^2, from the first week to the ninth week, respectively. The thickness of the skin layer was weekly measured by ultrasound images. The correlation between the thickness of the skin layer and the radiation energy density was analyzed by Pearson correlation analysis.

Results: The thickness of the skin layer demonstrated a significant increase in the 7th week of exposure during the injury process due to UV radiation, as compared to zero-day ($P<0.05$). Furthermore, it showed a 38 % increase in the 7th week. The obtained results illustrated a significant correlation coefficient of more than 0.97 between the thickness of the skin layer and the energy density of UV radiation. Microscopic sections in the long-term UV-irradiated group confirmed trichoepithelioma.

Conclusion: As evidenced by the obtained results, prolonged irradiation for 9 weeks induced an animal model of trichoepithelioma.

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Introduction

Ultraviolet (UV) radiation causes direct and indirect damage to the cell structure, thereby increasing the aging process. Ultraviolet B-rays (UVB) (with 280-320 nm wavelengths) cause direct damage to the skin, and the portion that reaches the epidermal cells induces biological damage [1] and the most important biological damage is inflicted by DNA damage. With UVB absorption, thymine bases in the DNA bind to adjacent thymine [2]. This evidence can affect protein synthesis. The accumulation of unrepaired damages can disrupt the cell cycle and create cell death. Moreover, these damages can sometimes destroy the cell apoptotic ability and increase the malignancies. The results depend on the type of tissue, UV dose, and its wavelength [3].

UV radiation initiates and activates a complex cascade of biochemical reactions in human skin. UVB destroys cellular antioxidants and antioxidant enzymes and DNA damages, which produces thymidine dimmers, activating the neuroendocrine system to suppress immunity [4]. Inflammatory mediators increase the permeability of the capillary membrane; therefore, neutrophils and other phagocytic cells are infiltrated in the skin. These effects create inflammation and free radical production [5]. Neutrophils release elastases and other proteases (cathepsin G) which cause inflammation and activate matrix metalloproteinases, leading to matrix degradation and accumulation of non-functional matrix components [6]. Inflammation and oxygen-free species cause oxidative damage to cellular proteins, fats, and carbohydrates, resulting in skin damages in the dermis and epidermal layers [7]. In the present study, we induced trichoepithelioma damage in an animal model. Thereafter, the correlation between the ultraviolet radiation dose and thickening of the skin layers resulting from ultrasonography was evaluated.

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Materials and Methods

A number of 25 male C57BL/6J mice aged 4-5 weeks weighing between 15-20 g were obtained from Pasteur Research Institute (Karaj, Iran). Animals were kept in a suitable environment away from direct light in laboratory animal cages for two weeks. During their maintenance, they were given free access to food, water, and standard temperatures, with 12-hours lighting and a 12-hours shutdown period at Tarbiat Modares University Animal Laboratory (Code of Ethics: 4284 Tarbiat Modares University). The animal's back hair was completely shaved the day before the imaging.

To achieve the animal model of UV damage [8], the mice were irradiated with UVB (Philips, 40 W, λ= 280 nm, Germany) as a group (due to a decrease in skin characteristics) 5 times a week for 9 weeks. The radiation intensity of 30 μW/cm^2 was measured by a UV meter (UV-3, Sibata ScientificTechnology Ltd, and Japan). The skin was irradiated with an energy density of 135, 270, 405, 540, 675, 810, 945, 1080, and 1215 J/m^2, from the first week to the ninth week, respectively (135×n; n=1-9).

To investigate the process of skin lesion formation, the histological study was performed with Hematoxylin and Eosin (H&E) Staining protocol. At the 9th week, fibroblast cells were examined, and an animal model of rare trichoepithelioma carcinoma was first induced by examining the skin lesions of a continuously irradiated animal.

High-frequency B-mode ultrasound imaging (Sonix TOUCH Ultrasound System, Ultrasonix Medical, Richmond, Canada) was performed with a linear array probe of 1.5 cm with the center frequency of 40 MHz ensuring that the layers of the skin are fully visible. After Calibrating "ImageJ" Software based on the resolution of the ultrasound images, the thickness of the skin layers was measured before and after UVB irradiation during the development of UV damage. Imaging protocols were performed weekly to examine the physical parameters of skin layer dimensions during the lesion process. The results of the physical parameters of skin layers were reported as mean and standard deviation, and the normality of the data distribution was assessed. One-way ANOVA with a 95% significance level was used to compare the mean skin layer thickness within the weeks under study. The correlation between the thickness of the skin layers and the energy density of UV radiation was analyzed by Pearson correlation analysis and linear regression. The obtained data were analyzed in SPSS software (version 17).

Results

Figure 1 illustrates the mean and standard deviation of skin thickness, including the sum of dermis thickness and epidermis thickness. The results indicated that before irradiation (zero-day), the thickness of skin layers (epidermis and dermis) was 0.62±0.05 mm with a coefficient of variance (CV)< 6%. On days 7, 14, 21, 28, 35, and 42 after UV irradiation, a significant increase was observed in mean thickness following UV-induced zero-day damage (P<0.05). On days 66 and 63 after the irradiation, the deep areas below the scar were not visible as the result of skin damage due to scarring and skin loss, as well as the high reflection of ultrasound by the scar. Figure 2 depicts the complete scar area.

An example of ultrasound and photographic images of animal skin during the skin irradiation process is displayed in Figure 2.

The comparison of ultrasound images demonstrated that skin ulcer developed in the group with long-lasting UV radiation. In the weekly assessments, some indicators, such as epidermal scaling, roughness of the skin, and tanning of the skin, were observed. UV waves exert marked effects on the skin, including tanning, redness, actinic keratosis, basal and squamous cell carcinoma, and telangiectasia. Accordingly, it can be concluded that the persistence of UV radiation can lead to some form of skin lesions. The results of the study of microscopic sections in week 9 are displayed in Figure 3.

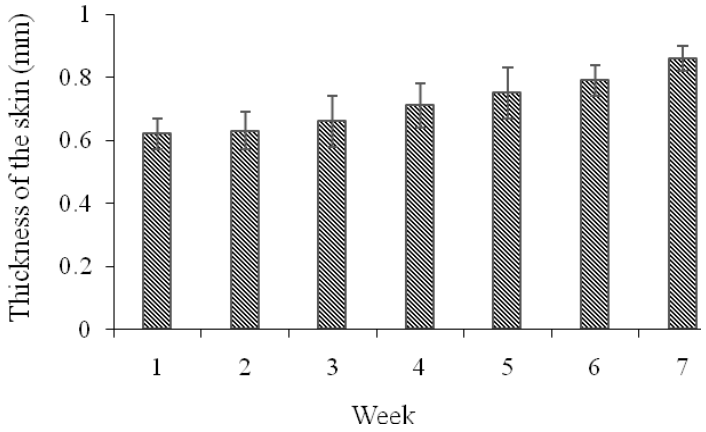
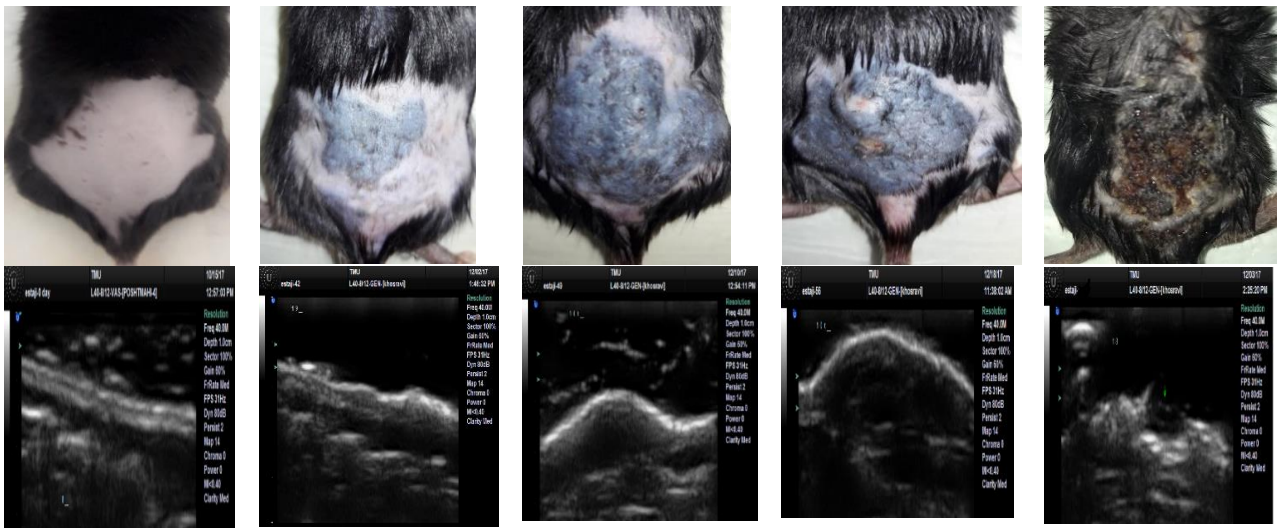
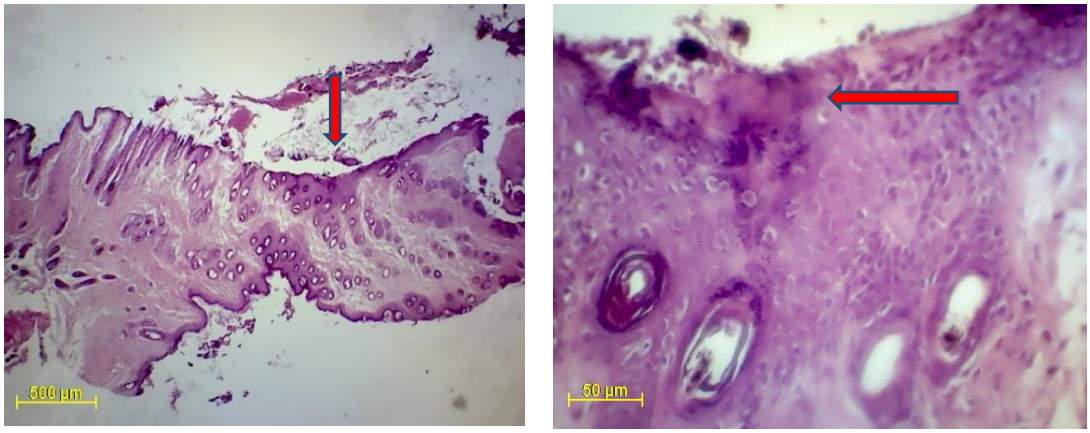


Figure 1. Mean and standard deviation of skin thickness (epidermal and dermis) by mm during the lesion process from the 1sth week (without UV irradiation) to the 7th week (after UV irradiation)



(a) (b) (c) (d) (e)

Figure 2. Photographic and high-resolution B-mode ultrasound (UV) images during the UVB irradiation process a) normal, b) 6th week of radiation, c) 7th week of radiation, d) 8th week of radiation, and e) 9th week of radiation



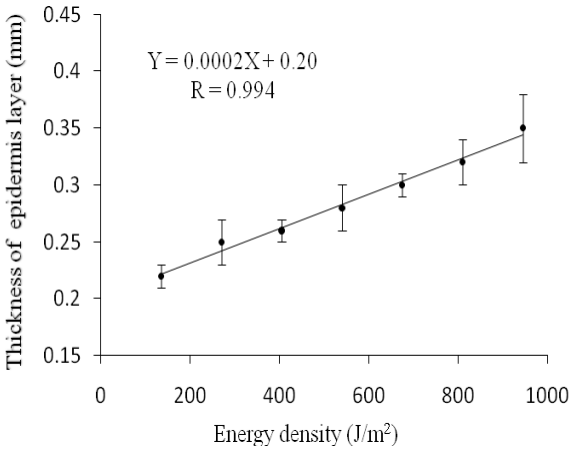
(a) (b)

Figure 3. Histological sections of trichoepithelioma skin lesion with hematoxylin-eosin staining, a) Specific small and minor lesion in superficial dermis (× 100), b) Tumor islands (× 400)

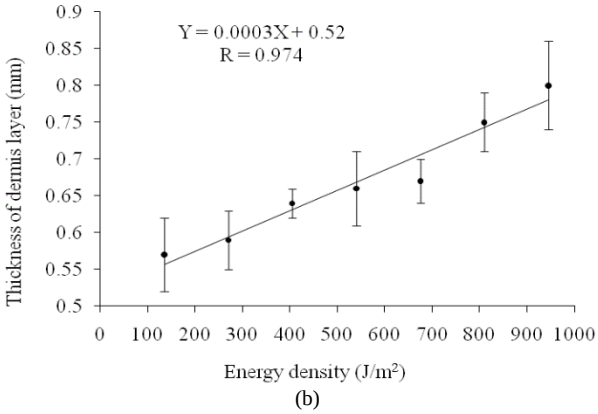
Based on the results, prolonged exposure to UV waves can develop some types of skin lesions. Trichoepitheloma is histologically similar to basal cell carcinoma in such a way that clinical and histological differentiation between them is difficult in some cases. The correct diagnosis of these lesions is of utmost importance for proper treatment decisions. This cutaneous lesion is composed of benign growth of epithelial-mesenchymal stromal cells. Morphologically, these lesions are prominent or numerous nodes 2-8 mm are scattered in the fully keratinized center with the lesion. There is a stroma containing a moderate amount of fibroblasts around these tumor islands. Histopathologically, trichoepithelioma consists of a set of basaloid cells, horn cysts, and hair papillae (Figure 3). Therefore, based on the histological findings, it can be argued that prolonged exposure to UV irradiation results in the occurrence of trichoepithelioma.

The results of Pearson correlation analysis and linear regression regarding the correlation of energy density of UV irradiation (135, 270, 405, 540, 675, 810, 945 J/m^2) with the thickening of epidermis and dermis layers are illustrated in Figure 4. Due to the high absorption of ultrasound by the scar, the deep areas below the scar were not visible; therefore, there exists no in-depth information on these energy density levels.

The results indicated that with a correlation coefficient of more than 0.97, there was a significant correlation between the thickening of the dermal and epidermal layers with the energy density of UV irradiation during 7 weeks of irradiation. The width of the origin of the regression functions corresponds to the thickness of the layers before UV radiation.



(a)



(b)

Figure 4. Linear correlation between thickening of the skin layer, a) epidermis and b) dermis during 7-week UVB irradiation and energy density of UV irradiation

Discussion

UV radiation to the skin can directly cause the formation of free radicals which cause a state similar to oxidative damage in the target cells and cancer in the epidermis. Therefore, there may exist a relationship between the thickening of the skin layer (and carcinogenicity) and the energy density of the UV radiation. The researchers stated that exposure to acute ultraviolet radiation increases the thickness of skin layers [9, 10]. The obtained results of the present study illustrated a significant correlation coefficient of more than 0.97 between the thickness of the skin layer and the energy density of UV radiation.

Ahmadi Ashtiani et al. [11] demonstrated that exposure to UV radiation can directly cause the formation of free radicals resulting in a state resembling oxidative damage in the target cells. Oxidative stress can damage cellular components and alter gene expression patterns. It ultimately leads to skin damages, such as melanoma and non-melanoma skin lesions, phototoxic effects, and aging. In the present study, due to prolonged exposure of mice to UV radiation, the thickness of the skin layers increased owing to the destruction of collagen fibers, their twisting and breaking, the accumulation of non-functional fibers, and the increase in inflammatory cells. In the current study, the thickness of skin layers (epidermis and dermis) on day 49 of UV irradiation showed a significant difference, as compared to zero-day (healthy mice). The percentage increase in mean thickness of the skin layers in the second, third, fourth, fifth, sixth, and seventh weeks was 1, 6, 14, 16, 27, and 38%, respectively, as compared to the pre-irradiated group.

Wang et al. [12] reported exposure to UV radiation due to increased expression of cyclic oxygenase, which in turn, increased the production of prostaglandin metabolites (PGs), known as tumor promoters in the skin. In the same context, Scott et al. [13] concluded that altering the thickness of epidermis by high-dose UVB light (MED 7.5) after 72 h impaired the structure of the epidermal cell, as well as its DNA and RNA. In another study, Raad et al. [14] irradiated white mice at the wavelengths of 292, 300, 307, 362 nm five times a week throughout a year. They observed that high-dose UV radiation increased the risk of carcinogenesis in basal cells and may cause melanoma.

In the same direction, Moon et al. [15] indicated that prolonged exposure to UVB is likely to cause precancerous and skin lesions in the epidermis. Therefore, there may be a link between the thickening of skin epidermis and skin carcinogenicity. The researchers also argued that a sharp increase in the thickness of the epidermis may cause basal cell cancer. In the present study, the thickness of the skin layers increased after UV irradiation. Trichoepithelioma is a benign skin lesion of follicular origin with histologically relevant, small, and distinctive lesions in the superficial dermis. The distribution of melanin in the basal layer is irregular which can be attributed to the destruction of pigment transfer from melanocytes to keratinocytes. Skin lesions, according to studies, have introduced an animal model for the induction of Trichoepithelioma.

Conclusion

In the present study, trichoepithelioma, which is a rare benign skin lesion, was induced in an animal model of C57BL6 mice. The obtained results pointed to the significant correlation (more than 0.97) of the energy density of UV irradiation with the thickening of the dermal layers and the epidermis during 7 weeks of irradiation.

Acknowledgment

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