Annals of West University of Timisoara

Series of Chemistry **20 (2)** (2011) 87-93

PROTEOMIC ANALYSIS OF IL-6 EXPRESSION IN UVB IRRADIATED RAT SKIN

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Received: 5 June 2011 Modified 12 June 2011 Accepted 22 June 2011

SUMMARY

The incidence of skin cancer has significantly increased over the past decade. It is important to define the accurate etiology and the mechanisms involved in development of skin cancer to apply the appropriate preventative measures. Interleukin-6 (IL-6) is a pleiotropic cytokine that contributes in the body to a multiple biological processes. IL-6 induces the final maturation of B cells into antibody-producing cells and can enhance or inhibit the proliferation of carcinoma cells. We try to determine the serum level of IL-6 in rat with irradiated with UVB. We have used Sprague-Dawley rats UVB irradiated in comparison with control (non-irradiated) lot. UVB radiation determined a cutaneous

inflammatory response at 24h after the last irradiation. The maximum inflammatory reaction was evident at 48h after the UVB exposure. Acute exposure to UVB radiations induced acute inflammatory response as evidenced by the increased level of IL-6 in serum both after 24 and 48 hours after the last irradiation. Inflammatory response to UVB radiation has been reflected clinically by apparition of erythema and local edema.
Keywords: IL-6; UVB radiation; rats skin.

INTRODUCTION

Ultraviolet radiations are injurious to the body especially to the skin, producing photodermatosis. Medium ultraviolet radiations (UVB) are between 290-320 nm. They can penetrate up to the papillary body causing vasodilatation and edema. Although only 1-2 % of the total UVB radiation reaches the earth surface they have a strong carcinogenic action being one of the main etiological causes of skin cancer via gene mutations and immunosuppression [1, 2, 3]. Skin exposure to UVB radiation determines a series of biological events, including the appearance of inflammatory response traditionally associated with erythema. The cause of erythema is the altered DNA directly exposed to photons. Chronic exposure may cause skin cancer in the absence of mechanisms to repair DNA macromolecule [4].

Interleukin-6 (IL-6) is a pluripotent cytokine, originally identified as a T-cell-derived cytokine, and synthesized by a variety of cells upon stimulation [5]. This cytokine induces final maturation of B cells into antibody-producing cells and contributes in the body to a multiple biological processes, in various types of tissues and cells [6, 7]. Il-6 plays an important role of this cytokine in the inflammatory reaction and immune response of the host organism to cancer by regulating immune responses, homeostasis, and the induction of the acute phase reaction [8, 9]. IL-6 regulates the growth, differentiation, and death of several cell populations including neurons, keratinocytes and melanocytes [10, 11, 12].

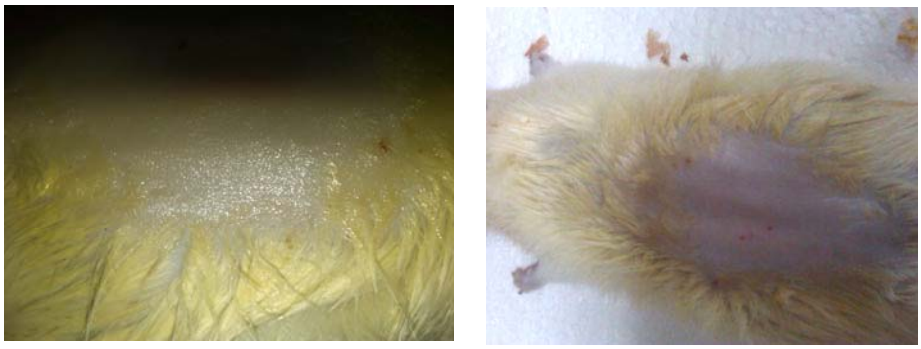
The UV irradiation is highly carcinogenic, being involved in initiation, promotion and progression of carcinogenesis process. One single exposure to UV causes a skin acute inflammatory response. Burns caused by acute exposure to UVB are characterized by erythema and edema due to increased vascular flow and vascular permeability [2, 13]. The inflammatory response is associated with an increased synthesis of prostaglandin E2 due to induction of cyclooxygenase 2 (COX2) [14]. In addition keratinocytes exposed to UVB causes the release of proinflammatory cytokines (interleukins) and reactive oxygen species (ROS) [4]. We try to determine the serum level of IL-6 in rat with irradiated with UVB.

MATERIALS AND METHODS

In this study, we used 15 male and female rats from Sprague-Dawley breed with an average weight of 409.06±30.56 grams. All animals were kept under standard conditions of humidity (45-55%), temperature (25º C) and light control (12 h light/12 h dark) following the European standards for protection of animals used in laboratory experiments [15]. Rats received normal diet and water ad libitum. To achieve the experiment (irradiation of dorsal area with UVB) and for collection of biological material, the rats were anesthetized with veterinary use ketamine (Calypso) 30 mg/kg body. At the end of the experiment, after anesthesia, blood was collected by venipuncture of portal vein. In order to determine the plasma concentration of interleukin-6 we used the ELISA (enzyme-linked immunosorbent assay) method using Rat IL-6 Immunoassay kit (Cytoscreen, Biosource). After collection the blood was rapidly centrifuged at 2500 rpm for 5 minutes and the samples were stored at -80º C until processed. The rats were divided in three lots: lot I - the control group, lot II animals irradiated with UVB and sacrificed in 24 hours after the last irradiation and lot III irradiated with UVB and sacrificed in 48 hours after the last irradiation. Irradiation was performed with a UVB lamp (290-320 nm), for 30 minutes, 3 days/week, from 30 cm of the skin. The skin from the dorsal part of the body was irradiated, which has been previously washed and shaved. After 24 h, 48 h from the last irradiation, the animals were sacrificed after a pre-anesthesia.

RESULTS AND DISCUSSION

At the animals from lot I (control) no macroscopic changes were observed (Fig. 1a). Macroscopic examination of the animals in the experimental groups showed that exposure to UVB radiation determined a cutaneous inflammatory response at 24 h after the last irradiation (Fig. 1b). The maximum inflammatory reaction was evident at 48 h after the exposure (Fig. 1c) both at female and male rats. The presence of UVB-induced skin inflammations was assessed by the presence of erythema and local edema and it was found at all animals from lot II and III).



a) lot I b) lot II

c) lot III

Figure 1. Macroscopic evaluation

Acute exposure to UVB radiation produces: an inflammatory response, DNA damage in skin cells, and a temporary and local immunosuppression. Inflammatory response includes synthesis of prostaglandins, release of cytokines, angiogenesis and an activated influx of neutrophils in the skin. Inflammatory response of the cells exposed to UVB, compared with controls, was analyzed by determining the serum values of IL-6 by Elisa technique. The obtained results represent the average and standard deviation, at control group and the experimental, at 24 h and 48 h from the last irradiation. In both experimental groups – lot II ($p<0.0001$) and lot III ($p <0.001$), we noted a significant increase in IL-6 protein expression. Plasma levels of IL-6 obtained at groups exposed at UVB were net superior to the serum values from the control lot, bot at animals sacrificed in 24 h from the last irradiation ($p< 0.0004$), and animals sacrificed in 48 h ($p< 0.00042$) (Fig. 2).

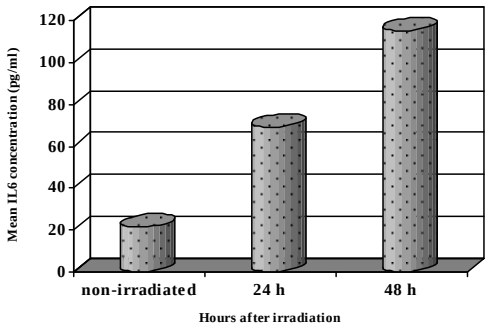


Figure 2. Serum level of IL6.

Acute exposure to ultraviolet light results was expressed by dermal inflammatory response, characteristic of classical sunburns. Sunburns caused by acute exposure to UVB are characterized by erythema and edema, due to the increased vascular flow and vascular permeability [13, 16]. Increased vascular flow and permeability causes itself infiltration of inflammatory cells in skin, including neutrophils and monocytes. The common effect of UV exposure is erythema assessed at 24 h after exposure, which is highly dose and wavelength dependent. Erythema is associated with molecular and cellular changes including the inflammatory cells in the skin layer [17]. Adhesion molecules expressed on vascular endothelial cells change their conformations to increase the adhesion of inflammatory cells and to reinforce their entrance in to the skin. Neutrophils are the first inflammatory cells recruited in the UVB exposed skin [18]. The primary function of the epidermis is to provide protection against numerous injuries present in external environment, including UV radiation. Protection measures, like apoptosis and inflammation, are considered to have a benefic role in protecting the epidermis, on long term, against propagation of potential tumorigenic cells, generated by acute exposure to a high dose of UV radiation [19]. However, these biological events can be detrimental to the architectural and functional integrity of the skin, due to the massive cell death and inflammatory response, which leads to epidermal erosion and consequently loss of barrier protective functions.

IL6 is a pro-inflammatory cytokine synthesized by many cell types: fibroblasts, endothelial cells, keratinocytes, in response to the action of other cytokines like TNF-α and Il-1. IL-6 is produced in cells of several organs, but the serum levels are very low at healthy people. Interleukin-6 was found to have a central role in defense mechanism by regulating the immune response, homeostasis and induction of acute phase reaction. Increased serum levels of IL-6 have been shown to be associated with progression of prostate cancer, independent androgen phenotype. Many types of cancer, like melanoma, renal carcinoma,

Kaposi's sarcoma, ovarian cancer, lymphoma, leukemia, multiple myeloma, was found to be activated by IL-6. Some study suggested that IL-6 stimulate the proliferation of cultured cerebral endothelial cells and plays an important role in the neovascularization that accompanies physiologic tissue remodeling [20-26].

CONCLUSION

Acute exposure to ultraviolet light results was expressed by dermal inflammatory response, characteristic of classical sunburns. Il-6 plays an important role in the inflammatory reaction and immune response of the host organism to cancer.

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