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Role of MMP-8 in photoaging and photocarcinogenesis

Rola MMP-8 w fotostarzeniu i fotokancerogenezie

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ABSTRACT

Introduction: Ultraviolet radiation (UVR) is especially harmful to human skin. It mostly contributes to skin photoaging that is associated with skin carcinomas, such as the most common skin cancer — basal cell carcinoma (BCC). It has been shown that significant role in skin carcinogenesis plays short-wavelength UVB radiation. What is more, UVR by changing the expression of matrix metalloproteinases (MMPs) in skin contributes to photoaging and plays an important role in photocarcinogenesis. MMP-8 is one of collagenases that is released during inflammatory process.
Material and methods: The study group consists of 85 individuals (22 patients with diagnosed BCC and 63 healthy volunteers that have been exposed to various doses of UVB radiation) in order to evaluate the expression of MMP-8 protein in skin biopsies. The Western-blot method was used to analyse the results.
Results: The expression of MMP-8 has been observed in all skin biopsies. The performed analysis did not present statistical significant difference in expression of MMP-8 between skin samples with BCC or exposed to UVR in comparison to healthy skin samples.
Conclusions: The role of MMP-8 in skin photoaging has little activity in skin following UVB radiation and it is slightly probable that MMP-8 contributes to photoaging and photocancerogenesis.

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Key words: ultra violet radiation, photocancerogenesis, matrix metalloproteinases-8

STRESZCZENIE

Wstęp: Promieniowanie ultrafioletowe (UVR, *ultraviolet radiation*) jest szczególnie szkodliwe dla skóry człowieka. Przyczynia się głównie do jej fotostarzenia, co w konsekwencji wiąże się z predyspozycją do tworzenia się nowotworów skóry, takich jak rak podstawnokomórkowy (BCC, *basal cell carcinoma*). Najważniejszą rolę w skórnej kancerogenezie odgrywa promieniowanie UVB, które powoduje zmianę ekspresji metaloproteinaz (MMP, *matrix metalloproteinases*) w skórze, przyczyniając się do rozwoju fotostarzenia. MMP-8 jest kolagenazą uwalnianą ze specjalnych ziarnistości, głównie wielojądrzastych neutrofili w miejscach stanu zapalnego.
Materiał i metody: Materiał badawczy stanowiło 85 osób (22 przypadki pacjentów ze zdiagnozowanym histopatologicznie BCC oraz 63 zdrowych ochotników, którzy zostali poddani naświetlaniom różnymi dawkami promieniowania UVB). U osób tych dokonano analizy poziomu ekspresji białka MMP-8 w bioptatach skórnych. Wyniki analizowano metodą *Western-blot*.
Wyniki: Ekspresja MMP-8 została zaobserwowana we wszystkich analizowanych bioptatach skórnych. Nie zauważono znaczącej statystycznie różnicy w ekspresji tej proteiny między próbkami ze zdiagnozowanym BCC oraz bioptatami poddanymi naświetlaniu w porównaniu ze zdrową skórą.
Wnioski: MMP-8 wykazuje niewielki udział w fotostarzeniu się skóry wywołanym pod wpływem promieniowania UVB. Rola tej metaloproteinazy w skórnej fotokancerogenezie nie została potwierdzona.

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Słowa kluczowe: promieniowanie ultrafioletowe, fotokancerogeneza, metaloproteinaza-8

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INTRODUCTION

Ultraviolet radiation (UVR) from the sun is especially harmful to human skin. UV-induced skin damage includes sunburn, altered pigmentation, immunosuppression as well as premature skin ageing (photoaging) [1, 2]. The last one is the most common form of skin damage and it is associated with skin carcinoma. Basal cell carcinoma (BCC) is the most common skin cancer that occurs in white population, arising particularly in sun-exposed areas of the body [3]. UVR is the most important environmental risk factor for development of this tumor. It has been shown that the most significant role in BCC carcinogenesis plays short-wavelength UVB radiation [4]. Photoaging affects all compartments and cellular components of the skin. Clinically visible features of altered skin include wrinkling and loss of elasticity. Loss of structural integrity of collagenous extracellular matrix (ECM) is probably primarily responsible for the wrinkled appearance of photodamaged skin [5]. Major alterations are seen in dermal connective tissue characterized by disorganized and damaged collagen fibrils and intense accumulation of altered elastic structures referred as "solar elastosis".

Matrix metalloproteinases (MMPs) are a well characterized family of proteases known not only to degradate ECM proteins, but also in activating cytokines such as tumor necrosis factor (TNF)-alpha and interleukin (IL)-1 beta [6]. UVR increases the expression of MMPs in skin and contributes to photoaging [7, 8]. MMPs by regulating different processes related to tumor progression such as tumor growth, angiogenesis and metastasis play an important role in photocarcinogenesis [9]. From the MMP-family we can distinguish a subgroup known as collagenase that is identified to have collagenolytic activities. Neutrophil collagenase (collagenase 2 or MMP-8) belongs to this group [10]. It is found in specific granules in neutrophils released during inflammatory processes but is also expressed by diverse cell types, including epithelial cells, fibroblasts, macrophages and endothelial cells [11–13]. MMP-8 is synthesized as latent proenzymes that requires proteolytic changing to become catalytically active and it cleaves type I collagen faster than type III [14].

However, whether UVB irradiation induces expression of MMP-8 in human skin, the samples with BCC have not been fully investigated. It has been observed that the increased risk of malignant melanoma and other cancers is correlated with the expression of MMP-8 [15]. However, its expression profile in BCC has not been clarified. In this study, we analyzed the expression of this protein in skin samples with diagnosed BCC and in healthy skin exposed to short and acute as well as chronic doses of UVB.

MATERIAL AND METHODS

The study group consists of 85 individuals. 22 patients with histologically confirmed BCC cases that were diagnosed at the Dermatology and Venerology Department in Łódź in years 2013–2015 and 63 healthy individuals as a control group divided according to gender and age. Healthy volunteers have been divided into subgroups that were exposed to various doses of ultraviolet radiations — different doses of minimal erythema dose (MED):

- control healthy group that was not exposed to any radiation (22 persons);
- volunteers that were exposed to short and acute ultraviolet radiation (3MED) (13 persons);
- volunteers that were exposed to chronic ultraviolet radiation (10 × 0,7 MED) (14 persons);
- volunteers that were firstly exposed to chronic ultraviolet radiation (10 × 0,7 MED) with the following of short and acute ultraviolet radiation (3MED) (14 persons).

The skin that was exposed to ultraviolet radiation and biopsied was the skin on buttock. The expression of chosen proteins in the specimens' skin was assessed applying Western blot method. Each research participant has been examined in details how he or she has been exposed to ultraviolet radiation previously. Each subject gave written informed consent before entering the study, which had been approved by the local Ethics Committee. The subjects were assessed by a dermatologist for their skin phototype according to the Fitzpatrick score and for hair and eye color. The tissues were immediately snap-frozen in liquid nitrogen and stored at –80°C until analysis. Statistical analysis was performed using Statistica software. The results comparing groups were analysed statistically by the Kruskal-Wallis test and considered statistically significant for $p < 0.05$.

RESULTS

The expression of MMP-8 has been observed in all skin biopsies. The performed analysis did not present statistical significant difference in expression of MMP-8 between various groups. The expression of MMP-8 is presented in Figure 1.

Western blot analysis of MMP-8 demonstrated different expression levels for various doses of radiation. The Figure 2 presents expression of MMP-8 assessed with Western blot method. The highest level of median expression was observed for control group that has not been radiated with UVR and was equal to 0.36×10^5 IDV (integrated density value). The metalloproteinase-8 expression was slightly lower for cases with diagnosed BCC (median value equal to 0.35×10^5 IDV) and the difference was not statistically significant. The expression observed in skin that was exposed to chronic ultraviolet radiation was the lowest one

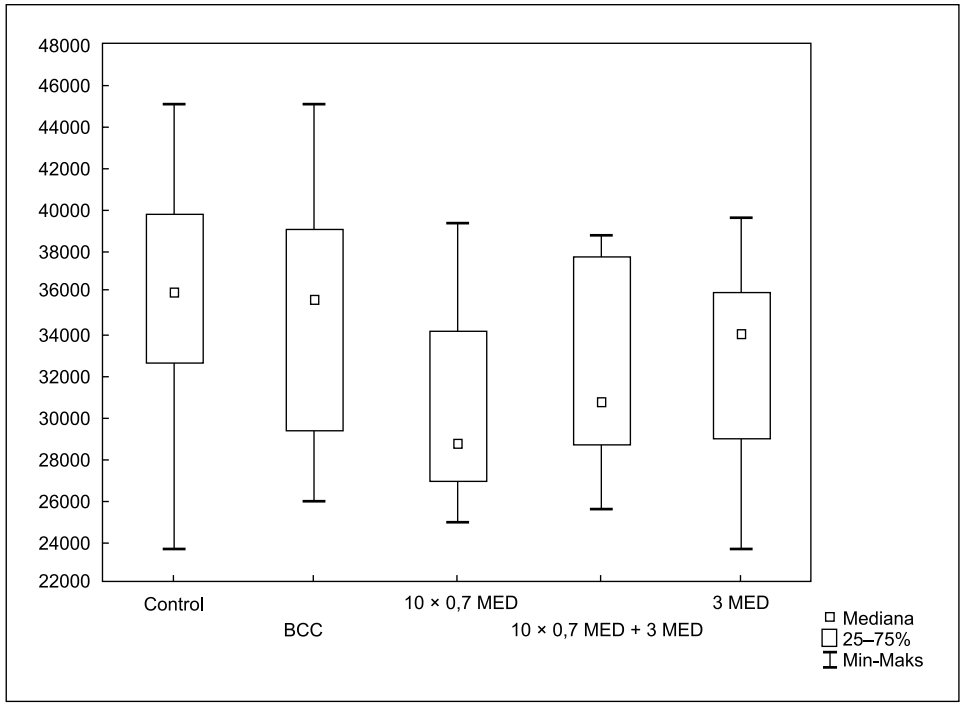

Figure 1. The expression of MMP-8 in various groups

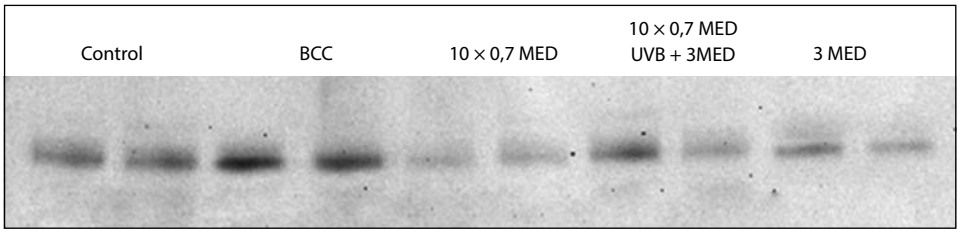

Figure 2. Expression of MMP-8 assessed with Western blot method

Table 1. Results obtained from Kruskal-Wallis test

	Control	BCC	10 × 0.7 MED	10 × 0.7 MED + 3MED	3MED
Control	X	1	0.092013	0.577351	0.808468
BCC	1	X	0.095893	1	1
10 × 0.7 MED	0.092013	0.095893	X	1	1
10 × 0.7 MED + 3MED	0.577351	1	1	X	1
3MED	0.808468	1	1	1	X

(median equals to 0.29×10^5 IDV). Slightly higher expression of MMP-8 was noticed in group of volunteers that were firstly exposed to chronic ultraviolet radiation (10 × 0.7 MED) with the following of short and acute ultraviolet radiation (median equals to 0.31×10^5 IDV), while the expression of MMP-8 in group of volunteers that were exposed to short and acute ultraviolet radiation was higher than in other radiated groups but lower than expression for control healthy group and patients diagnosed with BCC.

Kruskal-Wallis test has been performed in order to asses particular relationship between various levels of MMP-8 expression in different groups considered statistically significant for $p < 0.05$. The Table 1 presents results from Kruskal-Wallis test checking whether samples originate from the same distribution.

DISCUSSION

One of the major structural components of the ECM of dermal connective tissue is collagen. The accurate pathogenesis of the mechanisms by which collagen and elastic fibers are degradated and solar elastosis accumulated in skin remains incompletely understood. This process has been suggested to cause skin photoageing through acceleration of damage skin formation and impairment of the skin integrity [16]. Matrix metalloproteinases family comprise structurally related matrix-degrading enzymes that play a crucial role in destructive processes including inflammation, tumor invasion and skin aging. MMPs play a significant role in wrinkle formation, which is typical for photoageing. Degradation of type I collagen is initialized by one of three collagenases (MMP-1, MMP-8 or MMP-13). It has been proven that UVR induces MMP-1 in epidermis and dermis what is the cause of degradation of type I collagen in human skin [17]. MMP-13 presents higher cleavage specificity for type II collagen, which is characteristic for cartilage and it is five times less potent than MMP-1 in cleaving collagen types I and III [18]. The role of MMP-8 still remains ambiguous.

Fisher et al. [14] has demonstrated three independent methods — Western blot analysis, ELISA and immunohistology that UVR induces MMP-8 protein in human skin *in vivo*. They observed a significant increase of this protein within 8 hours after UVR which maintain for 24 hours. They demonstrated double staining immunofluorescence in neutrophils in UV-exposed skin. However, they found that neither normal human keratinocytes nor skin fibroblast have expressed MMP-8 mRNA after UVR exposure. It has been concluded that UVR of human skin does not indicate MMP-8 gene expression in skin cells. The increase of MMP-8 expression was caused by influx of neutrophils and macrophages from the circulation into the skin. However, the Western blot analysis has shown that in sample from nonirradiated human skin MMP-8 expression was minimally detected. Protein level was increased and remained elevated for 24 hours after irradiation. After 48 h the level of expression returned to the baseline. This observation is not consistent with our findings where the expression of MMP-8 was observed in all samples. We have not observed the increased level of MMP-8 expression in skin samples exposed to UVB in comparison to healthy skin samples. What is more, Fisher et al. [14] observed that MMP-8 was induced to similar levels of exposure to either a UVB-enriched source or the source that emits mostly UVA. The observed differences could be caused by the fact that patients' age probably differs. In our examination the average age was 58 y.o. for radiated healthy volunteers. The data presented by Fisher et al. [14] is only limited to information that the patients were adult.

Some other studies present a limited role of MMP-8 in UV-mediated collagen damage in the skin. However, this enzyme was observed to be induced by UV light, its upregulation is minimal [16]. It has been concluded that although MMP-8 was present after UVB radiation it probably contributed little to the overall structure damage to collagen in photoaging.

Despite the differences in various observations we can conclude that the role of MMP-8 in skin photoaging has little activity in skin following UV radiation. This is in accordance with results concluded by Fisher et al. [14]. No difference has been observed in our examination between the expression of MMP-8 in group that has been diagnosed with BCC and group of healthy volunteers. Thus it is very little probability that MMP-8 contributes to photoaging and photocancerogenesis.

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