Low-dose UVB irradiation prevents MMP2-induced skin hyperplasia by inhibiting inflammation and ROS

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Received April 23, 2015; Accepted June 11, 2015

DOI: 10.3892/or.2015.4072

Abstract. Skin cancer is one of the most common types of malignancy in the world. UV radiation is known as the primary environmental carcinogen responsible for skin cancer development. However, UV radiation is a ubiquitous substance existing in the environment and the physiological effect of UV radiation is consistently ignored. Therefore, in the present study, the physiological effect of UV radiation on inhibition of skin cancer was investigated. Normal mouse skin was processing by no pre-radiation or pre-radiation of low-dose UV before a medium or high dose of UV radiation. We found that the low-dose pre-radiated mouse skin tissue exhibited low skin inflammation, skin ROS production and consequently low skin epithelial hyperplasia after the medium-dose UV radiation compared with the no pre-radiated mouse. However, this inhibition was not indicated in the high-dose UV radiation group after low-dose pre-radiation. Furthermore, western blot analysis and gelatin zymography showed low expression and activation of MMP2 in the skin tissues processed following medium-dose radiation, but not in tissues treated with high-dose radiation after a low-dose pre-radiation. Further investigation of MMP2 inhibitors of TIMP2/TIMP4 showed an upregulated TIMP2 expression, but not TIMP4. Collectively, these data indicate that low-dose pre-radiation attenuates the skin inflammation and ROS production induced by medium-dose UV radiation and also elevates TIMP2 to withstand MMP2, therefore suppressing skin hyperplasia. The present study indicates a novel concept or prophylactic function of moderate UV radiation as a preventative strategy.

Introduction

Skin cancer has become a prevalent social and public health issue due to the fact that the incidence and mortality rates of skin cancer, including melanoma and non-melanoma skin cancers (NMSCs), consisting of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are rising in many countries (1,2). Skin cancer patients are associated with higher UV radiation exposure (3) and UVB susceptibility was reported to be significantly higher in skin cancer patients (4). In general, UV irradiation induces DNA damage, repair, skin inflammation and sunburn, gene mutation, post-inflammatory immunosuppression and eventually oncogenic pathway activity and is considered to be a major aetiological factor for skin carcinogenesis (5,6).

UVB exposure induces cutaneous inflammation. A single exposure to UVB may result in an acute inflammatory response, which is characterized by erythema (redness) and edema (swelling) due to increased vascular flow and vascular permeability (7,8). An inflammatory microenvironment aids in the proliferation and survival of malignant cells, promotes angiogenesis and metastasis, subverts adaptive immune responses and alters responses to hormones and chemotherapeutic agents, all of which promote the development of tumors (9,10). Inflammation is always accompanied by oxidative stress induced by reactive oxygen species (ROS) accumulation. ROS are believed to activate proliferative and cell survival signaling that alter apoptotic pathways that may be involved in the pathogenesis of a number of skin disorders. ROS act largely by driving several important molecular pathways that play important roles in diverse pathologic processes including inflammatory responses (11). Inflammation and ROS are inseparable and interact in the skin cancer process.

However, UV irradiation in sunlight is the most ubiquitous physical substance in our natural environment. Low-dose UVB irradiation prevents MMP2-induced skin hyperplasia by inhibiting inflammation and ROS.
4% of all neoplasms in Asians and 1 to 2% of all neoplasms in Blacks and Asian Indians (17-19). This fact indicates that skin cancer is less common in persons with skin color other than light-skin. Thus, melanin pigment is a UV screen that protects some deleterious sunlight compositions (20,21). Although the skin coloration in dark-skinned ethnic groups is the result of long-term evolution based on resistance and prevention of UVB irradiation-induced skin injury (22,23), in the short term, skin coloration namely pigmentation is due to solar irradiation. These studies indicate that a low dose of UV irradiation plays a protective role in skin cancer.

However, skin cancer still presents greater morbidity and mortality in colored-skinned individuals (17). It appears that the morbidity and mortality of skin cancer is not paralleled with incidence, since UVB irradiation depletes heavily pigmented skin of Langerhans cells (20). Once limited pigmented skin is depleted, the skin cancerization rate greatly increases. Therefore, the skin cancer protective role of melanin pigment is limited or UV-relative, but not an absolute prevention for skin cancer.

Collectively although excess UV irradiation certainly induces skin cancer, low-dose UV irradiation exhibits a protective role to prevent skin cancer yet is limited: i) to the most initial stage of cutaneous lesions or ii) to relative slight cutaneous lesions. However, we do not know how low-dose UV irradiation prevents these cutaneous lesions.

In the present study, we performed low-dose UVB pre-irradiation and then subsequent medium- or high-dose UVB irradiation. We found that low-dose UVB pre-irradiation inhibited medium-dose-induced skin inflammation, ROS production and initial keratinocyte hypertrophy while high-dose UVB irradiation did not induce change. This inhibition was associated with the balance of MMP2 and its inhibitor.

Materials and methods

Mice and UV irradiation. In the present experiment C57BL/6N mice were used and all the mice were purchased from Biocytogen Co., Ltd. (Beijing, China). The dorsal hair was removed by a depilatory in an area of 4 cm², and all mice were divided into 3 UV irradiation groups and 1 control group (n=8/group). The UV irradiation groups were exposed to low-, medium- and high-dose (10, 35 and 80 mJ/cm²) UV irradiation as reported previously (12). After pre-irradiation was performed from the age of 6 weeks and formal irradiation was carried out. All mice were fed ad libitum normal rodent chow and had access to water. The ECL Plus Western Blotting Detection System™ (Abnova, Taipei, Taiwan, China), mouse monoclonal IgG TIMP2 and TIMP4 antibodies (Abcam Trading Co. Ltd., Shanghai, China), mouse monoclonal IgG MMP2 antibody (Proteintech Group Inc., Wuhan, China), rabbit IgG MMP2 antibody (Proteintech Group Inc., Wuhan, China), mouse monoclonal IgG TIMP2 and TIMP4 antibodies (Abcam Trading Co. Ltd., Shanghai, China) in PBS containing 0.03% Tween-20 overnight at 4°C. The second reaction was performed using HRP-conjugated anti-rabbit IgG for 1 h at 22°C. The ECL Plus Western Blotting Detection System™ was used for enhanced chemiluminescence (ECL) detection of the protein bands.

SOD activity assay. SOD activity assay was performed as previously described (25). In brief, skin tissues were homogenized in cold buffer (0.1 M Tris/HCl, pH 7.4 containing 0.03% Tween-20, 0.1 M KCl, pH 7.4 containing 0.03% Tween-20 overnight at 4°C. The second reaction was performed using HRP-conjugated anti-rabbit IgG for 1 h at 22°C. The ECL Plus Western Blotting Detection System™ was used for enhanced chemiluminescence (ECL) detection of the protein bands.
0.5% Triton X-100, 5 mM \( \beta \)-ME and 0.1 mg/ml PMSF) and homogenates were centrifuged at 14,000 x g for 15 min at 4°C. The resulting supernatant that contained cytosolic SOD was subjected to an SOD activity assay with a commercial SOD activity assay kit (Biovision, Palo Alto, CA, USA).

Evaluation of keratinocyte hyperplasia. Dorsal skin tissues were excised 24 h after the last irradiation, and each harvested tissue sample was fixed in formalin and embedded in paraffin. Each sample was then cut into a section at 0.05-mm intervals. Each section was then stained with H&E staining. The thickness of the skin tissue keratinocytes was measured by SigmaPlot (Systat Software Inc. Chicago, IL, USA), and the average was calculated.

Gelatin zymography. To measure MMP2 activity, an extract of the supernatant of the skin tissue was collected. The gelatin zymography kit (Yagai Corporation, Tokyo, Japan) was used.
according to the manufacturer's instructions to perform gelatin zymography. In brief, 15 µl samples of conditioned supernatant were subjected to electrophoresis through gels provided in the gelatin zymography kit. Each gel was washed twice, once with each type of washing buffer for 30 min and then incubated for 40 h at 37˚C in the reaction buffer. Each gel was then stained with Coomassie blue and then destained three times so that the protein bands with gelatinolytic activity were easily identified.

**Statistical analysis.** Data are expressed as means ± SEM of triplicate runs. Each experiment was repeated as least twice. The Student's t-test and analysis of variants were used to assess differences and P<0.05 was considered to be significant.

**Results**

Effects of the different doses of UV irradiation on skin inflammation and ROS production. In order to confirm the role of various doses of UV irradiation on inflammation and ROS, we detected the time course of various classic inflammatory factors IL-1α, IL-1β and IL-6 mRNA from mouse dorsal skin tissues exposed to the three doses of UV irradiation. It was observed that low-dose irradiation did not induce any inflammation after a 4-week exposure compared to that before irradiation. A medium- or a high-dose irradiation induced a very significant gradual increase compared to that before irradiation (Fig. 1A). ROS accumulation detected by 4HNE also showed a similar result. Low-dose irradiation barely induced any ROS accumulation, while medium- or high-dose irradiation induced a significant increase in ROS accumulation (Fig. 1B). This result is consistent with the results of the ROS accumulation detection in the HaCaT keratinocyte cell line (Fig. 1D). However, different from inflammation, the difference in ROS accumulation induced between medium- and high-dose irradiation was not significant (Fig. 1B). This result forced us to consider that the imbalance of ROS accumulation and anti-ROS may be
the reason for the difference (26). Thus, we next detected the anti-ROS enzyme SOD1 and catalase. Both were increased in the low- and medium-dose irradiation groups but decreased in the high-dose irradiation group (Fig. 1C). These results indicate that although skin inflammation and ROS accumulation were correlated with the dose of UV irradiation, low-dose UV induced no inflammation and no ROS but increased anti-ROS ability, a potential protect effect.

Low-dose pre-irradiation protects skin epithelium against medium-dose UV irradiation-induced hyperplasia. Since low-dose irradiation presented a potential protective role, we aimed to ascertain whether low-dose UV irradiation may also suppress the medium- and high-dose induced skin epithelial proliferation and hypertrophy. Firstly, we confirmed that the skin epithelial hypertrophy was dependent on the dose of UV irradiation (Fig. 2Aa-c and B), while low-dose UV irradiation did not induce any hypertrophy compared with the no pre-irradiation group (Fig. 2Aa and d and B). However, compared to the medium-dose UV irradiation-induced epithelial hypertrophy (Fig. 2Aa and b and B), we found that medium-dose UV irradiation did not induce hypertrophy even in the low-dose...
pre-irradiation group (Fig. 2Ad and e and B). The inhibitory role was not observed when a high-dose UV irradiation was performed (Fig. 2Ac and f and B). These results indicated an inhibitory role of low-dose UV irradiation on medium-dose UV irradiation-induced epithelial hypertrophy, but not on high-dose UV irradiation-induced hypertrophy.

Low-dose pre-irradiation suppresses medium-dose UV irradiation-induced inflammation and ROS accumulation. As skin inflammation and ROS may lead to epithelial proliferation and even skin tumors (27) and low-dose pre-irradiation protected skin epithelial proliferation, we aimed to ascertain whether low-dose pre-irradiation suppresses UV irradiation-induced inflammation and ROS. All the mice were administered a medium- or high-dose re-irradiation after a low-dose UV pre-irradiation. We found that low-dose pre-irradiation suppressed medium-dose irradiation-induced inflammation but not high-dose irradiation-induced inflammation (Fig. 3A). Paralleled with this result, low-dose irradiation suppressed medium-dose irradiation-induced ROS accumulation but not high-dose irradiation-induced ROS accumulation (Fig. 3B). The same result was also found in
accumulation, which are the causes of skin epithelial hyperplasia, we did not know whether low-dose irradiation also affects MMP2 and TIMPs which are the direct mechanisms of skin epithelial hyperplasia (28). We found that low-dose irradiation did not affect the MMP2 expression and its activation, however, low-dose irradiation inhibited the increase in MMP2 induced by medium- but not high-dose irradiation (Fig. 4A and B).

Next, we aimed to ascertain how MMP2 inhibitor, TIMP2 and TIMP4, are affected after low-dose irradiation. We found that TIMP2 was increased following single low-dose irradiation and then decreased following medium- and high-dose irradiation and TIMP4 was not significantly altered following low-dose irradiation but increased following medium- and high-dose irradiation. However, after low-dose pre-irradiation, TIMP2 was highly expressed in the medium-dose irradiation group and TIMP4 was decreased. Both TIMP2 and TIMP4 were not significantly altered following high-dose irradiation (Fig. 4C). These results indicated that low-dose irradiation may elevate TIMP2 to inhibit MMP2 expression and activation but not TIMP4.

Discussion

In the present study, we demonstrated that a relative non-inflammation and non-ROS-inducing low-dose UV irradiation is a protective factor to suppress slight inflammation and ROS accumulation, which is induced by a medium-dose UV irradiation, further suppressing slight skin epithelial hyperplasia. This may be contributed to elevated SOD1 activity and suppressed skin epithelial MMP2 activity inhibited by increased TIMP2 activity. However, this type of suppression was not observed in high-dose UV irradiation-induced inflammation, ROS accumulation and skin epithelial hyperplasia with MMP2 activity. These results indicate that the protective role of low-dose UV irradiation is only limited to the most initial stage and for very slight lesions.

A relative low-dose UV irradiation induced no inflammation or ROS production (Fig. 1A and B), that was contributed to elevated anti-ROS enzymes (Fig. 1C). Although medium-dose UV irradiation also induced the same level of anti-ROS enzymes, which may not suppress the continuous accumulation of ROS. But with the increased intensity of UV irradiation, the anti-ROS system was absolutely damaged after high-dose UV irradiation (Fig. 1B and C). This indicates the dual character of UV irradiation on the ROS-antioxidant system (29,30). It is very easy for us to understand how ROS are portrayed as detrimental, as evidenced by the notable trend in the use of dietary and cosmetic antioxidants (31). However, recently, it was reported that increased ROS promote longevity and metabolic health, which may be explained by mitochondrial hormesis (mitohormesis) (32,33). ROS, derived from the mitochondrial electron transport system, may be necessary triggering elements for a sequence of events that result in benefits ranging from the transiently cytoprotective to organismal-level longevity. Basis on this concept, it is not difficult to understand that ROS also play an important role in the beneficial alterations in cellular physiology produced by caloric restriction, intermittent fasting, exercise and dietary phytoneutrients (34). Thus, the balance of ROS and antioxidants seems to be a key element (30).

Figure 5. Conclusive conceptual schemes of the different doses of UV irradiation inducing different reactions in the ROS and TIMP2/MMP2 system. (A) Moderate UV-irradiation elevated TIMP2 and anti-ROS ability, which suppressed MMP2 and ROS accumulation. (B) When an individual is exposed to a relative excessive UV irradiation, although TIMP2 and anti-ROS ability are increased there is failure to suppress the further increase in MMP2 and ROS accumulation. (C) When an individual is exposed to a moderate level of UV irradiation, the TIMP2 and anti-ROS ability are elevated. Then the elevated TIMP2 and anti-ROS ability suppresses more ROS accumulation and MMP2 induced by higher UV irradiation. (D) Absolute excessive UV irradiation not only induces MMP2 and ROS accumulation, but also impairs TIMP2 and anti-ROS ability. ROS, reactive oxygen species.
As the dual character of ROS, in the present study was stimulated by UV irradiation and in light of the importance of the ROS-antioxidant balance, it is difficult to judge whether it is beneficial or not for health. However, it seemed that low accumulation of ROS induced by low-dose UV irradiation, although the low accumulation of ROS was not suppressed by elevated antioxidants (Fig. 1C and D), inhibited further inflammation and further ROS accumulation induced by medium-dose UV irradiation (Fig. 3A, B and D). In the present study, there was an interesting phenomenon displayed. Low-dose UV irradiation inhibited medium-dose UV irradiation. It may be explained that an accumulation of good ROS inhibited the accumulation of bad ROS or the increasing antioxidants (SOD and catalase) inhibited further ROS accumulation (35). We also found another interesting phenomenon that both low- and medium-dose UV irradiation induced the same level of SOD expression (Fig. 1C). However, low-dose UV irradiation induced SOD-suppressed ROS accumulation and a further ROS induced by medium-dose UV irradiation, while the same level of SOD induced by medium-dose UV irradiation did not suppress ROS accumulation (Figs. 1B and 3B). This was because SOD activity was increased after low-dose UV irradiation but damaged after medium-dose UV irradiation (Fig. 3C). The protective role of low-dose UV irradiation contributed to the increased antioxidant activity more than simple antioxidant expression.

As inflammation and ROS induce skin epithelial hyperplasia (27,36), we also demonstrated that low-dose UV irradiation suppressed medium-dose UV irradiation induced skin epithelial hyperplasia (Fig. 2A and e), which was paralleled with its inhibition of inflammation and ROS accumulation. Furthermore, low-dose UV irradiation did not inhibit high-dose UV irradiation-induced inflammation and ROS, yet low-dose UV irradiation also did not inhibit high-dose UV irradiation-induced skin epithelial hyperplasia (Fig. 2C and f).

Skin epithelial hyperplasia, resulting from degradation of the basement membrane (BM) and extracellular matrix (ECM), is caused by epithelial cell proliferation and migration (37) and migration was reported to be correlated with the balance of MMP2 and its inhibitor TIMP2/MMP2 pathway. The present study indicates that not all UV exposure is bad or carcinogenic and moderate UV irradiation also has a beneficial role for increasing resistance to prevent cancer.

In conclusion as shown in Fig. 5, we demonstrated that low-dose UV irradiation, which does not induce any inflammation, protected skin against medium-dose UV irradiation-induced inflammation and ROS and further inhibited skin epithelial hyperplasia by regulating the balance of the TIMP2/MMP2 pathway. The present study indicates that not all UV exposure is bad or carcinogenic and moderate UV irradiation also has a beneficial role for increasing resistance to prevent cancer.

References


