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Control and Mechanism of Tumor Promotion in UV-Carcinogenesis

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Carcinogenesis can be theoretically divided to intiation step and promotion step. Intiation associates with genetic alterations including p53 tumor suppressor gene and ras oncogene. Promotion involves in clonal expansion of of an initiated cell by epigenetic mechanism, mainly through signal transduction and gene expression. Ultraviolet light (UV) acts as both initiator and promoter. Initiation is closely related with DNA damage induced by UV, including cyclobutane pyrimidine dimers, (6-4) photoproducts and 8-hydroxy-2'-deoxyguanosine. Cyclobutane pyrimidine dimers and (6-4) photoproducts are directly induced by UV, while 8-hydroxy-2'-deoxyguanosine is induced indirectly by the reactive oxygen species. Because initiation is an irreversal genetic event, while promotion is a reversal and epigenetic event, to know the molecular mechanisms of tumor promotion in UV-carcinogenesis is crucial to develop preventive medicine and suppress UV-carcinogenesis. Because ROS is also involved in signal transduction of the cell, anti-oxidant could be the good candidate of anti-promoting agent. Here, we describe the suppressive effect of UV-carcinogenesis by various anti-oxidant including olive oil. In addition, we discuss about the mechanism of UVB-induced expression of cyclooxygenase-2, which might be a representative molecule involved in promotion of UV-carcinogenesis.

Key words: tumor promotion, reactive oxygen species, antioxydant, olive oil, cyclooxygenase-2

INTRODUCTION

Several lines of evidence suggest that reactive oxygen species (ROS) are involved in skin cancer induced by UV. 1) UV increases the generation and decreases the degradation of ROS. 2) The exposure of skin to UV radiation causes significant depletion of antioxydant enzynes such as superoxide dismutase, glutathione peroxidase, catalase, and xanthine oxidase. 3) Depletion of physiological antioxidants

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antioxidants such as α -tocophenol and glutathione increases tumor induction. 4) Antioxidants and free radical scavengers such as β -carotene, α -tocophenol and ascorbic acid were shown to suppress UV-induced skin cancer. Fruits, some herbs and plants, as well as vegetables with various pharmacological properties were shown to be rich sources of cancer chemopreventive agents. Among these, particularly plant and vegetable extracts such as green tea, olive oil, rapeseed oil and safflower oil, have widely been considered to have antioxidant and free radical scavenging capabilities. For instance, UV-carcinogenesis was suppressed when green tea or green tea polyphenol fractions were topically applied to the mouse skin or were ingested orally in the drinking water [1]. Eidemiological study shows that the incidence of coronary heart disease and certain cencers such as breast, prostate and colon cancer is lower in Mediterranean areas where olive oil is the dietary fat of choice. However, since the effect of olive oil, which is rich in phenolic components, on UV-carcinogenesis has not been examined, we studied whether application of olive oil suppress UV-carcinogenesis and found the inhibitory effect [2]. Furthermore, we show a preliminary result indicating a candidate compoment of olive oil for suppression of UV-carcinogenesis. In addition, since prostaglandin may act for tumor promotion of UV-carcinogenesis, we show our attempt to reveal the mechanisms for UVB-induced cyclooxygenase-2 (COX-2) in keratinocytes.

PROTECTIVE EFFECT OF TOPICALLY APPLIEDOLIVE OIL AGAINST UV-CARCINOGENESIS OF MICE

Hairless mice were divided into three groups of 15 mice each as follows: group 1 (control mice receiving only UVB), group 2 (mice painted with olive oil before UVB exposure; pre-UVB group), and group 3 (mice painted with olive oil after UVB exposure; post-UVB group). Mice were irradiated three times per week at a dose of 3.43 kJ/m^2 each with a bank of six UVB lamps (Torex FL20SE-30/DMR fluorescent sun-lamps). Immediately before UVB irradiation (group 2) or immediately after irradiation (group 3) ~150 μl of olive oil was painted on to the dorsal skin of the mice. We repeated the experiment twice and had consistent results. The tumor incidence (percentage of mice with tumors) and the tumor multiplicity (mean number of tumors per mouse) in the post-UVB group were lower than those in control mice treated only with UVB or in mice in the pre-UVB group. The size of tumors did not differ between the three groups. There were no significant differences in the histopathological characteristics of tumors among these three groups, i.e. ~10% of tumors were papillomas, 30% were carcinoma *in situ* and 60%

were squamous cell carcinoma. The effects of olive oil on various types of DNA damage, including cyclobutane pyrimidine dimers (CPD), (6-4) photoproducts (6-4PP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG), were analyzed immunohistochemical technique using biopsies of mouse skin taken 30 min after single exposure of UVB with olive oil application immediately before or after the UVB. CPD and 6-4PP are formed by the direct absorption of UV energy to DNA, while 8-OHdG is a representative DNA base-modified product generated by ROS [3]. No significant difference in DNA damage formation was observed between samples from the UVB-exposed control mice and the pre-UVB group. In contrast, the post-UVB group showed less formation of 8-OHdG in the nuclei of epidermal cells, although the formation of CPD and 6-4PP did not differ from that in the control and pre-UVB groups. When olive oil previously irradiated with UVB exposure, the 8-OHdG formation was not reduced to the extent seen in the post-UVB group. These data indicated that the inhibition of UVB-carcinogenesis can be attributed to the scavenging activity of olive oil against ROS. In addition, it was suggested that olive oil has no sunscreen effect and its anti-oxidant effect could deteriorate upon UVB irradiation. Several components of olive oil, such as oleuropein, hydroxytyrosol, and squalene, have been shown to have anti-oxidant activity. We examined whether these components could suppress the 8-OHdG formation in UVB-exposed skin when applied to mouse skin immediately after single UVB irradiation. We found that oleuropein suppressed 8-OHdG formation to the same extent to olive oil itself, suggesting that oleuropein could be one of the effective component in olive oil for preventing UV-carcinogenesis.

MECHANISM OF UVB-INDUCED EXPRESSION OF COX-2 IN HACAT KERATINOCYTES

Plostaglandins (PGs) are known to have various biological effects including cell growth stimulation, immunosuppression, angiogenesis, and suppression of apoptosis. PGs are produced by phospholipids by phospholipase A_2 which release arachidonic acid and by COX which converts arachidonic acid to PGs. There are two isoforms of COX. COX-1 is constitutively expressed in most cell types, while COX-2 expression is induced by various endogeneous and exogeneous agents [4]. Since studies using COX-1 and COX-2 deficient mice showed a reduced skin carcinogenesis by chemicals and specific inhibitors for COX-2 suppressed photocarcinogenesis in mice [5], it is important to know the molecular mechanism of UVB-induced COX-2 expression. Previous studies have revealed that activations of tyrosine kinase, p38 MAP kinase and Akt were involved in UV-

induced COX-2 expression. Furthermore, inactivation of gycogen synthase kinase 3β is also associated with the increase of COX-2 by UV. Transcriptional factors CREB and ATF-1 were shown to be crucial for UVB-induced COX-2 gene upregulation. We are also attempting to examine the molecular mechanisms of UVB-induced COX-2 expression. UVB exposure to HaCaT cells induced expressions of COX-2 protein and mRNA biginning at 3~6 h after exposure and lasting until 60 h. PGE_2 production was also observed in parallel with COX-2 expression. The increase of COX-2 protein by UVB was partly suppressed by treating cells with anti-oxidant *N*-acetylcysteine, suggesting that ROS somehow involves in UVB-induced COX-2 expression. Because UV activates cell surface receptors and EGF is crucial for survival and proliferation of keratinocytes, we examined the effect of specific inhibitor of EGF receptor for UVB-induced COX-2 expression. The increase of COX-2 protein by UVB was suppressed in dose-dependent fashion by inhibitor of EGF receptor, indicating that activation of EGF signaling is deeply involved in UVB-induced COX-2 expression. This result was consistent with a study using squamous cell carcinoma cell line, which showed that the inhibitor of EGF-receptor suppressed constitutive expression of COX-2. Further studies attempting to reveal the detailed mechanisms of UVB-induced COX-2 expression will develop new modalities to prevent and suppress the UVB-carcinogenesis.

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