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# Preventive effect of antioxidant on ultraviolet-induced skin cancer in mice

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**Abstract**

Reactive oxygen species (ROS) have been shown to be responsible for inducing DNA damage after ultraviolet radiation (UV). Antioxidant, vitamin E and epigallocatechin gallate extracted from green tea, applied topically to the skin, delayed the onset of UV-induced skin cancer in mice. Since olive oil is reported to have a potent antioxidative effect in in vitro system, we asked whether, topical use of olive oil reduces the number and delays the onset of UV-induced skin cancer in mice. We found that super virgin olive oil painted immediately after UVB radiation significantly delayed the onset and reduced the number of skin cancer, but pretreatment of super virgin olive oil and pre- and/or post treatment by regular olive oil neither retarded nor reduced skin cancer formation in UV-irradiated mice. Further, 8-hydroxy-deoxyguanosine (8-OHdG) formation in mice epidermis was apparently reduced by super virgin olive oil painted immediately after UV radiation, although cyclobutane pyrimidine dimers and (6-4) photoproducts were not reduced by olive oil treatment. Our results suggest that daily topical use of super virgin olive oil after sun bathing may delay and reduce UV-induced skin cancer development in human skin, possibly by decreasing ROS-induced 8-OHdG which is responsible for gene mutation. 

*Keywords:* Ultraviolet radiation; Skin cancer; Olive oil; 8-OHdG; Active oxygen species

## 1. Introduction

Skin cancer is the most common type of cancer among the light-skin Caucasians in the western countries and Australia. It has been estimated that more than 700 000 cases of non-melanoma skin cancers (basal cell carcinoma; BCC, squamous cell carcinoma; SCC) are newly diagnosed in the United States every year [1]).

Solar ultraviolet radiation (UV) particularly UVB ranging from 290 to 320 m has been suggested epidemiologically and demonstrated experimentally to be the pivotal causal factor for NMCS development both in human and in animals.

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UVB radiation penetrates into the basal layer of epidermis (10% of the surface) and directly induces DNA damage, such as cyclobutane type pyrimidine dimers and (6-4) photoproducts (Fig. 1) which cause mutations at the opposite sites of these DNA damages.

Further, UVB and UVA radiations have been demonstrated to produce reactive oxygen species (ROS) in the cells and skin which cause DNA damage leading to gene mutation and abnormal cell proliferation [2,3]).

In addition to these tumor initiating effects, UV radiation also activates a number of transcription factors which induce the upregulation of genes involved in cell proliferation and function [4]). ROS can induce activation of the activator protein-1 transcription factor (AP-1) which may play an important role in tumor promotion.

A wide range of antioxidants have been shown to be effective in protection against photocarcinogenesis in murine skin [5]). Green tea polyphenol, a major antioxidant, was shown to be an anti-tumour agent in mice when administered systemically by drinking water and also topically by ointment to the skin [6]).

In the present study, we asked whether topical application of olive oil has preventive effect on UV-induced skin carcinogenesis in mice, since olive oil has been shown to have a potent antioxidant activity in vitro.

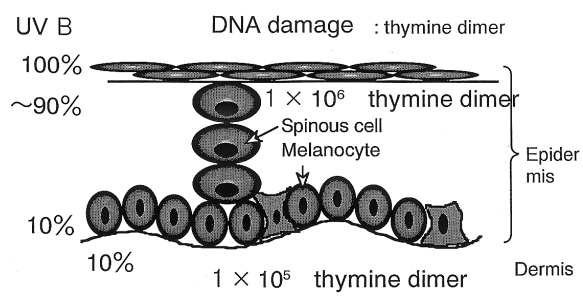


Fig. 1. Approximately 90 and 10% of UVB reach to the upper and basal layer of epidermis, respectively. One hour sunbathing at noon in summer induces approximately $1 \times 10^6$ and $1 \times 10^5$ of pyrimidine dimers per cell at the upper and basal layer of epidermis, respectively.

## 2. Materials and methods

### 2.1. Animals

Hairless female mice (BALB/cA Kud-hr) aged 6–8 weeks at the beginning of the experiment, were purchased from CLEA Japan, Inc. They were housed in plastic cages with wide meshed cover, and fed autoclaved mouse chow and water ad libitum. Room illumination was on, at an automatched cycle of 12 h light and 12 h dark and room temperature was maintained at 22–25°C.

### 2.2. Olive oils

Two kinds of olive oils, regular virgin olive oil and super virgin olive oil were supplied by DHC Co., Tokyo.

### 2.3. UVB irradiation

Total 75 mice were divided into five groups with 15 mice each, as follows; Group 1, control mice treated with UVB radiation; Group 2, mice painted with super virgin olive oil immediately after UVB exposure; Group 3, mice painted with regular virgin olive oil immediately after UVB exposure; Group 4, mice painted with super virgin olive oil 5 min before UVB exposure; Group 5, mice treated with regular virgin olive oil 5 min before UVB exposure. Five mice were sham irradiated with UVB radiation and five mice each were painted with super or regular virgin olive oil without UVB irradiation, respectively.

Mice skin were irradiated three times weekly at a dose of 3.43 KJ/m$^2$ each with a bank of six UVB lamps (Torex FL20SE/30DMR fluorescent sun-lamp peaking at 305 nm, Toshiba Medical Supply). UVB flux was measured by a UVB-305/365 D digital radiometer (Opto-Electromic Measuring Instruments, Toshiba Medical Supply, Tokyo).

Five minutes before or immediately after UVB irradiation, mice were painted with one of the two kinds of olive oil on the dorsal skin with a moist cotton swab.

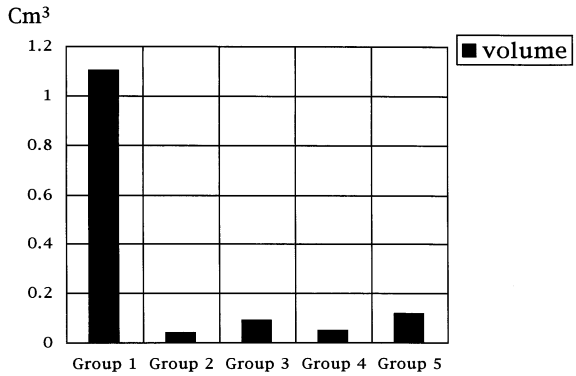


Fig. 2. Mice were treated as mentioned in Materials and Methods. Group I animals were UV irradiated without any other treatment (positive control). Non UV irradiated mice were free from skin tumors even at the end of the experiment (95th week of UV exposure).

### 2.4. Measurement of skin tumor

The number of tumors, larger than 1 mm in diameter was counted at 0, 5, 10, 15, 18, 20, 24, 25, 27, 28 and 33 weeks of repeated irradiations.

Tumors from each group were randomly biopsied after measuring the size of each tumor 48 h after the last UV-treatment.

### 2.5. Pathological analysis

Tumors biopsied were fixed with formalin and embedded in paraffin, then stained by hematoxylin and eosin. Tumors were classified as follows;

1. Papilloma: a tumor with papillornatous growth of epidermis without cellular atypicality and invasion of epidermal tumor cells into the dermis.
2. Precancerous lesion: a small tumor with acanthotic and papillornatous growth of epidermis with cells of various degrees of atypicality.
3. Squamous cell carcinoma: a tumor with nests of atypical cells invading into the dermis or deeper into subcutaneous fatty tissue.

### 2.6. lmmunohistochemistry

To detect photoproducts biopsied specimens were obtained at 1, 24 and 46 h after irradiation from tumors and their surrounding normal skin, and were reacted with antibodies for cyclobutane pyrimidine dimers (CPD), (6-4) photoproducts (6-4)PP, 8-hydroxy-2′-deoxyguanosine (8-OHdG) and p53 protein with TDM2, 64M-2, polyclonal antibody N45.1 and CM-5, respectively.

## 3. Results

### 3.1. The onset of UV-induced skin tumor

The onset of UV-induced skin tumor was 18 weeks ( a total of 58 exposures) for mice in group 1, 24 weeks (72 exposures) in group 2, 22.5 weeks (67 exposures) in group 3, 21.0 weeks (63 exposures) in group 4 and 23 weeks (75 exposures) in group 5.

### 3.2. Comparative study on the tumor incidence per mouse

The tumor incidence per mouse in group I was higher than those of any other four groups treated with olive oil after 24 weeks exposures. The mean number of tumor ( $>1.0$ mm in diameter) per mouse in group 1, 2, 3, 4 and 5 was 0.35, 0.06, 0.20, 0.13 and 0.26, respectively. The mean number of tumors of group 3 and 4 was smaller than that of group I, but became similar to that of group 1 during the additional 9 weeks exposures. The mean number of tumors of group 2, however, was significantly smaller than that of group 1. The mean tumor incidence per mouse 2 days after the final exposure (33 weeks, a total of 95 exposures) were 7.33, 2.64, 4.85, 6.69 and 7.57 in group 1, 2, 3, 4 and 5, respectively.

### 3.3. Mean tumor volume in each group mice after different times of exposures

Mean tumor volume per mouse of group 2 was significantly smaller than that of group 1, but those of other olive treated groups were not significantly smaller than that of group I (Fig. 2).

### *3.4. Semi-quantitative analysis of CPD, (6-4)PP 8-OHdG positive cells in mouse skin exposed to UV, UV + post olive oil, and pre olive oil + UV*

Larger number of cells having weak to strong CPD and (6-4)PP positivity were observed in UV-irradiated mouse epidermal cells compared to those of control (non-UV irradiated) mice. Any treatments with two kinds of olive oil did not affect the number of those photoproducts at 1, 12, 24, and 46 h after UV. 8-OHdG positive cells were significantly reduced in mice treated with super virgin olive oil immediately after a single UVB irradiation compared to other groups, although the number of 8-OHdG positive cells were still detected in mice treated with a single UV irradiation immediately followed by super virgin olive oil.

### *3.5. Histopathological study*

A total of 105 tumors ( group 1:23, group 2:17, group 3:23, group 4:21 and group 5:21) collected after repeated exposures for 28 weeks were examined histopathologically.

There was no significant difference of histopathological characteristics of tumors among five groups (Table 1).

### *3.6. p53 Protein expression*

A section of tumor containing more than 20% of p53 protein positive cells against CM-5-polyclonal antibody was regarded as p53 positive. When all five sections from one tumor showed positivity, the tumor was defined as p53 positive.

No significant difference of p53 positivity was found in tumors among five groups. Positivity in group 1, 2, 3, 4 and 5 was 74, 59, 65, 62 and 81%, respectively.

## 4. Discussion

Acute UVB effect on epidermal cells is DNA damage formation and activation of transcription factors through signal transduction from cellular surface possibly initiated by reactive oxygen species. These molecular alterations lead to cell death, cell cycle arrest in G1 phase for efficient repair of damaged DNA and cellular proliferation. Repair defect of DNA damage, such as CPD and (6-4)PP, has been shown to play a pivotal role in UV carcinogenesis through a number of clinical studied of xeroderma pigmentation patients [7,8] and photobiological studies using cultured XP cells [9] and XP gene knock out mice [10].

Cancer susceptibility of XP patients also indicated a role of DNA repair defect in initiation step of UV-induced carcinogenesis.

Antioxidants have been demonstrated to delay the onset of and to reduce the incidence rate of skin cancer development in UV irradiated mice [5,6,11,12].

Virgin olive oil has been reported to be responsible for low incidence of coronary heart disease

Table 1
Histopathological analysis of tumors developed in each experimental group[a]

| Experimental | Number of tumors | | | | |
|---|---|---|---|---|---|
| Group | Papilloma | SCC in-situ | SCC differenciated | SCC undifferenciated | Total |
| 1 | 3 | 5 | 11 | 4 | 23 |
| 2 | 2 | 5 | 9 | 1 | 17 |
| 3 | 2 | 9 | 8 | 4 | 23 |
| 4 | 2 | 6 | 11 | 2 | 21 |
| 5 | 0 | 5 | 15 | 1 | 21 |

[a] Tumors developed in mice exposed chronically to UVB radiation for 28 weeks were collected and studied histopathologically. There was no statistical difference of tumor types among mice treated differently.

and skin cancer in the Mediterranean where virgin olive oil is the main dietary fat source. Further, olive oil is shown to be a potent antioxidant [13]. We asked whether topical olive oil treatment is preventive for UV-induced skin cancer formation in relevance to 8-OHdG formation. For the first time, we have demonstrated that post-UV treatment with virgin olive oil after UV irradiation significantly retarded the onset time and reduced the number of tumor per mouse compared to the mice exposed to UV alone without any particular treatment. In addition, super virgin olive oil painted immediately after UVB radiation reduced the number of 8-OHdG positive cells in mice epidermis.

Regular olive oil painted before or after UV-Irradiation, and super virgin olive oil applied topically before UV irradiation neither retarded tumor development nor reduced the tumor incidence in mice significantly.

Our results indicate that virgin olive oil and regular olive oil had no sunscreen effect.

We speculate the mechanisms of anti-tumor effect of super virgin olive oil painted after UVB radiation as follows: (1) reduced formation of UV-induced 8-OHdG by UV radiation followed by topical painting of virgin olive oil may contributes to the delay of onset of cancer formation and reduction of skin tumors per mouse. (2) virgin olive oil has an anti-tumor effects in promotion step in UV carcinogenesis, which may be inactivated by previous UVB radiation. Hattori et al. [3] have already reported that UVinduced carcinoma in mice exhibited a high levels of 8-OHdG in both normal and cancer skin tissues.

In the present study, we have not analyzed base changes in tumor cells, yet, p53 overexpression is detected in many tumor tissues. Therefore, in a future work, mutation analysis of p53 gene, focussed on UV-specific $C \rightarrow T$ or $CC \rightarrow TT$ changes and ROS-induced GT mutation is remained to be done.

Our findings suggest that topical use of virgin olive oil after sun bathing may contribute to preventing of skin cancer on sun-exposed human skin.

It is expected that by reducing the amount of DNA photo damage and maintaining high antioxidant levels, it may be possible to reduce skin cancer formation by UV radiation. Berton et al. [5] showed that vitamin E acetate painted opically after UVB radiation scavenged ROS increased the repair rate of DNA damage and suggested a preventive role of vitamin E acetate in reducing UV induced skin cancer production. Taken together, topical use of anti-oxidants, such as super virgin olive oil after bathing may be effective in reducing and retarding skin cancer formation in human skin. But, to recommend the topical use of super virgin olive oil after sun bathing for preventing UV-carcinogenesis, it is required to clarify the ideal and practical time of olive oil application after UV radiation.

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