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Review article

Olive oil and skin anti-aging.

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Abstract

This study compiled functional components and effects of olive oil, focusing on the anti-aging effects in the field of dermatology. Olive oil is a type of vegetable oil that contains many monounsaturated fatty acids (MUFA) such as oleic acid. Olive extracts provide beneficial anti-oxidative effects, containing phenolic compounds such as tyrosol, hydroxytyrosol, caffeic acid, and oleuropein. Health promotion effects of olive oil are well-known in the Mediterranean diet, which has gained worldwide attention for its contribution to longevity. Olive oil reduces the risk of ultraviolet (UV)-induced carcinogenesis, affecting DNA damage with the formation of 8-OHdG due to UV exposure. Furthermore, olive oil can induce beneficial epigenetic modifications with effects of MUFA on DNA methylation and oleuropein on histone acetylation. It has been also reported that olive oil is effective for diabetic patients, characterized by severe glycative stress, to maintain the function of pancreatic β-cells, to lower blood glucose level, and to inhibit the formation and to promote the degradation of advanced glycation end products (AGEs). Therefore, olive oil plays a significant part in this rapidly aging society worldwide to help people retain and restore youth. Looking young could be inspiring and encouraging to live a positive life.

***KEY WORDS*:** Olive (*Olea europaea*), oleuropein, skin, ultraviolet exposure, photoaging

1. Introduction

Risk factors to accelerate aging are diversified. Current theories have demonstrated genomic instability, telomere attrition, epigenetic alterations, damage of nucleic acid by reactive oxygen species (ROS), accumulation of abnormal protein, mitochondrial dysfunction, stem cell exhaustion and altered intercellular signal pathways. A theory was presented in 2012 which stated that mitochondria, p53, and ROS are mutually related to inducing aging [1)].

Basic and clinical research has been continued over a long period with a great interest in photoaging, which is referred to as the acceleration of skin aging due to solar ultraviolet radiation. The risks of genetic injuries of skin cell nucleus due to ultraviolet radiation are unavoidable as long as humans live a life under the sun light. The theory has been accepted that DNA damage to skin tissues induces inflammation, which damages cells and tissues. The repetition of DNA damage induces gene mutations, and the repetition of inflammation leads to cellular senescence in skin [2, 3)]. Further, ROS are inevitably produced as a byproduct in minute cytoplasmic organs, mitochondria, which generate the energy necessary for cells to live. Therefore, excessive production of ROS, exceeding the anti-oxidant capacity of a cell, induces irreversible damage to cells and nearby tissues. Consequently, it is indicated that ROS are one of the major causes for skin aging [4-6)]. Recent studies have revealed that chemokine (C-X-C motif) ligand 1 (CXCL1) plays an important part in the process of inflammatory responses or carcinogenesis, which is caused by ultraviolet (UV)-induced DNA damage. Possibilities are suggested for the regulation of inflammation through the involvement of chemokine for the purpose of delaying aging [7)].

More than twenty years have passed since the remarkable effectiveness of olive, *Olea europaea*, have been realized. It was known that olive oil has a strong anti-oxidative effect. Focusing on 8-hydroxy-2'-deoxyguanosine (8-OHdG), which was formed due to the oxidation of a base, a gene component,

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examinations were conducted to discover whether 8-OHdG would be produced in skin cells under UV irradiation. It was clarified that 8-OHdG appeared in the human epidermis after UVB radiation at the degree of obtaining a suntan, using anti-8-OHdG antibody [8].

Experiments were performed with mice to clarify whether UV photocarcinogenesis was affected by oxidized bases in DNA, other than cyclobutane pyrimidine dimers (CPD) and 6-4PP, which have been regarded as the main causes of UV-induced skin cancer. To confirm the involvement of UV-induced 8-OHdG in UV-induced skin carcinogenesis, applying olive oil to the skin was assumed to reduce the formation of 8-OHdG and suppress carcinogenesis due to the strong anti-oxidative effect [9]. An increasing number of research laboratories have reported the effectiveness of the health promotion of olive oil since that time. Consequently, theories have been established to prove that olive oil is effective and has dietary benefits which contribute to good health in everyday life. This research reported the results of the experiments, which we conducted, with some recent literature reviews on the health effects of olive oil relating to lifestyle-related diseases such as cardiovascular diseases and diabetes.

2. Constituents and health effects of olive oil

The health effects of olive oil are well-known worldwide with a great deal of attention on the Mediterranean diet contributing to longevity. Olive oil is a type of vegetable oil that contains many monounsaturated fatty acids (MUFA), mainly oleic acid [10]. Olive oil is understood to reduce the incidence rate of cardiovascular diseases and cancers, which are the leading causes of death at present. Further, immunological surveillance involving human subjects showed that virgin olive oil (VOO) and extra virgin olive oil (EVOO) contain abundant phenolic compounds such as tyrosol, hydroxytyrosol (HT,) caffeic acid, and oleuropein, which are characterized by their anti-oxidative effects and other biological activities for good health. In particular, EVOO consumption for several months increases the expression of macrophages and promotes the decrease of cholesterol mediated by high-density lipoprotein (HDL) [11]. Furthermore, there is a possibility that olive oil can contribute to the alleviation of problems related to cognitive impairment or muscle weakness in the aging society, as results of animal experiments have suggested [12, 13]. Moreover, *in vitro* studies have indicated that olive oil phenols have beneficial effects to control the signal transmission related to inflammatory response, prevent apoptosis and regulate the energy metabolic pathway of the aging process [14, 15].

3. Anti-aging effects of olive oil

It is expected that DNA damage would be a major factor of inducing senescent cells and identifying the mechanism of DNA stabilization of olive oil would bring meaningful understanding of its anti-aging effects. In particular, mitochondrial DNA is more susceptible to oxidative attack than nuclear DNA, as is widely known. It has already been reported that the administration of olive oil lowered the probability of DNA damage in peripheral blood cells [16]. It is considered that H_2O_2 produced in cells by UVA is a cause of cellular senescence [17, 18]. It has been proven that oleuropein and other compounds contained in olive oil reduce DNA damage [19] and scavenge ROS (***Fig. 1***). However, it is assumed that a dose of olive oil that we ingest on a daily basis, which contains low density phenol compounds, can inhibit the cell damage induced by H_2O_2 [20]. Accordingly, oral administration of olive oil can be said to have similar effects of applying a sunscreen agent to prevent sunburn, although SPF (sun protection factor) is low as 1.5 to 2, as shown in other antioxidant materials orally administered.

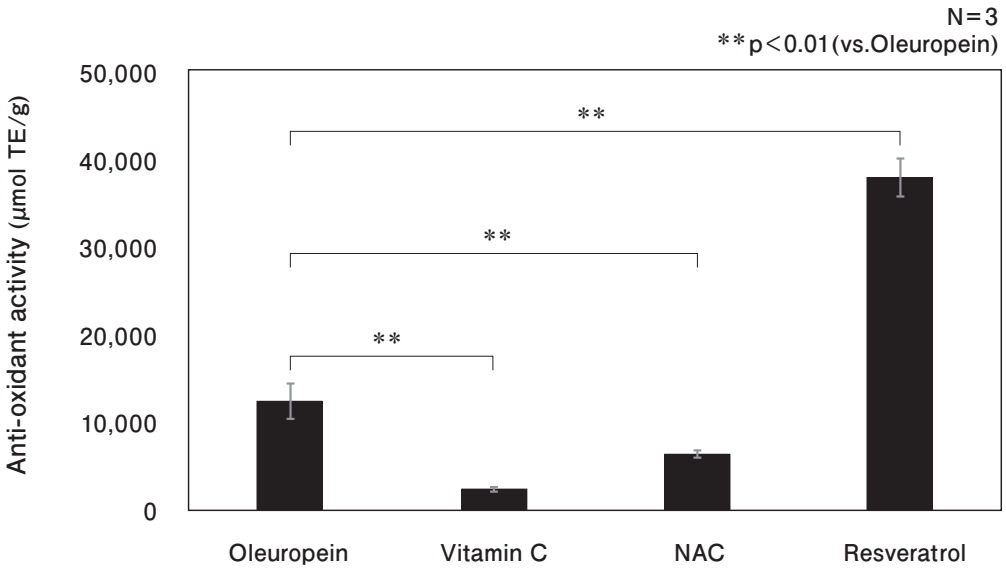

Fig. 1. **Anti-oxidant activities determined by ORAC assay**

Oleuropein showed a stronger radical scavenging activity compared with vitamin C, and NAC and resveratrol had the strongest ORAC among our studies. The values were expressed as μmol Trolox equivalents (TE) per gram of each anti-oxidant. The bars indicate mean values of triplicate determinations ± SD. ORAC, oxygen radical absorbance capacity; NAC, N-acetyl cysteine; SD, standard deviation.

While mitochondria produce ATP, ROS are inevitably produced as a byproduct. MUFA is less likely to be oxidized than polyunsaturated fatty acid, which is susceptible to ROS-induced damage of fatty acid in cell membranes. Therefore, olive oil is favorable to mitochondria.

Telomere attrition is one of the hallmarks of aging. Telomeres are shortened with aging and are deeply related to life-span, which is widely understood due to the findings that replicative DNA polymerases lack the ability to replicate completely the terminal ends of linear DNA molecules [21, 22]. Drinking alcohol and smoking accelerate telomere attrition, as was previously reported [23]. It has also been reported that Greek people, who consume a Mediterranean diet with abundant MUFA as a daily food, have longer telomeres in peripheral leucocyte than Dutch people, who have a different daily diet. Further research is awaited to examine the associations of telomere shortening with production of ROS or apoptosis [24, 25].

Recent investigations showed that telomeres were damaged and bases with a short GGATTG array appeared in cells exposed to ultraviolet radiation. Repair of DNA damage was induced via SOS response [26, 27]. It is considered that telomeres are always engaged in information transmission and contribute to the maintenance of life in cells [28].

Epigenetics has a great number of factors to influence cell functions. Among these factors, diet is regarded as an important factor [29]. It has been reported that MUFA affects the methylation of DNA and oleuropein affects histone acetylation, especially hyperacetylation of H3 histone in the Mediterranean diet [30]. In addition, lipid metabolism is important from the viewpoint of aging. It is said that with the total amount of Mediterranean food for one year, the methylation of SCD-1 (stearoyl CoA desaturase-1) gene promoter was accelerated [31]. This phenomenon means that the mechanism of the obesity inhibitor of the Mediterranean diet can be partly explained by epigenetic functions. Recent studies on the association between miRNA and cerebral apoplexy reported that ingestion of the Mediterranean diet reduced the degree of risks of people who had rs13702 Callele carriers of a high-risk factor of cerebral apoplexy [32]. It is also accepted that the Mediterranean diet and olive oil are involved in the prevention of coronary heart diseases, altering the expression of genes related to arteriosclerosis [33].

The accumulation of abnormal protein is regarded as a critical cause of Parkinson's disease and Alzheimer's disease (AD). Some *in vitro* studies of the influences on cells by phenolic materials reported that hydroxytyrosol, oleuropein and oleuropein aglycone, which are contained in olive oil, inhibited the fibril change of Tau protein [34]. Further, it is known that olive oil activates SIRT1 and exerts anti-aging effects [35, 36]. There was an encouraging report of an *in vitro* experiment where the administration of oleuropein enabled the subject to maintain proteasome functions for a long period, prolonging the life-span of cells and extending the period to reach senescence by approximately 15% [37]. Removal of abnormal protein is crucial to maintain the homeostasis of an organism. Olive oil is less susceptible to endoplasmic reticulum (ER) stress, which is induced by saturated fatty acid, as olive oil contains a large amount of short-chain unsaturated fatty acid. As a result, abnormality is unlikely to appear in protein folding [38]. Advanced glycation end products (AGEs) are a type of abnormal protein and are formed due to glycative stress. It has been reported recently that luteolin, which is an extract of olive oil, inhibits the formation of AGEs [39], and oleuropein, which is main compound of olive oil, promotes the degradation of α-diketone structure of AGEs and accelerates the metabolism of AGEs [40].

Developed and developing nations, including Japan, have health problems not only at present but also in the future, among which countermeasures against diabetes would be the greatest concern to focus on. Insulin resistance, which is strongly associated with aging, is both a reason for and a result of obesity. Countermeasures must be taken immediately. We are deeply and seriously concerned about the present medical environment for diabetes in Japan. Generally, it is not until people are diagnosed as diabetic that medical treatments start, such as pharmacotherapy. People with pre and preliminary diabetes must be encouraged to take preventive measures, eating a healthy diet and becoming physically active to control the progression of the disease. It is necessary to help people have a better understanding for the importance of prevention and treatment other than medications. Moreover, it is important to educate young children to realize that appropriate diet, and excise on a daily basis enables the prevention and the onset of lifestyle-related diseases such as diabetes. The decline of somatotropic hormone and insulin-like growth factor-I (IGF-I) with aging suggested that nutrition uptake similar to young generations has an abnormal metabolisms due to aging. Since approximately 1992, there have been reports that experiments with diabetic rabbits exhibited the anti-diabetes effects of olive oil. The point is that the administration of olive oil can lower blood glucose level and reduce oxidative stress [41,42]. Further, a randomized, double-blind, placebo-controlled trial was conducted in 2013 with patients with obesity among research participants. This trial recognized that the administration of olive leaf extracts for 12 weeks improved insulin sensitivity and accelerated the functions of pancreatic β-cells [43]. In addition, oleuropein reduced the oxidative stress related to diabetes [44]. Observing the relation between longevity and olive oil, some studies showed that EVOO regulated mTOR pathways and reduced cancer cell multiplication in cultured cells [45, 46]. It is considered that olive oil, which contains a large amount of short-chain unsaturated fatty acid, is favorable for the retention of mitochondria activity, and is expected to play a role as one of the foods for anti-aging.

Regarding cognitive impairment, olive oil is reported to reduce the deposition and increase the clearance of amyloid-β, which is regarded as a cause of Alzheimer's basket cells (AD) and a critical cause of the onset of AD [47]. There is a great deal of *in vitro* research regarding human skin, suggesting that polyphenol contained in olive oil is effective in the prevention and treatment of AD.

4. Preventive effects of olive oil on ultraviolet radiation carcinogenesis

UV radiation of sunlight is a major cause of skin cancer and photo-aging. The energy of UVB (wavelength, 290-320 nm) is directly absorbed by bases, which form genes, and induces cyclobutane pyrimidine dimer (CPD), a typical type of UV-induced DNA damage. The CPD formation by UVA (wavelength, 320-400 nm) is remarkably low in comparison with UVB, yet induces mutations, which is particular in UVA [48, 49]. However, the ultraviolet rays, which reach the surface of the earth, deliver its energy to energy-absorbing

materials, which exist in skin cells and tissues. The energy is transferred, via transfer of electron or activation of oxygen, to nucleic acid, protein, lipid, and carbohydrate and induces damage in these components of skin. As a result, skin cells have diverse reactions, such as inflammatory responses via signal transduction, leading to immunological abnormalities and destruction of dermal components which initiate photo-aging of the skin [50, 51].

We had an interest in reports that ROS were involved in UV-induced carcinogenesis. We studied the effect of UV on 8-OHdG, an oxidized guanine product, induced via electron transfer and ROS. Another inspiring piece of research suggested that oral administration of green tea with high anti-oxidant capacity or Vitamin E lowered the incidence of UV-induced skin cancer in experiments with mice [52, 53]. Mutations of *p*53 and *ras* in UV-induced cancer tissues of humans and mice were G : C → T : A transversion, and there was a possibility of 8-OHdG involvement. When we began investigating olive oil, some studies in epidemiology with human subjects had already reported that research participants who ingested ample vegetables with high anti-oxidative activity had low incidence of skin cancer [54]. However, as far as is known, there were no research data at that time which clearly showed that 8-OHdG (***Fig. 2***) was involved in ultraviolet radiation carcinogenesis. Firstly, we conducted an experiment with human and mouse skin, using artificial UVB radiation to clarify whether 8-OHdG would appear in the nucleus of skin, by immunohistochemical staining method with anti-8-OHdG antibody (***Fig. 3***) [8].

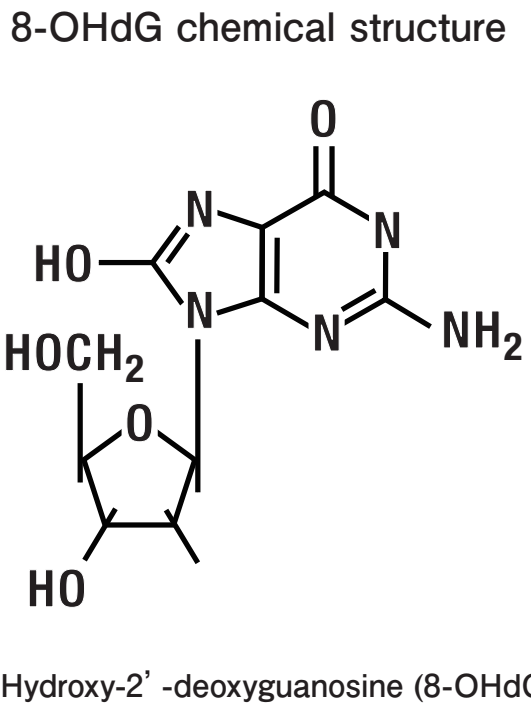

Fig. 2. **Oxidation of base Guanine (G)**

Base guanine exists as a gene component or gene pool in cell nucleus. Guanine is oxidized via electron transfer or oxygen molecule due to ultraviolet (UV) radiation, and 8-hydroxy-2'-deoxyguanosine (8-OHdG) is produced. Oxidative DNA damage is repaired by an enzyme, OGG1 (coding 8-oxoguanine-DNA glycosylase).

It was proven that three hours after UVB irradiation, a great number of 8-OHdG positive cells were observed in the UVB irradiated epidermis. Antibody positive cells, however, were not observed at 72 and 96 hours after UV irradiation, which indicated that 8-OHdG had been repaired. Next, to study the possible inhibitory effects of olive oil on UVB-induced 8-OHdG, olive oil was applied to mice skin before and after UV irradiation. To confirm its effects on DNA damage induced by UV light, CPD and 8-OHdG formed in epidermal cell nucleus were analyzed immunohistochemically, using antibodies against CPD and 8-OHdG. Furthermore, either before or after UV radiation, 150 µl of oleuropein (***Fig. 4***), hydroxytyrosol, or squalene, which were known as components of olive oil, were applied to mouse skin. Following this, the amount of CPD and 8-OHdG formation was compared among the groups. The inhibitory effects of olive oil application on the formation of CPD were not observed either before or after UV irradiation. However, in the formation of 8-OHdG, the group with olive oil applied immediately following UV irradiation revealed that the number of 8-OHdG positive cells had been distinctly reduced. Contrarily, as for the group with the olive oil application prior to UV irradiation, no effects were shown to decrease the number of 8-OHdG positive cells (***Fig. 5***). As for the examinations of olive oil component, only the oleuropein showed a reduction of 8-OHdG positive cells applied only after UV irradiation (***Fig. 6***, unpublished data).

Afterward, a comparative experiment was conducted on the number of days until the onset of skin cancer among three groups. Group I: UV irradiation, Group II: application of olive oil before UV irradiation, and Group III: application of olive oil after UV irradiation. Mice underwent UVB irradiation of 343.0 mJ/cm^2 on the skin three times a week for 32 consecutive weeks. Thirty-two weeks after the start of UV irradiation, the number of neoplastic cells per mouse was 8.53 in Group I of UV irradiation without olive oil application, 9.53 in Group II of application before UV irradiation, and 3.36 in Group III of application after UV irradiation. These data confirmed that the application of olive oil after UV irradiation significantly reduced the number of tumors (***Fig. 7***) [9].

From these data, we believed that oleuropein, which is contained in olive oil with strong anti-oxidant effects, inhibited the formation of UV-induced 8-OHdG. The effects of oleuropein application led to the reduction of skin tumor formation in mice. In addition, it was recognized that olive oil, which had been irradiated with UVB 343.0 mJ/cm^2 once, had lower anti-oxidant effects, because unlike fresh olive oil, the formation of 8-OHdG was not reduced by the application of UV-treated olive oil to the skin of UV-irradiated mice. However, in these experiments with mice, detailed examinations on different doses of UV or different periods between UV irradiation and application of olive oil were not performed.

These investigations are believed to have revealed that DNA oxidative damage by 8-OHdG was significantly involved in UV-induced carcinogenesis, in addition to CPD being regarded as the main cause of UV-induced carcinogenesis, which formed instantaneously with UV radiation. Subsequently Kunisada et al. conducted research on UV-induced skin cancer formation using OGG1 gene knockout mice, which were deficient in the repair of oxidative base damage, to show that 8-OHdG, oxidative DNA damage was deeply correlated with UV-induced carcinogenesis (***Fig. 8***) [55, 56].

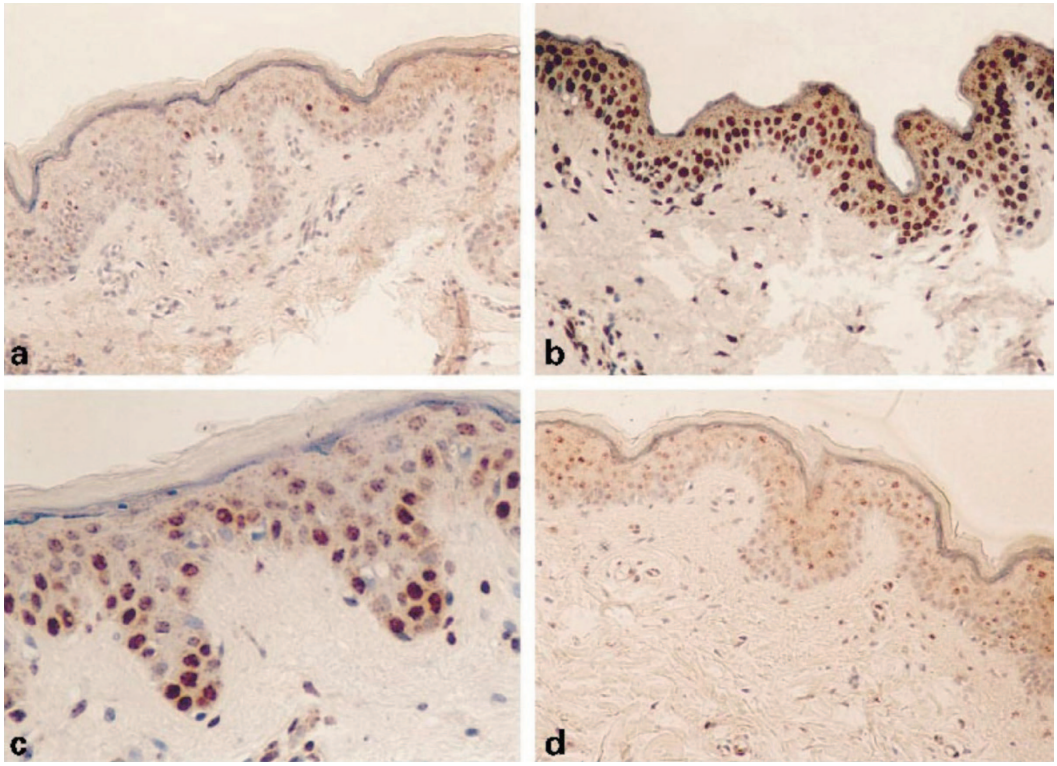

Fig. 3. **8-OHdG is formed in skin keratinocyte due to UV radiation)**

a: target skin without UV irradiation. **b:** skin at 30 minutes after UV irradiation. **c:** skin at 24 hours after UV irradiation; many positive cells remain. **d:** skin at 72 hours after UV irradiation; almost all 8-OHdG positive cells were not observed, which revealed that damage had been repaired. Findings of skin samples were collected by punch biopsy 30 minutes after UV radiation of a double dose of minimal erythema dose (MED) to the inside section of the upper arm of a human. Samples were processed with paraffin after formalin- fixation and was followed by being dyed with 8-OHdG antibody. Agreat number of 8-OHdG were formed in the nucleus dyed blown. Photos are quoted from Reference 8). 8-OHdG, 8-hydroxy-2'-deoxyguanosine; UV, ultraviolet.

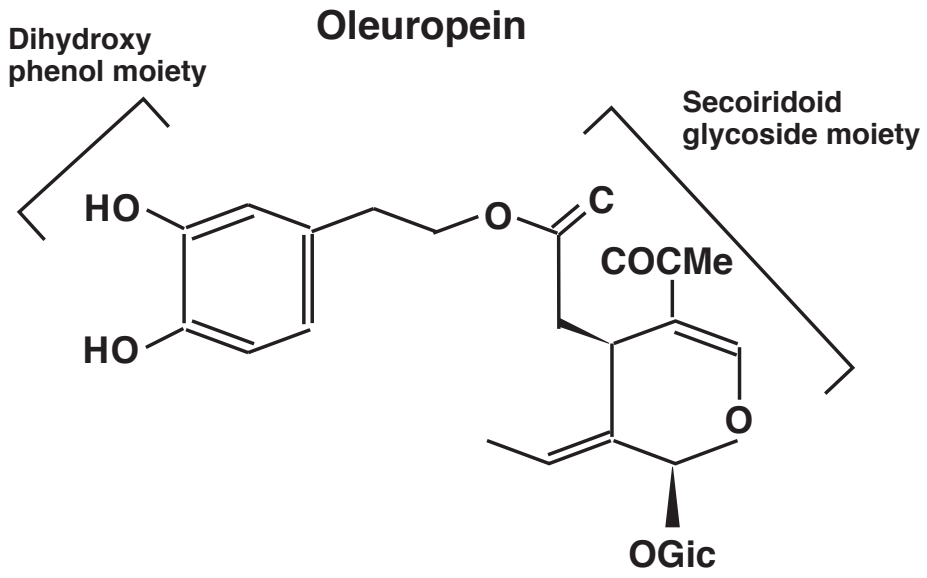

Fig. 4. **Chemical constitution formula of oleuropein**

Oleuropein is a smallmolecule contained in olive oil and has anti-oxidative activity. Gic, glucose.

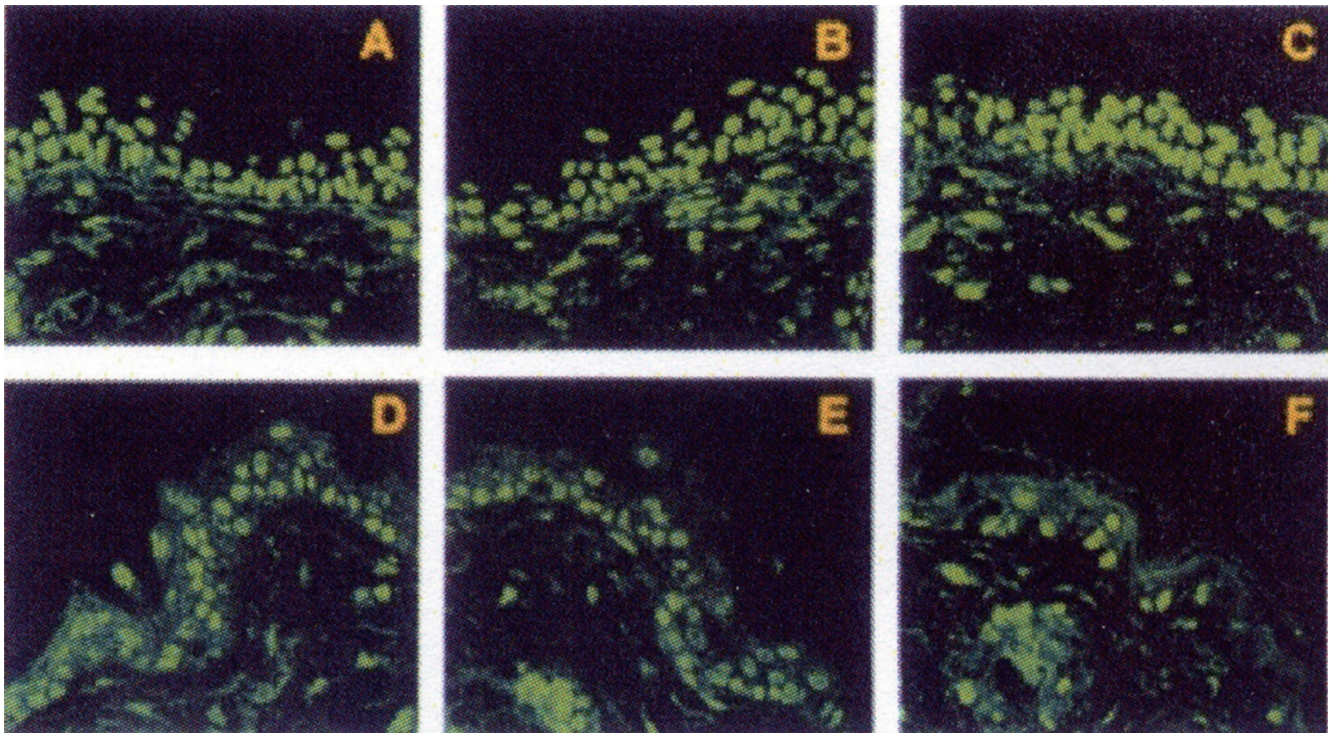

Fig. 5. **Effects of olive oil application on DNA damage formation (CPD: A-C and 8-OHdG: D-F) which appeared in the epidermal cell nucleus of mice back after UVB irradiation of 3.43 kJ/m^2**

Two sets of mouse skin samples were collected 30 minutes after irradiation. Samples were taken from mice skin where olive oil had been applied immediately before irradiation (**B**, **E**) and also from mice skin where olive oil had been applied immediately after irradiation (**C**, **F**). Skin samples treated with the olive oil immediately after UVB irradiation showed a significantly lower number of 8-OHdG positive cells, but no change in the number of CPD positive cells. Photos are quoted from Reference 9). CPD, cyclobutane pyrimidine dimers; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; UV, ultraviolet.

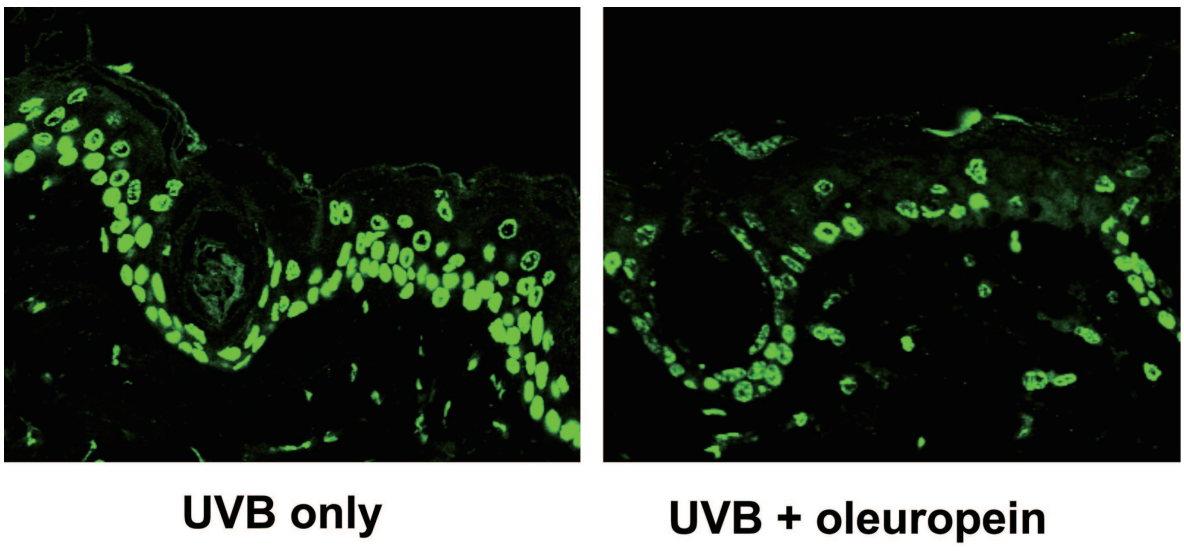

Fig. 6. **Inhibitory effects of oleuropein on the formation of 8-OHdG**

Oleuropein, in the amount of 0.1 mg (0.1 mL), was applied to the mice skin immediately after UVB irradiation of 3.4 kJ/m^2. The number of positive 8-OHdG cells and 8-OHdG antibody 30 minutes after UVB irradiation was distinctly smaller than the target skin without olive oil application. 8-OHdG, 8-hydroxy-2'-deoxyguanosine; UV, ultraviolet.

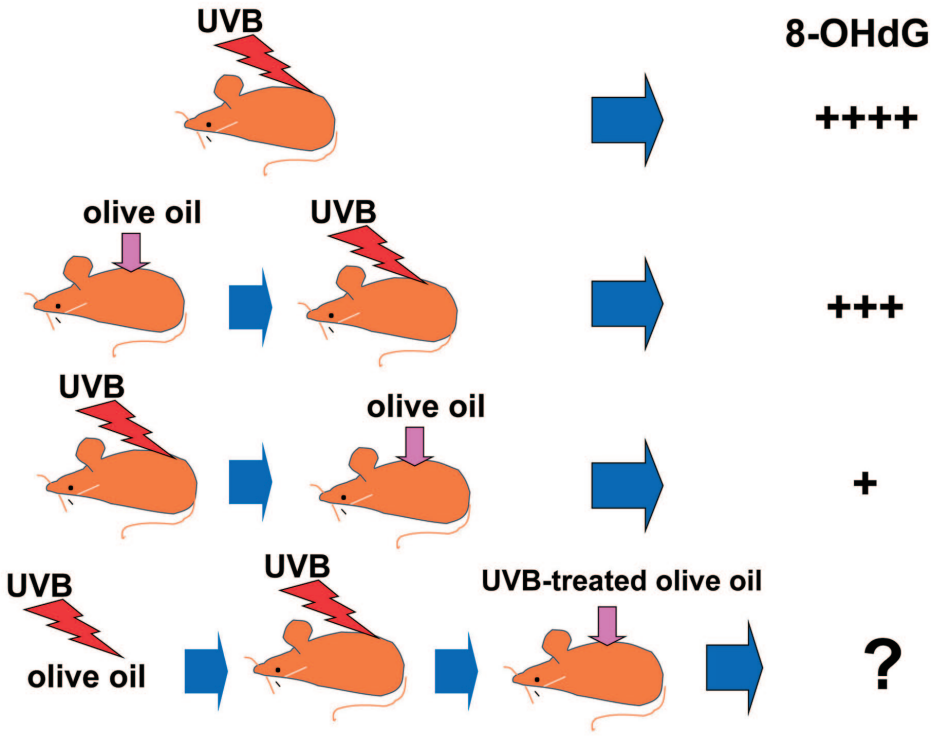

Fig. 7. **Influences of olive oil application on the UV-induced carcinogenesis in skin**

Mice were classified into four groups: 1) group with UVB irradiation of 3.43 kJ/m^2, 2) group with application with 150 µL of olive oil immediately prior to UV irradiation, 3) group with application of olive oil immediately following UV irradiation, and 4) group with application of UVB-treated olive oil (3.43 kJ/m^2) immediately following UV irradiation. All groups had 15 mice, compared in the shortest period of days by the formation of tumor, the number of tumor-bearing mice and the number of tumors per mouse 32 weeks after the irradiation. Groups with olive oil application before and after irradiation showed inhibitory effects of carcinogenesis, especially the group with application immediately after irradiation showed strong inhibitory effects. UV, ultraviolet.

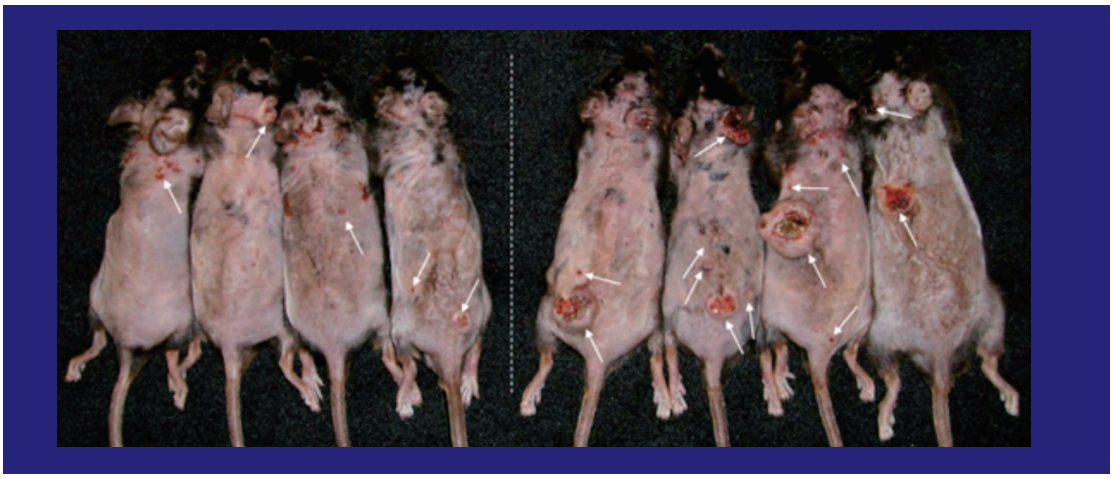

Fig. 8. **Influences on UV-induced skin carcinogenesis by repair enzyme on oxidative base damages**

Comparison experiments in carcinogenesis were performed between OGG1 knockout (KO) mice and normal mice, irradiated with UVB (2.5 kJ/m^2) three times a week for 40 weeks and observing the carcinogenesis process for 45 weeks after the start of irradiation. Tumors clearly appeared sooner in KO mice (30 weeks). As for normal mice, tumor-bearing mice appeared at 36 weeks. Comparing the time for each group when 60% of specimens exhibited tumors, the KO mice group was 35 weeks and normal mice group was 41 weeks. Forty-five weeks after irradiation, both groups had 100% tumor occurrence. Moreover, the mean of the number of tumors per mouse was 3.7 in the KO mice group and 1.71 in the normal mice group. The KO mice group had approximately twice as many tumor-bearing mice as the normal mice group. Photos are modified from References 5 and 6). UV, ultraviolet.

5. Future perspective: applying olive oil effects to the photo-aging inhibition in skin

It is believed that further studies are neccessary to examine influences to skin tissues by oleuropein and other small-sized materials contained in olive oil, focusing on the anti-oxidative and anti-glycative effects. Considering the rapidly aging society, the role of olive oil is significant, especially to help individuals retain and restore youth. Looking young may be inspiring and encouraging to live a positive life. We hope to fully understand the influences of UVA and UVB radiation on ROS formation in cells, mitochondria activities and apoptosis. How longevity of skin stem cells is affected by olive oil must be clarified. The researches that we have conducted strongly suggest the importance of ideal conditions to apply olive oil to skin against environmental stresses. A great number of researchers in the world also have found preventive effects for lifestyle-related diseases using oral administration of olive oil. By carefully observing and analyzing these research data, we would like to examine the optimum condition of olive oil for skin health both in oral administration and topical application to the skin.

Conflict of Interest Statement

We state that the performance of this study entailed no issues representing a conflict of interest.

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