Scoparone Inhibits Ultraviolet Radiation-Induced Lipid Peroxidation

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Antioxidant capabilities of scoparone, the component of *Artemisia scoparia* and other medicinal plants, against lipid peroxidation induced by ultraviolet radiation or Fenton reaction have been analyzed. Lipid peroxidation was monitored by measuring the absorption spectra of the conjugated dienes and quantified by the Klein oxidation index. Obtained results imply that scoparone is a very efficient inhibitor of ultraviolet radiation-induced lipid peroxidation and damage.

Key words: Artemisia scoparia, Ultraviolet Radiation, Lipid Peroxidation

Introduction

Acute and chronic exposure to solar radiation is linked to a number of types of skin damage, such as phototoxicity, photoallergy, aggravation of preexisting skin diseases, distinct photodermatoses, as well as to immunosupression, photosenescence and photocarcinogenesis (Guercio-Hauer *et al.*, 1994). Ultraviolet radiation is subdivided into four groups: UVA radiation (320–400 nm), UVB radiation (280–320 nm), UVC radiation (200–280 nm), and vacuum UV radiation with wavelengths less than 200 nm. The effect of UV radiation on multicellular organisms is localized in the skin. When a photon reaches the tissue-air boundary, part of the beam is reflected. The remaining light enters the tissue, where the absorption can begin. Radiation has to penetrate through the *stratum corneum* before reaching viable tissue, and therefore the thickness and composition of the *stratum corneum* always represents a modifying factor (Anderson and Parrish, 1981). Having reached viable tissue, the radiation can be absorbed by chromophores (melanin), whose amount varies between individuals. The effect of radiation on the cells mainly depends on its wavelength and on the amount of the radiation (Morliere *et al.*, 1995).

Peroxidation process leads to the damage of membranes and changes the biophysical properties. For example, an increased permeability of the lipid bilayer, decreased fluidity and lowered electric resistance of membranes has been observed as the results of peroxidation. Oxidation process can be initiated by the reactive oxygen species (ROS), such as the superoxide anion O_2^-, hydroxyl radical $\cdot OH$ or singlet oxygen $^1O_2$. Accumulation of ROS in aerobic organisms is thought to cause oxidative damage in cells. Oxidative damage is believed to be strongly associated with certain human pathological processes and diseases such as carcinogenesis, mutagenesis, aging, arthritis, and atherosclerosis. Peroxidation damage caused by UV radiation in the biological membranes and in the liposomes was confirmed in multiple studies (Ming-Kuei and Miao, 1999). Each organism has its own mechanism of protection against the free radicals activity, however, peroxidation can be also suppressed by natural inhibitors called antioxidants (Trommer *et al.*, 2001; Dobarganes and Velasco, 2002).

One of the potential antioxidants is scoparone (6,7-dimethoxy-2*H*-1-benzopyran-2-one), which belongs to the group of coumarins. More than 300 coumarins have been identified from natural sources, especially green plants. The pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution. Scoparone has been isolated from the hypolipidaemic Chinese herb *Artemisia scoparia* and shown to reduce the proliferative responses of human peripheral mononuclear cells, to relax smooth muscle, to reduce total cholesterol and triglycerides and to retard the characteristic pathomorphological changes in hypercholestero-

laemic diabetic rabbits. Various properties of scoparone were suggested to account for these findings, including the ability to scavenge the reactive oxygen species, to inhibite of tyrosine kinases and to potentiate of prostaglandin generation (Huang *et al.*, 1993; Liu *et al.*, 2001, 2002; Van Pelt *et al.*, 1989; Zhao *et al.*, 2000).

Material and Methods

Liposome preparation

Lipid soy-bean phosphatidylcholine (Sigma, St. Louis, USA) was dissolved in organic solvents (a mixture of chloroform and methanol 2:1 v/v). The lipid solution was evaporated *in vacuo*. After evaporation of the solvent, Tris [2-(hydroxymethyl)-2-amino-1,3-propanediol] buffer (pH 7.4) and scoparone, α-tocopherol and hyaluronic acid (all obtained from Sigma) with desired concentrations were added into a glass vessel with lipid film, and the solution was shaken mechanically. The suspension was then sonicated with a Labsonic 2000 sonicator (Braun Biotech, Göttingen, Germany) at 80 W for 15 min under nitrogen atmosphere in an ice-bath in order to obtain a clear suspension of liposomes with a phosphatidylcholine concentration of 3.5 mM.

Induction of lipid peroxidation by UV radiation

Peroxidative process was initiated by ultraviolet radiation (UVA) with a wavelength range 320–400 nm generated by a 75 W UV lamp (Philips, Hamburg, Germany). Homogenous liposome suspension with 2 mm thickness containing varying concentrations of scoparone or another antioxidant used (α-tocopherol or hyaluronic acid) was exposed to UVA radiation for 75 min.

Induction of lipid peroxidation by Fenton reaction

To generate the hydroxyl radical (·OH) so as to test the antioxidant efficacy of the prepared plant extracts, the Fenton (Haber–Weiss) reaction was used. $FeCl_2$ reacts with hydrogen peroxide in the following manner:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\cdot}OH + HO^{-}.$$

Fenton reaction was initiated by the addition of H_2O_2 and $FeCl_2$ with a final concentration of 100 mM and 2 mM, respectively, to the liposome suspension containing varying concentrations of antioxidants.

Determination of oxidation index

Absorption spectra of the conjugated dienes were recorded in the wavelength range 215–320 nm using a UV MINI 1240 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). The increase of the absorption at 233 nm was considered as an evidence of the formation of the conjugated dienes, and the oxidation index was calculated from the ratio of the absorbance values (A_{233}/A_{215}) (Klein, 1970; Babincová, 1994; Babincová and Sourivong, 2001; Babincová *et al.*, 1999, 2002).

Results and Discussion

Peroxidation of lipids is a measure of damage to the membrane lipids caused by the attack of the reactive oxygen species. Inhibition of lipid peroxidation by any external agent is often used to evaluate its antioxidant capacity. Peroxidation of the fatty acids of phospholipids occurs via a free radical chain mechanism. Formation of lipid free radicals is initiated by the abstraction of a hydrogen atom from the lipid chain. The most susceptible to degradation are lipids containing double bonds, since unsaturation permits delocalization of the remaining unpaired electrons along the lipid chain. Polyunsaturated lipids are thus particularly prone to oxidative degradation. In the presence of oxygen, the process further proceeds via the formation of the hydroperoxides, which degrade spontaneously to form aldehydes with concomitant fission of the fatty acid chain. Natural phospholipids contain only non-conjugated double bonds and, therefore, have a UV absorbance peak at a very short wavelength (200–205 nm). Removal of a hydrogen atom from the methylene group located between two double bonds spreads the unsaturation over five carbon atoms and results in the formation of a conjugated diene which is energetically more favourable than the two isolated double bonds. As a result, the second absorbance maximum at 233 nm appears.

At first we have studied lipid peroxidation induced by UVA radiation. Until not long ago, UVA radiation was considered to be beneficial to the skin, and prevention against acute sunburn mainly concentrated on protection against the harmful effects of exposure to UVB rays (De Gruijl, 2000). However, upon deeper investigation, it was discovered that UVA radiation is most cytotoxic to human skin cells. These rays penetrate deeper into the dermis, where they provoke dermal connective

tissue alterations associated with photoaging and many other subchronic to chronic skin disorders (Schaefer *et al.*, 2000). In fact, UVA radiation has been shown to induce lipid peroxidation in liposomal, micellar, and natural systems (Bose and Chatterjee, 1995; Bose *et al.*, 1989).

The main role in lipid peroxidation induced by UV radiation is played by singlet oxygen $^1O_2$. Singlet oxygen can be generated *e.g.* by the reaction

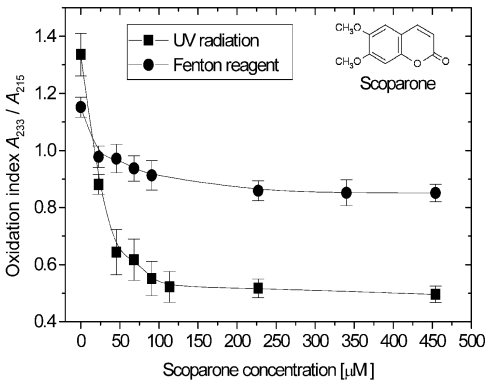

Fig. 1. Dependence of the Klein oxidation index on the scoparone concentration. Lipid peroxidation was initiated with 75 min exposition to ultraviolet radiation. Error bars represent standard deviations of a mean value (n = 10).

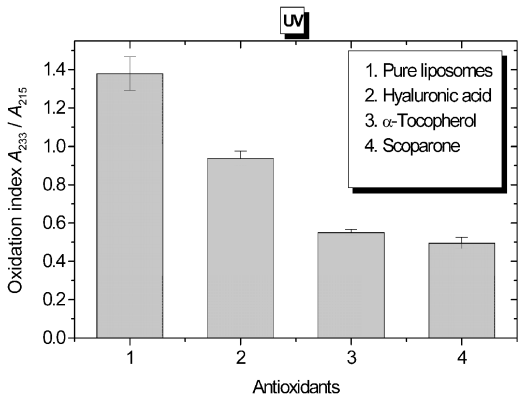

Fig. 2. Comparison of antioxidative activities of all used substances (with the following concentrations: 200 μM hyaluronic acid, 800 μM α-tocopherol, and 120 μM scoparone). Lipid peroxidation was initiated with 75 min exposition to ultraviolet radiation. Error bars represent standard deviations of a mean value (n = 10).

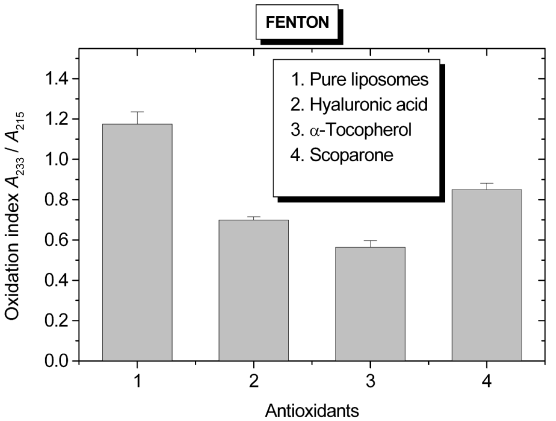

Fig. 3. Comparison of antioxidative activities of all used substances (with the following concentrations: 200 μM hyaluronic acid, 800 μM α-tocopherol, and 120 μM scoparone). Lipid peroxidation was initiated by the Fenton reaction. Error bars represent standard deviations of a mean value (n = 10).

of two peroxyl radicals. UVA radiation can also induce the formation of hydroxyl radicals (Halliwell and Gutteridge, 1993).

The dependence of the Klein peroxidation index on scoparone concentration is shown in Fig. 1. From the dose-response curve of scoparone-scavenging activities it was found that the scavenging activity increased with the increase of the scoparone concentration and that the scavenging effect is most pronounced starting from the concentration 120 μM for both UVA- as well as Fenton reaction-induced free radical formation. For a comparison we have also monitored conjugated diene formation in the presence of α-tocopherol, which is known to be incorporated into the lipid bilayer, and in the presence of hyaluronic acid, which is a water-soluble polysaccharide (Figs. 2, 3). It is clear, scoparone is a very potent antioxidant, also effective at very low concentrations, with capabilities even better than that of these two well-known free radical scavengers. We found that scoparone is less efficient when the free radical formation was initiated using the Fenton reaction (Fig. 3).

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