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Influence of astaxanthin, zeaxanthin and lutein on DNA damage and repair in UVA-irradiated cells

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Abstract

In order to gain more knowledge about the antioxidant role of the predominant carotenoids (lutein and zeaxanthin) of the human retina, this study investigated their antioxidant activity and capacity. Astaxanthin was also studied, because its structure is very close to that of lutein and zeaxanthin. The antioxidant activity of these molecules was evaluated using chemiluminescence techniques, with lucigenin and luminol as chemiluminogenic probes for the superoxide radical and hydrogen peroxide, respectively. It was found that all three carotenoids have similar superoxide-scavenging activity. The effect on the reduction of H_2O_2–luminol chemiluminescence was present in the following order, zeaxanthin > astaxanthin $\geqslant$ lutein.

Possible antioxidant capacity of these three compounds was sought using a biological system consisting of SK.N.SH human neuroblastoma and rat trachea epithelial cells subjected to oxidative stress from exposure to UVA radiation. In particular, we determined whether these compounds were capable of minimizing DNA damage and influencing the kinetics of DNA repair.

DNA damage was assessed using the Comet assay, a rapid and sensitive single-cell gel electrophoresis technique used to detect primary DNA damage in individual cells. Neuroblastoma cells appeared more resistant to oxidative irradiation insult. The presence of carotenoids reduced DNA damage when rat epithelial cells were exposed to UVA radiation for 2 min. A different result was obtained in experiments performed on neuroblastoma cells; in this case, the presence of carotenoid during UVA exposition increased the damage.

The addition of carotenoids to epithelial cells after 2 min of UVA exposition did not seem to improve the kinetics of DNA repair; on the contrary, zeaxanthin (after 60′ incubation) and lutein (after 180′ incubation) showed a genotoxic effect.

The addition of carotenoids to neuroblastoma cells after 30′ UVA exposition positively influences the kinetics of DNA repair in the first 15 min of incubation. At longer exposition times, while the behaviour measured was not constant, a genotoxic effect was not observed. The data from this study provide additional information on the antioxidant and pro-oxidant activities of the predominant macular pigment carotenoids of the human retina.

Keywords: Carotenoids; ROS; DNA; Comet assay; UV radiation

1. Introduction

It is now well established that solar radiation is a genotoxic agent; damage is wrought by the UV region of the spectrum (for a recent review [1]).

Ultraviolet radiation induces oxidative stress because it produces reactive oxygen species [2] and modulates the level of antioxidants [3–6]. Oxidative stress leads to DNA, protein and lipid damage. In DNA, hydroxyl radicals ($OH^{\bullet}$) have been observed to cause strand breaks, and singlet oxygen ($^1O_2$) has been observed to oxidize bases [2].

UV spectrum radiation encompasses UVA (315–400 nm), UVB (280–315 nm) and UVC (100–280 nm).

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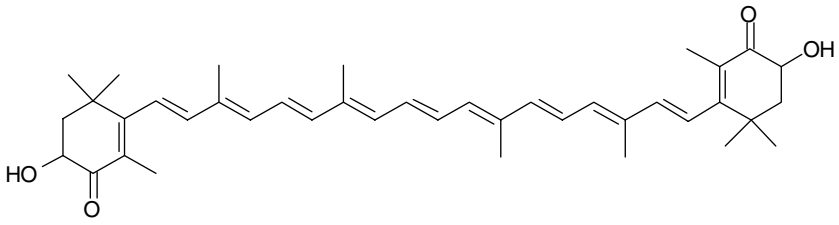
However, since UVC radiation is filtered out, for the most part, by atmospheric ozone, both UVA and UVB radiation play a more significant role in the initiation of photo-carcinogenesis [7]. At a molecular level, UVA and UVB differ in the sites of action in the generation of pre-mutagenic lesions. UVB radiation is site specific and is absorbed directly by cellular DNA. The most frequent photolesions resulting from UVB-induced DNA alterations are cyclobutane pyrimidine dimers (CPDs) capable of interfering with DNA replication. However, these can be removed by several repair mechanisms, including excision repair.

Conversely, UVA radiation does not attack the DNA directly, but is adsorbed by intracellular chromophores such as riboflavin and membrane bound enzymes. This results in an altered cellular redox potential via the generation of reactive oxygen species (ROS) and/or nitric oxide, causing photosensitization.

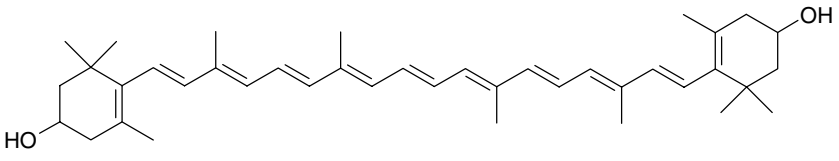
Ultraviolet radiation provokes overproduction of free radicals, which damages biological systems. This observation has stimulated research into the role of natural antioxidants that can mitigate photobiologic damage. Our interest in this field has focused on carotenoids, a group of natural pigments that have been recently used in biological systems for their antioxidant capacity [8,9]. It now appears that important actions can be attributed to some carotenoids, and evidence indicates that they may reduce development rates of some human cancers [10]. However, there are increasing concerns that carotenoids such as β-carotene and lycopene may also exert pro-oxidant properties under certain conditions (for recent reviews [11,12]).

In the present study, three different carotenoids – lutein, zeaxanthin and astaxanthin – were used (Scheme 1). The xantophylls lutein and zeaxanthin are the predominant carotenoids of the macular pigment of the human retina and the levels of their concentration in the retina have functional and pathological consequences (i.e. age-related macular degeneration). Individuals suffering from age-related eye disease have inferior xantophyll densities throughout their retinas, and dietary zeaxanthin and lutein levels are inversely linked to the risk of AMD as well as cataracts. Lutein and zeaxanthin cannot be synthesized by humans and must be obtained through diet via consumption of fruits and vegetables [13,14]. It has been reported that macular pigments might prevent a wide range of human diseases, including various cancers and other conditions [15]. Although astaxanthin has never been isolated from the human eye, it was included in this study because its structure is very close to that of lutein and zeaxanthin and because it affords protection from UV-light [16]. Like zeaxanthin and lutein, it cannot be synthesized by mammals and must be acquired through diet. Astaxanthin is found in salmon, trout, shrimp and other aquatic animals, where it has several biological functions.

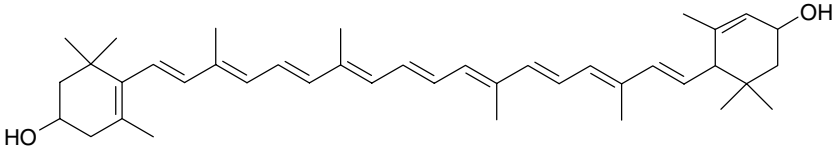
Using chemiluminescence [17] with two different chemiluminogenic probes, lucigenin and luminol, we investigated the antioxidant activity of these carotenoids versus superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). These compounds were also studied to determine whether they can minimize DNA damage inflicted on SK.N.SH human neuroblastoma and rat trachea epithelial cells following exposure to UVA radiation, and whether they can influence the kinetics of DNA repair. DNA damage inflicted by UVA radiation was compared to H_2O_2-induced DNA damage.



ASTAXANTHIN



ZEAXANTHIN



LUTEIN

Scheme 1. Chemical structures of the compounds studied.