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Review

Bioactivity and protective effects of natural carotenoids

Wilhelm Stahl*, Helmut Sies

Heinrich-Heine-University Duesseldorf, Institute of Biochemistry and Molecular Biology I, P.O. Box 101007, D-40001 Duesseldorf, Germany

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Abstract

Carotenoids comprise a class of natural fat-soluble pigments which are found in numerous fruits and vegetables. The consumption of a diet rich in carotenoids has been epidemiologically correlated with a lower risk for several diseases. The antioxidant activity of carotenoids and biochemical properties influencing signaling pathways have been discussed as basic mechanisms of prevention. Conflicting data from intervention studies with β-carotene to prevent cancers and cardiovascular disorders have challenged the concept. However, there is convincing evidence that carotenoids are important components of the antioxidant network. Photooxidative damage is suggested to be involved in the pathobiochemistry of several diseases affecting the skin and the eye, and carotenoids may protect light-exposed tissues. Lutein and zeaxanthin are the predominant carotenoids of the retina and are considered to act as photoprotectants preventing retinal degeneration. The unique distribution, localization and high levels of both carotenoids within the macula lutea as well as their physicochemical properties make them suitable candidates for photoprotection. β-Carotene is used as an oral sun protectant for the prevention of sunburn and has been shown to be effective either alone or in combination with other carotenoids or antioxidant vitamins. Protective effects are also achieved with a diet rich in lycopene.

Keywords: Carotenoid; Prevention; Disease; Signaling; Antioxidant

1. Introduction

Adequate nutrition is a key element of a healthy lifestyle associated with a lowered risk for chronic illnesses. The consumption of five portions of fruits and vegetables per day ("five-a-day") is proposed to sustain optimal health and especially colored food items are recommended. Data from epidemiological studies consistently show an inverse correlation between the intake of fruits and vegetables and the incidence of several diseases such as cardiovascular, ophthalmological, gastrointestinal or neurodegenerative disorders and some types of cancer [1]. It has been postulated that among the different dietary components of fruits and vegetables, the so-called secondary plant constituents play a major role in disease prevention [2]. Some of these phytochemicals are responsible for the bright colors of plants. Among the various natural pigments, carotenoids comprise an important group of more than 600 structurally different compounds [3–5].

2. Carotenoids

Carotenoids are tetraterpenoids and synthesized in plants, and other photosynthetic organisms as well as in some non-photosynthetic bacteria, yeasts, and molds. Most of the carotenoids are composed of a central carbon chain of alternating single and double bonds and carry different cyclic or acyclic end groups. Their major biochemical functions are determined by the extended system of conjugated double bonds which is also responsible for their color [6]. Selected carotenoids are components of the light harvesting system in chloroplasts and play an important role in the protection of plants against photooxidative damage [7]. The yellow, orange and red color of many fruits and flowers is caused by carotenoid-containing chromoplasts

* Corresponding author. Tel.: +49 211 811 2711; fax: +49 211 811 3029.
E-mail address: wilhelm.stahl@uni-duesseldorf.de (W. Stahl).

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which are usually devoid of chlorophyll. Considerable amounts are present in green parts of the plants including leaves where chlorophyll masks the carotenoids. Carotenoids can also be found in many animal species and are important colorants in birds, insects, fish, and crustaceans. However, animals and humans cannot synthesize carotenoids de novo and depend on dietary supply. According to their chemical composition they are categorized as either carotenes or xanthophylls [4]. β-Carotene, α-carotene, and lycopene are prominent members of the carotene group which includes carotenoids composed only of carbon and hydrogen atoms. Xanthophylls, however, carry at least one oxygen atom. Zeaxanthin, lutein, α-and β-cryptoxanthin, canthaxanthin and astaxanthin are important xanthophylls with hydroxy- and keto groups as structural elements.

Depending on the number of double bonds, an array of *cis*/*trans* (E/Z) configurations are possible for a given carotenoid [8]. In homogenous solutions carotenoids tend to isomerize and form a mixture of mono- and poly-*cis* isomers in addition to the all-*trans* form. Energetic and stereochemical aspects determine the isomer pattern. When incorporated into the food matrix the compounds are more resistant towards isomerization. In general, the all-*trans* form is thermodynamically most stable and predominant in nature but several *cis* isomers of carotenoids are present in blood and tissues [9]. The majority of carotenoids contains chiral centers, and thus they may occur in various stereoisomeric forms. In most plants one stereoisomer dominates because of stereospecific biosynthesis. However, there is evidence that optical isomers may be interconverted in animals; two stereoisomeric forms of zeaxanthin have been identified in the human macula lutea [10].

3. Biological effects of carotenoids

3.1. Provitamin A activity

Carotenoids can also be divided into provitamin A and non-provitamin A compounds [4,11]. The major provitamin A carotenoid in the Western diet is β-carotene, but also α-carotene, and β-cryptoxanthin contribute to vitamin A supply and may prevent vitamin A deficiency. Vitamin A is essential for the promotion of growth, embryonal development and visual function. The contribution of provitamin A carotenoids to the daily vitamin A intake depends on dietary habits and available food sources. It has been estimated that carotenoids from fruits and vegetables provide more that 70% of the vitamin A intake in Third World countries; in Western societies the contribution is much less. However, intake must be corrected for bioequivalence, evaluating what portion of the ingested provitamin A carotenoid is absorbed, cleaved, reduced and finally available as retinol or retinyl ester [12]. Several studies have shown that the bioequivalence of plant-derived provitamin A carotenoids is much less than anticipated from studies using dietary supplements. Bioavailability and metabolism are affected by several factors including food matrix properties, preparation of the food, coingestion of fat and fiber, diseases of the gastrointestinal tract, vitamin A status or malnutrition. There is evidence that different enzymes targeted to different cleavage sites are operative to metabolize carotenoids to apo-carotenoids and retinal. Cloning and sequencing of enzymes with β,β-carotene 15,15′-oxygenase activity from *Drosophila*, chicken, mouse and human has been reported [13,14]. The efficacy of cleavage, substrate specificity for various provitamin A compounds, as well as genetic variations and factors with impact on the expression of carotenoid-metabolizing enzymes are individual variants determining vitamin A supply via carotenoids.

3.2. Carotenoids and cellular signaling

Intercellular signaling is a prerequisite for coordinating biochemical functions in multicellular organisms, and it has been demonstrated that dietary carotenoids exhibit biological activities with impact on signaling pathways [15]. Carotenoids and/or their metabolites influence the expression of certain genes or may act as inhibitors of regulatory enzymes which has been discussed in context with cancer preventive properties of these compounds.

In vitro, the inhibitory effects on the growth of leukemic cells were observed when lycopene was applied in combination with 1,25-dihydroxy-vitamin D3 [16]. Lycopene and tocopherol inhibited the growth of human prostate carcinoma cell lines [17]. The inhibition of cell proliferation by lycopene is associated with a delay in cell cycle progression and apparently related to diminished IGF-I receptor signaling [18]. Lycopene lowers cyclin D levels, leading to diminished phosphorylation of the retinoblastoma protein [19], a process associated with growth arrest.

Particular attention has been given to the stimulatory effects exerted by carotenoids on gap junctional communication (GJC) [20,21]. Gap junctions are water-filled pores, connecting the cytosol of two neighboring cells, allowing the exchange of low-molecular mass compounds. Among the carotenoids, β-carotene and canthaxanthin belong to the most effective stimulators of GJC. In cell culture, carotenoids reversibly inhibit the progression of carcinogen-initiated fibroblasts to the transformed state. This inhibitory effect has been found to be related to an increased GJC induced by these compounds. GJC is implicated in the regulation of cell growth, differentiation and apoptosis. Non-tumorous cells are contact-inhibited and have functional GJC; most tumor cells, however, have dysfunctional homologous or heterologous GJC [22]. The regulation of gap junctional communication is complex and the mechanisms related to carotenoid activity are not fully understood.

Carotenoids induce phase I and phase II metabolic enzymes which play a role in the detoxification of carcinogens [15]. There is evidence that lycopene stimulates

the expression of phase II enzymes via pathways dependent on the transcription factor Nrf2 and antioxidant responsive elements (ARE) in the promoter region of the respective genes [23]. Such effects on gene expression may be related to the protective or adverse properties of carotenoids.

3.3. Antioxidant activities

Reactive oxygen and nitrogen species are generated during aerobic metabolism and pathological processes. They are damaging biologically important molecules like lipids, DNA or proteins and are involved in the pathobiochemistry of degenerative diseases [24–26]. Among the various defense strategies, carotenoids are most likely involved in the scavenging of two of the reactive oxygen species, singlet molecular oxygen ($^1O_2$), and peroxyl radicals. Further, they are effective deactivators of electronically excited sensitizer molecules which are involved in the generation of radicals and singlet oxygen [27,28].

Singlet oxygen quenching by carotenoids occurs via physical or chemical quenching [29,30]. Physical quenching involves the transfer of excitation energy from $^1O_2$ to the carotenoid, resulting in ground state oxygen and an excited triplet state carotenoid. The energy is dissipated between the excited carotenoid and the surrounding solvent to yield the ground state carotenoid and thermal energy. In the process of physical quenching the carotenoid remains intact and can undergo further cycles of singlet oxygen quenching. The rate constants for the reaction of carotenoids with singlet oxygen are in the range of 10^9 M^{-1} s^{-1}, i.e. near diffusion control. β-Carotene and other carotenoids are the most efficient natural $^1O_2$-quenchers; their quenching activity is closely related to the number of conjugated double bonds present in the molecule [31–34]. Carotenoids efficiently scavenge peroxyl radicals, especially at low oxygen tension, and contribute to the defense against lipid peroxidation [35]. Under specific conditions carotenoids may also act as prooxidants. Such properties have been determined in vitro and discussed in context with adverse effects observed upon β-carotene supplementation at high levels.

The antioxidant defense system of the organism is a complex network and comprises several enzymatic and non-enzymatic antioxidants [36]. It has been suggested that interactions between structurally different compounds with variable antioxidant activity provides additional protection against increased oxidative stress. Synergistic interactions against UVA-induced photooxidative stress have been described in cultured human fibroblasts when combinations of antioxidants were applied with β-carotene as main component [37]. In comparison to the individual antioxidants, vitamins E, C and β-carotene exhibited cooperative synergistic effects scavenging reactive nitrogen species [38]. The cooperative interaction between β-carotene and α-tocopherol was also examined in a membrane model [39]. A combination of both lipophilic antioxidants resulted in an inhibition of lipid peroxidation significantly greater than the sum of the individual effects. The antioxidant activity of carotenoid mixtures was assayed in multilamellar liposomes, measuring the inhibition of the formation of thiobarbituric acid-reactive substances [40]. Mixtures were more effective than single compounds, and the synergistic effect was most pronounced when lycopene or lutein was present. The superior protection of mixtures may be related to the specific positioning of different carotenoids in membranes.

3.4. Health effects of carotenoids

In vitro and animal studies provide evidence that carotenoids may protect against several kinds of cancer [41]. These studies are in line with epidemiological studies demonstrating that an increased consumption of a diet rich in carotenoids is associated with a diminished risk for different kinds of cancer. Additionally, there is a correlation between β-carotene serum levels and a diminished cancer risk as shown e.g. for lung cancer. However, most intervention trials with β-carotene as a component of a supplement did not show any effects regarding the risk for cancer [42,43]. One exception is a study performed in China where protective effects were observed [44]. There is evidence from two intervention trials that the risk for lung cancer is increasing when high doses of β-carotene are supplemented to a population at high risk, such as smokers or asbestos workers [45,46]. The unexpected results from these intervention trials have been broadly discussed, and several hypotheses were raised to explain their outcome. It should be noted, that the applied doses exceeded by far the normal dietary intake of β-carotene, and extremely high blood levels were determined at the end of the intervention, higher than in studies where no adverse effects were observed. Interactions of β-carotene with other compounds, either carotenoids or other dietary constituents, might play a role. It has been suggested that among the biological conditions related to the risk, the prooxidant effects of β-carotene are implicated in the development of lung cancer [28,47,48].

Recently, it has been shown in animal models that high doses of β-carotene affect the expression of a retinoic acid receptor subtype that might be important in context with carcinogenesis; these effects were more pronounced when the animals were additionally exposed to cigarette smoke [49,50].

The relationship between carotenoids and prostate cancer has been examined in observational studies with varied results [51]. A decreased risk for prostate cancer was associated with high lycopene consumption from tomatoes and tomato products. Two of three studies in which blood lycopene levels were measured reported an association between higher lycopene levels and a lowering of prostate cancer risk [51]. Based on intervention studies it has been suggested that supplementation with lycopene or a

lycopene-rich diet may decrease the growth of prostate cancer [52,53]. The contribution of β-carotene and other carotenoids to cancer prevention associated with a carotenoid-rich diet remains unclear. Mixtures of compounds may be more efficient than single components.

Also with respect to the prevention of cardiovascular diseases, data for β-carotene are conflicting [54]. The compound is capable to inhibit lipid peroxidation in LDL, a process suggested to be involved in the pathogenesis of atherosclerosis. Up until now there is no convincing evidence from intervention studies that β-carotene or carotenoids in general are protective.

3.5. Carotenoids and age-related macular degeneration

A focus of current carotenoid research regards the possible role of this group of compounds in the protection against age-related macular degeneration (AMD). AMD is a major cause for irreversible blindness among the elderly in the Western world and it affects about 20% of the population above the age of 65 [55]. The macula lutea ("yellow spot") is part of the retina, and the area of maximal visual acuity. Lutein and zeaxanthin are the pigments responsible for the coloration of this tissue; other carotenoids such as lycopene, α-carotene or β-carotene are not found in the macula lutea [56]. Lutein and zeaxanthin are also dominating the carotenoid pattern of the entire retina, but the concentration in the macula lutea is considerably higher than in the rest of the retina [57]. The uptake, transport and metabolism of xanthophylls in the retina are likely mediated by specific xanthophyll-binding proteins. An isoform of glutathione *S*-transferase (GSTP1) has been shown to act as a zeaxanthin-binding protein in the human macula [58].

Epidemiological data support the concept that the macular pigments have a protective role; a strong association was found for lutein [59]. In comparison to the controls unaffected by AMD, lower levels of lutein and zeaxanthin were found in the retina from donors suffering from AMD [60]. There is also some evidence from a small intervention study (LAST) that visual function is improved in patients suffering from atrophic AMD when lutein alone or lutein together with other nutrients is supplemented [61].

Molecules protecting occular tissue against photooxidative damage may act in two ways: first as filters for damaging blue light, and second as antioxidants quenching excited triplet states or singlet molecular oxygen and scavenge secondary ROS like lipid hydroperoxides or the superoxide radical anion [55]. In homogenous solution all major dietary carotenoids including β-carotene and lycopene are efficient blue-light filters. However, the spectral properties of carotenoids as well as antioxidant activities change with the environment. The filtering effects of lutein and zeaxanthin were superior to those of lycopene and β-carotene when investigated in a membrane model using unilamellar liposomes [62]. The xanthophylls were incorporated in higher amounts into the membrane and, thus, showed better filtering efficacy.

In a large scale intervention trial the effects of a combination of high-dose β-carotene together with vitamins C and E and zinc on AMD progression and visual acuity were investigated. Interestingly, the data revealed a statistically significant decrease for the development of advanced AMD with antioxidants plus zinc [63].

3.5.1. Skin protection

β-Carotene supplements are widely used as a so-called oral sun protectants [64]. However, studies demonstrating the protection of oral treatment with β-carotene against skin responses to sun exposure are scarce. The protective effects are thought to be related to the antioxidant properties of the carotenoid. Upon UV-irradiation, the skin is exposed to photooxidative damage which is induced by the formation of reactive oxygen species. Photooxidative damage affects cellular lipids, proteins and DNA and is considered to be involved in the pathobiochemistry of erythema formation, premature aging of the skin, development of photodermatoses, and skin cancer [65,66]. Several studies in humans have shown that carotenoid levels in plasma and skin decrease upon UV-irradiation; lycopene is lost preferentially as compared to other carotenoids [67]. Thus, the beneficial effects of supplementation have been postulated. It was demonstrated that β-carotene levels in skin and serum can be increased by supplementation with carotenoids derived from the alga *Dunaliella salina* [68]. Protective effects where investigated with the carotenoid supplement (24 mg total β-carotene/day) and a combination of the supplement with vitamin E when erythema was induced by illumination with a solar simulator [69]. Erythema formation was significantly diminished and erythema suppression was more pronounced with the combination of carotenoids and vitamin E. In order to lower the dose of β-carotene, the compound was partially substituted by other carotenoids [70]. The photoprotective effect of β-carotene alone (24 mg/day) was compared to that of a carotenoid mixture consisting of β-carotene, lutein and lycopene (8 mg each/day). Supplementation was for 12 weeks, and carotenoid levels in the serum and skin, as well as erythema intensity after irradiation with a solar light simulator were determined at baseline and after 6 and 12 weeks of treatment. The intensity of erythema was diminished to a similar extent in both groups. Hence, supplementation for 12 weeks with 24 mg of a carotenoid mixture supplying 8 mg each of β-carotene, lutein and lycopene ameliorates UV-induced erythema in humans [70].

Protection against UV-induced erythema was observed after dietary intervention, as opposed to supplementation with isolated compounds. Tomato paste contains high amounts of the tomato-specific carotenoid lycopene and was selected as a natural dietary source providing carotenoids to protect against UV-induced erythema in humans [71]. The ingestion of tomato paste (40 g/day, equivalent to

16 mg lycopene/day) over a period of 10 weeks led to elevated serum levels of lycopene and an increase of total carotenoids in skin. After 10 weeks of treatment, erythema formation was significantly lower in the group consuming the tomato paste than in the controls. This study demonstrates that UV-induced erythema can be ameliorated by dietary intervention.

Protection was also determined when carotenoids were provided with a carotenoid-enriched drink. The photoprotective effects of synthetic lycopene were compared with a supplement derived from a tomato extract and a drink containing a solubilized form of the supplement [72]. With the different sources, similar amounts of lycopene (about 10 mg/day) were provided and after 12 weeks, significant increases in lycopene serum levels and total skin carotenoids were observed in all groups. Sensitivity towards UV light was determined after irradiation with a solar simulator determining the degree of erythema before and after supplementation. The protective effect was most pronounced in the groups that ingested the supplement or the drink.

4. Conclusion

Carotenoids are unique constituents of a healthy diet and play an important role the network of antioxidant vitamins and phytochemicals. They are good blue light filters and are efficient quenchers of singlet oxygen and excited triplet state molecules. The lipophilicity of carotenoids determines their subcellular distribution; they are enriched in membranes and other lipophilic compartments. Taken together, this makes them suitable photoprotectants, not only for plants but also for humans.

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Two monographs on carotenoids appeared recently:

L. Packer, K. Kraemer, U. Obermüller-Jevic, H. Sies (Eds.), Carotenoids and Retinoids—Molecular Aspects and Health Issues, AOCS Press, Champaign, Illinois, 2004.

N.I. Krinsky, S.T. Mayne, H. Sies (Eds.), Carotenoids in Health and Disease, Marcel-Dekker, New York, 2004.

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