Chapter 11
Provitamin A Carotenoids and Cancer Prevention

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Key Points

- Clinical intervention trials conducted to determine the chemoprotective effect of large doses of β-carotene as a potential chemopreventive agent on the incidence of lung cancer in smokers found either no protective effect or a harmful effect. However, evidence for a protective role of whole fruits and vegetables rich in provitamin A carotenoids (β-carotene, α-carotene, and β-cryptoxanthin) in the prevention of certain cancers and other chronic diseases (e.g., atherosclerosis, diabetics, age-related macular degeneration, UV damage in skin) continues to be reported in human epidemiological studies and small intervention trials, as well as in mechanistic studies using cell culture and animal models.
- These findings have led to an increased effort to better understand the role of carotenoids and their derivatives in the process of these chronic diseases, with special attention to their metabolism and biological actions, dose effects, organ-specific effects, and the oxidative environment especially in smokers and alcohol drinkers.
- Greater knowledge has been gained in the biological effects of provitamin A carotenoid derivatives on the potential for beneficial effects of small quantities or harmful effects of large quantities of the resulting metabolic products. Provitamin A carotenoids may have certain unique beneficial effects against cancer risks.
- The molecular biological properties of provitamin A carotenoids, such as β-cryptoxanthin, and their metabolites remain to be determined through further more detailed research.

Keywords Provitamin A carotenoids • Metabolism • Molecular mechanisms • Cancer prevention

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Introduction

More than 700 carotenoids have been identified but only 40–50 of them are present in the typical human diet and can be absorbed, metabolized, and utilized by the body [1]. All carotenoids possess a polyisoprenoid structure, a long conjugated chain of double bonds with a near bilateral symmetry around the central double bond [2]. The conjugated double bond makes carotenoids susceptible to oxidative cleavage and geometric (*trans*/*cis*) conversion. Some carotenoids such as α-carotene, β-carotene, and β-cryptoxanthin can be cleaved to form vitamin A; therefore, they are called provitamin A carotenoids (Fig. 11.1). Provitamin A carotenoids have at least one β-ionone ring. β-Carotene, a strongly colored red-orange pigment, is one of the most well-known and well-studied provitamin A carotenoids. Much research on carotenoids to date has concentrated on β-carotene. β-Cryptoxanthin is another provitamin A carotenoid which has drawn more attention of researchers in recent years. Some carotenoids such as lutein, zeaxanthin, and lycopene cannot form vitamin A, so they are called non-provitamin A carotenoids.

The richest dietary sources of β-carotene are yellow, orange, and leafy green fruits and vegetables, such as carrots, spinach, sweet potatoes, and cantaloupe. β-Cryptoxanthin is closely related to β-carotene in structure, with only the addition of a hydroxyl group. It is a member of the class of carotenoids known as xanthophylls. In a pure form, cryptoxanthin is a red crystalline solid with a metallic luster. β-Cryptoxanthin is mainly derived from orange fruits like tangerine and papaya. α-Carotene is a form of carotene with a β-ring at one end and an ε-ring at the other. It is the second most common form of carotene. The following vegetables are rich in α-carotene: carrots, sweet potatoes, pumpkin, winter squash, and broccoli.

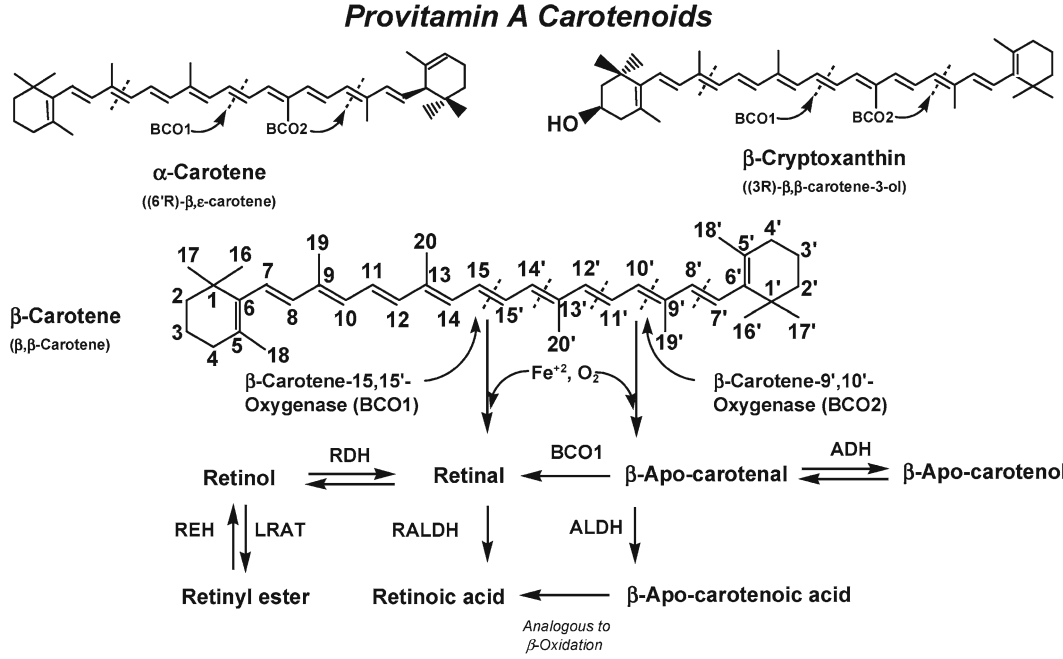

Fig. 11.1 Chemical structures of provitamin A carotenoids (β-carotene, α-carotene, and β-cryptoxanthin) and metabolic pathway of β-carotene. Figure adapted from Mernitz H, Wang XD. The bioconversion of carotenoids into retinoids: implications for cancer prevention. In: Vitamin A: New Research, editors, Loessing: KARGER press; 2007. p. 39–57

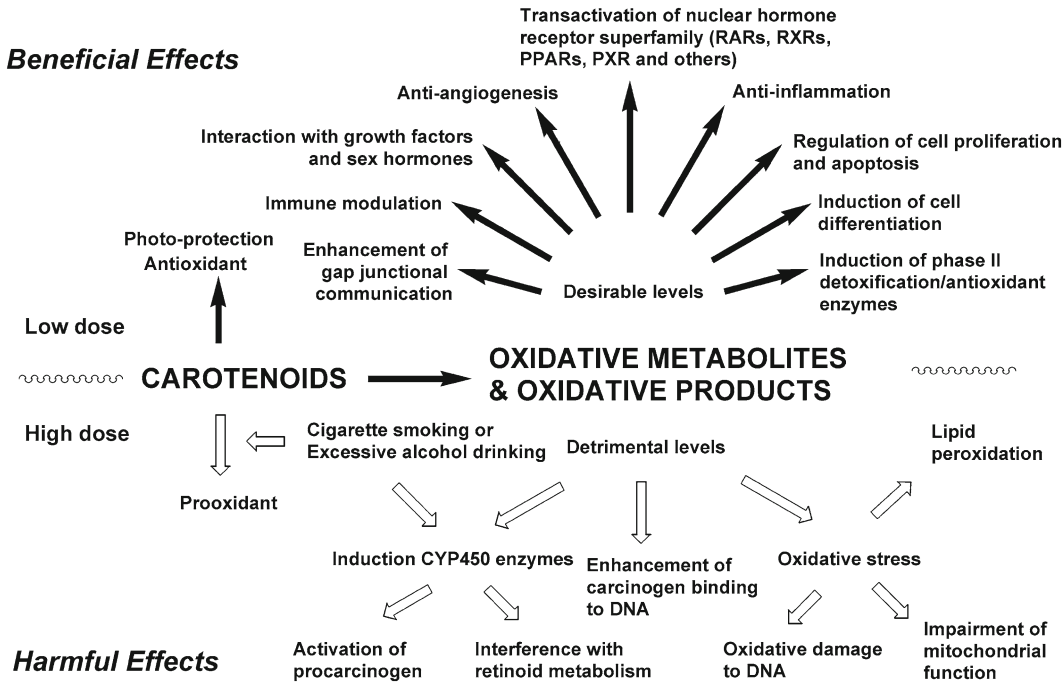

Fig. 11.2 Schematic illustration of beneficial and harmful effects of carotenoids on human health, including possible mechanisms related to carotenoid dose and oxidative metabolite formation. The biological activities of carotenoids could be related to the function of intact carotenoids or their metabolic products, which can possess either more or less activity than their parent compounds, or have entirely different functions. It appears that while small quantities of carotenoids can offer protection against certain cancers and chronic diseases related to free radical oxidation, larger amounts of carotenoid metabolites may actually be harmful, especially when coupled with a highly oxidative environment, such as the lungs of a cigarette smoker or liver of an excessive alcohol drinker. Oxidative destruction of β-carotene results in the formation of metabolites that may facilitate the carcinogenic process. Strong interactions among β-carotene, vitamin E, and vitamin C, and the capability of these compounds to "recycle" each other or antioxidant "network," regenerating efficient antioxidants from their radical cations, have led researchers to speculate about the potential utility of combined antioxidant therapy *in vivo*. It is possible that this additional protection against oxidative degradation may increase the utility of nutritional interventions targeting lung cancer in smoke-exposed models, surpassing effects seen in single-agent intervention studies. The combination of carotenoids and other antioxidants, such as vitamins E and C, which provide complementary or synergistic protective effects, would be a valuable strategy against cancer risk. Figure adapted from Wang XD. Carotenoid oxidative/degradative products and their biological activities. In: Krinsky NI, Mayne ST, Sies H, editors. Carotenoids in health and disease. New York: Marcel Dekker, Inc. press. 2004. p. 313–335

Epidemiological studies show that a high dietary intake of carotenoids may offer protective effects against the development of certain cancers [3]. However, other reports show that β-carotene alone or in combination with vitamin A could increase the risk of lung cancer in smokers [4, 5]. These observations have led to extensive research efforts to better understand the mechanisms involved in the action of carotenoids on carcinogenic processes. This chapter will focus on the roles of β-carotene specifically and other provitamin A carotenoids, especially β-cryptoxanthin, in cancer prevention as well as illustrate the potential mechanisms (Fig. 11.2).

Bioavailability and Metabolism of Provitamin A Carotenoids

The bioavailability of β-carotene from vegetables and fruits is generally not high [6] and is covered extensively in Section I of this book. The half-life of plasma carotenoids is 12 days and under for β-carotene, α-carotene, and β-cryptoxanthin. Food processing and cooking cause breakdown of the food matrix and release of embedded carotenoids increasing absorption and bioavailability [7, 8]. After release from the food matrix, ingested carotenoids must be emulsified and solubilized into micelles before they are absorbed into the intestinal mucosa. The efficiency of absorption of a moderate dose of β-carotene in oil is about 9–22%, so dietary fat promotes β-carotene absorption [9]. Both the cellular uptake and secretion of β-carotene are saturable, concentration-dependent processes. After β-carotene is taken up by the mucosa of the small intestine, it is either cleaved by β-carotene 15,15′-monooxygenase (CMO1 or BCO1) or β-carotene 9′,10′-monooxygenase (CMO2 or BCO1) into vitamin A and other metabolites, or packaged into chylomicrons and secreted into the lymphatic system for transport to the liver and other peripheral tissues.

The two metabolic pathways for β-carotene to convert to vitamin A include central and excentric cleavage (Fig. 11.1). For provitamin A carotenoids, central cleavage is the main pathway to form vitamin A. β-Carotene, α-carotene, and β-cryptoxanthin are cleaved symmetrically at their central double bond by CMO1. The excentric cleavage pathway [10, 11] was confirmed by the molecular identification of an excentric cleavage enzyme, CMO2, in mice, humans, zebrafish, and ferrets [12, 13]. CMO2 has been demonstrated to have the ability to catalyze the asymmetric cleavage of β-carotene to produce β-*apo*-10′-carotenal and β-ionone [12]. Although the contribution of CMO2 in vitamin A biosynthesis remains controversial, a quantitative trait locus associated with yellow adipose and milk color was identified to contain a premature stop codon mutation in the bovine CMO2 gene. This results in increased adipose, serum, and milk β-carotene concentrations and decreased liver retinol compared to wild types, yet no developmental or physiologic abnormalities in CMO2 mutants were observed [14]. β-*Apo*-carotenals can be cleaved further by CMO1 to produce retinol and retinoic acid [15], or oxidized to their corresponding *apo*-β-carotenoic acids. β-*Apo*-carotenoic acids may then undergo a process similar to β-oxidation of fatty acids, until further oxidation is blocked by the methyl group at the C13 position [16]. Recently, utilizing HPLC, LC-MS, and GC-MS, we have identified both volatile and non-volatile *apo*-carotenoid products including 3-OH-β-ionone, β-ionone, 3-OH-β-*apo*-10′-carotenal, and β-*apo*-10′-carotenal, indicating cleavage at both the 9,10 and 9′,10′ carbon–carbon double bond of β-cryptoxanthin [17]. Furthermore, in the presence of NAD^+, *in vitro* incubation of 3-OH-β-*apo*-10′-carotenal with ferret hepatic homogenates resulted in dose-dependent formation of 3-OH-β-*apo*-10′-carotenoic acid. Since *apo*-carotenoids serve as important signaling molecules in a variety of biological processes, enzymatic cleavage of β-cryptoxanthin by mammalian CMO2 represents a new avenue of research regarding vertebrate provitamin A carotenoid metabolism and biological function.

Biological Activity of β-Carotene on Cancer

Beneficial Effects and Potential Mechanisms

Regulation of Transcriptional Receptors

Provitamin A carotenoids can produce all-*trans*-retinoic acid and 9-*cis*-retinoic acid [18], the ligands for retinoic acid receptors (RARs) and retinoid X receptors (RXRs), respectively. β-Carotene and its oxidative metabolite, *apo*-14′-carotenoic acid, can reverse the downregulation of RARβ by smoke-borne

carcinogens in normal bronchial epithelial cells [19], and the transactivation of the RARβ2 promoter induced by β-*apo*-14′-carotenoic acid is through its metabolism to all-*trans*-retinoic acid [19]. So the bioactivities of β-carotene may be mediated through transcriptional activation of a series of genes associated with antiproliferative or proapoptotic activities.

Antioxidant Function

In the early 1980s, two key publications [20, 21] revealed that β-carotene could be an antioxidant and anti-cancer agent. This greatly stimulated the field of carotenoid research. Cancer development has been linked to DNA damage, which could result from an increased level of oxidative stress. Provitamin A carotenoids are scavengers of singlet oxygen and other reactive oxygen species [22]. Therefore the antioxidant activities of provitamin A carotenoids may be one mechanism underlying their beneficial effects against carcinogenesis. β-Carotene is able to neutralize singlet oxygen ($^1O_2$) and interrupt lipid peroxidation chain reactions (see Chap. 4). Based on that activity, β-carotene can reduce the harmful effects of solar radiation on photosensitive individuals [23], decrease DNA oxidative damage in lymphocytes [24], and reduce the MDA level in human plasma [25]. In rats, β-carotene also exhibited antioxidant and anti-apoptotic properties to prevent ethanol-induced cytotoxicity in isolated hepatocytes by decreasing oxidative stress and inhibiting caspase-9 and caspase-3 expression [26].

β-Carotene and Antioxidant Combinations

The interactions among β-carotene, α-tocopherol (vitamin E), and ascorbic acid and the capability of these compounds to "recycle" each other, led researchers to characterize their combined antioxidant activities. α-Tocopherol enhances lymphatic transport of β-carotene and central cleavage of β-carotene to form vitamin A (rather than oxidative by-products) *in vivo* [27]. Further, α-tocopherol and ascorbic acid were able to decrease the production of undesirable oxidative metabolites and increase the formation of retinoids from β-carotene in lung tissues of smoke-exposed ferrets *in vitro* [28]. The formation of excentric cleavage products in ferret lung post-nuclear fractions after incubation with β-carotene was greatly increased while the formation of retinoic acid was decreased in animals that had been exposed to cigarette smoke. Retinoic acid reduction was reversed by addition of α-tocopherol or ascorbic acid both *in vitro* [29] and *in vivo* [30]. These studies suggest that α-tocopherol and ascorbic acid act synergistically to prevent the oxidative excentric cleavage of β-carotene induced by exposure to cigarette smoke and enhance vitamin A formation.

Combined antioxidant (i.e., β-carotene, α-tocopherol, and ascorbic acid) supplementation reversed smoke-induced changes of lung protein levels related to cellular proliferation and apoptosis [30, 31] and reversed the increased labeling of proliferating cellular nuclear antigen observed in smoke-exposed, carcinogen-injected ferrets. Supplementation also reversed smoke- and carcinogen-induced phosphorylation of mitogen-activated protein kinase (MAPK), c-jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK), which subsequently induced phosphorylation of p53 tumor suppressor protein and activated its downstream apoptotic protein Bax. In addition, the combined antioxidants also suppressed smoke-induced oxidative stress [31]. A human study showed that the beneficial effects of combined β-carotene, vitamin E, and selenium supplementation on mortality were still evident up to 10 years after the cessation of supplementation [32]. These data provide *in vivo* evidence of the utility of combined nutrients as a chemopreventive strategy to reduce the risk of lung cancer in smokers.

Inhibition of Proliferation and Induction of Apoptosis

β-Carotene was able to inhibit the activation of MAPK pathways, cell proliferation, and phosphorylation of p53 [31]. β-Carotene suppressed proliferation of some cancer cells, such as human squamous cells (SK-MES lung carcinoma or Scc-25 oral carcinoma) [33], and this inhibitory effect was accompanied by a rapid appearance of a unique 70 kDa protein, analogue to heat shock proteins. Moreover, β-carotene suppressed the growth of prostate tumor cells xenografted in nude mice [34]. β-Carotene inhibited cyclin D1-associated cdk4 kinase activity, along with a decrease in the levels of the hyperphosphorylated form of retinoblastoma protein in human fibroblasts [35], which may partially explain the inhibitory effect of β-carotene on cancer cell proliferation.

Genetic loss or functional aberration of cellular control mechanisms of apoptosis is considered to be a critical event in the initiation, promotion, or progression of cancer [36]. Apoptosis represents a protective mechanism against neoplastic transformation and development of tumors by eliminating genetically damaged cells or cells that may have been inappropriately induced to divide by mitogenic and proliferative stimuli. β-Carotene exhibits potential roles in the induction of apoptosis of human cervical cancer cells [37], colon adenocarcinoma [38, 39], gastric cancer cells [40], and leukemic cells [41]. One possible underlying mechanism for the proapoptotic effect of β-carotene could be its potential regulation on caspase cascade activation in tumor cells. For example, β-carotene induced apoptosis by the activation of caspase-8, caspase-9, and caspase-3 via cytochrome c release from mitochondria. Concomitantly, a dose-dependent decrease of B-cell lymphoma 2 and increase of BID protein cleavage were also observed [42].

Inhibition of Malignant Transformation

The potential tumor preventive effects of provitamin A carotenoids might associate with the inhibition of malignant transformation. For example, β-carotene can inhibit malignant transformation induced by 3-methylcholanthrene or X-ray treatment in a fibroblast cell line [43]. All-*trans*-β-carotene is consistently more active in suppressing neoplastic transformation in both murine and human keratinocytes as compared with 9-*cis*-β-carotene [44].

Harmful Effects and Potential Mechanisms

During 1994–1996, the human trials with β-carotene concluded no evidence of beneficial effect and actually showed an increased risk of lung cancer in heavy smokers and asbestos workers [4, 5, 18, 45]. These unexpected findings brought carotenoid researchers back to experimental research in animal and cell culture models in an attempt to find insight into this contradiction. The effects of dose responses, the antioxidant/pro-oxidant effect, and the coexistence of the central and excentric cleavage pathways reveal the complexity of carotenoid metabolism in organisms and raise questions regarding the potential effects of interaction between exogenous factors (e.g., tobacco smoking and chronic alcohol consumption) with carotenoids and their metabolites (Fig. 11.2) [46].

Dosage

Small quantities of β-carotene can offer protection against certain cancers related to free radical oxidation, while larger amounts might be harmful, especially when coupled with a highly oxidative

environment. The dosages of β-carotene used in two human intervention studies mentioned above were 20–30 mg per day for 2–8 years. This is tenfold higher than the intake of β-carotene in the typical American diet (~2 mg per day) and resulted in accumulation of relatively high levels of β-carotene and its oxidative excentric cleavage metabolites in lung tissue. Research in animal and cell culture models suggest that β-carotene is unstable in the free radical-rich environment of lungs exposed to cigarette smoke, which will alter β-carotene metabolism to produce undesirable excentric cleavage metabolites (Fig. 11.2). These metabolites facilitate a number of changes associated with the carcinogenic process, including induction of carcinogen-activating enzymes, binding of carcinogen metabolites to DNA, interference with vitamin A metabolism, downregulation of tumor suppressor genes, upregulation of oncogenes, induction of oxidative stress, and enhanced induction of cell transformation by carcinogens [46]. Given that β-carotene in the diet is less bioavailable than supplemental β-carotene, no harmful effects have been associated with high levels of dietary β-carotene from natural food sources, aside from the occasional appearance of carotenodermia.

Pro-oxidant Effect

Some evidence indicates that carotenoids might behave as pro-oxidants in certain circumstances. At higher oxygen concentrations, carotenoid peroxy radicals such as Car-OO$^{\bullet}$ or ROO-Car-OO$^{\bullet}$ could be formed, which could act as pro-oxidants, promote hydrogen abstraction and oxidation of unsaturated lipids, and hence exacerbate membrane damage. β-Carotene at a concentration of 0.2 μM augmented UVA-induced heme oxygenase-1 expression [47]. In another study, β-carotene at a concentration of 10 μM increased the production of reactive oxygen species and the levels of cellular oxidized glutathione in leukemia and colon adenocarcinoma cell lines *in vitro* [48]. Lowe et al. demonstrated that β-carotene can protect HT29 cells against oxidative DNA damage at relatively low concentrations (1–3 μM), but lose this capacity at higher concentrations (4–10 μM) [49]. Based on the evidence obtained from the clinical trials of β-carotene supplementation in lung cancer, it appears that β-carotene may act as a protective antioxidant against cancer at physiological levels, but may lose its effectiveness or even exert pro-oxidant effects at pharmacological levels, especially in highly oxidative compartments of the body. The interactions among β-carotene, α-tocopherol, and ascorbic acid *in vitro* and their potential capability to transform each other, led researchers to their combination studies to eliminate potential pro-oxidant effects by a single agent. In animal studies, α-tocopherol and ascorbic acid decreased the production of undesirable oxidative metabolites and increased the formation of retinoids from β-carotene in lung tissue of smoke-exposed ferrets *in vitro* and *in vivo*.

Induction of Phase I Enzymes

In laboratory studies, tobacco smoking and chronic, excessive alcohol consumption, especially when coupled with a high dose of carotenoids, induced the expression of cytochrome P450 enzymes [29, 50]. These enzymes may activate procarcinogens present in alcoholic beverages, tobacco smoke, and diet, leading to increased formation of carcinogen-DNA adducts. If not repaired or repaired incorrectly, these adducts may eventually lead to mutations and ultimately cancer, especially, if the adducts are located in tumor suppressor genes. In addition, these same cytochrome P450 enzymes can break down retinoic acid and lead to significantly decreased tissue retinoic acid levels [29]. These studies provide possible mechanistic explanations for the discordance between the results of observational epidemiological studies and intervention trials using carotenoids as a potential beneficial agent.

Other Provitamin A Carotenoids and Their Cancer-Preventive Effects

β-Cryptoxanthin

Recently, several epidemiological studies have brought attention to β-cryptoxanthin for its potential benefits against lung cancer [51–53]. In two cohort studies involving Chinese populations, among all carotenoids examined, only serum levels of β-cryptoxanthin or the dietary intake of β-cryptoxanthin were significantly associated with a reduced risk of lung cancer [51, 52]. Similarly, in a pooled analysis of data from seven large cohorts in North America and Europe involving 3,155 incident cases of lung cancer, β-cryptoxanthin was the only dietary carotenoid significantly associated with a reduction of lung cancer risk (RR = 0.76; 95% confidence interval, 0.67–0.86; highest versus lowest quintile) [53]. Data from our lab showed that β-cryptoxanthin inhibited the proliferation of premalignant human bronchial epithelial cells, which was associated with a decrease of cells in S phase, lowered protein levels of cyclin D and E, and increased levels of the cell cycle inhibitor p21, without inducing apoptosis [54]. β-Cryptoxanthin significantly increased RARβ mRNA in these cells. The effect of β-cryptoxanthin is, in part, due to the transactivation of RARs, supported by further observation that β-cryptoxanthin dramatically increased RARE-dependent promoter activity in cells co-transfected with RAR expression vector [54].

Recently, studies indicated that β-cryptoxanthin provides a beneficial effect against cigarette smoke-induced inflammation, oxidative DNA damage, and squamous metaplasia in the lungs of ferrets [55]. The effects of β-cryptoxanthin supplementation were evaluated on cigarette smoke-induced squamous metaplasia, inflammation, and changes in protein levels of pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) and transcription factors nuclear factor kappa B (NF-κ B) and activator protein-1 (AP-1), as well as on smoke-induced oxidative DNA damage [8-hydroxy-2′-deoxyguanosine (8-OHdG)] in the lung tissue of ferrets. β-Cryptoxanthin supplementation dose-dependently increased plasma and lung β-cryptoxanthin levels. Both low-dose (7.5 μg/kg body weight per day) and high-dose (37.5 μg/kg body weight per day) β-cryptoxanthin lowered cigarette smoke-induced lung squamous metaplasia in ferrets. Lung squamous metaplastic lesions were observed in all ferrets in the smoke-exposed group, but only in two of the six ferrets in the low-dose β-cryptoxanthin group with smoke exposure and only in one of the six ferrets in the high-dose β-cryptoxanthin group with smoke exposure. No lung squamous metaplasia was found in the control group, the low-dose or high-dose β-cryptoxanthin alone groups. Cigarette smoke significantly increased inflammation in ferret lungs with the median grade of 3, and both low- and high-dose β-cryptoxanthin significantly lowered smoke-induced inflammation with the median grade of 2 (range: from 1 to 3). β-Cryptoxanthin substantially reduced smoke-elevated TNFα levels in alveolar, bronchial, bronchiolar, and bronchial serous/mucous gland epithelial cells and in lung macrophages. Moreover, β-cryptoxanthin decreased smoke-induced activation of NF-κB, expression of AP-1, c-Jun, and c-Fos, and levels of 8-OHdG in the same epithelial cells. The beneficial effects of β-cryptoxanthin were stronger by high-dose β-cryptoxanthin than those by low-dose β-cryptoxanthin [55].

Recent studies demonstrated that cancer-preventive effects of β-cryptoxanthin may depend on the enhancement of DNA repair as well as antioxidant protection against damage [56]. At low concentrations, close to those found in plasma, β-cryptoxanthin protects transformed human cells (HeLa and Caco-2) from damage induced by H_2O_2 or by visible light in the presence of a photosensitizer. In addition, it has a striking effect on two kinds of DNA repair—SB rejoining and excision repair of oxidized bases [56].

Several recent studies indicate that β-cryptoxanthin could suppress α7-nicotinic acetylcholine receptor (α7-nAChR) expression and its mediated PI3K signaling pathways in human immortalized lung cells and lung cancer cells [57]. The nicotinic acetylcholine receptors (nAChRs) were initially believed to exist only in the nervous system. However, emerging studies showed that nAChRs, their physiological agonist acetylcholine, and its synthesizing enzyme choline acetyltransferase are widely expressed in mammalian cells, including cancer cells [58–60]. Some tobacco components like nicotine and nicotine-derived nitrosamino ketone (NNK) are high-affinity nAChR agonists. The α7-nAChR is the main subunit of nAChRs in lung cancer cells, and numerous studies have reported that α7-nAChR plays critical roles in lung carcinogenesis and lung cancer development by regulation of multiple cellular signaling pathways [60, 61]. Nicotine and NNK can enhance lung cancer cell proliferation and motility through stimulation of α7-nAChR as well as upregulation of α7-nAChR expression [61, 62]. Therefore, α7-nAChR represents a valuable molecular target for prevention or therapy of tobacco-related lung cancers [60, 63]. The suppression of α7-nAChR expression and its downstream signaling, especially PI3K signaling pathways, by β-cryptoxanthin might provide mechanical explanation to the inhibitory effect of β-cryptoxanthin on lung cancer cell proliferation and survival *in vitro*, and the chemopreventive effects of β-cryptoxanthin among current smokers in human epidemiologic studies. Moreover, we found that β-cryptoxanthin is effective at inhibiting migration and invasion of α7-nAChR positive lung cancer cells by suppressing actin remodeling, ruffling/lamellipodia formation, but not in α7-nAChR negative cells. In addition, β-cryptoxanthin could suppress angiogenesis through inhibiting endothelial cell migration, invasion, tube formation, and microvessel outgrowth in aortic ring sprouting experiments. These results provided additional mechanical support for the anti-proliferation and anti-motility activities of β-cryptoxanthin.

α-Carotene

Several studies showed that α-carotene possesses higher activity than β-carotene to suppress tumorigenesis in skin, lung, liver, and colon [64, 65]. In the skin tumorigenesis experiment [64], the percentage of tumor-bearing mice in the control group was 69%, whereas the percentages of tumor-bearing mice in the groups treated with α- and β-carotene were 25% and 31%, respectively. The average number of tumors per mouse in the control group was 3.7, whereas the α-carotene-treated group had 0.3 tumors per mouse ($p < 0.01$). β-Carotene treatment also decreased the average number of tumors per mouse (2.9 tumors per mouse), but the difference from the control group was not significant. The higher potency of α-carotene than β-carotene in the suppression of tumor promotion was further confirmed in this study. For example, in a 4-nitroquinoline 1-oxide (4NQO)-initiated and glycerol-promoted mouse lung carcinogenesis model, the average number of tumors per mouse in the control group was 4.1, whereas the α-carotene-treated group had 1.3 tumors per mouse ($p < 0.001$). β-Carotene treatment did not show any suppressive effect on the average number of tumors per mouse, but rather induced a slight increase (4.9 tumors per mouse). In a liver carcinogenesis experiment [64], C3H/He mice, which have a high incidence of spontaneous liver tumor development, were treated for 40 weeks with α- and β-carotene with a 0.05% emulsion in drinking water or vehicle control. The mean number of hepatomas was significantly decreased by α-carotene treatment as compared with that in the control group; the control group developed 6.3 tumors per mouse, whereas the α-carotene-treated group had 3.0 tumors per mouse ($p < 0.001$). On the other hand, the β-carotene-treated group only showed a tendency toward a decrease of tumors, as compared with the control group [64].

Conclusion and Future Directions

Many epidemiological studies show the benefit of provitamin A carotenoid-rich fruits and vegetables on the risk of chronic diseases; however, clinical supplementation trials have returned null findings or evidence of harm in certain populations. Based on these results, high-dose carotenoid supplementation is not recommended for the general population, and smokers and consumers of alcohol are warned to avoid high-dose carotenoid supplements. However, the metabolism and molecular biological properties of many carotenoids remain to be determined through further research. Recent studies indicated that provitamin A carotenoids other than β-carotene may be active in several important cellular signaling pathways in lung carcinogenesis, and excentric cleavage carotenoid metabolites could have greater biological roles than their parent compounds in several molecular targets. In particular, studies from seven large well-implemented cohort studies consistently show that among all of the specific carotenoids examined, increased dietary intake or elevated blood levels of β-cryptoxanthin is strongly associated with a reduced risk of lung cancer. The experimental evidence shows that β-cryptoxanthin inhibits the growth of both premalignant and malignant lung cell lines, and decreased dose-dependently the tobacco smoke-induced lung inflammation, TNFα levels, and squamous metaplasia in animal studies. These studies indicate that each of the provitamin A carotenoids has certain unique beneficial effects against cancer risks. In particular, whether there are great differences between β-carotene and β-cryptoxanthin in their biological activities and whether β-cryptoxanthin is a potentially effective chemopreventive agent against the development of lung cancer need further studies.

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