The Multifunctional Carotenoids: Insights into Their Behavior

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Expanded Abstract

Because it is widely held that damage to DNA by reactive species is a significant contributor to carcinogenesis, it has been postulated that antioxidants in supplements or in foods containing them, which decrease oxidative DNA damage, should have a protective effect against cancer. In antioxidant-cancer risk studies the most common oxidative biomarkers that have been used are: 8-hydroxyguanine in urine, the comet assay in leukocytes, various measures of “total” antioxidant capacity in blood, and serum levels of antioxidant nutrients.

It has been shown in many studies that DNA strand breaks can be reduced by antioxidant supplements or by various combinations of fruits and vegetables that are high in antioxidant nutrient content. It is noteworthy, however, that in a study by Duthie et al., the combination of 100 mg of vitamin C, 280 mg of vitamin E, and 2.5 mg of β-carotene reduced DNA damage, as reflected by the comet assay, only in smokers but not in non-smokers (1). Mixed results have also been seen when urinary 8-hydroxyguanine was used, and observed effects may not be long lasting. The question remains, therefore, do these biomarker assays of oxidative stress have any predictive value with regard to cancer incidence? The simple answer is that we don’t know. Even if we disregard the technical problems of lack of standardization of the assays in various published studies, no prospective epidemiologic or intervention studies have been done in human populations to show that individuals with lower levels of DNA strand breaks have a lower risk of cancer, or indeed that diets or antioxidant supplements can lower the number of DNA strand breaks over the long run (e.g., years). What is known is that antioxidants can alter short-term oxidation status however it is assayed, but we have no idea of the cancerlowering predictive value of these assays.

In this article β-carotene will be used as a model antioxidant in an attempt to answer the question of the clinical significance of diet and changes in oxidative biomarkers with regard to cancer risk. It is now realized that different oxidative effects (antioxidant, pro-oxidant) of β-carotene can be seen, depending on its concentration and other conditions (e.g., the oxygen concentration) (2). Epidemiologic studies have shown an inverse relation between dietary or serum values of β-carotene and the incidence of lung cancer (3). Thus, it was against all expectations that β-carotene given in high doses to smokers resulted in an increased incidence of lung cancer in 2 intervention trials (ATBC, CARET) (4,5). The original interpretation of these trials was that dietary β-carotene in epidemiologic studies was simply acting as a surrogate for some other factor, or that case control studies (the design used in most of the epidemiologic studies on β-carotene) were inherently biased. However, another bother-some explanation was that β-carotene at high dose could be working to promote cancer. It was noted that in the 2 β-carotene intervention trials that resulted in a higher incidence of cancer, the serum β-carotene levels achieved were significantly higher than that in another intervention trial in which there was no increase in lung cancer in response to supplementary β-carotene (Physicians Health Trial) (4–6). These disparate findings could be accounted for by the different concentrations of β-carotene reached in tissue.

Although the β-carotene intervention trial results were shocking at the time, other studies have also shown that supplemental antioxidants can increase the incidence of cancer. For example, it has been reported that vitamin E supplementation in patients with treated head and neck cancers resulted in an increased occurrence of secondary primary cancers during the supplementation period (7). This study included 522 patients with stage 1 head and neck cancers who were treated by radiation. These patients were supplemented with 400 international units of vitamin E and 30 mg of β-carotene or placebo, which began on the first day after radiation therapy, and these treatments were continued for 3 years. During the course of the trial, β-carotene was discontinued after 156 patients had been enrolled in the study. The remaining patients received only vitamin E. After a median follow up of 52 mo, second primary cancers and recurrences of the first tumor were counted. It was found that patients receiving the vitamin E supplements had a higher rate of second primary cancers during the supplementation

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period but reverted to a lower rate after supplementation was discontinued. Thus, in this study, α-tocopherol supplementation produced adverse effects on the occurrence of secondary primary cancers as well as on cancer-free survival. In addition to this study, an animal study by Fiala was recently published entitled “Induction of preneoplastic lung lesions in guinea pigs by cigarette smoke inhalation and their exacerbation by high dietary levels of vitamin C and E” (8). Preneoplastic lung lesions in this study included bronchial hyperplasia, bronchial dysplasia, and squamous metaplasia, which are analogous to the pathologies found in human smokers. The high-dose antioxidant supplements in this study appeared to have cocarcinogenic activity with cigarette smoke for inducing the various lung lesions.

Thus, it appears that low-dose antioxidants might protect against cancer, whereas with high-dose supplementation, there is an induction of more neoplasms. In in-vitro studies, β-carotene quenches singlet oxygen, and singlet oxygen is known to impair or destroy membranes, enzymes, and DNA and lead to the formation of free radicals (9). However, it should be realized that the effect of β-carotene dosage on DNA oxidative damage has not been well studied in vivo. The key question that needs to be answered is whether DNA base damage is related to the dose of antioxidant to which it is exposed. The unexpected results mentioned above using high-dose antioxidants could result from the fact that antioxidants in high dosage can act as pro-oxidants (9). For example, when β-carotene acts as an antioxidant, a resonant, stable, carbon-centered, carotenoid radical is formed (10). However, in the presence of high oxygen tension, oxygenation of this carotenoid radical is possible, which would result in pro-oxidant activity. In this case, the carotenoid radical undergoes auto-oxidation to form eccentrically cleaved β-apocarotenals, which could be indirectly responsible for the higher incidence of lung cancer seen in the 2 intervention trials (ATBC, CARET) (4,5,11).

A study on ferrets showed that high-dose supplementary β-carotene plus smoke can induce squamous metaplasia in the lung (11). Moreover, it was shown that incubation of all-trans-β-carotene with postnuclear fraction from lung tissue of ferrets that were exposed to smoke or not exposed to smoke demonstrated that the formation of carotenoid breakdown products (auto-oxidation) was 3 times higher in lung extracts from ferrets exposed to smoke (11). These data indicate that the free radicals-rich atmosphere in the lungs of cigarette smoke–exposed ferrets leads to conditions that destroy the highly concentrated β-carotene (auto-oxidation) to form an abundance of eccentric metabolites that are structurally similar to retinoids. These eccentric cleavage products were subsequently shown to interfere with the metabolism of retinoic acid and thus with retinoid signaling. Further, Salgo et al. also showed that fractions containing such metabolites facilitated the binding of the smoke-derived carcinogen benzopyrene to DNA, and Poroco et al. (12,13) showed that the induction of cell transformation by benzopyrene was markedly enhanced by the addition of β-carotene. Importantly, subsequent studies using the ferret model showed that low-dose β-carotene in the presence of smoke did not induce lung lesions or the eccentric cleavage products of β-carotene (14). One cannot get away from the message that dose and tissue concentration of the antioxidant does matter as to the clinical outcome.

Part of the problem of assessing whether antioxidants play a chemopreventive role in cancer is that there has not (until recently) been an appropriate assay for measuring true antioxidant capacity or performance in vivo. The assays being used to test total antioxidant capacity in blood (for example, TRAP, ORAC, FRAP)³ have shown almost no contribution of fat-soluble nutrients such as vitamin E and carotenoids (15). This is because these assays are performed in water-soluble systems; thus, the contribution of fat-soluble antioxidants, such as carotenones or vitamin E, would be minimal if any. Recently, an antioxidant capacity assay that uses a lipid-soluble free radical initiator and probe has been designed that is able to reflect total antioxidant capacity of both the lipid and hydrophilic compartments of plasma (16). In such a system, fat-soluble antioxidants (e.g., carotenoids and vitamin E) clearly have been shown to have antioxidant activity; but more importantly, synergistic protective effects between the lipid-soluble antioxidants and water-soluble antioxidants, such as ascorbic acid and polyphenols, have been demonstrated using this system. Such an assay should now be used to correlate with health outcomes (cancer incidence, mortality, etc.) to truly determine the importance of oxidative stress in carcinogenesis and how best to reduce oxidative stress by combinations of low-dose antioxidants.

Future randomized, controlled studies on the clinical significance of diet and changes in oxidative markers should include a standardized assay for serially measuring oxidative damage to DNA as well as true antioxidant capacity. In addition, site-specific cancer incidence should be studied, not just overall cancer incidence, and genetic variations should be documented (for example, genetic variations in the endogenous rates of free radical production and levels of antioxidant defenses). Finally, the other effects of the antioxidants should be considered, such as inhibition of DNA adduct formation and the suppression of P450 enzyme activity, as potentially useful biomarkers (17,18).

### Literature Cited


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