Antioxidants and Lung Cancer Prevention

Xiang-Dong Wang and Robert M. Russell

Jean Mayer United States Department of Agriculture,
Human Nutrition Research Center on Aging at Tufts University, Boston, Mass., USA

Worldwide, tobacco use causes an estimated 3 million deaths per year, and this number is expected to rise to 10 million deaths annually by 2020. Tobacco smoking and exposure to environmental (second-hand) tobacco smoke are the most important risk factors for lung cancer. Lung cancer is the most common type of cancer worldwide and also is currently the leading cause of cancer death in the United States. It is well documented that smoke contains both carcinogen and free radicals which can cause a variety of damage to DNA, including mutation of tumor-suppressor genes. If smoke-induced DNA damage cannot be repaired, this will lead to cell transformation and, ultimately, to lung cancer. The best protection against lung cancer is the avoidance of tobacco smoke. However, the number of current smokers remains alarmingly high, especially in the teenage population. Therefore, nutritional intervention seems to be an appropriate way to rationally and realistically modify cancer risk. This review will focus on resent research on the chemopreventive activity of β-carotene against lung cancer and formulates a hypothesis for using the combination of antioxidant supplementation (β-carotene, vitamin C and vitamin E) in future studies.

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Smoke Contains both Carcinogen and Free Radicals Which Can Cause a Variety of Damage to DNA

How could a lung epithelial cell, under certain risk conditions such as smoke exposure, escape the normal proliferative controls to become a malignant tumor? Lung tumorigenesis is complex, and involves multiple mutations in the genome of the tumor cell that confer a growth advantage [1]. It has been documented that the major mechanism for lung carcinogenesis is via interactions between carcinogens from the cigarette smoke and the genes. Cigarette smoke contains a mixture of chemical carcinogens, such as polycyclic aromatic hydrocarbons and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone which become important carcinogens after metabolic activation. The activated carcinogens bind covalently to DNA, leading to miscoding and gene mutations. If a gene mutation takes place in an oncogene (e.g., Kirsten-ras) or tumor suppressor gene (e.g., p53), lung epithelial cells will escape the normal proliferative controls, and ultimately result in a lung tumor (Fig. 1). At least 20 compounds in cigarette smoke have been identified which induce genetic changes and lung tumors in animal models.

The potential role of free radicals and oxidative damage in tobacco-associated lung carcinogenesis has also been proposed. Cigarette smoke contains free radicals such as quinones and hydroquinone, and induces reactive oxygen species including the hydroxyl radical, the superoxide anion radical, hydrogen peroxide and the nitric oxide radical, etc. Increased circulating products of lipid peroxidation (e.g., F_2-isoprostanes) in smokers provide evidence of oxidative damage by cigarette smoke. The reactive oxygen species can also cause DNA nicking and single-strand breakage in cultured cells. It has been demonstrated that increased levels of miscoding adducts (8-hydroxyguanine and 8-hydroxydeoxyguanosine) occur in DNA from smokers’ lungs and urine. Although there is no direct evidence that oxidative damage is the cause of cigarette smoke-induced lung cancer, it is possible that antioxidants (e.g. β-carotene, vitamin C and vitamin E) could play a chemopreventive role via prevention of oxidative stress.

Controversy Regarding the Chemopreventive Activity of β-Carotene against Lung Cancer

A large body of observational epidemiologic studies has consistently demonstrated that individuals eating more fruits and vegetables, which are rich in antioxidants, and people having higher serum antioxidant levels have a lower risk of lung cancer. The consistency of the results from observational studies suggests that dietary carotenoids, particularly β-carotene, might be the component responsible for the protective effect against lung cancer. Dietary β-carotene and high fruit and vegetable intake has been significantly associated with a reduction in lung cancer risk in both smoking and non-smoking men and women [2]. A num-
Fig. 1. Possible mechanisms by which high-dose β-carotene increased lung cancer risk in smokers, while low-dose β-carotene with combined vitamins C and E may provide some modest protection against lung cancer risk. Adapted from Wang and Russell [4].

A number of animal and laboratory studies has also shown that β-carotene can block the carcinogenic process and inhibit specific tumor cell growth [3]. However, the biochemical and molecular mechanisms underlying the inhibitory effects of lung carcinogenesis by β-carotene are still not understood [4]. Some proposed mechanisms are that β-carotene may: (1) function as an antioxidant, although there is insufficient evidence to conclude that β-carotene is a strong antioxidant; (2) be a precursor of vitamin A and retinoic acid; (3) enhance intercellular gap junction communication; (4) increase immunologic function, and (5) induce carcinogen-metabolizing enzymes that detoxify carcinogens.

In contrast to the results from observational studies, two intervention studies, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study and the Beta-Carotene and Retinol Efficacy Trial (CARET), using high-dose β-carotene supplements in humans have demonstrated an increased risk of lung cancer among smokers. The third trial (the Physicians’ Health Study) reported that there was no significant benefit or harm due to β-carotene supplementation to mostly
non-smokers in terms of malignant neoplasms or death. These studies have been reviewed recently [5]. The failure of these intervention studies to demonstrate a protective effect of supplemental β-carotene could be due to many factors, including interference with the absorption or action of other nutrients in the diet, the wrong duration or dose (too little or too much) of supplementation, or an inappropriate study population, etc. Nevertheless, it is difficult to explain the apparent enhancement of lung carcinogenesis by β-carotene supplementation in smokers, since the ingestion of large amounts of β-carotene apparently did not produce toxic side effects in patients treated chronically with high-dose β-carotene for skin disease. This harmful effect in smokers could be due to (1) β-carotene itself; (2) metabolites of β-carotene, or (3) a different metabolism of β-carotene in the lungs of smokers vs. non-smokers. Recent studies provide useful information on the controversy regarding the chemopreventive activity of β-carotene and will be reviewed below.

**High-Dose β-Carotene Supplementation May Result in the Formation of Large Quantities of Undesirable Oxidative Metabolites in the Free Radical-Rich Atmosphere of Lungs of Cigarette Smokers**

Understanding the molecular details of β-carotene metabolic pathways can yield insights into possible physiological and/or pathophysiological mechanisms. β-Carotene can be cleaved by mammalian tissues mainly at the central double bond (C-15,15′) but also at eccentric double bonds (e.g., C-13′,14′, C-11′,12′, C-9′,10′, and C-7′,8′) to form retinoids and β-apo-carotenoids, which have structures that are similar to retinoids [6]. Since low levels of cleavage products of β-carotene can, in and of themselves, give rise to retinoic acid [7], dietary sources of β-carotene from carotenoid-rich fruits and vegetables could be beneficial and antiproliferative, as evidenced by the results of a large number of epidemiologic studies. However, if high-dose β-carotene supplementation results in the formation of large quantities of undesirable oxidative metabolites in the cell (e.g., due to β-carotene accumulation in the highly oxidative conditions of the lung), they may promote lung carcinogenesis via several possible mechanisms (Fig. 1).

Recently, we carried out an animal study using ferrets exposed to smoke while at the same time receiving high-dose β-carotene supplements (equivalent to 30 mg/day in the human) [8]. Ferrets, an appropriate animal model for studying the absorption and tissue metabolism of β-carotene [9], were subjected to either cigarette smoke exposure (equivalent to smoking 1.5 packs of cigarettes/day in the human), β-carotene supplementation, or both for 6 months. The results showed that, in ferrets, exposure to cigarette smoke decreased β-carotene levels in plasma and in lung tissue, in both the β-carotene-supplemented and unsupplemented animals (Table 1). To assess whether the dramatic decrease in β-carotene was due to the enhanced breakdown of the molecule (especially to β-carotene eccentric cleav-
Table 1. The comparison of concentrations of β-carotene and retinoids in four groups of ferrets after six months of treatment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Smoke-exposed β-Carotene-supplemented</th>
<th>Smoke-exposed and β-carotene-supplemented</th>
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<tbody>
<tr>
<td>Plasma (nmol/l)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>β-Carotene</td>
<td>5 ± 1a</td>
<td>4 ± 1a</td>
<td>109 ± 9c</td>
</tr>
<tr>
<td>Retinol</td>
<td>754 ± 30a</td>
<td>716 ± 22a</td>
<td>805 ± 41a</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>1.36 ± 0.08a</td>
<td>1.23 ± 0.07a</td>
<td>1.43 ± 0.09a</td>
</tr>
<tr>
<td>Lung tissue (pmol/100 mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Carotene</td>
<td>9 ± 0.4a</td>
<td>2 ± 0.1b</td>
<td>2,618 ± 70c</td>
</tr>
<tr>
<td>Retinol</td>
<td>41 ± 3a</td>
<td>37 ± 4a</td>
<td>44 ± 6a</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>1.7 ± 0.3a</td>
<td>ND</td>
<td>0.4 ± 0.1b</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n = 6). ND = Not detected. Values in each line with different superscript letters are significantly different (p < 0.05). Adapted from Wang et al. [8].

age products) by smoke exposure, in vitro incubations of all-trans-β-carotene were performed with the post-nuclear fractions of lung tissue from either smoke-exposed or non-smoke-exposed ferrets. The results showed that the formation of β-apo-14′-, 12′-, 10′-, and 8′-carotenals was 3-fold higher in lung extracts from the smoke-exposed ferrets as compared to the non-smoke-exposed ferrets (Fig. 2) [8]. These data suggest that the free radical-rich atmosphere in the lungs of cigarette smokers can modify β-carotene metabolism in the lung epithelia to form an abundance of oxidative metabolites. However, the question remains as to whether β-carotene itself or its metabolites promote lung carcinogenesis.

**β-Carotene Itself Acts as an Anticarcinogen, but Its Oxidized Products Can Facilitate Carcinogenesis**

Recently several in vitro and in vivo experiments have studied the question of whether β-carotene metabolites can act as cocarcinogenic agents [10–12]. The in vitro experiment reported by Salgo et al. [10] compared the effects of β-carotene and its oxidation products (β-apo-carotenals, β-carotene-5,6-epoxide and 11,12,15,15′-tetrahydro-β-carotene) catalyzed by the microsomal mixed function oxygenase system on the binding of benz[a]pyrene metabolites to calf thymus DNA. The investigators used the hepatic microsomal fractions from either untreated or treated (with Aroclor 1254 and 3-methylcholanthrene) rats. The latter two compounds can cause marked increases in cytochrome P4501A1 and 1A2
activity, the major isoforms associated with benzo[a]pyrene oxidation in human liver and lung tissue. The results showed that intact β-carotene decreased the level of binding of metabolites of benzo[a]pyrene to DNA, whereas β-carotene in its oxidized form and its oxidative metabolites (using fractions of β-carotene oxidation products separated by HPLC) facilitated the binding of benzo[a]pyrene metabolites to DNA. Similarly, an in vivo study by Perocco et al. [11], using BALB/c 3T3 cells, showed that induction of cell transformation by benzo[a]pyrene was markedly enhanced by the presence of β-carotene, although it was not clear whether the enhancement of cell transforming activity of β-carotene was due to β-carotene itself or whether it was due to its metabolites. However, the authors showed that intact β-carotene does not have any cell-transforming activity in BALB/c 3T3 cells. Since high levels of cytochrome P450 enzymes (CYPs) could predispose an individual to an increased cancer risk from bioactivated tobacco-smoke procarcinogens, Paolini et al. [12] carried out a study which showed a significant increase in carcinogen-metabolizing enzymes (CYP1A1/2, CYP2A1, CYP2B1, and CYP3A1/2) in the lungs of rats supplemented with very high doses of β-carotene (500 mg/kg body weight). It is uncertain whether this booster effect of β-carotene on phase-I carcinogen-bioactivating enzymes is due to β-carotene itself or its metabolites; however, it has been reported that β-apo-8′-carotenal, an eccentric cleavage product of β-carotene, but not β-carotene itself, is a strong inducer of CYP4501A1 and 1A2 in rats [13]. It is interesting that the formation of β-apo-8′-carotenal was 2.5-fold higher in lung extracts incubated with β-carotene from our smoke-exposed ferrets as compared to the non-smoke-exposed ferrets (Fig. 2). These studies support the hypothesis that intact β-carotene can act as an anticarcinogen, but that its oxidized products can facilitate carcinogenesis, e.g., by facilitating DNA damage or by inducing carcinogen-metabolizing enzymes (Fig. 1).

**Chronic Smoke Exposure and Excessive β-Carotene Supplementation Result in Lung Cell Proliferation and Squamous Metaplasia**

In our recent ferret study [8], we observed a strong pulmonary proliferative response, which was assessed by examining squamous metaplasia (a precancerous lesion) in animals which were subjected to either β-carotene supplementation, cigarette smoke exposure, or both for 6 months. The results show that smoke

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**Fig. 2.** HPLC analysis of carotenoids in ferret lung post-nuclear fraction (LPNF) after incubation with 10 μM β-carotene. **A** Without LPNF. **B** With normal LPNF. **C** With smoke-exposed LPNF. The results show that the formation of β-apo-14′-, 12′-, 10′-, and 8′-carotenals [14′-NAL (11.5 min), 12′-NAL (16.2 min), 10′-NAL (17.1 min), and 8′-NAL (19.9 min)] were 3-fold higher in lung extracts from the smoke-exposed ferrets as compared to the non-smoke-exposed ferrets. Adapted from Wang et al. [8].
exposure caused a mild aggregation and proliferation of macrophages in the lung tissue of ferrets. However, localized proliferation of alveolar cells (type II pneumocytes) and alveolar macrophages, and keratinized squamous epithelium were observed in all ferrets in the high-dose β-carotene-supplemented group. Furthermore, severe focal proliferation of alveolar cells, squamous metaplasia and destruction of alveolar walls were observed in all smoke-exposed ferrets which were given high-dose β-carotene supplementation. To assess a potential increase in cell proliferation in the whole lung, we analyzed proliferating cellular nuclear antigen (PCNA) expression from the four groups of ferrets. PCNA expression increased 4-fold or 2-fold (Fig. 3) in the lungs of β-carotene-supplemented ferrets with or without smoke exposure, respectively. These observations support the hypothesis that high-dose β-carotene either alone or with additional smoke expo-

Fig. 3. Effect of high-dose β-carotene (BC) feeding, smoke (SM) exposure or the combination of both on PCNA expression. Upper panel: Densitometry analysis, n = 6/each group. Lower panel: Representative Western blot analysis by using anti-PCNA antibody. Lane 1 = Control; lane 2 = smoke-exposed; lane 3 = high-dose β-carotene feeding, and lane 4 = smoke-exposed with high-dose β-carotene feeding. The size of the detected PCNA was 36 kD. Adapted from Wang et al. [8].
Fig. 4. Effect of β-carotene (BC) feeding, smoke (SM) exposure, or the combination on AP-1 (c-Jun and c-Fos) expression in the lungs of ferrets. Upper panel: Densitometry analysis, n = 6/each group. Lower panel: Representative Western blot analysis using either anti-c-Jun or anti-c-Fos antibody. The sizes of the detected c-Jun and c-Fos were 39 and 62 kD, respectively. Lane 1 = Control; lane 2 = smoke-exposed; lane 3 = high-dose β-carotene supplementation, and lane 4 = smoke-exposed with additional high-dose β-carotene supplementation. Adapted from Wang et al. [8].

sure alters retinoid signaling and causes proliferation of alveolar cells and squamous metaplasia (a precancerous lesion). To study further the mechanisms involved, we tested if protooncogene (c-Jun and c-Fos) gene expression in ferret lung tissue was altered either by smoking, high-dose β-carotene supplementation, or both together, by using Western blot analysis (Fig. 4). The products of the two protooncogenes, c-Fos and c-Jun, form a complex in the nucleus, termed AP-1 that binds to a DNA sequence motif that is referred to as the AP-1 response element (AP-1 RE). By binding to this sequence, AP-1 mediates signals from growth factors, inflammatory peptides, oncogenes, tumor promoters and oxidative stress, usually resulting in cell proliferation. In our ferret study, both c-Jun and c-Fos protooncogene expression were up-regulated 3- to 4-fold in the smoke-
exposed ferrets that were fed β-carotene as compared with the control animals (Fig. 4). Recently, it has been reported that c-Jun is required for progression through the G1 phase of the cell cycle by a mechanism that involves direct transcriptional control of the cyclin D1 gene [14]. It is conceivable that the overexpression of c-Jun by chronic smoke exposure and excess β-carotene supplementation cause abnormal cell cycle regulation to drive cells into a premature S phase (PCNA; Fig. 3), resulting in cell proliferation and squamous metaplasia.

Vitamin A deficiency is known to result in replacement of the mucociliary epithelium with keratinized squamous epithelium in the tracheobronchial mucosa and has been associated with enhanced lung carcinogenesis. In our recent study [8], we also examined retinoic acid level (Table 1). The results showed that the concentration of retinoic acid in lung tissue was significantly lower in all three treatment groups (smoke, β-carotene, β-carotene plus smoke) after 6 months, as compared with the control group (Table 1). This decreased retinoic acid concentration could be due to increased catabolism of retinoic acid into more polar metabolites by a cytochrome P450-dependent process (Fig. 1). It is known that smoke is a strong inducer of cytochrome P450 enzymes in lung tissue. Also as mentioned above, two groups of investigators have shown that an induction of cytochrome P450 enzymes (CYP1A1/2, CYP2A1, CYP2B1, and CYP3A1/2) can occur with high-dose β-carotene supplementation. Although there is little known about the specific enzymes that are induced by either smoke exposure or high-dose β-carotene and whether they could be responsible for increased retinoic acid catabolism, it is possible that, after prolonged β-carotene supplementation, the free radical-rich atmosphere in the lungs of cigarette smokers or non-smokers increases β-carotene breakdown to form oxidative metabolites which could interfere with retinoid signal transduction, thus causing malignant transformation. To confirm our hypothesis, we examined retinoic acid receptors (RARs) gene expression in our ferrets, and showed that RARβ gene expression (which may function as a tumor suppressor), but not RARα, was downregulated between 18 and 73% in the three active treatment groups, compared with the control group (Fig. 5). Our result supports a role for RARβ as a tumor-suppressor gene, which has already been proposed by several investigators [15, 16], although the molecular mechanism through which RARβ expression is lost is uncertain. Recent in situ hybridization studies confirm that up to 50% of primary lung tumors lack RARβ expression and that loss of expression is an early event in lung carcinogenesis [17]. Thus, loss of tumor-suppressor function of RARβ, by deletion, mutation or transcriptional repression, may lead to enhanced cell proliferation and potentially to tumor formation.

In summary, we hypothesize that one of the biologic bases for the harmful effects of high-dose β-carotene supplements in smokers is the free radical-rich atmosphere in the lungs of cigarette smokers. This environment alters β-carotene metabolism, and produces oxidative metabolites which accelerate malignant transformation by downregulating the RARβ gene and upregulating AP-1 (c-Jun
Fig. 5. Effect of β-carotene (BC) feeding, smoke (SM) exposure, or the combination on RARs (RARα and RARβ) gene expression in the lungs of ferrets. Upper panel: Densitometry analysis, n = 6/each group. Lower panel: Representative Western blot analysis using either anti-RARα or RARβ antibody. The size of the detected RARα and RARβ was 53 kD. Lane 1 = Control; lane 2 = smoke-exposed; lane 3 = high-dose β-carotene supplementation, and lane 4 = smoke-exposed with additional high-dose β-carotene supplementation. Adapted from data from Wang et al. [8].

and c-Fos) activity. Conversely, if low levels of eccentric cleavage products are produced in the cell, as would be the case when one eats dietary levels of β-carotene or with low-dose β-carotene supplementation, this could be beneficial and antiproliferative, since low levels of eccentric cleavage products can, in and of themselves, give rise to retinoic acid. Very recently, Lowe et al. [18] demonstrated that intact β-carotene protects against oxidative DNA damage (induced by xanthine/xanthine oxidase) in HT29 cells at relatively low concentrations (1–3 μM), but rapidly loses this capacity at higher doses (4–10 μM).
Antioxidants and Lung Cancer Prevention

**Adequate Amounts of Vitamin C and Vitamin E Must Be Present to Prevent β-Carotene from Being Oxidized in Order That β-Carotene Has a Chemopreventive Effect**

Although the mechanism of enhanced β-carotene oxidation or instability of the β-carotene molecule in the lungs of cigarette-exposed animals is not clear, a possible mechanism is that exposure to smoke in lung cells results in decreased levels of other lung tissue antioxidants, such as ascorbate and α-tocopherol. Recently, Bohm et al. [19, 20] raised the possibility that oxidized vitamin E could be recycled by β-carotene and oxidized vitamin E could be recycled by vitamin C, and reported that a combination of β-carotene, α-tocopherol and vitamin C provides synergistic protection against free radical damage in an in vitro cell system. These investigators observed that the combination of β-carotene and vitamin E offered additive protection against cell damage. However, in the presence of ascorbic acid, they observed a synergistic rather than an additive effect, which can be explained by an electron transfer reaction in which β-carotene radicals are repaired by vitamin C to maintain β-carotene in its unoxidized form [21]. These studies support the hypothesis that the carcinogenic response to high-dose β-carotene supplementation reported in the human intervention trials is related to the instability of the β-carotene molecule in the free radical-rich environment of the lungs of cigarette smokers and to the lower levels of other antioxidants (vitamin C, vitamin E) in the lungs of smokers. Therefore, investigations on the effectiveness of a combination of antioxidants (β-carotene, vitamin C and α-tocopherol) as an effective chemopreventive strategy against lung cancer should be initiated.

Vitamin C is a strong reducing agent which is known to act as a major circulating water-soluble antioxidant, both in vitro and in vivo. Vitamin C protects lipids in human plasma against peroxidative damage by scavenging oxygen-derived free radicals in both smokers and non-smokers. However, smokers have significantly lower plasma levels of vitamin C compared with non-smokers. Even non-smokers exposed to passive smoke have reduced ascorbic acid concentrations in their plasma. This may help to explain why diets high in fruit and vegetables, and hence high in vitamin C, have been found to be associated with a lower risk for cancers of the lung, oral cavity, esophagus, stomach, and colon [22]. It is also known that vitamin C can regenerate vitamin E from the vitamin E radical during lipid peroxidation, and that the toxicity of vitamin C and vitamin E is very low.

Vitamin E is a major lipid-soluble antioxidant. α-Tocopherol (the most active form of vitamin E) is the major peroxyl radical scavenger in biological lipid phases such as membranes or low-density lipoproteins. Several lines of evidence also demonstrate that interactions occur between β-carotene and tocopherols. β-Carotene, or its metabolites, can exhibit prooxidant properties [23], which may depend on the high oxygen tensions and/or a high dose of β-carotene. Although one recent report [24] indicates that a prooxidant effect of β-carotene is unlikely in biologically relevant conditions, there is an interaction between tocopherols
Antioxidants and Lung Cancer Prevention

and β-carotene with respect to pro- or antioxidant effects: in vitro in the absence of tocopherols or in vivo under conditions of vitamin E deficiency, β-carotene appears to act as a prooxidant. Since the prooxidant effect of β-carotene may be attributed to its oxidative degradation products, tocopherol may limit the prooxidant effects of carotenoids in biologic systems by protecting carotenoids from oxidation. Conversely, β-carotene may be capable of regenerating α-tocopherol from its radical. Pretreatment of human lung cells with vitamin E and β-carotene together has been shown to provide significant protection against DNA strand breakage induced by tobacco-specific nitrosamines [25]. In this regard, it should be mentioned that a trial in Linxian County, China, conducted in about 30,000 men and women showed that, after an intervention period of 5 years, those given a combination of β-carotene (15 mg/day), vitamin E, and selenium had a 13% reduction in cancer deaths [26]. We had shown previously that α-tocopherol can protect β-carotene from oxidation and can enhance vitamin A formation in vivo [27].

Recently, the possible protective effects of combined antioxidant supplements in humans exposed to environmental tobacco smoke have been reported [28–30]. Howard et al. [29] demonstrated that the increased oxidative stress induced by environmental tobacco smoke can be reduced in humans by supplementation of antioxidant vitamins and trace minerals (3 mg of β-carotene, 60 mg of vitamin C, 30 IU of α-tocopherol, 40 mg of zinc, 40 μg of selenium, and 2 mg of copper) in humans. Duthie et al. [30] carried out a double-blind antioxidant supplementation study with vitamin C (100 mg/day), vitamin E (280 mg/day), and β-carotene (25 mg/day) in 50 smokers and 50 non-smokers for 20 weeks. These investigators reported that the supplementation of the combined antioxidants resulted in a significant (p < 0.002) decrease in endogenous oxidative base damage in lymphocyte DNA of both smokers and non-smokers. These findings, therefore, support the role of combination of antioxidants (β-carotene, vitamin E and vitamin C) against human lung cancer.

**Conclusion**

Studies suggest that the carcinogenic response to high-dose β-carotene supplementation reported in the human intervention trials is related to the instability of the β-carotene molecule in the lungs of cigarette smokers which is a free radical-rich but antioxidant-poor environment. The presentation of high doses of β-carotene via supplements to the highly oxidative environment of the lung will result in increased levels of oxidative metabolites of β-carotene. The increased β-carotene oxidative metabolites may promote carcinogenesis by: (1) inducing carcinogen-bioactivating enzymes and bioactivating tobacco smoke procarcinogens; (2) facilitating the binding of metabolites of benzo[a]pyrene to DNA; (3) enhancing retinoic acid catabolism and downregulating RARβ, and (4) acting as a prooxidant, causing damage to DNA. Since the stability of β-carotene is dependent on other
Antioxidants and Lung Cancer Prevention

antioxidants, particularly vitamin C and vitamin E, investigations on the effectiveness of a combination of antioxidants (β-carotene, vitamin C and α-tocopherol) as an effective chemopreventive strategy against lung cancer should be undertaken.

References


Discussion

Dr. Albanes: As you probably are aware, we observed an interaction between alcohol and the beta-carotene in the Finland trial, such that the beta-carotene effect was more harmful among the men who had claimed to consume larger amounts of alcohol. Based on your data and your familiarity with the systems, do you see any possible mechanisms by which this interaction may have occurred?

Dr. Wang: We are currently doing another study using a rat model to investigate this. We are keeping the rats on a liquid, alcohol-supplemented diet, and examining the retinoic acid concentration and also the retinoic acid receptor and AP-1 protein expression. We have found that the retinoic acid concentrations are very low after the rats have received the alcohol diet for 1 month, which is similar to what we have observed in the smoke-exposed ferret with high dose beta-carotene supplementation. We have also found that the retinoic acid signaling, such as retinoic acid receptor-DNA binding activity, is decreased by alcohol treatment. Right now the problem is that we have been unsuccessful in developing this model in the ferret because rats are not a good animal model to use for studying the interaction of beta-carotene and alcohol. We have tried giving the ferrets an alcoholic drink to take with a solid diet, but they pass out straight away. What we are doing right now is to establish the ferret model using different doses of alcohol.

Dr. Mason: This is a question more for Dr. Albanes than for Dr. Wang. Dr. Albanes, given these results and the fact that this might be a dose-related effect, is it feasible to do a subanalysis of your ATBC trial, and look at those individuals who were not particularly compliant over 10 years – those who perhaps only took their beta-carotene supplements once a week or whatever – and to see whether there was any protective effect, given the nature of these observations?

Dr. Albanes: We had excellent compliance overall, so we had relatively few people who only occasionally took pills. We did not see any relation, though, between the level of compliance and the beta-carotene effect on lung cancer. We did another analysis in which the blood levels were stratified by tertiles – in other words, from the lowest in the beta-carotene group to the very highest. Unfortunately all the levels were exceedingly high and we didn’t see much of a dose-response. There was a suggestion that the men who had the very highest blood levels within the beta-carotene group had a slight reduction in risk, but these levels were exceedingly high, even compared with the models being described. So I don’t think we have more information to clarify the issue of the dose-response observed here.
Dr. Heimburger: One of my colleagues has just submitted a paper in which she has found accumulation of vitamin C in the lung tissues of smokers. Of course it’s well known that the diets of smokers are lower in vitamin C, there are the interactive oxidation effects, and the blood levels of vitamin C are lower in smokers, so we were surprised to find an accumulation, in fact a really marked increase, in vitamin C in the lung tissues. These were resected samples of lung from people who had lung cancer. Have you observed that, or are there reports of other nutrients in lung tissue?

Dr. Wang: Vitamin C levels are usually decreasing in plasma, both in smokers and even in non-smokers exposed to environmental smoke. We didn’t analyze vitamin C concentration in the lung tissues when we conducted the study. Right now, we are studying possible protective effects of a combined antioxidant (vitamin C, vitamin E and β-carotene) supplementation in the smoke-exposed ferrets. We are going to examine the levels of these antioxidants in the lung tissues.

Dr. Riboli: In the American Journal of Epidemiology we report a prospective study in New York where blood was collected from 14,000 women, who were then followed up for 10 years. We measured β-carotenoids and compared the results between women who later developed breast cancer and those who did not develop breast cancer. We found that women who developed breast cancer had prediagnosis levels of β-carotene, α-carotene, and β-hydroxyxanthine that were significantly lower than in the women who did not develop breast cancer. Our interpretation was not that high levels of β-carotene protect against breast cancer but rather that very low levels of β-carotene or carotenoids may be indicators of a high risk of breast cancer by virtue of the fact that they are biomarkers of a very low intake of vegetables. The relative risk was in the order of 2 for the lowest quartile of intake. I would strongly insist that we do not interpret this result as meaning that by giving high doses of β-carotene one could protect women against breast cancer.

Dr. Go: In examining β-carotene metabolism you focused on the phase I cytochrome P450-metabolizing enzyme pathway. I wonder why you did not look at the phase-II metabolizing enzyme, given that the pathway going through the liver, the glutathione pathway, could probably handle a lot of those metabolites that you’re interested in. In essence, what I’m driving at is that if the human body has two metabolizing enzymes we should not be seeing the effect you have presented to us today.

Dr. Wang: Our data and those from other groups suggest that the phase-I enzymes are involved. At present we do not know what happens to the phase-II enzymes. We believe phase I enzymes are the principal enzymes involved, but it’s a good question, maybe we should look at phase II enzymes also.

Dr. Bloch: In the slides you presented, the high doses of β-carotene seem to be more risk-producing than the smoke. Is smoking enhancing the effect of the β-carotene? We keep emphasizing smoking, but is it the smoking in combination with the β-carotene that’s really the risk factor?

Dr. Wang: We showed that there was an increase in metabolites of β-carotene when we incubated β-carotene with smoke-involved tissue. These increased β-carotene by-products may promote tobacco smoke-induced carcinogenesis, especially given high dose of β-carotene supplementation. We think that the instability of the β-carotene is the big problem in the smoker, because smoke also causes decreased tissue levels of other antioxidants which may protect β-carotene from being oxidized.