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Procarcinogenic and Anticarcinogenic Effects of ß-Carotene

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A large body of observational epidemiologic studies has consistently demonstrated that individuals who eat more fruits and vegetables, which are rich in carotenoids, and people who have higher serum ß-carotene levels have a lower risk of cancer, particularly lung cancer. In contrast to these observations, two human intervention studies that used high-dose ß-carotene supplements reported an increased risk for lung cancer among smokers. Recently, in vitro and in vivo studies have shed light on the present conundrum regarding the potential chemopreventive activity of ß-carotene; that is, ß-carotene itself may act as an anticarcinogen, but its oxidized products may facilitate carcinogenesis. These studies support the hypothesis that the carcinogenic response to high-dose ß-carotene supplementation reported in the human intervention trials is related to the instability of the ß-carotene molecule in the free radical–rich environment in the lungs of cigarette smokers. This is especially possible because smoke also causes decreased tissue levels of other antioxidants, such as ascorbate and α-tocopherol, which normally have a stabilizing effect on the unoxidized form of ß-carotene. Nutritional intervention using a combination of antioxidants (ß-carotene, α-tocopherol, and vitamin C) as anticarcinogenic agents could be an appropriate way to rationally and realistically reduce cancer risk.

Introduction

Tobacco use in the United States is responsible for more than 450,000 total deaths and 170,000 cancer deaths every year. Smoking is the single most important risk factor for lung cancer, the most common type of cancer worldwide and currently the leading cause of cancer death in the United States. It is well documented that smoking can damage DNA, including mutations of the *p53* tumor suppressor gene and down-regulation of retinoic acid receptor ß (RARß). Although the best protection against lung cancer is the avoidance of tobacco smoke, the number of current smokers remains alarmingly high (approximately 25% of the U.S. population), especially among teenagers. Nutritional intervention could be an appropriate way to rationally and realistically modify cancer risk.

Several observational epidemiologic studies have consistently demonstrated that individuals who eat more fruits and vegetables, which are rich in carotenoids, and people who have higher serum ß-carotene levels have a lower risk of cancer.[1,2] The consistency of the results from observational studies is particularly strong for lung cancer.[3] Dietary ß-carotene and high fruits and vegetable intake has been significantly associated with a reduction in lung cancer risk in both smoking and nonsmoking men and women.[2,3] Animal and laboratory studies have also shown that ß-carotene can block the carcinogenic process and inhibit specific tumor cell growth.[4,5] However, the mechanisms of action of ß-carotene in inhibiting lung carcinogenesis are still unknown. Some proposed mechanisms are that ß-carotene may function as an antioxidant,[6] be a precursor to retinoic acid,[7–9] enhance gap junction communication,[10,11] increase immunologic function,[12–14] and induce carcinogen-metabolizing enzymes that detoxify carcinogens.[15,16]

In contrast to the results from observational studies, two intervention studies, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC)[17,18] and the Beta Carotene and Retinol Efficacy Trial (CARET),[19–21] which used high-dose ß-carotene supplements in humans, demonstrated an increased risk of lung cancer among smokers. The ATBC trial, a Finnish randomized, double-blind, placebo-controlled study that used daily supplementation of a-tocopherol (50 mg), ß-carotene (20 mg), or both in 29,133 smokers, observed a significant 16% excess incidence of lung cancer among participants who received the ß-carotene supplements for an average of 6 years (with a range of 5–8 years). CARET yielded similar results. CARET studied 18,314 men and women, 60% of whom were current smokers and 39% of whom were former smokers, including 22% asbestos-exposed smokers. Daily doses

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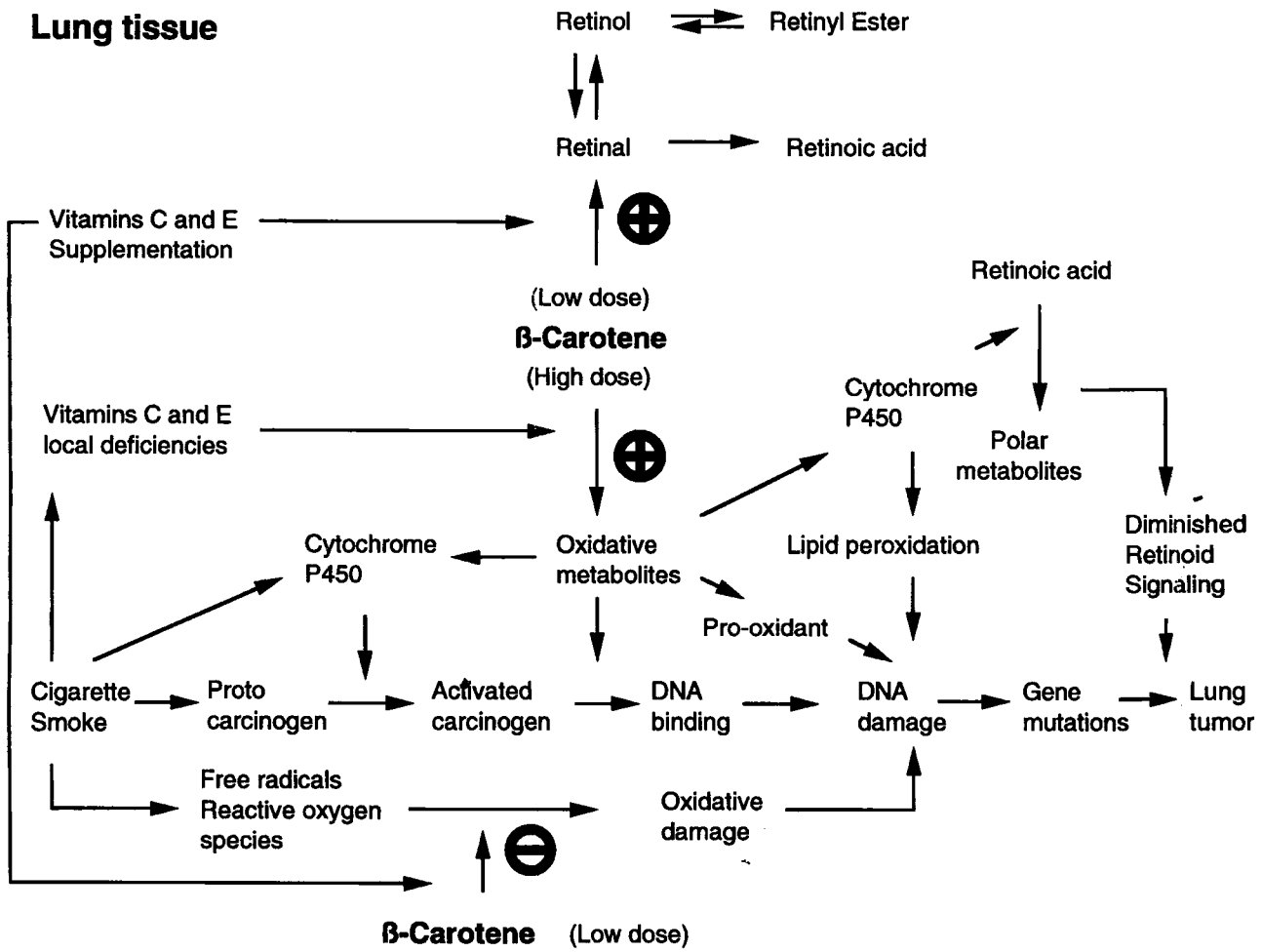

Figure 1. Possible mechanisms by which high-dose ß-carotene increased lung cancer risk in smokers, whereas low-dose ß-carotene with combined vitamins C and E may provide modest protection against lung cancer risk.

of ß-carotene (30 mg) and retinyl palmitate (25,000 IU), or a placebo, were given for approximately 4 years of intervention. The CARET active intervention was stopped 21 months early because of clear evidence of no benefit and evidence of possible harm: there were 23% more lung cancers and 17% more deaths in the active intervention group. A third trial, the Physicians' Health Study by Henneken et al.,[22] involved 22,071 male physicians (only 11% of whom were current smokers, although 39% were former smokers at the beginning of the study, in 1982) who took ß-carotene (50 mg) or a placebo every other day for 12 years. In this group of subjects, there was no significant benefit or harm owing to ß-carotene supplementation in terms of malignant neoplasms or death.

The failure of these intervention studies to demonstrate a protective effect of supplemental ß-carotene could be explained by many factors, including interference with the absorption or action of other nutrients in the diet, the wrong duration or dose of supplementation (too little or too much), or an inappropriate population. Nevertheless, it is difficult to explain the apparent exacerbation of lung carcinogenesis by ß-carotene supplementation in smokers. This harmful effect in smokers could be due to ß-carotene itself, metabolites of ß-carotene, or a different metabolism of ß-carotene in the lungs of smokers versus nonsmokers. The mechanism(s) of the harmful effect of ß-carotene is even more difficult to understand because the ingestion of large amounts of ß-carotene apparently did not produce toxic side effects in patients treated chronically with high-dose ß-carotene for skin disease.[23,24]

Lung tumorigenesis is complex and involves multiple mutations in the genome of the tumor cell that confer a growth advantage (Figure 1). How could a lung epithelial cell under certain risk conditions, such as chronic high-dose ß-carotene and smoke exposure, escape the normal proliferative controls and become a malignant tumor? It is possible that chronic high doses of ß-carotene alter normal ß-carotene (or other nutrient) metabolism and result in a significant increase in the formation of its oxidized form or its oxidative metabolites, which damage DNA directly and/or provide a promoting environment for carcinogenesis. Recently, several reports from both in vitro and in vivo studies have provided useful information regarding the potential chemopreventive activity of ß-carotene and a mechanistic understanding of what might have gone wrong in the ß-carotene trials.

The Free Radical–rich Atmosphere in the Lungs of Cigarette Smokers Can Modify ß-Carotene Metabolism in the Lung Epithelium to Form an Abundance of Oxidative Metabolites

Understanding the molecular details of ß-carotene's metabolic pathways can yield insight into possible physiologic and/or pathophysiologic mechanisms. ß-Carotene can be cleaved by mammalian tissues, mainly at the central double bond (C-15,15′), but also at excentric double bonds (e.g., C-13′,14′; C-11′,12′; C-9′,10′; and C-7′,8′) to form retinoids and ß-apo-carotenoids, which have structures similar to those of retinoids.[25] Because low levels of products of ß-carotene cleavage can, in and of themselves, give rise to retinoic acid,[7–9] dietary sources of ß-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 ß-carotene supplementation results in the formation of large quantities of undesirable oxidative metabolites in the cell (e.g., owing to high ß-carotene concentrations in the highly oxidative conditions of the lung), they may promote lung carcinogenesis via several possible mechanisms (Figure 1).

In a recent study in ferrets,[26] exposure to cigarette smoke decreased ß-carotene levels in plasma and lung tissue in both the ß-carotene supplemented and unsupplemented animals (Table 1). To assess whether the dramatic decrease of ß-carotene was due to the enhanced breakdown of the ß-carotene molecule (especially ß-carotene excentric cleavage products) by smoke exposure, in vitro incubations of all-*trans*-ß-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 ß-apo-14'-, 12'-, 10'-, and 8'-carotenals were threefold higher in lung extracts from the smoke-exposed ferrets than those in those from the non-smoke-exposed ferrets.[26] These data suggest that the free radical–rich atmosphere in the lungs of cigarette smokers can modify ß-carotene metabolism in the lung epithelia to form an abundance of oxidative metabolites.

Although the mechanism of enhanced ß-carotene oxidation or instability of the ß-carotene molecule in the lungs of animals exposed to cigarettes is not clear, a possible mechanism is that exposure of lung cells to smoke results in decreased levels of other lung tissue antioxidants, such as ascorbate and α-tocopherol. Vitamin C and vitamin E normally have a stabilizing effect on the unoxidized form of ß-carotene.[27,28] We had previously shown that α-tocopherol can protect ß-carotene from oxidation and enhance vitamin A formation in vivo.[29] To examine the effects of smoke on ß-carotene metabolism in the presence of vitamin E, we conducted in vitro incubations of all-*trans*-ß-carotene in the presence or absence of vitamin E with the postnuclear fraction of lung tissue from either smoke-exposed or non-smoke-exposed ferrets (Liu C, Wang X-D, Russell RM, unpublished data, November 1998) (Table 2). The results from this in vitro incubation study showed that the formation of ß-carotene oxidative metabolites (ß-apo-carotenals and ß-carotene-5,6-epoxide) increased two- to fivefold in the smoke-exposed ferrets versus the non-smoke-exposed ferrets (Table 2). However, ß-carotene oxidation by smoke exposure decreased 42–70% when α-tocopherol was added the incubation mixture. Thus, these data indicate that smoke exposure alone enhances the oxidative cleavage of ß-carotene in ferret lung tissue, which produces increased amounts of ß-carotene oxidative metabolites. However, the oxidation of ß-carotene by smoke can be inhibited by the presence of α-tocopherol.

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

Very recently, several in vitro and in vivo experiments have studied the question of whether ß-carotene metabolites can act as cocarcinogenic agents.[30–32] The in vitro experi-

Table 1. Concentrations of ß-Carotene and Retinoids in Four Groups of Ferrets After 6 Months of Treatment[a]

	Control Group	Smoke-exposed Group	ß-Carotene-supplemented Group[b]	Smoke-exposed and ß-Carotene-supplemented Group[b]
ß-Carotene				
Plasma (nmol/L)	5 ± 2*	4 ± 2*	109 ± 21**	40 ± 12***
Lung (pmol/100 mg)	9 ± 1*	Trace	2618 ± 171**	171 ± 22***
Retinol				
Plasma (nmol/L)	754 ± 73	716 ± 54	805 ± 99	749 ± 53
Lung (pmol/100 mg)	41 ± 7	37 ± 10	44 ± 14	38 ± 5
Retinoic acid				
Plasma (nmol/L)	1.36 ± 0.19	1.23 ± 0.17	1.43 ± 0.22	1.25 ± 0.15
Lung (pmol/100 mg)	1.70 ± 0.7*	ND	0.4 ± 0.2**	ND

Source: Adapted from data from reference 26.

[a]Values are means ± standard deviations ($n = 6$). ND = not detected. Different asterisks indicate that values are significantly different from other groups ($P<0.05$).

[b]ß-Carotene was given in an amount equivalent to 30 mg/day in humans.

Table 2. Appearance of Excentric Cleavage Products After Incubation of 10 μM ß-Carotene with the Postnuclear Fraction of Lung Tissue in Non-Smoke-exposed and Smoke-exposed Ferrets in the Absence or Presence of α-Tocopherol (10 μM)[a]

Incubation	ß-Carotene Recovered	ß-Apo-Carotenals 14′-	12′-	10′-	8′-	ß-Carotene-5,6,-Epoxide
Normal lung						
ß-Carotene	7736	36	120	82	23	32
ß-Carotene plus α-tocopherol	7812	31	96	72	0	22
Smoke-exposed lung						
ß-Carotene	5472	85	410	186	51	140
ß-Carotene plus α-tocopherol	6051	62	289	124	21	58

[a]Data represent an average of two to three separate experiments. All units are given in pmol/mL incubation mixture.

ment with calf thymus DNA reported by Salgo et al.[30] compared the effects of ß-carotene and its oxidation products (ß-apo-carotenals, ß-carotene-5,6-epoxide, and 11,12,15,15′-tetrahedron-ß-carotene) on the binding of benzo[a]pyrene metabolites. Benzo[a]pyrene is an important smoke-borne carcinogen. The activation of benzo[a]pyrene is mainly conversion to its 7,8-diol-9,10-epoxide, which is highly carcinogenic and reacts with DNA to form adducts. The investigators used the hepatic microsomal fractions from rats that were either treated with Aroclor 1254 and 3-methylcholanthrene or untreated. These two compounds can cause marked increases in cytochrome P4501A1 and P4501A2 activity, the major isoforms associated with benzo[a]pyrene oxidation in human liver and lung tissue. The results showed that intact ß-carotene decreased the level of binding of metabolites of benzo[a]pyrene to DNA, whereas ß-carotene in its oxidized form and its oxidative metabolites, using fractions of ß-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.,[31] which used BALB/c 3T3 cells, showed that induction of cell transformation by benzo[a]pyrene was markedly enhanced by the presence of ß-carotene, although it was not clear that the enhancement of cell-transforming activity of ß-carotene was due to ß-carotene itself or metabolites. The authors demonstrated, however, that ß-carotene itself does not have any cell-transforming activity in BALB/c 3T3 cells. Because high levels of cytochrome P450 enzymes (CYPs) could predispose an individual to an increased risk of cancer from bioactivated tobacco smoke procarcinogens, Paolini et al.[32] carried out a study that 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 ß-carotene (500 mg/kg body weight). It is uncertain whether this booster effect of ß-carotene on phase I carcinogen-bioactivating enzymes is due to ß-carotene itself or its metabolites; however, it has been reported by one investigation that ß-apo-8′-carotenal, an excentric cleavage product of ß-carotene, but not ß-carotene itself, is a strong inducer of CYP4501A1 and CYP4501A2 in rats.[33] Interestingly, the formation of ß-apo-8′-carotenal was 2.5-fold higher in lung extracts incubated with ß-carotene from smoke-exposed ferrets than in those from non-smoke-exposed ferrets.[26]

In general, these studies support the hypothesis that intact ß-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 (Figure 1).

Cigarette Smoke, Oxidative Metabolites of ß-Carotene, or Both May Enhance Retinoic Acid Catabolism in Lung Tissue

Because little information is available regarding the possible effect of ß-carotene supplementation on retinoid signal transduction pathways in smokers, we carried out an animal study in which ferrets were exposed to smoke and simultaneously received high-dose ß-carotene supplements that were equivalent to 30 mg/day in humans.[26] Ferrets, an appropriate animal model for studying the absorption and tissue metabolism of ß-carotene, were subjected to either cigarette smoke exposure, ß-carotene supplementation, or both for 6 months. The results (Table 1) showed that the concentration of ß-carotene in lung tissue (171 pmol/100 mg) from the smoke-exposed and ß-carotene-supplemented group of ferrets was comparable to the ß-carotene level in the lung (76 pmol/100 mg) of two active participants from CARET intervention study.[34] However, the concentration of retinoic acid in lung tissue was significantly lower in all three treatment groups after 6 months, 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. It is known that smoke strongly induces cytochrome P450 enzymes in lung tissue.[35,36]

As mentioned above, two groups of investigators[32,33] showed that induction of cytochrome P450 enzymes (CYP1A1/2, CYP2A1, CYP2B1, and CYP3A1/2) can occur

with high-dose ß-carotene supplementation. Little is known, however, about the specific enzymes that are induced by either smoke exposure or high-dose ß-carotene and whether they could be responsible for increased retinoic acid catabolism. Because retinoic acid 4-hydroxylase is a cytochrome P450 enzyme that is induced by retinoic acid,[37,38] and because the chemical structure of some ß-carotene oxidative metabolites are structurally similar to retinoic acid, it is possible that retinoic acid 4-hydroxylase can be induced by ß-carotene oxidative metabolites. This enzyme was not induced, however, in the lungs or livers of ferrets after treatment with ß-carotene, smoke, or a combination of both for 6 months (Wang X-D, Sonneveld E, Liu C, et al., unpublished data, March 1998). Thus, the role of cytochrome P450 enzymes in the catabolism of retinoic acid in lung tissue, after either smoke exposure or high-dose ß-carotene supplementation, needs further investigation.

High-Dose ß-Carotene Exposure and Its Metabolites Could Potentially Interfere with Retinoid Signal Transduction to Cause Lung Cell Proliferation and Carcinogenesis

It is possible that after prolonged ß-carotene supplementation, the free radical–rich atmosphere in the lungs of cigarette smokers or nonsmokers increases ß-carotene breakdown to form oxidative metabolites that could interfere with retinoid signal transduction, thus causing malignant transformation. The background for this hypothesis is based on the following information.

First, retinoids play an important role in the control of cellular proliferation and differentiation in lung epithelial cells.[39] In vivo, vitamin A deficiency results in a replacement of the mucociliary epithelium with keratinized squamous epithelium in the tracheobronchial mucosa. Moreover, squamous metaplasia is reversed if vitamin A is restored to the diet.[40] Retinoic acid derived from vitamin A acts on normal bronchial epithelium by inducing mucus and blocking squamous differentiation.[39,41] Because squamous metaplasia occurs during the early stages of lung carcinogenesis, it is possible that perturbations in retinoid signaling contribute to lung carcinogenesis.[42,43]

Second, retinoid signaling occurs through nuclear receptors, which appear to act as transcription factors.[44] Both retinoic acid receptors (RAR) and retinoid X receptors (RXR) are members of the nuclear hormone receptor superfamily of sequence-specific, ligand-activated transcription factors. Retinoid receptors regulate gene expression by binding as dimeric complexes to specific DNA sites known as retinoic acid response elements (RARE), which are located in the 5' promoter region of susceptible genes, thereby transcriptionally activating a series of genes with distinct antiproliferative activity. Several lines of evidence suggest that RARß plays an important role in normal lung development; e.g., it is highly expressed in adult lung tissue and upper airway epithelium of developing mice.[45,46] In vitro, one of the earliest events to occur during airway epithelial cell exposure to retinoic acid is the induction of RARß mRNA.[47]

Third, a role for RARß as a tumor suppressor gene has been proposed.[48] RARß is abnormally expressed in human lung cancer cell lines and a number of primary tumors.[46–49] Most RARß expression abnormalities involve reduced or absent expression of the RARß2 isoform, the most abundant isoform in normal human lung tissue.[46,47,50] Recent in situ hybridization studies confirm that up to 50% of primary lung tumors lack RARß expression and that loss of expression occurs early in lung carcinogenesis,[51,52] although the molecular mechanism by which RARß expression is lost is uncertain. Four types of lung cancer (adenocarcinoma and squamous cell, large cell, and small cell carcinoma) are characterized by a nonrandom heterozygous loss of a region of the short arm of chromosome 3, the distal region of which contains the RARß gene.[49,53–55] The frequency of this loss was found to be higher in epidermoid (squamous) tumors. Restoration of RARß2 in a RARß-negative lung cancer cell line has been reported to inhibit tumorigenicity in nude mice.[48] Moreover, introduction of RARß2 into RARß-negative HeLa cells inhibits cell proliferation in vitro in a retinoic acid–dependent manner.[56] Thus, loss of tumor suppressor function of RARß by deletion, mutation, or transcriptional repression may lead to enhanced cell proliferation and potentially to tumor formation.

Finally, the chemoprotective effects of retinoids are thought to be mediated through transrepression of activator protein 1 (AP-1), which involves inhibition of AP-1 protein induction.[44] 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 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, and tumor promoters, usually resulting in cell proliferation. It was reported that RARα acts as a negative regulator of AP-1–responsive genes.[57] Moreover, retinoid receptors and the transcription factor AP-1 (Jun/Fos) can inhibit each other's activities. Recently, a protein-protein interaction mechanism for an antiproliferative effect of retinoic acid receptors has become of interest, as suggested in a recent study by Kamei et al.,[58] who showed that the interaction between RAR and cAMP response element–binding protein (CBP) is responsible for some forms of repression of AP-1 activity. Recent studies have also revealed a novel posttranscriptional mechanism by which all-*trans*-retinoic acid antagonizes the ultraviolet-induced c-Jun protein by stimulating its breakdown through the ubiquitin-proteasome pathway.[59,60] In a very recent study, Lee et

Table 3. Effect of ß-Carotene Feeding, Smoke Exposure, or the Combination on Proliferating-cell Nuclear Antigen (PCNA) Expression, Retinoic Acid Receptors (RARα and RARß) Gene Expression, and Activator Protein 1 (Encoded by c-Jun and c-Fos) Expression in the Lungs of Ferrets[a]

	Control Group	Smoke-exposed Group	ß-Carotene-supplemented Group	Smoke-exposed and ß-Carotene-supplemented Group
PCNA	100 ± 11	130 ± 11	180 ± 21	366 ± 60
RARα	100 ± 8	94 ± 10	98 ± 17	97 ± 9
RARß	100 ± 10	82 ± 14	38 ± 11	27 ± 12
c-Jun	100 ± 23	128 ± 21	121 ± 18	416 ± 52
c-Fos	100 ± 11	119 ± 21	194 ± 29	392 ± 43

Source: Adapted from data from reference 26.

[a]The relative protein values in the three treatment groups were calculated as percent ± standard deviation (n=6) compared with the untreated group as 100%.

al.[61] provided the first evidence that all-*trans*-retinoic acid suppresses Jun N-terminal kinase (JNK) activity by inhibiting JNK phosphorylation. Because AP-1 sites are found in a number of genes important to the control of cell proliferation, this type of interaction is almost certainly responsible for some of the antiproliferative and anticancer properties attributed to retinoic acid.

In a ferret study,[26] a strong pulmonary proliferative response, which was assessed by examining proliferating cellular nuclear antigen expression (PCNA) (Table 3) and squamous metaplasia (Figure 2), was observed in animals subjected to either ß-carotene supplementation, cigarette

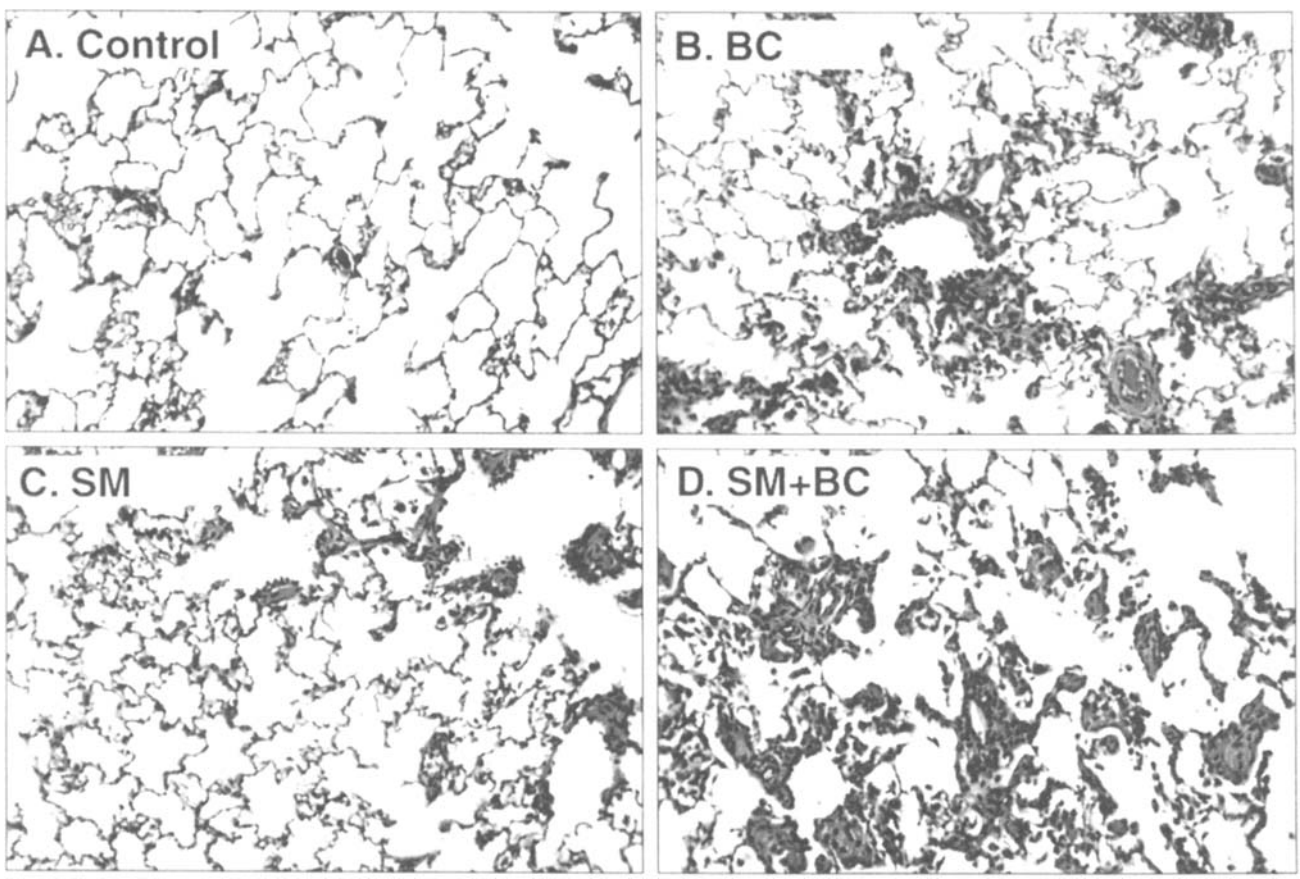

Figure 2. Pathologic changes of high-dose ß-carotene feeding, smoke exposure, and both on ferret lung tissue (hematoxylin-eosin × 50). The results show that smoke exposure caused mild localized squamous metaplasia, in addition to aggregation and proliferation of macrophages (Figure 2C). More severe proliferation of alveolar cell (type II pneumocytes) and alveolar macrophages was observed in both the high-dose ß-carotene–supplemented group (Figure 2B) and the smoke-exposed group with high-dose ß-carotene supplementation (Figure 2D), compared with the group exposed to smoke alone or with the untreated control group (Figure 2A). Adapted from data from reference 26.

smoke, or both for 6 months. RARß gene expression, which may function as a tumor suppressor, but not RARα and RARγ, was down-regulated by 18% to 73% in the three active treatment groups compared with the control group (Table 3). Furthermore, both c-Jun and c-Fos protooncogene expressions were up-regulated three- to fourfold in the smoke-exposed ferrets fed ß-carotene compared with the control animals (Table 3).

Very recently, it was 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.[62] The all-*trans*-retinoic acid–induced proteolysis of cyclin D1 leads to the arrest of human bronchial epithelial cells in the G1 phase of the cell cycle.[63] It is conceivable that the down-regulation of RARß and overexpression of c-Jun by chronic smoke exposure and excess ß-carotene supplementation cause abnormal cell cycle regulation to drive cells into a premature S phase (PCNA) (Table 3), which results in cell proliferation and squamous metaplasia (Figure 2).

In summary, we hypothesize that one of the biological bases for the harmful effects of high-dose ß-carotene supplementation in smokers is the free radical–rich atmosphere yet antioxidant-poor environment in the lungs of cigarette smokers. This environment alters ß-carotene metabolism and produces oxidative metabolites that accelerate malignant transformation by down-regulating the RARß gene and up-regulating AP-1 (c-Jun and c-Fos) activity. Conversely, if low levels of excentric cleavage products are produced in the cell, as would be the case when one eats high dietary levels of ß-carotene or receives low-dose ß-carotene supplementation, this could be beneficial and antiproliferative, because low levels of excentric cleavage products can, in and of themselves, give rise to retinoic acid.

Very recently, Lowe et al.[64] demonstrated that intact ß-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). In a recent hamster study, a strong inhibitory effect of various doses of ß-carotene on the development of upper respiratory tract tumors was observed in animals treated with diethylnitrosamine and cigarette smoke.[65] It is interesting that ß-carotene levels in the serum of the ß-carotene–supplemented animals were not different in the control animals.[65]

To Achieve the Potential Chemopreventive Activity of ß-Carotene, Vitamin C and Vitamin E Must Be Present to Prevent ß-Carotene Oxidation

Recent in vitro studies on the protection of the ß-carotene molecule with vitamin E and vitamin C, along with several human epidemiologic investigations, support the hypotheses that adequate amounts of vitamins C and E must be present to prevent ß-carotene from being oxidized and for ß-carotene to have a chemopreventive effect.[27,28,66,67]

Vitamin C is a strong reducing agent known to act as an antioxidant both in vitro and in vivo.[68] Vitamin C protects lipids in human plasma against oxidative damage by scavenging oxygen-derived free radicals in both smokers and nonsmokers.[68] Recent studies suggest that vitamin C is able to convert the ß-carotene radical back to ß-carotene and can help maintain ß-carotene in its unoxidized form.[27,66] However, smokers have significantly lower plasma levels of vitamin C compared with nonsmokers.[69] Even nonsmokers exposed to passive smoke have reduced ascorbic acid concentrations in their plasma.[70] Thus, it seems that the anti- or procarcinogenic response to carotenoid supplementation rests on the stability of the carotenoid molecule and the presence of other antioxidants. This may help to explain why diets high in fruits and vegetables, and hence high in vitamin C, are associated with a decreased risk for cancers of the lung, oral cavity, esophagus, stomach, and colon.[71]

Several lines of evidence also demonstrate that interactions occur between ß-carotene and tocopherols. ß-Carotene or its metabolites can exhibit prooxidant properties[72] that may depend on the high oxygen tensions or the dose of ß-carotene.[6,72] Although one recent report[73] indicates that a prooxidant effect of ß-carotene is unlikely in biologically relevant conditions, there is an interaction between tocopherols and ß-carotene with respect to pro- or antioxidant effects: ß-carotene appears to act as a prooxidant in vitro in the absence of tocopherols[74,75] or in vivo under conditions of vitamin E deficiency.[76,77] Because the prooxidant effect of ß-carotene has been attributed to its oxidative degradation products,[78] tocopherols may limit the prooxidant effects of carotenoids in biological systems by protecting carotenoids from oxidation. Conversely, ß-carotene is capable of regenerating α-tocopherol from its radical.[28,66] Pretreatment of human lung cells with vitamin E and ß-carotene together was shown to provide significant protection against DNA strand breakage induced by tobacco-specific nitrosamines.[79] In this regard, it should be mentioned that a trial in Linxian County, China, involving approximately 30,000 men and women showed that after an intervention period of 5 years, those given a combination of ß-carotene (15 mg/day), vitamin E, and selenium had a 13% reduction in cancer deaths.[80]

It was recently reported that a combination of ß-carotene, α-tocopherol, and vitamin C provides synergistic protection against free radical damage in an in vitro cell system.[66] Investigators observed that the combination of ß-carotene and vitamin E offered additive protection against cell damage. In the presence of ascorbic acid, however, they observed a synergistic rather than an additive effect, which can be explained by an electron transfer reaction in which ß-carotene radicals are repaired by vita-

min C. Possible protective effects of combined antioxidant supplements in humans exposed to environmental tobacco smoke were also recently reported.[67,81,82] Howard et al.[67] demonstrated that the increased oxidative stress induced by environmental tobacco smoke can be reduced in humans by supplementation with antioxidant vitamins and trace minerals (3 mg ß-carotene, 60 mg vitamin C, 30 IU α-tocopherol, 40 mg zinc, 40 µg selenium, and 2 mg copper). Duthie et al.[82] carried out a double-blind antioxidant supplementation study with vitamin C (100 mg/day), vitamin E (280 mg/day), and ß-carotene (25 mg/day) in 50 smokers and 50 nonsmokers for 20 weeks.[82] The investigators reported that supplementation with the combined antioxidants resulted in a significant ($P<0.002$) decrease in endogenous oxidative base damage in the lymphocyte DNA of both smokers and nonsmokers. These findings support the role of supplementation with a combination of antioxidants (ß-carotene, vitamin E, and vitamin C) against human lung cancer.

Conclusion

Studies suggest that the carcinogenic response to high-dose ß-carotene supplementation reported in human intervention trials is related to the instability of the ß-carotene molecule in the lungs of cigarette smokers, which is a free radical–rich yet antioxidant-poor environment. The presentation of high doses of ß-carotene via supplements to the highly oxidative environment of the lung results in increased levels of oxidative metabolites of ß-carotene. The increased ß-carotene oxidative metabolites may promote carcinogenesis by inducing carcinogen-bioactivating enzymes and bioactivating tobacco smoke procarcinogens, facilitating the binding of metabolites of benzo[a]pyrene to DNA, enhancing retinoic acid catabolism and down-regulating RARß, and acting as a prooxidant, causing damage to DNA. Because the stability of ß-carotene is dependent on other antioxidants, particularly vitamins C and E, investigations regarding the effectiveness of a combination of antioxidants (ß-carotene, vitamin C, and α-tocopherol) as an effective chemopreventive strategy against lung cancer should be studied. Furthermore, animal studies under highly controlled experimental conditions should be conducted to investigate dose-dependent relationships between combined antioxidants, smoke exposure, and gene expression and mutation before the initiation of any more human intervention trials.

1. Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? Nature 1981;290:201–8
2. Ziegler RG, Mayne ST, Swanson CA. Nutrition and lung cancer. Cancer Causes Control 1996;7:157–77
3. Mayne ST. Beta-carotene, carotenoids, and disease prevention in humans. FASEB J 1996;10:690–701
4. Krinsky NI. Action of carotenoids in biological systems. Annu Rev Nutr 1993;13:561–87
5. De Flora S, Bagnasco M, Vainio H. Modulation of genotoxic and related effects by carotenoids and vitamin A in experimental models: mechanistic issues. Mutagenesis 1999;14:153–72
6. Burton GW, Ingold KU. Beta-carotene: an unusual type of lipid antioxidant. Science 1984;224:569–73
7. Napoli JL, Race KR. Biogenesis of retinoic acid from ß-carotene: differences between the metabolism of ß-carotene and retinal. J Biol Chem 1988;263:17372–7
8. Wang X-D, Krinsky NI, Benotti PN, Russell RM. Biosynthesis of 9-*cis*-retinoic acid from 9-*cis*-ß-carotene in human intestinal mucosa. Arch Biochem Biophys 1994;313:150–5
9. Wang X-D, Russell RM, Liu C, et al. ß-Oxidation in rabbit liver in vitro and in the perfused ferret liver contributes to retinoic acid biosynthesis from ß-apocarotenoic acid. J Biol Chem 1996;271:26490–8
10. Zhang LX, Cooney RV, Bertman JS. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. Carcinogenesis 1991;12:2109–14
11. Sies H, Stahl W. Carotenoids and intercellular communication via gap junctions. Int J Vitam Nutr Res 1997;66:119–25
12. Bendich A. Carotenoids and the immune response. J Nutr 1989;119:112–5
13. Santos MS, Meydani SN, Leka L, et al. Natural killer cell activity in elderly men is enhanced by ß-carotene supplementation. Am J Clin Nutr 1996;64:772–7
14. Hughes DA, Wright AJA, Finglas PM, et al. The effect of ß-carotene supplementation on the immune function of blood monocytes from healthy male nonsmokers. J Lab Clin Med 1997;129:309–17
15. Edes TE, Thornton W Jr, Shah J. ß-Carotene and aryl hydrocarbon hydroxylase in the rat: an effect of ß-carotene independent of vitamin A activity. J Nutr 1989;119:796–9
16. Gradelet S, Le Bon AM, Berges R, et al. Dietary carotenoids inhibit aflatoxin B1-induced liver preneoplastic foci and DNA damage in the rat: role of the modulation of aflatoxin B1 metabolism. Carcinogenesis 1998;19:403–11
17. Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029–35
18. Albanes D, Heinonen OP, Taylor PR, et al. Alpha-tocopherol and ß-carotene supplements and lung cancer incidence in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study: effects of baseline characteristics and study compliance. J Natl Cancer Inst 1996;88:1560–70
19. Omenn GS, Goodman G, Thornquist M, et al. The Beta-Carotene and Retinol Efficacy Trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. Cancer Res 1994;54(suppl):2038–43
20. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of beta-carotene and vita-

min A on lung cancer and cardiovascular disease. N Engl J Med 1996;334;115–55
21. Omenn GS, Goodman GE, Thornquist MD, et al. Risk factor for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. J Natl Cancer Inst 1996;88:1550–9
22. Henneken CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with beta-carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996;334:1145–9
23. Mathews-Roth MM. Beta-carotene therapy for erythropoietic protoporphyria and other photosensitivity diseases. Biochimie 1986;68:875–84
24. Mathews-Roth MM. Carotenoids in erythropoietic protoporphyria and other photosensitivity disease. Ann N Y Acad Sci 1993:691;127–38
25. Wang X-D, Krinsky NI. The bioconversion of ß-carotene into retinoids. Subcell Biochem 1998;27:159–80
26. Wang X-D, Liu C, Bronsen R, et al. Retinoid signaling and activator protein-1 expression in ferrets given ß-carotene supplements and exposed to tobacco smoke. J Natl Cancer Inst 1999;91:60–6
27. Black HS. Radical interception by carotenoids and effects on UV carcinogenesis. Nutr Cancer 1998;31:212–7
28. Bohm F, Edge R, Land EJ, et al. Carotenoids enhance vitamin E antioxidant efficiency. J Am Chem Soc 1997;119:621–2
29. Wang X-D, Marini RP, Hebuterne X, et al. Vitamin E can enhance lymphatic absorption of ß-carotene and its conversion into vitamin A in the ferret. Gastroenterology 1995;108:719–26
30. Salgo MG, Cueto R, Winston GQ, Pryor WA. Beta-carotene and its oxidation products have different effects on microsome mediated binding of benzo[a]pyrene to DNA. Free Radic Biol Med 1999;26:162–73
31. Perocco P, Paolini M, Mazzullo M, et al. Beta-carotene as enhancer of cell transforming activity of powerful carcinogens and cigarette-smoke condensate on BALB/c 3T3 cells in vitro. Mutat Res 1999;440:83–90
32. Paolini M, Cantelli-Forti G, Perocco P, et al. Co-carcinogenic effect of beta-carotene. Nature 1999;398:760–1
33. Gradelet S, Leclerc J, Siess MH, Astorg PO. Beta-apo-8'-carotenal, but not beta-carotene, is a strong inducer of liver cytochromes P4501A1 and 1A2 in rat. Xenobiotica 1996;26:909–19
34. Redlich CA, Blaner WS, Van Bennekum AM, et al. Effect of supplementation with ß-carotene and vitamin A on lung nutrient levels. Cancer Epidemiol Biomarkers Prev 1998;7:211–4
35. Pelkonen O, Maenpaa J, Taavitsainen P, et al. Inhibition and induction of human cytochrome P450 (CYP) enzymes. Xenobiotica 1998;28:1203–53
36. Villard PH, Seree EM, Re JL, et al. Effects of tobacco smoke on the gene expression of the Cyp1a, Cyp2b, Cyp2e, and Cyp3a subfamilies in mouse liver and lung: relation to single strand breaks of DNA. Toxicol Appl Pharmacol 1998;148:195–204
37. White JA, Guo YD, Baetz K, et al. Identification of the retinoic acid-inducible all-*trans*-retinoic acid 4-hydroxylase. J Biol Chem 1996;271:29922–7
38. Sonneveld E, van den Brink CE, van der Leede BJM, et al. Human retinoic acid 4-hydroxylase (CYP26) is highly specific for all-*trans* RA, and can be induced through retinoic acid receptors in human breast and colon carcinoma cells. Cell Growth Differ 1998;9:629–37
39. Wu R, Martin WR, Robinson CB, et al. Expression of mucin synthesis and secretion in human tracheobronchial epithelial cells grown in culture. Am J Respir Cell Mol Biol 1990;3:467–78
40. Chopra DP. Squamous metaplasia in organ cultures of vitamin A–deficient hamster trachea: cytokinetic and ultrastructural alterations. J Natl Cancer Inst 1982;69:895–905
41. Jetten AM, George AM, Pettit GR, Rearick JI. Effects of bryostatins and retinoic acid on phorbol ester- and diacylglycerol-induced squamous differentiation in human tracheobronchial epithelial cells. Cancer Res 1989;49:3990–5
42. Zhang XK, Liu Y, Lee MO. Retinoid receptors in human lung cancer and breast cancer. Mutat Res 1996;350:267–77
43. Lotan R. Retinoids in cancer prevention. FASEB J 1996;10:1031–9
44. Pfahl M, Chytil F. Regulation of metabolism by retinoic acid and its nuclear receptor. Annu Rev Nutr 1996;16:257–83
45. Dolle P, Ruberte E, Leroy P, et al. Retinoic acid receptors and cellular retinoid binding proteins. 1. A systematic study of their differential pattern of transcription during mouse organogenesis. Development 1990;11:1133–51
46. Gebert JF, Moghal N, Frangioni JV, et al. High frequency of retinoic acid receptor ß abnormalities in human lung cancer. Oncogene 1991;6:1859–68
47. Nervi C, Vollbert TM, George MD, et al. Expression of nuclear retinoic acid receptors in normal tracheobronchial cells and in lung carcinoma cells. Exp Cell Res 1991;195;163–70
48. Houle B, Rochette-Egly C, Bradley WEC. Tumor-suppressive effect of the retinoic acid receptor-ß in human epidermoid lung cancer cells. Proc Natl Acad Sci U S A 1993;90:985–9
49. Zhang XK, Liu Y, Lee MO, Pfahl M. A specific defect in the retinoic acid response associated with human lung cancer cell lines. Cancer Res 1994;54:5663–9
50. Moghal N, Neel BG. Evidence for impaired retinoic acid receptor-thyroid hormone receptor AF-2 cofactor activity in human lung cancer. Mol Cell Biol 1995;15:3945–59
51. Xu XC, Ro JY, Lee JS, et al. Differential expression of nuclear retinoid receptors in normal premalignant, and malignant head and neck tissues. Cancer Res 1994;54:3580–7
52. Xu XC, Sozzi G, Lee JS, et al. Suppression of retinoic acid receptor ß in non-small cell lung cancer in vivo: implications for lung cancer development. J Natl Cancer Inst 1997;89:624–9
53. Carney DN, De Leij L. Lung cancer biology. Semin Oncol 1988;15:199–214
54. Brauch H, Johnson B, Hovis J, et al. Molecular analysis of the short arm of chromosome 3 in small-cell and non-small cell carcinoma of the lung. N

Engl J Med 1987;317:1109–13
55. Geradts J, Chen JY, Russell EK, et al. Human lung cancer cell lines exhibit resistance to retinoic acid treatment. Cell Growth Differ 1993;4:799–809
56. Frangioni JV, Moghal N, Stuart-Tilley A, et al. The DNA binding domain of retinoic acid receptor ß is required for ligand-dependent suppression of proliferation. J Cell Sci 1994;107:827–38
57. Schule R, Rangarajan P, Yang N, et al. Retinoic acid is a negative regulator of AP-1 responsive genes. Proc Natl Acad Sci U S A 1991;88:6092–6
58. Kamei Y, Xu L, Heinzel T, et al. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 1996;95: 403–14
59. Fisher GJ, Talwar HS, Lin J, et al. Retinoic acid inhibits induction of c-Jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin in vivo. J Clin Invest 1998;101:1432–40
60. Fisher GJ, Talwar HS, Lin J, Voorhees JJ. Molecular mechanisms of photoaging in human skin in vivo and their prevention by retinoic acid. Photochem Photobiol 1999;69:154–7
61. Lee H-Y, Sueoka N, Hong W-K, et al. All-*trans*-retinoic acid inhibits Jun N-Terminal kinase by increasing dual-specificity phosphatase activity. Mol Cell Biol 1999;19:1973–80
62. Wisdom R, Johnson RS, Moore C. c-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. EMBO J 1999;18:188–97
63. Boyle JO, Langenfeld J, Lonardo F, et al. Cyclin D1 proteolysis: a retinoid chemoprevention signal in normal, immortalized, and transformed human bronchial epithelial cells. J Natl Cancer Inst 1999;91: 373–9
64. Lowe GM, Booth LA, Young AJ, Bilton RF. Lycopene and ß-carotene protect against oxidative damage in HT29 cells at low concentrations but rapidly lose this capacity at higher doses. Free Radic Biol Med 1999;30:141–51
65. Furukawa F, Nishikawa A, Kasahara K, et al. Inhibition by ß-carotene of upper respiratory tumorigenesis in hamsters receiving diethylnitrosamine followed by cigarette smoke exposure. Jpn J Cancer Res 1999;90:154–61
66. Bohm F, Edge R, McGarvey DJ, Truscott TG. ß-Carotene with vitamins E and C offers synergistic cell protection against NO_x. FEBS Lett 1998;436:387–9
67. Howard DJ, Ota RB, Briggs LA, et al. Oxidative stress induced by environmental tobacco smoke in the workplace is mitigated by antioxidant supplementation. Cancer Epidemiol Biomarkers Prev 1998;7:981–8
68. McCall MR, Frei B. Can antioxidant vitamins materially reduce oxidative damage in humans? Free Radic Biol Med 1999;26:1034–53
69. Schectman G, Byrd JC, Gruchow HW. The influence of smoking on vitamin C status in adults. Am J Public Health 1989;79:158–62
70. Tribble DL, Giuliana LJ, Fortmann SP. Reduced plasma ascorbic acid concentrations in nonsmokers regularly exposed to environmental tobacco smoke. Am J Clin Nutr 1993;58:886–90
71. Byers T, Guerrero N. Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. Am J Clin Nutr 1995;62(suppl):1385–92
72. Palozza P. Prooxidant actions of carotenoids in biological systems. Nutr Rev 1998;56:257–65
73. Baker DL, Krol ES, Jacobsen N, Liebler DC. Reactions of beta-carotene with cigarette smoke oxidants. Identification of carotenoid oxidation products and evaluation of the prooxidant/antioxidant effect. Chem Res Toxicol 1999;12:535–43
74. Haila KM, Lievonen SM, Heinonen MI. Effects of lutein, lycopene, annatto, and gama-tocopherol on autoxidation of triglycerides. J Agric Food Chem 1996;44:2096–100
75. Terao J, Yamauchi R, Murakami H, Matsushita S. Inhibitory effects of tocopherols and ß-carotene on singlet oxygen-initiated photoxidation of methyl linoleate and soybean oil. J Food Processing Preserv 1980;4:79–93
76. Lomnitski L, Bergman M, Schön I, Grossman S. The effect of dietary vitamin E and ß-carotene on oxidation processes in the rat testis. Biochim Biophys Acta 1991;1082:101–7
77. Handelman GJ, Packer L, Cross CE. Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. Am J Clin Nutr 1996;63:559–65
78. Jørgensen K, Skibsted LH. Carotenoid scavenging of radicals: effect of carotenoid structure and oxygen partial pressure on antioxidative activity. Z Lebensm Unters Forsch 1993;196:423–9
79. Weitberg AB, Corvese D. Effect of vitamin E and beta-carotene on DNA strand breakage induced by tobacco-specific nitrosamines and stimulated human phagocytes. Clin Cancer Res 1997;16:11–4
80. Blot WJ, Li JY, Taylor PR, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J Natl Cancer Inst 1993;85:1483–91
81. Comstock GW, Alberg AJ, Huang HY, et al. The risk of developing lung cancer associated with antioxidants in the blood: ascorbic acid, carotenoids, alpha-tocopherol, selenium, and total peroxyl radical absorbing capacity. Cancer Epidemiol Biomarkers Prev 1997;6:907–16
82. Duthie JS, Ma A, Ross MA, Collina AR. Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. Cancer Res 1996;56: 1291–5