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Beta-Carotene and Vitamin E in Oral Cancer Prevention

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Abstract The ultimate proof that a putative chemopreventive agent does prevent cancer is a demonstration of reduced cancer incidence in a targeted population. However, because of practical and logistical considerations, such trials are virtually impossible to conduct for the majority of cancers. Therefore, a conclusion regarding the efficacy of chemopreventive activity is based on consideration of a variety of indirect lines of evidence, including laboratory studies, animal model systems, epidemiologic surveys, intervention trials involving reversal of premalignant changes, and the prevention of malignancies in particularly high risk subjects. Furthermore, the only agents worth testing are those with limited, or preferably, no toxicity, since the final use will be prevention in a generally healthy population. Beta-carotene and vitamin E both fulfill all the criteria for suitable chemopreventive agents; several lines of evidence point toward preventive roles for them in oral cancer. In numerous epidemiologic studies, low intake of beta-carotene has been associated with higher cancer risk. Both intake and supplemental use of vitamin E have been associated with a lowered risk of cancer. Smokers, whose habit is a major risk factor, have lower beta-carotene levels in oral mucosal cells when compared with non-smokers. In several laboratory and animal model systems, including the very relevant hamster cheek pouch model, these agents strongly inhibit oral cavity carcinogenesis. Beta-carotene and vitamin E produce regression of oral leukoplakia, a premalignant lesion for oral cancer. This has now been shown in seven clinical trials: five with beta-carotene alone, one with vitamin E, and one with a combination of both. Actual cancer incidence reduction trials in high risk groups have targeted the prevention of second malignancies in patients cured of an oral cancer. Such trials are now in progress. These data, together with the lack of any significant side effects, and an emerging role for these agents in the prevention of other life-shortening chronic diseases such as atherosclerosis, are strongly supportive of a very significant disease-preventive role for these nutrients, including a chemopreventive role in oral cancer.

Key words: antioxidant nutrients, cancer prevention, beta-carotene, oral cancer, vitamin E

Oral cavity cancer is a common malignancy whose regional incidence varies from one part of the world to another. Overall, these cancers are the sixth most frequent cancers in the world. Some of the highest rates occur in developing countries, where up to 25% of all malignancies are found in the oral cavity [1]. In the United States, there are approximately 42,000 new cases of head and neck cancer annually, leading to 12,000 deaths [2]. Most of these malignancies are caused by tobacco and alcohol use [3,4]. In developing countries, tobacco and betel quid chewing, usually mixed with other toxins such as slaked lime, is a common custom resulting in most of the oral cancers. Tobacco, either smoked or chewed, causes more than 75% of oral cavity cancer [3]. Thus, the major risk factor for oral cancer is the same as that for some other common diseases, such as lung can-

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cer and heart disease. Consequently, primary prevention strategies for oral cancer, such as discontinuing tobacco use, will have an impact on many life-threatening diseases. Other prevention modalities, including beta-carotene, may also be beneficial for a number of cancer sites linked by a common etiology, namely tobacco.

Even though the treatment of oral cancer has improved over the past few decades from the standpoint of reducing morbidity and disfigurement, there has been no demonstrable improvement in the survival of patients afflicted with this disease. In fact, National Cancer Institute surveillance data, comparing outcomes in the 1980s to those that were achievable in the 1970s, are essentially identical [5]. Advanced-disease patients have a dismal 5 year survival rate in the range of 25% or less. Early disease can generally be cured by local treatment modalities, such as surgery and/or radiotherapy, but there remains a significant risk of developing a second malignancy of the upper aerodigestive tract, often resulting in death despite having cured the primary lesion [6,7]. In the 1950s, the concept of "field cancerization" was proposed as an explanation for the coexistence of malignant and premalignant changes frequently seen in the same patient [8]. This refers to diffuse changes in the mucosa, presumably by exposure to carcinogens, that lead to increased neoplastic growth.

The approach most likely to reduce morbidity and mortality from oral cancer is prevention. Cessation of tobacco use is clearly a major objective in this endeavor. Additionally, there is now considerable evidence suggesting a role for nutritional agents in preventing this disease, particularly the antioxidants beta-carotene and vitamin E.

The most convincing and direct proof of an intervention's cancer-preventive activity would be to demonstrate an actual reduction in cancer incidence via a clinical trial. Such an approach is impossible for most cancers for logistical and practical reasons. Most individual types of cancer, although common, are infrequent events in an otherwise healthy population. Long trials lasting decades and involving several thousands of subjects would be necessary for each malignancy. A more practical approach regarding putative chemopreventive activity would be to consider an accumulation of other, admittedly indirect, lines of evidence for or against each agent. Table I lists the various lines of evidence supporting such a role for beta-carotene and vitamin E in oral cancer prevention. Less work has been completed with vitamin E, greater attention having initially been given to beta-carotene. Nevertheless, vitamin E is equally, if not more, promising in preclinical work; a finding borne out by the results of early clinical trials. Interest in a disease-preventive role for vitamin E has consequently increased considerably in recent years and will be reflected in the completion of more clinical trials using this agent in the near future.

CARCINOGENESIS AND CANCER PREVENTION

The disease process leading to invasive cancer is called carcinogenesis. Carcinogenesis proceeds through a series of sequential steps: initiation, promotion, and progression. Our efforts in the area of cancer therapeutics have concentrated primarily on invasive cancer, the final stage of this disease. Although this has resulted in an occasional success story, such an approach is inherently limited in its impact because it focuses on the last and final stage of the chronic

TABLE I. Beta-Carotene and Vitamin E in Oral Cancer Prevention

1.	Laboratory studies, animal models (hamster cheek pouch).
2.	Epidemiology: Risk correlations with diet and use of supplements.
3.	Pharmacology: Low levels in high risk groups (smokers).
4.	Ability to decrease micronucleated cells in very high risk groups (beta-carotene).
5.	Ability to reverse oral leukoplakia, a premalignant lesion.
6.	Effect on incidence of second malignancies?

process of carcinogenesis. As is the case with emphasis on the final stage of any chronic, ultimately fatal, disease, this approach can result in only modest, if any, effect on its control and eradication. In cardiovascular heart disease, for example, emphasis on the management and treatment of myocardial infarction, the final step, will have considerably less impact on morbidity from heart disease than effective prevention of the underlying disease process of atherosclerosis. Such a re-focus on carcinogenesis as the chronic disease, rather than invasive cancer as the endpoint, is particularly important when one considers strategies for cancer prevention, since these strategies will undoubtedly involve interventions that prevent, inhibit, or reverse the multiple steps involved in carcinogenesis.

EPIDEMIOLOGIC AND LABORATORY EVIDENCE FOR AN INHIBITORY ROLE FOR BETA-CAROTENE AND VITAMIN E IN ORAL CARCINOGENESIS

Numerous epidemiologic studies have linked low intake of carotenoids with an increased risk of cancer, including that of the oral cavity [9]. Because of the difficulty in quantitating vitamin E in the diet, studies with this antioxidant are fewer in number than those with beta-carotene, but also suggest protection [10]. A very important epidemiologic study was published recently by Gridley *et al.* [11], reporting that subjects taking supplemental vitamin E had approximately half the risk of oral cavity cancer than those not taking the supplement. This study is important because it is the first epidemiologic study demonstrating a beneficial effect from supplemental vitamin E intake.

Another epidemiologic approach has been to study the pharmacology of these agents in subjects at risk for oral malignancy. Although it has been known for some time that heavy cigarette smokers have lower plasma levels of carotenoids and beta-carotene than non-smokers, Stich *et al.* [12], and more recently Peng *et al.* [13], have now shown that oral mucosal cell levels of beta-carotene are also lower in smokers compared to non-smokers. At our own institution Peng *et al.* [13] have demonstrated that this difference exists despite similar dietary intakes and that the magnitude of the difference is likely to be too large for smokers to achieve non-smoker levels simply by diet modification. In another series of studies, Kaugars and colleagues [14,15] from the Medical College of Virginia have studied plasma levels of carotenoids in tobacco chewers. In preliminary results, they found lower dietary intakes and lower plasma levels in those subjects who developed premalignant lesions versus those who did not. These latter findings need further study since, in a subsequent report, the same group failed to confirm a statistically significant difference [16].

In laboratory studies, carotenoids have been shown to have antimutagenic activity in bacterial systems. Similarly, in many cell culture systems, they have a profound effect in preventing transformation induced by chemicals and radiation [17,18]. Of direct relevance to oral carcinogenesis are observations on the capacity of these compounds to block genotoxic damage in Chinese hamster ovary cells caused by tumor promoters such as areca nut extracts and other oral carcinogens [19]. The precise mechanism of action of retinoids and carotenoids in cancer inhibition has not yet been determined. They produce effects on cell differentiation, immunologic function, interaction of cells with growth factors, such as epidermal growth factor, and changes in gene expression; all mechanisms which may be important in their anticarcinogenic activity.

An animal model of particular relevance to head and neck cancer is the hamster cheek pouch model, in which precancerous and cancerous lesions are produced after application of the carcinogen 7,12-dimethylbenz(*a*)anthracene. This model was first described in 1954 and has been extensively studied by Shklar, Schwartz, and their colleagues [20–23]. The retinoids (13-*cis*-retinoic acid, retinyl acetate) and beta-carotene are all very active in inhibiting the formation of cancerous lesions in this system. Vitamin E also has significant inhibitory activity which is synergistic with beta-carotene [24]. This system has proven to be very useful for the study of oral carcinogenesis and its inhibition.

MICRONUCLEATED CELL FREQUENCY

Increased frequency of micronucleated cells is thought to reflect genotoxic damage produced

TABLE II. Beta-Carotene and Micronuclei Frequency

Population	Dose	Result	Reference
India	180 mg/week	Decrease	[25]
Philippines	180 mg/week	Decrease	[26]
Canada (Inuits)	180 mg/week	Decrease	[27]

by carcinogens. Stich and colleagues [25–27] have reported a series of studies showing that beta-carotene, alone or in combination with vitamin A, can decrease the incidence of micronucleated cells in exfoliated oral mucosal cells from populations considered to be at high risk for oral cancer (Table II). Preliminary results from studies in the West, where the lesion is primarily from smoking and not chewing tobacco, have shown a much lower initial frequency of micronucleated cells than in the trials by Stich *et al.* [25,27]. No results on changes with treatment have yet been reported in "non-chewing" subjects; such changes may be difficult to demonstrate because of the low pretreatment frequency [Garewal *et al.*, these proceedings].

BETA-CAROTENE AND VITAMIN E REVERSE ORAL CAVITY PREMALIGNANCY

The reversal or suppression of premalignant lesions is an important strategy to prevent cancer. The basis for this approach is that premalignant lesions are usually the first clinically identifiable clues that allow recognition of a mucosa affected by carcinogenesis. It should be emphasized that the ultimate goal of this strategy is to develop interventions applicable to the prevention of cancer and not merely eradication of premalignant lesions. In general, the latter are not lethal or even morbid by themselves, and they are associated with rather low rates of transformation to cancer. Therefore, it is imperative that agents selected for trials using premalignant lesions, with the ultimate goal of cancer prevention, should have minimal or preferably, no toxicity; a large number of subjects (whose lesions are unlikely to progress to cancer in their lifetimes) will necessarily be exposed to the intervention. Clearly, the type and number of side effects considered acceptable for any therapy depends on the severity of the condition being treated. High levels of toxicity are acceptable in treatments for overt malignancy. Similarly, a moderate degree of toxicity can be tolerated for some premalignant diseases, such as familial polyposis of the colon, which are associated with a very high cancer risk. However, for the majority of the more common premalignant lesions, the cancer risk is often very low and almost any side effects produced by a drug will generally be unacceptable.

Most oral cavity premalignant lesions come under the category of leukoplakia, *i.e.*, a white patch or plaque on the mucosa that cannot be rubbed off and is not attributable to a specific disease entity [28]. In general, they have a rather low malignant potential [29]. Oral erythroplakia and speckled leukoplakia have a higher transformation rate, but are relatively rare lesions [29,30]. Similarly, the presence of severe dysplasia demands a more aggressive treatment strategy [29].

The objectives of intervention trials involving leukoplakia must therefore be kept in mind when designing chemoprevention studies. If the objective is to develop a treatment applicable to the small minority of patients with erythroplakia and/or high grade dysplasia that are not amenable to such standard treatments as reduction in local irritants, surgical excision, or cryosurgery, then some degree of toxicity in the therapy may be acceptable. In this category are toxic agents such as topical bleomycin, 5-fluorouracil, high dose vitamin A, and synthetic retinoids such as 13-*cis*-retinoic acid, all of which have been known to be active for over two decades [31–35]. However, if the objective is to develop agents for generalized, population-based use in the primary prevention of oral cancer, then the non-toxic antioxidants, such as beta-carotene and vitamin E, are clearly preferred.

Interest in testing beta-carotene arose from the accumulated epidemiologic, laboratory, and

TABLE III. Oral Leukoplakia Trials Using Beta-Carotene and Vitamin E

Investigator	Agent	CR (%)	PR (%)	OR (%)	Country	Reference
Stich	BC	15	NS	NS	India	[25]
Stich	BC + vit A	27	NS	NS	India	[25]
Garewal	BC	8	63	71	USA	[37]
Toma	BC	33	11	44	Italy	[41]
Malaker	BC	28	22	50	Canada	[38]
Kaugars	BC + vit E + vit C	–	–	60	USA	[39]
Garewal	BC	–	–	56	USA	[42]
Benner	Vit E	23	23	46*	USA	[43]

BC = beta-carotene, **CR** = complete response, **PR** = partial response, **OR** = overall response, **NS** = not stated

*Response rate was 65% if based on "evaluable" patients rather than all subjects entered.

animal data. Furthermore, the activity of vitamin A and several synthetic retinoids was known, but their applicability was limited. Consequently, a series of trials in the 1980s tested beta-carotene; the results are summarized in Table III.

As shown in Table III, Stich *et al.* [25,36] have reported clinical results on a series of trials in India using vitamin A and beta-carotene, alone or in combination. It should be emphasized that this study population differs from that in other trials in that the lesion in India is primarily related to chewing betel nuts and other noxious substances. Furthermore, the study population may have had some degree of pre-existing vitamin A deficiency. In one study, treatment consisted of beta-carotene (180 mg/week, Group I) or beta-carotene plus vitamin A (100,000 IU/week, Group II) or placebo (Group III) given twice weekly for 6 months. At 6 months, 15% of patients in Group I and 27.5% in Group II had complete remissions of their lesions as compared with only 3% in Group III [25]. Furthermore, the appearance of new lesions was strongly inhibited in the treatment groups. In a more recent trial using 200,000 IU of vitamin A alone per week for 6 months, Stich *et al.* [36] reported a 57% complete response rate with total suppression of new lesions. Although this moderately high dose of vitamin A did not produce clinically overt toxicity, potentially serious side effects such as liver function abnormalities were not specifically monitored.

Studies with beta-carotene in Western populations have been more recent. We reported a pilot trial of beta-carotene given at a dose of 30 mg/day daily for 3–6 months [37]. A response rate of 71% (95% confidence interval 53–89%) was observed in 24 patients. Of particular importance was the fact that no clinically significant toxicity that could be attributed to beta-carotene was observed during this trial.

In a carefully conducted cross-over phase II trial by Malaker *et al.* [38] in Canada, in which patients were initially treated with beta-carotene for 6–9 months with non-responders then receiving 13-*cis*-retinoic acid, a response rate of approximately 50% was noted with beta-carotene.

Another study using a combination of antioxidant agents, including beta-carotene, is being conducted by Kaugars *et al.* [39]. In this trial, patients are supplemented with a combination of beta-carotene, alpha-tocopherol, and vitamin C. This combination is virtually non-toxic and a 60% response rate was recently reported [40]. Toma *et al.* [41] in Italy have reported a response rate of 44% in 18 evaluable subjects treated with beta-carotene alone at a dose of 90 mg/day.

We are presently conducting a multi-institutional trial that involves the University of California (Irvine), the University of Connecticut (Farmington), and the University of Arizona [42]. In this trial, subjects are treated with beta-carotene at a dose of 60 mg/day for 6 months at which point responding subjects are randomized in a blinded fashion to either continue beta-carotene or receive a placebo for another 12 months. There are two main clinical objectives in this study: (1) confirm the response rate to beta-carotene in a multi-institutional setting, and (2) establish whether or not continuation of beta-carotene will produce sustained remissions. It is well known from all previous studies that discontinuation of the intervention agent results in rapid recurrence of lesions, presumably because the initiating factors are still present. However, it needs to be established whether or not continued treatment will produce lasting remissions. The initial phase of this study was a feasibility trial to confirm whether or not a high response rate could be obtained with this type of treatment in a multi-institutional setting. This was accomplished with a response rate of 56% (95% confidence interval 41–72%) in the first 39 subjects who completed the 6 month "induction."

Although the laboratory data are equally convincing for vitamin E, clinical intervention trials with vitamin E have been started more recently. In addition to the study by Kaugars *et al.* [40] mentioned above, Benner *et al.* [43] recently reported a multi-center study with a response rate of 46% (95% confidence interval 32–61%) in 43 subjects treated with 400 IU of vitamin E twice daily for 24 weeks. The response rate for this study was 65% if calculated on the basis of 31 evaluable subjects. This result is extremely encouraging in that another nontoxic, nutritional agent, vitamin E, has been shown to be active in reversing oral leukoplakia.

CONCLUSIONS AND FUTURE TRIALS

In summary, numerous lines of evidence suggest a potential role for beta-carotene and other antioxidants in preventing oral cavity malignancy. Though it is recognized that the "ultimate" proof would be actual demonstration of a reduction in oral cavity cancer incidence, trials with this as an endpoint will never be feasible. Therefore, all alternative, indirect lines of evidence need to be considered in arriving at a conclusion regarding a potential chemopreventive role. In this regard, the cumulative evidence in favor of these agents is quite strong and is derived from a wide range of specialties, including epidemiology, laboratory studies, pharmacology, and clinical intervention trials.

Another important group of subjects targeted for testing chemopreventive approaches are those who have had an early primary head and neck cancer which is considered cured. As mentioned earlier, these patients have a high risk for developing a second primary cancer of the aerodigestive tract [6,7,44]. It is reasonable to speculate that agents active in reversing preneoplastic lesions may be active in reversing the "field cancerization" effect thought to underlie this increased incidence of second cancers. Nontoxic agents are again preferred in this setting, inasmuch as prolonged treatment is anticipated and many of these patients will have received radiation treatment to the oral cavity resulting in chronic mucosal injury. Hence, they may be unable to tolerate the mucocutaneous toxicity associated with other active agents such as retinoids. Trials using beta-carotene, alone or in combination with low doses of retinol, have recently been initiated in multi-center settings to test whether the incidence of second primaries can be reduced.

Finally, it is important to consider these results in the context of the ability of these agents to prevent other life-threatening, chronic diseases, particularly cardiovascular disease. The early results of recent studies are indeed very encouraging [45,46]. The unifying mechanism underlying these diseases could very well be accumulated oxidative damage, thereby providing a theoretical basis for the potential of antioxidants to prevent a variety of seemingly unrelated diseases. The remarkable consistency of results from the various clinical trials reported thus far, such as those in oral leukoplakia or heart disease incidence reduction, has generated tremendous enthusiasm for conducting prospective trials to add to the array of evidence for a chemopreventive role for antioxidants. The potential for making a significant impact on morbidity and mortality reduction is of such magnitude that these innocuous, non-toxic, dietary components

could very well emerge as one of the most important disease-preventive modalities of the decade.

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