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Authors

Garewal, H
Meyskens, F, Jr
Friedman, S
et al.

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Oral Cancer Prevention: The Case for Carotenoids and Anti-Oxidant Nutrients[1,2]

HARINDER GAREWAL, M.D., PH.D,[*,3] FRANK MEYSKENS, JR., M.D.,[†]
SHERRY FRIEDMAN, R.N.,[*] DAVID ALBERTS, M.D.,[*] AND LOIS RAMSEY, B.S.[*]

*Section of Hematology–Oncology, Tucson VA Medical Center and University of Arizona Cancer
Center, Tucson, Arizona 85723; and †University of California, Irvine, California 92717*

The most convincing evidence for a preventive role for any modality is obviously dem-
onstration of incidence reduction produced by that modality. However, cancer prevention
trials with cancer incidence as an endpoint have logistic problems rendering them essentially
impossible to conduct for most malignancies. Hence a workable strategy often involves
analysis of other, indirect lines of evidence to reach conclusions. For oral cancer, dietary
epidemiologic evidence points to a protective role for foods rich in carotenoids. Other
anti-oxidants, such as vitamin C, are also implicated. Similarly, laboratory evidence points
to a carcinogenesis inhibitory role for both retinoids and carotenoids. Clinical studies have
targeted premalignant lesions, i.e., oral leukoplakia. For over two decades the efficacy of
retinoids, natural and synthetic, has been known. Nevertheless, it has been difficult to
translate this into a recommendation for prevention because of the toxicity of retinoids. The
synthetic retinoid most often used in these trials is 13-cis-retinoic acid. This compound is
toxic even at very low doses (0.1 mg/kg/day), particularly when given over several weeks to
months. Hence, although effective, it cannot be advocated for prevention of oral cavity
cancer. Studies with nontoxic antioxidants, such as β-carotene, are much more recent.
Early results are promising in that β-carotene, alone or in conbination with other nutrients,
can reverse oral leukoplakia without toxicity in short-term trials. Studies currently under
way will demonstrate whether durable remissions can be obtained using this strategy. It
should be emphasized that such long-term trials are problematic to conduct with the toxic
retinoids because the risks of prolonged exposure to them outweighs the chance of cancer
development in the usual leukoplakia lesion. © 1993 Academic Press, Inc.

INTRODUCTION

Squamous cell cancers of the oral cavity are common neoplasms with an inci-
dence that varies from region to region in the world (1). Some of the highest rates
are found in developing countries, particularly India; Sri Lanka; South Vietnam;
Papua, New Guinea; Philippines; and parts of Brazil, where up to 25% of all
malignancies originate in the oral cavity (1–3). There have been recent increases
in some types of oral cavity cancer in Scandinavia, the United States, and Scot-
land, especially among younger males with increased use of chewing tobacco
being implicated as a potential cause (4–6). In the United States there are approx-
imately 43,000 new cases annually, resulting in about 11,600 deaths (7). This

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variation in regional incidence is related to variation in use of known etiologic agents. Tobacco and alcohol are strongly implicated as causative agents, particularly the former (8). In developing countries tobacco and betel nut chewing is a widespread custom resulting in most of these cancers (9). Consequently, in the 1990s, efforts at preventing this disease must focus primarily on reduction or elimination of these noxious habits. Other putative preventive modalities, such as those discussed in this paper, should be viewed as complimenting the control of tobacco use, rather than as a substitute for it. Because of the known link to carcinogen exposure as well as increasing evidence that several potential chemopreventive agents may inhibit the carcinogenesis process in the oral cavity, the potential for controlling this malignancy by preventive efforts is an achievable goal to be vigorously pursued.

It is obvious that the most convincing approach to proving efficacy of a chemopreventive agent would be to conduct a trial that shows reduction in incidence of the malignancy in an intervention versus a control group. However, it is equally obvious that for most cancers such studies are logistically impossible to undertake because of the low frequency of the event in the general population. The size and duration of the study would be prohibitive. Consequently, it becomes necessary to rely on a body of evidence from a variety of other types of studies in order to draw conclusions pertaining to cancer preventive activity. Such studies include epidemiologic data, laboratory evidence, animal models, and human intervention trials using surrogate endpoints for cancer risk. In this paper the evidence for a putative chemopreventive role for β-carotene and anti-oxidant nutrients in oral cavity cancer is reviewed, with emphasis on the recent clinical intervention trials.

When evaluating any agent for chemopreventive potential, it is important to keep its eventual application in perspective, i.e., prevention of a cancer in a population. With this in mind, it hardly needs emphasizing that for chemopreventive modalities to be considered realistic they need to be essentially nontoxic. Cancer is a low-frequency event. If chemoprevention or other preventive strategies are to have a meaningful impact on mortality and morbidity from the disease, they will have to be applied or recommended to large populations without the luxury of toxicity monitoring and surveillance. Although subgroups with markedly increased cancer risk can be identified in the population, they account for a small minority of all cancers. Some level of toxicity, compatible with the increased cancer risk, can be justified in interventions aimed at these small subgroups. However, if cancer prevention is to make a significant contribution toward reducing overall cancer mortality, the proposed preventive modalities must have wide applicability. Hence, the need for almost total lack of toxicity.

EPIDEMIOLOGIC, LABORATORY, AND ANIMAL STUDIES

As is true for several other malignancies, a low intake of vegetables and fruits has been associated with increased risk for upper aerodigestive tract cancers including those of the oral cavity (10–12). Some of these trials have actually been conducted in areas where oral cavity malignancy is endemic, in other words in situations where carcinogen exposure is intense (13). Although correlations with individual nutrients are difficult from such epidemiologic trials, the evidence

points more toward carotenoid intake than preformed retinol, as well as toward vitamin C and, more recently, supplemental vitamin E (14). In the laboratory, these compounds have been shown to have antimutagenic activity in bacterial and tissue culture systems (15, 16). For example, β-carotene can block genotoxic damage induced in Chinese hamster ovary cells by tumor promoters, including extracts of areca nut, which is an integral part of betel quid and has been linked to oral cancer causation (17). Although the precise mechanism of action remains to be delineated, beneficial effects on cell differentiation, immunologic function and interaction of cells with growth factors, such as epidermal growth factor, are all potential mechanisms.

Of particular relevance to oral cancer is the ability of these agents to produce profound inhibition of the formation of precancerous and cancerous lesions in the hamster cheek pouch model of oral carcinogenesis (18–22). This model was first described in 1954 and has been extensively used for screening potentially useful agents. The retinoids, β-carotene, vitamin E, and vitamin C have all produced marked inhibition of tumor formation in this model (18–22).

CLINICAL INTERVENTION TRIALS

Clinical trials in humans have used three types of target lesions as their endpoints in order to study the ability of putative chemopreventive agents to influence the carcinogenic process (Table 1).

Intermediate Marker Studies (Micronuclei)

There is intense interest in defining surrogate markers for cancer risk, termed intermediate markers. Use of these markers would make it feasible to conduct trials demonstrating changes in a favorable direction. Conceptually these markers serve as substitute endpoints in place of cancer incidence. Although numerous markers have been proposed in the recent literature, currently there is no single one that has been adequately tested for validity or quality controlled for methodology.

Among the postulated markers for oral cavity lesions, the frequency of micronucleated exfoliated oral mucosal cells is one that has had the most extensive clinical application. Micronucleated oral cells have been postulated to result from genotoxic damage induced by carcinogens (23). The test suffers from numerous technical problems, not the least of which is that it is based on visual identification of micronuclei in smears that contain numerous other cytologic abnormalities. It is perhaps meaningful when applied to populations that demonstrate a clearly increased frequency of micronuclei compared with controls. In areas where carcinogenic exposure is intense, such as the betel quid and tobacco chewers in

TABLE 1
TARGET LESIONS IN HUMANS

1. Intermediate markers: micronuclei
2. Premalignant lesions: leukoplakia
3. Second primary malignancies

South Asia, the reported frequency of micronucleated cells is several-fold higher, about 4 to 5%, than in the "normal" population where it is usually less than 1%. In populations with lower oral cancer risk, such as the tobacco chewers among the Inuits in Canada, the frequency is increased, but only to about the 1 to 2% range. In other situations, including most populations in the United States, where smoking tobacco predominates as the risk factor for oral cancer, the percentage of micronucleated cells is considerably lower.

In support of a protective role for β-carotene, intervention studies conducted in populations with definitely increased micronuclei frequency have all demonstrated a significant decrease in micronuclei frequency with β-carotene supplementation (24–26, Table 2). Some of these study patients had premalignant conditions, i.e., leukoplakia, while others simply chewed tobacco, but were free of lesions (24). In fact, in the Canadian Inuit population trial, none of the patients had leukoplakia despite chewing (24). The reason for this is unclear, although the authors speculate that there are possible nutritional etiologies. Nevertheless, this feature of the study is emphasized because this trial has recently been misquoted as showing a 0% response rate for β-carotene in oral leukoplakia (27). Since leukoplakia did not exist in the study population to begin with, the basis of such an interpretation is unclear. The trial itself was a positive study demonstrating reduction in micronuclei frequency, which was its objective.

At the University of Arizona, ongoing studies are evaluating micronuclei frequency in conjunction with a β-carotene intervention trial in oral leukoplakia (see below). Owing to the low frequency noted among Western, non-tobacco-chewing populations, we have conducted quality control experiments to evaluate the variability inherent in the method in order to determine its applicability. Using triplicate counts of the same slides by the same laboratory technician, the average difference in successive counts was found to be 3 per 1000 cells (range 0–5). This must be taken into account when interpreting trials in populations whose baseline frequency is often in the range of 0 to 10 per 1000 cells (0–1%). How the variability will influence the applicability of the test in this setting remains to be fully determined.

Premalignant Lesions: Oral Leukoplakia

Oral cavity premalignant lesions are generally classified as leukoplakia or erythroplakia. Their reversal is an important strategy in identifying agents that might be useful in preventing oral cancer. Leukoplakia is defined as a whitish patch that cannot be removed by scraping and cannot be classified clinically or microscopically as any other disease entity. It should be emphasized here that the ultimate

TABLE 2
β-Carotene Supplementation and Micronuclei Frequency

Reference	Study population	Dose	Result
26	Philippines	180 mg/wk	↓
25	India	180 mg/wk	↓
24	Canada (Inuits)	180 mg/wk	↓

goal of targeting leukoplakia in chemoprevention studies is to find interventions that will be applicable to preventing oral cancer and not merely eradicating premalignant lesions. Oral leukoplakia, like the vast majority of premalignant lesions, such as colon polyps, is not lethal or even morbid by itself and is generally associated with rather low rates of transformation to cancer (28). The transformation rate is higher if certain clinical features, such as a reddish component (erythroplakia) or speckled leukoplakia, are present (28). Similarly, if high-grade dysplasia is found histologically the transformation rate increases. Nevertheless, such clinically "serious" lesions are relatively rare and, when present, demand more aggressive treatment. Furthermore, premalignant lesions such as leukoplakia usually do not produce symptoms and therefore do not demand treatment purely on this basis. Hence, care must be taken to avoid administering toxic agents to subjects with premalignant lesions who are participants in chemoprevention studies designed to find strategies for cancer prevention. Use of agents with toxicity is only justified if the objective is to develop a treatment that is applicable to a small minority of cases with more serious lesions that are not amenable to current treatments such as reduction in local irritants, surgical excision, or cryosurgery. Examples of this for oral leukoplakia would be a treatment such as topical bleomycin which has been shown to be effective (29). However, if the objective is to target leukoplakia as a lesion in which to screen agents for the prevention of oral cancer, then toxicity is unacceptable.

Retinoid trials in leukoplakia. The activity of retinoids in reversing the hyperkeratosis associated with leukoplakia has been known since the early 1960s, when high doses of vitamin A was shown to be effective (30). Later, in the 1970s, the activity of synthetic retinoids, such as 13-*cis*-retinoic acid, *trans*-retinoic acid, and etretinate was demonstrated with an approximately 60 to 90% response rate (31–33). However, toxicity was again considerable at the doses employed.

The retinoid that has received the most attention is 13-*cis*-retinoic acid. A trial conducted in the 1980s confirmed its activity in leukoplakia, but again demonstrated major toxicity (34). Trials with lower doses of 13-*cis*-retinoic acid (0.5 mg/kg/day) are being planned. Nevertheless, it should be pointed out that 13-*cis*-retinoic acid has been shown to be toxic at doses much lower than this, i.e., 0.1 to 0.2 mg/kg/day (35, 36). Although this has been known from dermatologic studies for many years, a recent, well-controlled skin cancer prevention trial in which toxicity monitoring was carefully performed, also noted considerable toxicity at a dose of 10 mg per day, i.e., 0.1 to 0.2 mg/kg/day (37). Thus, a role for this agent in oral cancer prevention is unlikely.

β-*carotene and anti-oxidant nutrients.* In view of the toxicity of retinoids and the considerable preclinical evidence in support of a possible preventive role, attention has recently focused on the use of β-carotene, vitamin E, and vitamin C in oral leukoplakia. In contrast to the retinoid studies, trials with these agents were started more recently. Studies using vitamins E and C are currently ongoing with results likely to be available shortly. Studies with β-carotene are further along, with early phase II trials having been completed. The β-carotene trials are summarized in Table 3, which also includes a recent 13-*cis*-retinoic acid trial for comparison.

TABLE 3
TRIALS IN ORAL LEUKOPLAKIA

Author	Ref	Agent	Dose	Response %		
				CR	PR	OR
Garewal	39	BC	30 mg/day	8	63	71
Toma	38	BC	90 mg/day	33	11	44
Stich	25	BC	180 mg/week	15	NS	NS
		BC + Vit A	180 mg/week + 100,000 IU/week	28	NS	NS
Hong	34	13-cRA	1–2 mg/kg/day	8	59	67

Note. CR, complete response; PR, partial response; OR, overall response; NS, not stated; BC, β-carotene; 13-cRA, 13-*cis*-retinoic acid.

Stich *et al.* (25) have conducted trials in India using β-carotene, alone or in combination with vitamin A. In one study, treatment consisted of β-carotene (180 mg/week) group I; β-carotene (180 mg/week) plus vitamin A (100,000 IU/week) group II; and placebo group III given twice weekly for 6 months. Only the *complete* remission rate was reported. This was about 15% in group I, 28% in group II, and only 3% in group III. Development of new leukoplakias was strongly inhibited in group II (8%) and inhibited to a lesser degree in group I (15%) in comparison to group III (21%).

In a recent trial which has been reported in abstract form only, Toma *et al.*, using 90 mg/day of β-carotene found a 44% overall response rate (38).

Our group has conducted trials using β-carotene as a single agent. An initial pilot study produced a response rate of 71% (8% complete, 63% partial) in 24 patients treated with β-carotene at a dose of 30 mg/day (39). Biopsies of all lesions were done at entry, with dysplasia present in 11 of the 24 cases. This pilot experience has been followed by an ongoing multicenter trial involving the University of Arizona, University of California (Irvine), and University of Connecticut (Farmington). The trial design is shown in Fig. 1. The objectives of this study are to confirm the previous response rate in a multicenter setting as well as to establish whether maintenance β-carotene will be successful in keeping subjects in remission. Previous experience suggests that when intervention agents are discontinued, leukoplakic lesions tend to recur. Nevertheless, it remains to be demonstrated whether nontoxic treatments can maintain these lesions in remission. This trial also involves monitoring a variety of potential intermediate endpoints including micronuclei frequency, labeling index, and flow cytometric abnormalities.

A preliminary analysis of the feasibility phase of this study has been completed. The principal objective of the feasibility phase was to demonstrate whether the response rate noted in the pilot study could be reproduced in a multicenter trial to justify continuing with the full study. As of March, 1992, 32 patients had completed the initial 6 month "induction" phase with 19 responses (59% response rate, 95% confidence interval 40 to 76%). Another 10 patients remained stable, while 3 had disease progression.
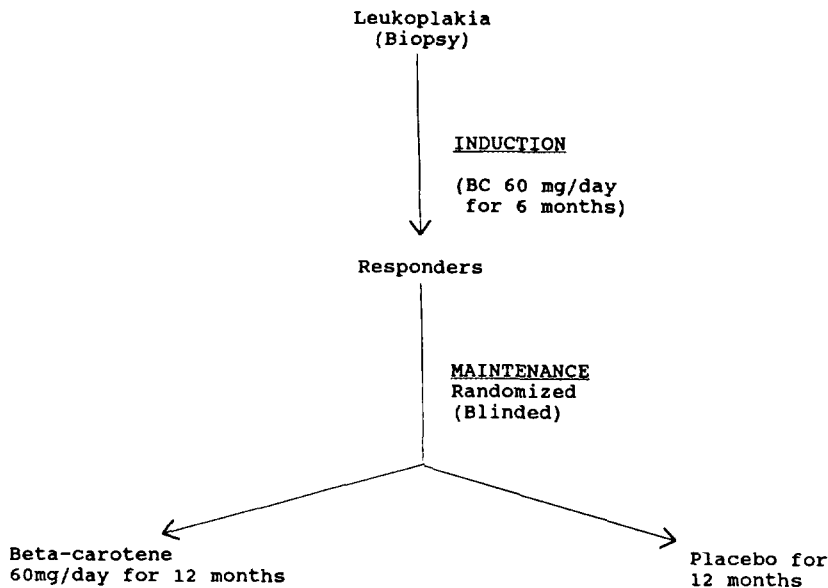
Leukoplakia
(Biopsy)

INDUCTION

(BC 60 mg/day
for 6 months)

Responders

MAINTENANCE
Randomized
(Blinded)

Beta-carotene
60mg/day for 12 months

Placebo for
12 months

FIG. 1. Design of ongoing β-carotene study in oral leukoplakia. All patients receive β-carotene at 60 mg/day (induction) and responders are randomized to continue β-carotene or placebo for 12 months (or until progression).

We are studying the pharmacology and tissue distribution of β-carotene as a component of this trial. A procedure for reliably collecting oral cavity cells and assaying carotenoid content has been developed. Initial results show significant accumulation of β-carotene in exfoliated oral cavity cells with suggestions of a correlation between plasma levels and oral cavity cell levels. A significant increase in plasma β-carotene levels was observed as expected (0.217 ± 0.180 μg/ml at baseline versus 4.247 ± 1.895 μg/ml at 6 months, $P = 0.0001$). β-Carotene levels in exfoliated buccal mucosal cells were also significantly increased (1.09 ± 0.80 ng/million cells at baseline versus 22.25 ± 10.28 ng/million cells at 6 months, $P = 0.004$). Plasma alpha-tocopherol levels did not change significantly (10.81 ± 3.23 μg/ml versus 11.43 ± 3.58 μg/ml, $P = 0.53$) nor did exfoliated cell alpha-tocopherol levels (94.17 ng/million cells versus 118.8 ng/million cells, $P = 0.55$). These data from the pilot phase will be updated as the study progresses.

Prevention of Second Primary Tumors

Patients with squamous cell head and neck cancer have an increased incidence of second primary tumors, either synchronous or metachronous, of the upper aerodigestive tract (40–43). If habits such as tobacco use continue, the incidence of second primaries approaches 3 to 5% per year. In fact, in patients who present with early cancers, the second primary cancer is often the cause of their mortality, since a high cure rate of the primary tumor is usually achievable. Hence, strategies for diminishing the incidence of second primaries are of importance to this patient group. Agents active in reversing premalignant lesions might indeed prove

to be effective in reversing the field defect thought to underlie the increased incidence of second malignancies. Nontoxic agents are to be preferred in this setting also, since prolonged treatment is anticipated and many of these patients will have received radiation treatment resulting in oral mucosal injury. Therefore, their tolerance of mucocutaneous toxicity associated with the retinoids is often compromised. Nevertheless, a somewhat greater degree of toxicity is acceptable in this population, since cancer risk is considerably higher than in the usual patient with leukoplakia. A recent report of an adjuvant trial using high-dose 13-*cis*-retinoic acid in patients with all stages (I to IV) of cancer showed a remarkable reduction in second primary malignancies, although it failed to show a significant adjuvant affect, i.e., reduction in recurrence (44). Toxicity was a major problem leading to premature discontinuation of therapy in about one-third of the study patients even though all had had serious cancers to begin with.

Second primary prevention trials using lower doses of synthetic retinoids or β-carotene are currently either ongoing or planned. In the Southwest Oncology Group we are planning a trial that will only include early stage (I or II) cancer patients who have entered remission after their primary treatment. They will be randomized to receive β-carotene versus a control group receiving placebo (Fig. 2).

Future Directions

Early results have been extremely encouraging, suggesting that there may be a real potential for using nontoxic chemopreventive agents in preventing oral cavity cancer. Initial studies with β-carotene have yielded positive results. Whether these nontoxic agents are as active as the toxic retinoids is an area of theoretical interest, but, from the practical standpoint, this may not be so critical. Even if these agents are half as active, they are much more likely to be useable as chemopreventive modalities than the toxic compounds. Results of ongoing studies with vitamin E are awaited as are the results of the longer duration β-carotene trials.

There is also a need to test combinations of agents. In this context, the early results of an ongoing trial using a combination of β-carotene, vitamin E, and vitamin C were reported in abstract form (45). A response rate of about 60% is being observed (45). Particularly important will be an analysis of the nature of the
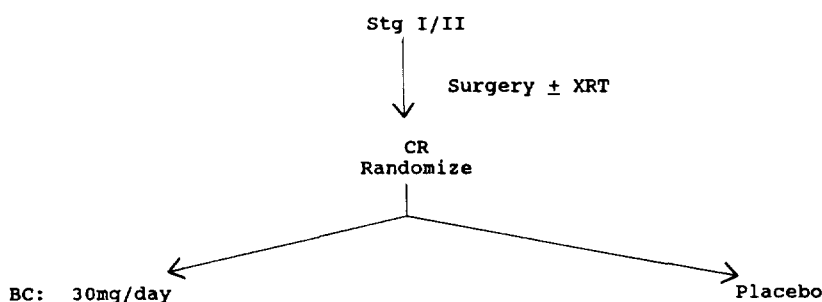
FIG. 2. Design of proposed Southwest Oncology Group trial for prevention of second primary malignancies in patients successfully treated for their initial cancer.

responses, i.e., the distribution of complete versus partial response, to assess whether the combinations are more active than the single agents. Since the pilot, single institution studies have been encouraging, multi-institutional or cooperative group studies need to be conducted in order to verify the results in a more heterogeneous, but perhaps more clinically applicable, setting.

These results in oral cavity cancer prevention need to be considered in the context of other trials assessing these agents for preventive effect against other life-shortening diseases such as cardiovascular disease (46). Clearly, the potential for widespread applicability of such agents will be greatly enhanced if the results of such trials indicate a beneficial effect for the prevention of several major diseases. The encouraging early results are a reason to be optimistic that this may indeed turn out to be the case. We hope that the results of ongoing trials will bear this out in the not too distant future.

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