Premalignant lesions: role of antioxidant vitamins and $\beta$-carotene in risk reduction and prevention of malignant transformation$^{1,2}$

Vishwa N Singh and Suzanne K Gaby

ABSTRACT Epidemiological studies have shown that diets rich in one or more antioxidant nutrients may reduce the risk of cancers of the lung, uterine cervix, mouth, and gastrointestinal tract. Study of premalignant lesions offers a comparatively expedient approach to identifying and evaluating the efficacy of the cancer chemopreventive components of foods. Some recent findings suggest roles for $\beta$-carotene and/or vitamin C in reversing or reducing the risk of cervical dysplasia and oral leukoplakia. There are some indications that vitamin C and $\beta$-carotene may reduce the risk of atrophic gastritis and gastric cancer. Additional epidemiological and molecular biology studies and clinical intervention trials using premalignant lesions as the marker of specific cancer risks should become an important component of future research in the area of cancer chemoprevention. Am J Clin Nutr 1991;53:386S–90S.

KEY WORDS Ascorbic acid, cervical, diet, dysplasia, gastric, leukoplakia, metaplasia, precancerous, tocopherol

Introduction

The transformation of a normal cell into a neoplastic cell is considered to proceed through three phases: initiation, promotion, and progression (Fig 1). Initiation is the permanent alteration of the genetic information (mutation) in the cell. Although initiation does not necessarily lead to cancer, it is indicative of increased risk of neoplasia.

The second step, promotion, can be a very slow process in humans. It has been estimated, eg, that the latency period between initiation and the appearance of prostatic cancer could be $>40$ y (1). The latency period for many common cancers is 10–30 y. Because promotion is generally reversible, and because it takes place over a long period of time, it is a very attractive target for intervention with cancer chemopreventive agents. Potentially, a premalignant lesion can be either made to regress, or its conversion to neoplasia significantly slowed, so that cancer does not develop in the lifetime of the subject. There is also a large time window during which one can identify a premalignant lesion.

Broadly speaking, precancerous lesions could include a whole range of events leading to the development of malignancy, such as the formation of DNA adducts, micronuclei, and sister chromatid exchanges. Conventionally, however, pathologists use the term “premalignant lesions” for abnormal growth of tissue with altered nuclei, such as dysplasia and carcinoma in situ. A suggestion has been made to define premalignant lesions as “atypical intraepithelial proliferation with varying degrees of architectural and nuclear atypia” (2). Premalignant lesions and the cancer sites with which they are associated are listed in Table 1.

Antioxidant vitamins and premalignant lesions

Evidence linking nutrition and cancer risk continues to accumulate. Doll and Peto (3) suggested that perhaps 35% (range 10–70%) of all cancer mortality in the United States could be attributable to dietary factors. Wynder and Gori (4) offered the figure of 40% in men and 60% in women. Although certain nutrients, such as fat, seem to contribute to the risk of some types of cancer, high consumption of foods rich in other substances, such as fiber, carotenoids, and some vitamins and minerals has been associated with reduced risk of certain cancers. Despite an overwhelming body of epidemiological evidence establishing these associations, the cancer inhibiting mechanism(s) of action of these nutrients is not yet fully understood. One promising hypothesis attempting to explain the potential chemopreventive actions of vitamins C and E and of $\beta$-carotene is their antioxidant and, in the case of $\beta$-carotene, singlet oxygen quenching properties.

The study of premalignant lesions, epidemiologically shown to be associated with eventual development of cancers, offers an ideal opportunity for identification and evaluation of efficacy of cancer inhibitory agents. Compared to large-scale intervention trials with cancer as the endpoint, a study of premalignant lesions in a selected population can be economical and of shorter duration. In addition, a clear understanding of premalignant lesions can help in understanding the process of carcinogenesis. Currently, epidemiological and biochemical studies of premalignant lesions are limited in number. However, cervical dysplasia and oral leukoplakia have been the focus of some recent studies.

Cervical dysplasia

Cervical dysplasia, a premalignant lesion of the uterine cervix, is associated with increased risk of cervical cancer (5). It is gen-

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eraly agreed that early detection of this precancerous lesion may significantly reduce the incidence and mortality from cervical cancer (5).

In a number of epidemiological studies (Table 2), significantly increased risks of cervical dysplasia/carcinoma in situ have been found to be associated with low dietary intakes and/or low blood levels of \( \beta \)-carotene (7–9, 11, 12) and vitamin C (6, 8–10).

One study reported that women consuming \( \beta \)-carotene in amounts below the study median intake had a significantly increased risk of severe cervical dysplasia or carcinoma in situ (7). The risk was two to three times higher than that for women with the highest dietary \( \beta \)-carotene intake. Similarly, relative to healthy controls, serum \( \beta \)-carotene level was found to be lowered in women with preinvasive cervical lesions (11). In another study, plasma \( \beta \)-carotene levels in the highest quartile were associated with about an 80% reduction in the risk of having cervical carcinoma in situ (9). An evaluation of plasma \( \beta \)-carotene levels in patients with cervical dysplasia of varying severity, carcinoma in situ and cancer revealed a progressive decrease in the carotenoid level with increasing severity of disease (12).

A significantly lower dietary intake of vitamin C was associated with the presence of disease in a case-control study of cervical dysplasia (6). Mean daily intake was 80 mg for cases and 107 mg for control. An intake of < 30 mg vitamin C/d was estimated to carry a relative risk of cervical dysplasia ten times higher than an intake > 30 mg/d. A comparison of plasma vitamin C levels also showed that higher vitamin C status was associated with reduced risk of dysplasia (10). Subjects with uterine cervical dysplasia had plasma vitamin C levels less than one-half those of control subjects (mean levels 0.36 vs 0.75 mg/dL). This difference was highly significant.

Several epidemiological studies have also demonstrated a correlation between high intakes of foods rich in \( \beta \)-carotene (13–15) and vitamins E and C (13) and reduced risk of cervical cancer. In one study, women with an estimated intake of < 2 mg dietary

**TABLE 1**

Important premalignant lesions known to be associated with increased cancer incidence

<table>
<thead>
<tr>
<th>Premalignant lesion</th>
<th>Cancer site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinic keratosis</td>
<td>Skin</td>
</tr>
<tr>
<td>Gastric dysplasia</td>
<td>Stomach</td>
</tr>
<tr>
<td>Barrett’s esophagus, dysplasia</td>
<td>Esophagus</td>
</tr>
<tr>
<td>Cervical dysplasia, carcinoma in situ</td>
<td>Cervix</td>
</tr>
<tr>
<td>Oral leukoplakia, erythroplakia</td>
<td>Oral cavity</td>
</tr>
<tr>
<td>Bronchial metaplasia (in smokers)</td>
<td>Lung</td>
</tr>
<tr>
<td>Papillomas</td>
<td>Bladder</td>
</tr>
<tr>
<td>Colonic polyps</td>
<td>Colon</td>
</tr>
</tbody>
</table>

**TABLE 2**

Studies showing a negative association between dietary and/or blood levels of \( \beta \)-carotene and vitamin C and cervical dysplasia, carcinoma in situ, and cancer

<table>
<thead>
<tr>
<th>Diet</th>
<th>Vitamin C</th>
<th>( \beta )-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>carcinoma in situ</td>
<td>Romney et al (8)</td>
<td>Romney et al. (8)</td>
</tr>
<tr>
<td>Blood</td>
<td>Romney et al (10)</td>
<td>Harris et al (11)</td>
</tr>
<tr>
<td></td>
<td>Brock et al (9)</td>
<td>Palan et al (12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brock et al (9)</td>
</tr>
<tr>
<td>Diet</td>
<td></td>
<td>LaVecchia et al (15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Verreault et al (13)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orr et al (16)</td>
</tr>
</tbody>
</table>

* Becomes insignificant when corrected for confounding factors.
β-carotene/d were found to have a sixfold greater risk of develop-
ing cervical cancer, as compared with those consuming > 3 mg/d (15). Similarly, high blood levels of β-carotene and vitamin C have been found to be associated with lower incidence of cervical cancer (16). Another study found low serum levels of carotene in cervical cancer patients, but the difference from controls was not statistically significant (17). In that study, however, the serum level of total carotene rather than β-carotene was measured. More recently, measurements of both plasma β-carotene and total carotene showed that the β-carotene level of women with in situ cervical cancer was markedly lower than that of matched controls (9). Yet, as in the previous study, the plasma level of total carotene did not differ significantly between cases and controls.

In studies where the intakes of carotenoids and preformed vitamin A could be evaluated separately, only the carotenoid intake was associated with reduced risk of cervical cancer/carcinoma in situ (9, 13–15). In none of these studies was performed vitamin A intake associated with a reduction in the risk of cervical cancer. Similarly, measurement of β-carotene and retinol in the plasma showed a markedly lower plasma level of β-carotene in the cancer-dysplasia patients in comparison with controls, but vitamin A was found to be unchanged. These findings suggest that the protective role of β-carotene is independent of its provitamin A role (Table 3).

The epidemiological evidence for a protective effect of antioxi-
dant nutrients, particularly β-carotene, against cervical dys-
plasia (as well as cancer), makes this an interesting premalignant lesion to be studied in intervention trials. Furthermore, the fact that Papanicolaou smear screening is done regularly (and is not associated with any morbidity) make such studies highly prac-
ticable.

Precancerous lesions of the oral cavity

This topic is discussed in detail elsewhere in these proceedings (18, 19). Briefly, however, it should be noted that supple-
mentation with carotenoids as well as vitamin A has been associated with a reduction in the frequency of micronucleated cells in the buccal mucosa of betel nut chewers (20, 21) and with complete or partial regression of oral leukoplakia, a precancerous lesion (22, 23). In fact, case-control studies have shown that con-
sumption of carotene-rich vegetables and vitamin C–rich fruits markedly reduced risk of oral cancer (24–26).

Atrophic gastritis and gastric cancer

The development of gastric cancer is considered to proceed through a series of stages (27). In the initial stages, the mucosa undergoes various degrees of gastritis. These changes render the tissue more susceptible to the damaging action of genotoxic agents such as N-nitroso compounds. Following the development of intestinal metaplasia of the stomach, further damage leads to a precancerous lesion (dysplasia) and finally gastric cancer.

It has been repeatedly demonstrated that nitrates and nitrates can be converted to carcinogenic nitrosamines and nitrosamides and that vitamins C and E can block these conversions in vitro and in vivo (28). In addition, supplementation with vitamin C as been associated with decreased mutagenicity of gastric juice (29, 30). This provides a strong scientific rationale for vitamins C and E reducing the risk of gastric cancer.

Indeed, several epidemiological studies have shown reduced risk of gastric cancer in the populations consuming high levels of foods rich in vitamin C (31) (Table 4). For example, it has been consistently found that gastric cancer incidence is associated with low intakes of fruits and vegetables, particularly those that are rich in vitamin C (33–35, 37, 42, 43). Similarly, populations with high risk of this cancer have been shown to consume less fresh fruit and vegetables that those with low risk (39, 44). In a prospective study, gastric cancer was associated with low plasma levels and low dietary intake of vitamin C and β-carotene (34).

Atrophic gastritis (AG) is not itself a precancerous condition, but may develop to metaplasia and dysplasia (35). In one study, a history of chronic gastritis and gastric ulcer was associated

### TABLE 3
Risk of cervical dysplasia and cancer: comparative effects of β-carotene, total carotenes, and retinol

<table>
<thead>
<tr>
<th>Study</th>
<th>Reduced risk</th>
<th>No effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical dysplasia and carcinoma in situ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brock et al (1)</td>
<td>Plasma β-carotene</td>
<td>Dietary retinol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dietary total carotenes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasma retinol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasma total carotenes</td>
</tr>
<tr>
<td>Palan et al (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>Plasma β-carotene</td>
<td>Plasma retinol</td>
</tr>
<tr>
<td>LaVecchia et al (15)</td>
<td>Dietary β-carotene</td>
<td>Serum total carotenes</td>
</tr>
<tr>
<td>Heinonen et al (17)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 4
Vitamin C and gastric cancer risk: epidemiology

<table>
<thead>
<tr>
<th>Study</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graham et al (32)</td>
<td>Frequency of citrus fruit or juice consumption was the same in cases and control subjects</td>
</tr>
<tr>
<td>Bjelke (33)</td>
<td>In people below age 60 y, a high intake of vitamin C was associated with reduced risk</td>
</tr>
<tr>
<td>Stahelin et al (34)</td>
<td>Low vitamin C intake and below average citrus fruit consumption in cases, compared with control subjects</td>
</tr>
<tr>
<td>Correa et al (35)</td>
<td>Strong protective effect of fruit and dietary vitamin C</td>
</tr>
<tr>
<td>Risch et al (36)</td>
<td>Some protective effect from citrus fruit intake, but less effect of total vitamin C</td>
</tr>
<tr>
<td>Trichopoulos et al (37)</td>
<td>Less frequent consumption of lemons and oranges among cases</td>
</tr>
<tr>
<td>LaVecchia et al (38)</td>
<td>Fresh citrus fruit protective</td>
</tr>
<tr>
<td>Burr et al (39)</td>
<td>Low intake of fruit and low plasma ascorbate in subjects in a town with a high incidence of gastric cancer, relative to subjects in a low-risk town</td>
</tr>
<tr>
<td>You et al (40)</td>
<td>Greater intake of vitamin C associated with reduced risk</td>
</tr>
<tr>
<td>Buiatti et al (41)</td>
<td>Reduced gastric cancer risk associated with increasing intake of citrus fruits</td>
</tr>
</tbody>
</table>
with two- to threefold increased risk of gastric cancer (40). Several epidemiological studies have observed a high incidence of AG and/or intestinal metaplasia of the gastric mucosa in populations with a high risk of gastric cancer (27, 39, 45). The presence of AG may contribute to cancer further by creating an environment conducive to the growth of nitrate-reducing bacteria.

As compared to normal controls, subjects with chronic gastritis and, especially, hypochlorhydria were found to have significantly lower concentrations of vitamin C in gastric juice (30, 46, 47). The lowest levels were found in individuals considered to be at greatest risk for developing gastric cancer. Burr et al (39) found a lower average plasma ascorbate level among men in an area with a high incidence of atrophic gastritis and a high stomach cancer death rate, relative to a population in a low-risk area. In a case-control study, dietary vitamin C was found to be protective in reducing risk of chronic atrophic gastritis (48).

In cases of gastric dysplasia, serum levels of carotenoids were low in men and women, and vitamin E levels were lower in men, in comparison with healthy controls (42). However, it is difficult to establish a cause and effect relationship from these findings.

Antioxidant nutrients, particularly vitamin C, appear to be associated with reduced risk of gastrointestinal cancers. Precancerous lesions of the gastrointestinal tract would seem to be especially susceptible to the promoting or antineoplastic effects of ingested substances, thereby representing a fruitful area for intervention research.

Bronchial metaplasia

One of the most consistent epidemiological findings in nutrition research has been an association between β-carotene intake and status and reduced lung cancer risk, particularly risk of squamous cell cancer (49). Similarly, studies have shown that high intake/status of vitamins C (31, 50) and E (51, 52) may also be protective against lung cancers.

Bronchial metaplasia, a potentially premalignant lesion, is considered to be an indicator of lung cancer risk in smokers. Thus, study of bronchial metaplasia could provide a useful marker for evaluation of lung cancer risk in clinical intervention trials with limited cost and shorter duration than measurement of lung cancer as the endpoint.

Large-scale NCI-sponsored intervention trials with subjects at high risk of developing lung cancer are currently underway using β-carotene (53). One of these studies will measure an intermediate endpoint in carcinogenesis, sputum atypia. However, additional antioxidant nutrient intervention trials in populations with bronchial metaplasia would be prudent. Such studies are feasible and have in fact shown regression of metaplasia after treatment with other agents: retinoids (54, 55) and combined folate and vitamin B-12 (56). Similarly, in an experimental model in vitro asbestos-induced squamous cell metaplasia of the hamster trachea was made less severe by incubation in a high concentration of ascorbic acid (57).

References

19. Stich HF. Remission of precancerous lesions in the oral cavity of tobacco chewers and maintenance of the protective effect by β-carotene and vitamin A. Am J Clin Nutr 1991;53(suppl);2985-304S.