Correlates of Mutagen Sensitivity in Patients with Upper Aerodigestive Tract Cancer

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Abstract

Although tobacco and alcohol use are the major determinants of upper aerodigestive tract carcinogenesis, not all smokers develop cancer. This phenomenon is due to individual variations in genetic susceptibility to carcinogens. One explanation may be differences in mutagen sensitivity (as measured by the in vitro bleomycin-induced mutagen sensitivity assay) in patients with squamous cell carcinoma of the upper aerodigestive tract. Antioxidant supplementation has also been shown to decrease DNA damage and thus may also inhibit carcinogenesis. In this study, we examined whether smoking, alcohol intake, and dietary antioxidant intake were correlated with mutagen sensitivity. The 612 patients evaluated are part of an ongoing multicenter Phase III trial of 13-cis retinoic acid for the prevention of second primary tumors. We found that patients with pharyngeal cancers were more likely than patients with oral cavity or larynx cancers to be mutagen sensitive. There were no significant differences in the distribution of mutagen sensitivity by sex or alcohol use. Never smokers were significantly more likely (61.1%) to be mutagen sensitive than current smokers (35.6%). Dietary consumption of the micronutrients α-carotene, β-carotene, lutein, lycopene, and vitamin C was not correlated with mutagen sensitivity. Therefore, we suggest that mutagen sensitivity is an independent marker of cancer risk not affected by other known risk factors.

Introduction

The etiologic role of tobacco and alcohol exposure in upper aerodigestive tract cancers is unquestioned. However, only a fraction of exposed individuals will develop neoplastic lesions. Genetically determined modulation of environmental exposures is an attractive possible mechanism for the variation in host susceptibility (1). Therefore, the concept of genetic susceptibility to carcinogenic exposures must be factored into the risk-assessment process.

Hsu et al. (2) have developed an in vitro mutagen sensitivity assay based on the quantification of bleomycin-induced chromatid breaks in short-term cultured lymphocytes to measure human susceptibility to environmental carcinogens. In two previous retrospective case-control studies, we demonstrated that bleomycin-induced mutagen sensitivity (either as a continuous or dichotomous variable) was an independent risk factor for head and neck cancers, after adjustment for tobacco and alcohol use, with adjusted odds ratios of 4.3 and 2.5 (3, 4). Our data also showed that mutagen sensitivity was a significant predictor of multiple primary cancer risk subsequent to an initial head and neck cancer (5, 6).

Considerable epidemiological evidence suggests that carotenoids are associated with a decreased risk of epithelial cancers. Because antioxidant supplementation has been shown to decrease endogenous oxidative DNA damage in the lymphocytes of smokers and nonsmokers (7), it is important to assess the correlation (if any) between the dietary intake of these micronutrients and the level of induced mutagen sensitivity.

This report presents baseline data on smoking status, bleomycin-induced mutagen sensitivity, and micronutrient intake from patients with upper aerodigestive tract cancers enrolled in an ongoing multicenter Phase III placebo-controlled trial of 13-cis retinoic acid for the prevention of second primary tumors. The purpose of this analysis was to evaluate the association of baseline mutagen sensitivity values with smoking status, clinical variables, and self-reported dietary micronutrient and alcohol intake.

Materials and Methods

Each eligible patient for the chemoprevention trial was required to have had a confirmed diagnosis of squamous cell carcinoma of the upper aerodigestive tract (oral cavity, pharynx, or larynx) and presented with stage I or II disease (as defined by the American Joint Committee Staging criteria), diagnosed and treated within the previous 3 years. The patients were identified from M. D. Anderson Cancer Center, the Radiation Therapy Oncology Group, and the Clinical Community Oncology Program. These data are derived from baseline examinations for entry into the trial that took place from November 1991 to July 1995.

Questionnaires were administered at entry to the trial by a research nurse were the primary method of collecting risk-factor data. These questionnaires comprehensively deter-
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Each patient. This time frame for baseline dietary data collection was determined to capture ongoing measures of usual intake while they are being followed in the trial. The methods used for the development of the food frequency questionnaire are well documented (8–10). The foods included on the food list were selected from the published literature, and appropriate additions were made to the final food list, which had 126 foods (11–13) and consisted of almost all (93%) of the food items on the Health Habits and History Questionnaire (8). The changes in the questionnaire included the addition of Hispanic foods, separation of fruits and vegetables to allow the analysis of individual carotenoids, and the addition of nutritional supplements. Validity and reliability studies in several populations have documented the utility of the Health Habits and History Questionnaire for use in American populations (9, 10). The patients were also asked to report their use of vitamin, mineral, and fiber supplements.

Ten ml of blood were drawn into heparinized tubes for cytogenetic analyses and express mailed or hand delivered to our laboratory at the M. D. Anderson Cancer Center. The methodology for the bleomycin assay was described in detail previously (14). Briefly, 1 ml of whole blood was cultured in 9 ml of RPMI 1640 blood medium (Gibco, Grand Island, NY) with 20% FBS and 12.5 ml/liter of phytohemagglutinin (Murex Diagnostic). After 67 h of cultivation, bleomycin (final concentration of 30 μg/ml; Blenoxane; Nippon Kayaku Co., Ltd.) was added to the culture. Four h later, 0.04 μg/ml colcemid was added to induce mitotic arrest. At 72 h, the cells were harvested by conventional cell harvesting procedures. The cells were treated with hypotonic KCl solution for 12 min, fixed, washed with freshly prepared Carnoy’s mixture (methanol:acetic acid, 3:1 v/v) and air dried on coded wet slides. The slides were then stained with Giemsa solution. For each sample, the chromosome breaks in 50 metaphases were counted, and the results were averaged to determine the number of breaks/cell. A minimum of 50 well-spaced metaphases per sample was read under a ×100 dry objective to determine the frequency of spontaneous aberrations. Gaps or attenuated regions were disregarded (2). We have demonstrated previously that scoring a minimum of 50 metaphases yields an acceptable reliability (15).

Descriptive statistics, including percentages, mean, and SD were reported when appropriate. Patients were classified into quintiles to study the age effect. χ² statistics were computed to assess the correlation between the categorical variables. Logistic regression analysis was applied for adjusting the effect of multiple covariates on mutagen sensitivity. All Ps reported were based on two-sided tests. The trial is still ongoing and blinded as to treatment arm.

Results

Baseline epidemiological data at entry into the trial have now been evaluated on 612 patients. Of these, 134 (21.9%) were current smokers, 113 (18.5%) had quit within the previous year (recent quitters), 292 (47.7%) had quit more than 1 year previously (long-term quitters), and 73 (11.9%) reported that they had never smoked (never smokers; Table 1). The oldest stratum of patients was significantly more likely to be long-term quitters (62.1%) than was the youngest stratum, of whom only 40.8% were long-term quitters (P < 0.001). The highest proportions of never smokers were in the youngest (22.2%) and oldest (20.4%) age strata.

Although there were no sex differences in the proportions of current smokers, only 10% of the male patients were never smokers, compared with 20% of the female patients (Table 1). African-American patients were more likely to be current smokers (38.9%) than were subjects of other ethnic groups, although these differences were not statistically significant due to the small numbers in some strata. The self-reported smoking status at baseline was validated by measuring serum cotinine.
levels (data not shown). Over 95% of the self-reported never and current smokers were validated. Ninety percent of long-term quitters were also biochemically confirmed to be non-smokers. However, only 30% of those who reported quitting within the past 3 months were biochemically confirmed to be quitters. Forty-three (7%) of the patients reported using chew-pipes (data not shown).

Break/cell data were available at the time of this report on a subset of these 612 patients (n = 490) in this ongoing chemoprevention trial. The overall percentage of sensitive individuals (those having 1.0 break/cell) was 41.8% (Table 2). Patients with pharyngeal cancers and patients younger than 40 years of age were more likely than patients with other subsites of cancer and older patients, respectively, to be mutagen sensitive (Table 2). The lowest breaks/cell were noted for patients of stage I cancers and older patients, respectively, to be mutagen sensitive.

There was a tendency (P = 0.05) for patients with stage II disease to exhibit higher mean breaks/cell (0.99) compared with 0.90 for stage I patients. There were also significant differences in the distribution of mutagen sensitivity by prior treatment modality (Table 2) Patients who were surgically treated were significantly more likely to exhibit lower breaks/cell (mean 0.90 for stage I patients. There were also significant differences in the distribution of mutagen sensitivity by smoking status. It is interesting that 61% of never smokers were mutagen sensitive compared with only 35.6% of current smokers, and 40.2% and 41.9% for long-term and short-term quitters, respectively (P = 0.02).

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cant inverse association between years smoked and lycopene intake ($P = 0.04$; data not shown). The prevalence of current alcohol use at baseline was 48%. Men, on average, drank twice as much alcohol as women (21 versus 11 g/day; tabular data not shown). The prevalence of vitamin use at baseline was 53.9%. Patients who reported taking vitamin supplements tended to have higher mean dietary intakes of vitamin C than nonusers did, (188 versus 159 $\mu$g, $P = 0.04$), respectively. These differences probably reflect a greater health awareness in the former group. There was a similar, but not statistically significant, pattern for lutein intake. Our eligibility requirements precluded participants from taking more than 25,000 I.U. of vitamin A or 30 mgs or more $\beta$ carotene, so that supplement intake was generally in the form of multiple vitamins.

There were no significant associations between baseline mean intake of any of the micronutrients and breaks/cell values (Table 3). In fact, individuals who were mutagen sensitive reported higher mean intakes of $\alpha$-carotene, $\beta$-carotene, and lutein than did nonsensitive patients.

**Discussion**

Like lung cancers, upper aerodigestive tract cancers can be considered paradigms of tobacco-induced diseases. Although approximately 90% of patients with upper aerodigestive tract cancers have ever smoked, a substantial proportion do quit either at diagnosis or during therapy. Only 22% of our patients (who were diagnosed within the previous three years) were still smoking, and 19% claimed to have recently quit but exhibited high rates of recidivism based on cotinine assessments. We have shown previously that the fear of recurrent disease, the effects of treatment, and physician advice were the most common incentives for successful smoking cessation in recently treated patients (16). Contrary to commonly held perceptions, Gritz et al. (17) suggested that head and neck cancer patients are willing to modify health-endangering behavior and that diagnostically are conducive to behavioral change.

There are epidemiological data to support the hypothesis that the dietary intake of certain micronutrients has an independent protective effect on the development of head and neck cancer (18–21). A nested case-control study by Zheng et al. (22) reported an inverse association between serum carotenoids (especially $\beta$-carotene) and $\alpha$-tocopherol and cancer risk. A consistent protective effect was noted for each of the individual carotenoids, including $\beta$-carotene, cryptoxanthin, lutein, and lycopene. In a study of 871 cases in four different areas of the United States, fruit consumption had a dose-dependent protective effect on oral and pharyngeal cancer (23). A nested case-control study evaluating the nutritional correlates of second primary cancers reported nonsignificant, but similar, trends (24).

The overall mean dietary intake of vitamin C among our patients appears to be higher than that reported in NHANES $^3$ 88–91 (170 versus 100–110 mg/day; Ref. 25). The mean intake of vitamin A was also higher (1847 versus 1006–1193 RE/day among the NHANES population). However, current cigarette smokers tended to have the lowest intake. There are no population-based data on the dietary intake of the individual carotenoids to compare these data. The fat calories consumed by our population were equal to those reported in NHANES 88–91 for adults of this age range.

In vitro chromosomal analyses have been used fairly commonly to study individual sensitivity to genotoxicity and cancer risk and are gaining wider approval for formal hypothesis testing by classic epidemiological methods. In a recently published long-term cohort study of 3182 workers occupationally exposed to mutagenic agents and evaluated for chromosomal aberrations at entry into the study, Hagmar et al. (26) reported a statistically significant increase in cancer risk (relative risk = 2.1) in the highest stratum of baseline aberrations. Studies such as this confirm the value of using chromosomal aberrations in peripheral lymphocytes as a marker of cancer risk. Recently, we participated in a multicenter meta-analysis of three case-control studies of head and neck cancers that confirmed the role of bleomycin-induced mutagen sensitivity as a predictor of these cancers and demonstrated that there were no differences across institutions in the distribution of mutagen sensitivity and that age and tobacco and alcohol use did not influence the mutagen sensitivity values (27).

This chemoprevention trial included only early-stage patients and patients who had been treated previously with surgery and/or radiotherapy. Our published retrospective analysis of a different series of 298 patients with previously untreated upper aerodigestive-tract cancers of all stages at the M. D. Anderson Cancer Center (6) documented a mean break/cell value of 0.98, identical to the mean value in the present series of patients, all of whom had been treated previously. This finding suggests that prior radiotherapy does not influence the mutagen-sensitivity values. Thus, it would appear that this new series of early-stage patients is fairly representative of all patients with upper aerodigestive-tract cancer.

There were no differences in the mean breaks/cell for laryngeal patients across any treatment arm; therefore, the significantly lower-sensitivity values for patients treated surgically are intriguing. Oral cavity patients tended to have lower mutagen sensitivity scores and were most likely to be treated surgically. Furthermore, patients with oral cavity ($n = 96$) and pharyngeal cancers ($n = 7$) who were treated with surgery alone had lower mutagen-sensitivity values (0.79 and 0.67, respectively) compared with the group of patients treated either with radiotherapy alone or with combined modalities (1.11 for 60 oral cavity cancer patients and 1.09 for 31 pharyngeal cancer patients). This observation that the patients deemed most likely to be curatively treated (by surgery) had the lowest mutagen sensitivity scores needs to be confirmed in larger series of patients and by correlating mutagen sensitivity with disease outcome.

Our observation that cancer patients who were nonsmokers had a higher prevalence of mutagen sensitivity than current or former smokers suggests that nonsmokers constitute a particularly susceptible subgroup of head and neck cancer patients. This intriguing finding was also noted in our previous head and neck study (6), as well as in the case series reported by Schantz.

**Table 3** Mean (SD) of micronutrients by mutagen sensitivity

<table>
<thead>
<tr>
<th>Micronutrients (µg)</th>
<th>&lt;1.0 Break/cell</th>
<th>≥1.0 Break/cell</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-carotene</td>
<td>776 (727)</td>
<td>848 (877)</td>
<td>0.33</td>
</tr>
<tr>
<td>$\beta$-carotene</td>
<td>4337 (2992)</td>
<td>4539 (3893)</td>
<td>0.53</td>
</tr>
<tr>
<td>Lutein</td>
<td>2434 (1853)</td>
<td>2480 (2039)</td>
<td>0.80</td>
</tr>
<tr>
<td>Lycopene</td>
<td>4560 (4207)</td>
<td>4726 (5050)</td>
<td>0.51</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>169 (106)</td>
<td>165 (104)</td>
<td>0.68</td>
</tr>
</tbody>
</table>

$^3$ The abbreviation used is: NHANES, National Health and Nutrition Examination Survey.
et al. (28). Free radical oxygen is generated from a variety of both endogenous and exogenous sources besides tobacco, and individuals with the mutagen-sensitive phenotype may be less able to repair this free-radical oxygen damage. Our previous studies have shown that mutagen sensitivity was a risk factor independent of smoking status (3, 4). Furthermore, in our lung cancer studies, we have noted higher risks associated with mutagen sensitivity for former smokers compared with current smokers and lighter versus heavier smokers (29). At low- or no-exposure levels, a susceptible genotype or phenotype may be more etiologically relevant than in heavily exposed patients in whom exposure could overwhelm even a nonsusceptible genotype.

It is also important to consider the effect of antioxidants on chemicals that cause genetic damage by generating oxygen radicals. The cytotoxicity of bleomycin is due to its forming a complex with ferrous iron and molecular oxygen. The complex intercalates into DNA, principally between GT and GC dinucleotides, and releases oxygen radicals. 8-Hydroxy guanosine residues also form. DNA is degraded by the bleomycin-ferrous complex when a reducing agent (e.g., vitamin C) is added. Trizna et al. (30, 31) reported two in vitro studies demonstrating that vitamins E and C protected against bleomycin-induced breakage in a dose-dependent manner. Pohl and Reidy (32) showed a significant reduction in the number of bleomycin-breakage in a dose-dependent manner.

Historically, benzene and/or its metabolite benzene has been the most frequently studied as an exogenous mutagen and is present in the environment and in the tobacco smoke (33). Benzene is a major component of gasoline (33). In addition, benzene is a component of cigarette smoke and is a recognized eicosanoid precursor (33). Benzoic acid and benzoic aldehyde are metabolized in the liver to benzoate, a metabolite that is cleaved by hepatic esterases to form benzoic acid, a compound that may be a mutagen (34). Benzoic acid is a mutagen and may be mutagenic in the mouse and human embryo (35).

It is unlikely that head and neck cancer is caused by the interaction of a single gene and the environment; one gene may not have a strong effect but in conjunction with other genes may shift the risk profile in an unfavorable direction. Therefore, multiple susceptibility factors must be assessed to determine the true dimensions of gene environmental interactions. This knowledge is essential for the design of future epidemiological and intervention studies. In the near future, integrated multi-disciplinary programs will seek to evaluate chemopreventive strategies in cohorts of phenotypically normal individuals deemed to be genetically susceptible to cancer development.

References


Unpublished data.
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