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 \( \alpha \)-carotene, \( \beta \)-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 that were administered at entry to the trial by a research nurse were the primary method of collecting risk-factor data. These questionnaires comprehensively deter-
mined tobacco and alcohol consumption at baseline, as well as before diagnosis. A self-administered food frequency questionnaire (unquantified as to portion size) was used to determine total nutrient intake during the month before the registration of each patient. This time frame for baseline dietary data collection is appropriate for estimating usual intake in a clinical trial. Patients have subsequent dietary assessments at years 3, 5, and 7 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 24-h recall data collected during an assessment of dietary intake in South Texas populations and from patients eligible for the study who were selected from the Radiation Therapy Oncology Group clinics. Other important sources of retinol and carotenoids in the American diet were identified 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 dietary 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-spread 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 whenever 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.
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 chewing tobacco and 12 (2%) using snuff, and 11 (2%) smoked 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 with oral cavity cancers. There were no significant differences in the break/cell score for patients assessed more than 6 months after completion of radiotherapy (mean of 0.99) compared with those who had been treated more recently (0.92). There was also no correlation between break/cell score and months since radiotherapy. In a logistic regression model, with multiple covariates, smoking status was a significant predictor of the mutagen sensitivity phenotype (OR = 3.2; P = 0.01) after adjusting by stage (P = 0.03), prior treatment (surgery versus other treatments; P = 0.07), and smoking × prior treatment interaction (P = 0.89).

Current smokers had a significantly lower mean intake of α-carotene (633 μg) than did never-smokers (1017 μg) and long-term quitters (819 μg). Current smokers also reported lower mean intakes of β-carotene, lutein, and vitamin C than did never-smokers and long-term quitters, although these differences were not statistically significant. This trend was not apparent for lycopene intake. There was a statistically signifi-

| Table 2. No. (percentage) of mutagen-sensitive and -insensitive subjects by select variables |
|----------------------------- | ------ | -------- | ------- | | |
| Variable                  | n     | Mean     | Breaks/cell <1.0 | ≥1.0 | p     |
| Cancer site               |       |          |                |      |       |
| Larynx                    | 296   | 0.93     | 169 (57.1)     | 127 (42.9) | 0.69 |
| Oral cavity               | 156   | 0.91     | 95 (60.9)      | 61 (39.1)  |      |
| Pharynx                   | 38    | 1.01     | 21 (55.3)      | 17 (44.7)  |      |
| Age (years)               |       |          |                |      |       |
| ≤40                       | 23    | 1.01     | 10 (43.5)      | 13 (56.5)  | 0.40 |
| 41–50                     | 63    | 0.88     | 38 (60.3)      | 25 (39.7)  |      |
| 51–60                     | 122   | 0.91     | 76 (62.3)      | 46 (37.7)  |      |
| 61–70                     | 199   | 0.97     | 110 (55.3)     | 89 (44.7)  |      |
| 71+                       | 83    | 0.89     | 51 (61.4)      | 32 (38.6)  |      |
| Sex                       |       |          |                |      |       |
| Male                      | 397   | 0.93     | 230 (57.9)     | 167 (42.1) | 0.83 |
| Female                    | 93    | 0.94     | 55 (59.1)      | 38 (40.9)  |      |
| Cigarette smoking         |       |          |                |      |       |
| Never smoked              | 54    | 1.02     | 21 (38.9)      | 33 (61.1)  | 0.02 |
| Long-term quitter         | 239   | 0.95     | 143 (59.8)     | 96 (40.2)  |      |
| Recent quitter            | 93    | 0.88     | 54 (58.1)      | 39 (41.9)  |      |
| Current smoker            | 104   | 0.88     | 67 (64.4)      | 37 (35.6)  |      |
| Alcohol                   |       |          |                |      |       |
| Infrequent/none           | 95    | 0.97     | 51 (53.7)      | 44 (46.3)  | 0.39 |
| Quit                      | 158   | 0.92     | 89 (56.3)      | 69 (43.7)  |      |
| Current                   | 237   | 0.92     | 145 (61.2)     | 92 (38.8)  |      |
| Stage                     |       |          |                |      |       |
| I                         | 330   | 0.90     | 202 (61.2)     | 128 (38.8) | 0.05 |
| II                        | 160   | 0.99     | 83 (51.9)      | 77 (48.1)  |      |
| Prior treatment           |       |          |                |      |       |
| Surgery                   | 120   | 0.80     | 86 (71.7)      | 34 (28.3)  | 0.00 |
| Radiotherapy              | 313   | 0.97     | 170 (54.3)     | 143 (45.7) |      |
| Both                      | 57    | 1.01     | 29 (50.9)      | 28 (49.1)  |      |
The overall mean dietary intake of vitamin C among our patients appears to be higher than that reported in NHANES3 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.
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-Hydroxyguanosine 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. Pohli and Reidy (32) showed a significant reduction in the number of bleomycin-induced chromatid breaks in eight volunteers given supplemental vitamin C (100 mg for 2 weeks and 1 g for another 2 weeks). More recently, Kucuk et al. (33) studied serial mutagen-sensitivity scores monthly in 25 healthy nonsmokers for 12 months and noted significant correlations between certain plasma nutrient levels and the mutagen-sensitivity values.

However, our data, which are based on much larger numbers of patients, show no correlation between dietary intake of any nutrient and mutagen-sensitivity scores. In a case-control study of 167 head and neck cancer patients and 177 age- and sex-matched controls, Schantz (34) also observed no association between mutagen sensitivity and dietary intake of vitamins C and E, cryptoxanthin, or lycopene in either cases or controls. It must be remembered that these micronutrient data are derived from dietary intake, not from plasma measures, and that dietary intake of these antioxidants is not directly correlated with serum measures. Plasma concentrations of carotenoids reflect short- to medium-term intake, and interindividual variation in the plasma response to intake is substantial (34). A recent report from the United States Department of Agriculture-National Cancer Institute database (12, 13) on the relationship between dietary carotenoid and plasma concentrations of carotenoids indicated a significant correlation for α, β, and total carotene (0.49; P < 0.05) (35). Vitamin C intake and plasma levels of ascorbate typically have an S-shaped association curve. Higher intakes of vitamin C are not well correlated with plasma levels because excess vitamin C is excreted in the urine. Thus, these estimates of dietary intake may better reflect true intake of vitamin C than plasma ascorbate levels do. Recently, Cloos et al. showed no effect on mutagen-sensitivity levels by the administration of N-acetylcyesteine in 19 treated patients compared with 14 untreated subjects (36).

The underlying mechanism for mutagen sensitivity associated with cancer proneness may reflect in part an altered repair process. Pandita and Hittelman (37) suggested that the mutagen-sensitivity phenotype may also involve an inherent chromatin alteration that increases the efficiency of translating DNA damage into chromosome damage after mutagen exposure. Wei et al. (38) examined DNA repair capacity using a host cell reactivation assay in parallel with the mutagen-sensitivity assay in 16 established lymphoblastoid cell lines, including 3 head and neck cancer cell lines. Using UV radiation and nitroquinoline-4-oxide as the test mutagens for both assays, they reported that reduced cellular DNA repair capacity was significantly correlated with increased frequency of mutagen-induced chromatid breaks. This finding suggests that repair fidelity may be impaired in hypersensitive persons. 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 multidisciplinary programs will seek to evaluate chemopreventive strategies in cohorts of phenotypically normal individuals deemed to be genetically susceptible to cancer development.

References
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