Short Communication

Mutagen Sensitivity Exhibits a Dose-Response Relationship in Case-Control Studies

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Abstract

We have been quantitating, as a marker of cancer susceptibility, induced chromatid breaks in lymphocyte cultures exposed to chemical mutagens. This report highlights the consistency of the results from two case-control studies, using different methods of presenting the data. In both the lung cancer case-control study, which used bleomycin, a radiomimetic agent, as the test mutagen, and the melanoma study, which used 4-nitroquinoline-oxide, an UV-mimetic agent, the mean number of breaks/cell was significantly higher in the cases compared with the controls. When the data were dichotomized at the 75th percentile of breaks in the control populations, significantly elevated odds ratios (3.7 and 5.0, respectively) were detected. Dose-response relationships were evident in both studies when the data were categorized by quartiles of breaks/cell in the controls, with highest risk estimates being in the top quartile of induced breaks. The potential for extending this assay to other cancer sites, using a variety of test mutagens, is exciting.

Materials and Methods

The assay methods used primary cultures of human peripheral lymphocytes for mutagen exposure and enumerated the number of chromatid breaks induced as an estimate of mutagen sensitivity. From 335 normal blood donors assayed for bleomycin clastogenicity (2), the mean number of b/c was 0.60, with a SD of 0.35. We used 1.00 b/c as the arbitrary demarcation line for separating individuals who were hypersensitive to bleomycin from those who were less so. Using this breakpoint, less than 13% of normal individuals could be classified as hypersensitive, whereas approximately 48% of patients with head and neck cancers were in this category (2). Similarly, from 103 normal individuals assayed for 4NQO sensitivity, the mean + 1 SD value was 0.80 (3). Approximately 15% of normal individuals were hypersensitive, whereas 44% of melanoma patients were hypersensitive (3).

Because responses to mutagen-induced chromatid breakage rates represented a continuous variable, dichotomizing the breakage data may not be the optimal approach. One of the reasons for this inadequacy is inherent to the method employed; namely, instead of using the entire cell population to estimate the samples for mutagen sensitivity, we usually examine only 50 metaphases per sample. Lee et al. (4) have shown that scoring 50 metaphases yields adequate statistical reliability. However, one or two metaphases containing a high number of chromatid breaks (e.g., seven or eight breaks each) or having no breaks could skew the data considerably. If mutagen sensitivity values could be stratified into more quantitative grades to represent degrees of susceptibility to environmental carcinogenesis, the test results would be more meaningful. In the present report, we calculated ORs to assess the dose-response relationship for chromatid breakage frequencies of both bleomycin and 4NQO exposure as an improvement to our previous dichotomous definition of mutagen sensitivity.

For the bleomycin assay, the cases and controls were derived from a molecular epidemiology study of lung cancer in minority populations (African- and Mexican-Americans) described previously (5, 6). There were 113 of the former and 67 of the latter with newly diagnosed, previously untreated lung cancer and 270 controls matched for age, ethnicity, and sex. Epidemiological data were collected by personal interview with informed consent.

For the 4NQO assay, there were 71 melanoma cases and 137 controls. The majority of cases were referred by Dr. Lynn Feun (University of Miami Sylvester Comprehensive Cancer Center, Miami, FL), and the control individuals were healthy volunteers, most of whom were spouses of patients with head and neck cancers.

The blood culture, mutagen treatment, and cell harvest procedures have been reported previously (2). Briefly, primary

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3 The abbreviations used are: 4NQO, 4-nitroquinoline-1-oxide; b/c, breaks per cell; OR, odds ratios; CI, confidence interval.
blood cultures on day 3 of incubation were treated with bleomycin (0.03 units/ml) for 5 h before harvest. For 4NQO (1 × 10^5 m), the treatment time was 24 h. Therefore, cell harvest for the 4NQO study was performed on day 4 of culture. All cultures were given a 1-h Colcemid arrest before harvest. The harvest procedure and slide preparation method followed the conventional cytogenetic protocol. For each sample, 50 well-spread metaphase figures were critically examined for recording chromatid breaks, and the mean number of b/c was used to estimate sensitivity to the mutagen under investigation.

Mutagen sensitivity was analyzed as a continuous variable, dichotomized at the level of 1 b/c for bleomycin sensitivity and 0.62 b/c for 4NQO sensitivity (the value of the 75th percentile of b/c in controls) and categorized by quartiles of b/c values in the respective controls. The linear trend was estimated by the χ² test. The difference in continuous variables was evaluated by the Student’s t test. ORs were used to estimate the relative risks. Ninety-five % CIs were computed according to Woolf (7). Logistic regression was conducted to estimate risks, which were adjusted for multiple factors, with STATA statistical software (8).

Results and Discussion

Table 1 presents the results for bleomycin sensitivity analyzed all three ways. Used as a continuous variable, the mean b/c value of cases was significantly higher than that of the controls (P < 0.0001). When bleomycin sensitivity was dichotomized by the 75th percentile of b/c according to its distribution in the controls, we found that bleomycin sensitivity was associated with a 3.7-fold adjusted significantly elevated risk for lung cancer. When we categorized bleomycin sensitivity by quartiles of b/c according to the distribution in the controls, we noted a dose-response relationship between bleomycin sensitivity and lung cancer risk. The trend test was significant at the P < 0.0001 level, with a 5-fold elevated risk in the highest stratum of b/c (5).

Table 2 summarizes the results with similar statistical analysis of 4NQO-induced chromatid aberrations, again expressed as b/c values. The mean + 1 SD value of the control population was considerably lower than that for the bleomycin study (0.48 versus 0.78, respectively). Nevertheless, the mean b/c value for cases was significantly higher than that of the controls.

The risk estimate associated with 4NQO sensitivity was 5.0 (CI, 2.7, 9.3). There was a similar dose-response relationship for quartiles of b/c and an even greater risk (OR, 9.3) for the highest stratum of b/c. Fig. 1 presents the graphic representation of the OR values listed in Tables 1 and 2 for easy inspection. We believe that this method of analysis provides a more quantitative assessment for estimating an individual’s susceptibility to environmental carcinogenesis based on genetic background.

These data support the utility of the mutagen sensitivity assay as a predictor of cancer risk. The consistency of the dose-response relationship with different mutagens is impressive. The potential for applying the assay to different cancer sites using site-specific mutagens is highlighted. The long-term goal is to refine epidemiological methods to identify susceptible subgroups for cancer prevention strategies.

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