Mutagen Sensitivity and Risk of Gliomas: A Case-Control Analysis

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Abstract

Although the risk factors contributing to the etiology of brain tumors remain largely unknown, this pilot study suggests that genetically determined sensitivity to environmental carcinogens may play a role in the pathogenesis of these tumors. In this study, we examined short-term lymphocyte cultures from 45 adult malignant glioma patients and 117 age-, sex-, and ethnicity-matched healthy controls for mutagen-induced chromatid breaks and evaluated their family history of cancer, smoking, and demographic variables to ascertain the association between mutagen sensitivity and risk of brain tumors. The mutagen selected was γ-radiation. The mean number of induced breaks/cell was 0.72 (SD = 0.45) for the cases and 0.45 (SD = 0.35) for the controls (P < 0.0001). Using the median number of induced breaks/cell in the controls as the breakpoint for defining mutagen sensitivity, we observed an unadjusted odds ratio of 5.36 (95% confidence interval = 2.12-13.69) for mutagen sensitivity and brain tumor risk and an adjusted odds ratio of 5.79 (2.26-14.83), when we controlled for epidemiological risk factors including smoking, race, income, and education. Although a larger study is needed to confirm this intriguing result, these preliminary findings suggest that increased sensitivity to radiation is an independent risk factor for gliomas.

Introduction

In the United States in 1995, approximately 12,100 people will have died from primary brain tumors and 16,500 people will have been diagnosed with the disease, which usually causes rapid death or debilitation (1). The etiology of and risk factors for brain cancer remain unknown (2, 3), although variation in cancer susceptibility is well documented and is the focus of intensive epidemiological research. Such studies have found that one determinant of cancer susceptibility is DNA repair capability. Hsu et al. (4) and Spitz and Hsu (5) hypothesized that genetic instability is not an all-or-none phenomenon but exists in varying degrees within the general population, and they developed an in vitro lymphocyte assay to provide an indirect measure of DNA repair capability.

Previous case-control studies using a variety of mutagens have implicated mutagen sensitivity (as quantified by induced chromatid breaks) as a risk factor for a variety of cancers including lung (6, 7), upper aerodigestive tract (8-10), colon (4), melanoma, non-melanoma skin (4), and multiple primary cancers (11, 12). Studies of skin fibroblasts or peripheral blood lymphocytes from individuals with a genetic condition predisposing them to cancer have shown a higher frequency of chromatid breaks and gaps after X-irradiation in the G2 phase of the cell cycle compared with cells from normal controls (13).

Materials and Methods

Study Population. Cases were defined as patients who registered at The University of Texas M. D. Anderson Cancer Center from November 1994 through July 1995 with histologically confirmed and previously untreated malignant gliomas. They were participants in an ongoing family study of gliomas, and each case donated 10 ml of blood in heparinized tubes before the initiation of therapy. For convenience in this pilot study, the controls were blood donors who either came in to the M. D. Anderson Blood Bank to donate blood or participated in an off-site employee blood drive. The cases were matched for age, sex, and ethnicity with the controls.

Each subject completed either an in-person or telephone interview that included questions on details of family history of cancer, prior radiation treatment, tobacco use, and demographic data. Family history of cancer was categorized as having no or one or more first-degree relatives with cancer; a smoker was classified as someone who had smoked at least 100 cigarettes in his or her lifetime. A former smoker was defined as one who had smoked cigarettes in the past but had stopped smoking >1 year previously.

Chromosome Analysis. γ-Radiation was selected as the test mutagen to induce chromosome breaks, because it induces single-stranded and double-stranded DNA breaks, and radiation exposure is a documented risk factor for brain tumors (15). We established the dose level based on the results of experiments with at least six normal cell lines. The dose-response curves were calculated by methods to be described in a subsequent paper. Using doses ranging from 0.5 to 2.0 Gy, we determined that the optimal dose at which intact metaphases were observed was 1.5 Gy for peripheral lymphocytes. At a dose of 2.0 Gy, no countable breaks were observed because the cells died. The dose for a fractionated treatment in the clinic is generally 1.2-2 Gy/fraction.

Standard lymphocyte cultures were established as described elsewhere (4, 16). Briefly, 1 ml of fresh blood from each study subject was inoculated into a T-25 plastic culture flask with 9 ml RPMI 1640 containing 11.5 μg/ml phytohemagglutinin (Murex Diagnosis, Norcross, GA), and two cultures were prepared for each subject. Within 72 h of incubation, vigorous cellular growth was obtained. At ~67 h of incubation, the cells were then irradiated with a γ-source from a 137Cs source (Cesium Irradiator Model 1, model 30; J. L. Shepherd and Associates, Glendale, CA). The flask containing cell cultures were exposed directly to incident γ-radiation at the rate of 15.58 Gy/min for 5.8 s or ~0.26 Gy/s, and the cells were allowed to grow for another 5 h before they were harvested. They were then treated with Colcemid (0.04 μg/ml) to arrest the cell cycle in mitosis before they were harvested for preparation of conventional air-dried slides. The slides were coded and stained with 4%...
Giemsma without banding. The prepared slides were fixed according to the protocol and read in batches. The fixed slides remain intact without affecting the reading and can, therefore, be read even years after fixation. One of us (J. G.), unaware of the disease status of each individual, counted the number of chromatid breaks in 50-metaphase samples from each individual. Only frank chromatid breaks or exchanges were recorded; chromatid gaps or attenuated regions were disregarded. The average number of breaks/cell was then calculated. Any individual who expressed a value higher than the median of the controls (0.34 breaks/cell) was considered radiation sensitive.

Statistical Analysis. To test for significant associations between mutagen sensitivity, family history, and smoking, univariate ORs were calculated as estimates of the relative risks. Ninety-five % CIs were computed according to the method of Woolf as detailed in Schlesselman (17). Multiple logistic regression was conducted with S-Plus Software (18) to estimate risks adjusted for multiple factors. We considered mutagen sensitivity and age as continuous variables, and then we tested the data with mutagen sensitivity as a discrete variable. The $\chi^2$ test was used to test for significant differences between cases and controls. CIs for the adjusted ORs were calculated by using the estimated logistic coefficient and the corresponding SE.

Results

The study included 45 glioma patients, of whom 23 (51.1%) had glioblastoma multiforme, 15 (33.3%) had anaplastic astrocytomas, 5 (11.1%) had oligodendroglioma, 1 (2.2%) had a juvenile pilocytic astrocytoma, and 1 (2.2%) had an ependymoma. The cases and controls ($n = 117$) were well matched on sex and ethnicity and differed little in smoking status, family history of cancer, and the socioeconomic variables income and education. The median age was 44 years for the cases and 45 years for the controls ($P = 0.62$; Table 1). Sixty-two % of the cases and 73% of the controls had completed high school or college ($P = 0.27$). The household income of the cases was slightly lower, with 22% of the cases and 13% of the controls having incomes <$25,000 ($P = 0.21$; Table 1).

Fig. 1 shows the distribution of mutagen sensitivity by case and control status. The range of the number of breaks/cell is shown. The range was 0.08–3.08 for the controls and 0.2–2.30 for the cases. One control had a high number of breaks/cell. The mean number of breaks/cell was 0.72 (SD = 0.45) for the cases and 0.45 (SD = 0.35) for the controls ($P < 0.0001$). Mutagen sensitivity was the only risk factor associated with gliomas; none of the other risk factors showed an excess risk among the cases (Table 2). The univariate OR for mutagen sensitivity was 5.36 (95% CI = 2.12–13.69). When we controlled for all of the variables (age, sex, ethnicity, and family history) by using robust multiple regression, the adjusted OR for mutagen sensitivity was 5.79 (95% CI = 2.26–14.83), which showed that mutagen sensitivity was associated significantly with the risk of gliomas (data not shown). We also examined the cases to determine whether mutagen sensitivity was related to any of the histological subtypes of glioma. We found that patients with glioblastoma had the highest risk (OR = 1.45; 95% CI = 0.53–3.57) compared with all others, and the OR for anaplastic astrocytomas compared with the others was 0.36 (95% CI = 0.20–1.84) (data not shown).

Discussion

Data from this preliminary study suggest that sensitivity to $\gamma$-radiation is associated significantly with the risk of gliomas. This finding supports those of other studies of lung and upper aerodigestive cancers, which used bleomycin as the mutagen and a different cutoff value for defining mutagen sensitivity, and found that mutagen sensitivity was an independent risk factor for developing cancer (8–10).

Because the study presented here was the first to use $\gamma$-radiation to induce breaks, a $\gamma$-radiation cutoff point for mutagen sensitivity had not been established. We, therefore, defined mutagen sensitivity as the median value of the number of breaks/cell in the controls. In other studies of lung and head and neck cancer in which bleomycin was used to induce chromosome breaks, between 65 and 69% of the cases were deemed mutagen sensitive (8–10) compared with 85% in the study presented here.

It should be noted that the cutoff value for mutagen sensitivity is dose dependent rather than arbitrary, i.e., the higher the dose of mutagen tested, the higher the cutoff point, because of the shifting in the distribution of the data. Given interlaboratory differences, a recommended absolute value based on the dose of a given mutagen in one laboratory may, therefore, not be applicable in others. Without prior information about the optimum dose for a new mutagen and the dose curves of the test mutagen, using a statistical parameter such as the median of the distribution of actual data seemed most reasonable.

In this study, we controlled for factors that might modify mutagen sensitivity by matching the cases with the controls for age, sex, and ethnicity. Cases and controls differed in the socioeconomic variables, family income, and level of education; however, one would not expect these factors to have an influence on inherited mutagen sensitivity.

In a previous study, we found that family history of cancer was associated with mutagen sensitivity in upper aerodigestive cancer patients (OR = 2.63; 95% CI = 1.06–6.53) for patients who were mutagen sensitive and had one first-degree relative affected with cancer (19). For mutagen-sensitive patients with two or more affected relatives, the OR increased to 6.59 (95% CI = 1.69–25.2). Because the patients were participants in a family study, in the study presented here, we wanted to duplicate our earlier findings. However, many of the controls in the study presented here had donated blood because their relatives were affected with cancer, and 56% of the cases and 51% of the controls had at least one first-degree relative with cancer. Therefore, by choosing the blood donors as controls, we actually also

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<td>≥$25,000</td>
<td>35 (78)</td>
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Fig. 1. Distribution of mutagen-induced breaks by case and control status.
matched for family history of cancer. Even in this situation, the mutagen sensitivity was still an independent risk factor for gliomas. In one of the few investigations of cells irradiated with ionizing radiation, Knight et al. (13) examined the cytogenetic damage to G2-phase, X-irradiated lymphocytes and found that individuals with a deficiency in the repair of DNA in the G2 phase reported having first- and second-degree relatives with 3.6- and 2.2-fold higher risks of cancer, respectively. These findings suggest that a deficiency in G2 DNA repair after X-irradiation is associated with a high risk of cancer.

DNA repair deficiency and predisposition to cancer are hallmarks of rare chromosome instability syndromes (20). These genetic syndromes have also been related to differences in radiosensitivity (21). It has also been shown in in vitro studies that individuals vary in lymphocyte radiosensitivity and that mutagen sensitivity correlates with DNA repair capacity (22). Therefore, it is reasonable that individuals sensitive to γ-radiation because of their inability to repair radiation damage may also have an increased risk of developing brain tumors. This hypothesis needs to be tested in a larger study to investigate the roles of mutagen sensitivity, radiation exposure, and risk of developing gliomas.

In summary, this study is the first to suggest that increased sensitivity to radiation may be associated with risk of developing gliomas. Our findings warrant a larger study with an appropriate control group to study the epidemiological risk factors together with markers of susceptibility and risk of gliomas.

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References

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