ABSTRACT

The identification of genetic traits that predispose individuals to environmentally induced cancers is one of the most important problems in cancer risk assessment. Genetic deficiency in the μ-isozyme of the glutathione S-transferases (EC 2.5.1.18) has recently been associated with increased lung cancer risk. To test whether this association could arise from a metabolically mediated sensitivity to mutagenic substrates, cytogenetic damage in lymphocytes from 21 isozyme-deficient and 24 nondeficient individuals was induced. Cells were treated with trans-stilbene oxide, an excellent substrate for GSH S-transferase μ, or cis-stilbene oxide, a poor substrate for the isozyme. Sister chromatid exchange induction was measured as an indicator of cytogenetic damage. A trimodal distribution of trans-stilbene oxide-induced sister chromatid exchanges was observed in the population, including resistant, moderate, and highly sensitive groups. Glutathione S-transferase deficiency was associated with both moderate and high sensitivity to trans-stilbene oxide-induced damage but had no effect on cis-stilbene oxide-induced sister chromatid exchange. The results indicate that GSH S-transferase μ, a proposed marker of cancer susceptibility, is also a marker of susceptibility to the induction of cytogenetic damage by a certain class of mutagens. The differential effects of the cis- and trans-isomers of stilbene oxide illustrate that the stereoselectivity of GSH S-transferase μ toward various alkene epoxide substrates can be an important factor affecting individual sensitivity to DNA-damaging epoxides.

INTRODUCTION

GSH S-transferases (EC 2.5.1.18) are a family of multifunctional enzymes that bind organic anions and detoxify reactive electrophiles capable of damaging DNA (1–4). In humans, the three main classes of glutathione S-transferases, α, μ, and π, are the products of three separate gene families located on different chromosomes (5–11). Approximately 50% of Caucasians are genetically deficient in one isozyme of this family, GSH S-transferase μ. This isozyme is highly efficient in the detoxification of trans-stilbene oxide (12–16), a potent epoxide mutagen. Recent evidence indicates that hereditary differences in the expression of glutathione transferase μ are due to a gene deletion (17). Persons deficient in the μ-isozyme have been reported to be at high risk for smoking-induced lung cancer (18). If the enzymatic deficiency in epoxide detoxification causes the observed increase in carcinogenic risk, then somatic cells having this defect could be more susceptible to chromosomal damage induced by epoxide substrates of the enzyme. Hypersensitivity to the induction of cytogenetic damage is associated with increased cancer risk in rare genetic chromosomal instability syndromes (19). However, there has been no known example of a genetic polymorphism in human carcinogen metabolism that both is associated with increased cancer risk and has been shown to lead to increased cellular sensitivity to mutagens.

In this study, we used the induction of SCEs in peripheral blood lymphocytes to study the relationship between concentration-dependent increases in cytogenetic damage and genetic GSH S-transferase μ deficiency. The SCE method was used as an endpoint of cytogenetic damage because it is rapid and highly sensitive for determining the actions of mutagens and carcinogens (20). To test the specificity of GSH S-transferase μ deficiency on the induction of SCEs by epoxide mutagens, peripheral blood cultures were treated with TSO or CSO, and the number of SCEs induced was measured after 72 h. TSO was used because it is an excellent substrate for GSH S-transferase μ in human lymphocytes (14–18). To test the specificity of GSH S-transferase μ deficiency on SCE induction, we also treated lymphocytes from some of the subjects with CSO, a poor substrate for GSH S-transferase μ (16). We found that persons whose lymphocytes are deficient in GSH S-transferase μ were more sensitive to the induction of SCEs by TSO. GSH S-transferase μ deficiency, however, had no effect on the sensitivity of lymphocytes to SCEs induced by CSO.

MATERIALS AND METHODS

Study Population. The study group consisted of 45 unrelated normal adult volunteers (19 males; 26 females). Venous blood samples were drawn into 10-ml heparinized Vacutainer tubes (Becton-Dickenson) with the participants' informed consent. Information regarding the subjects' age, sex, smoking history, and occupation was collected. Eight of the 45 subjects were current smokers. Aside from cigarette smoking, none of the subjects was known to have been exposed to agents that might affect SCE frequencies.

Cell Culture and Cytogenetics. Whole heparinized blood (0.5 ml) was added to 4.5 ml of RPMI 1640 culture medium containing 2 mM glutamine, 10% fetal calf serum, 100 μg/ml penicillin, 100 units/ml streptomycin, and 2% phytohemagglutinin (Difco, PHA-M). Lymphocytes were cultured for 21 h at 37°C, in 5% CO2 in 1-oz glass prescription bottles. Cells were treated from 21 to 72 h with TSO or CSO (Aldrich Chemical Co., Milwaukee, WI) dissolved in ethanol (0.4% v/v). Control cultures were treated with ethanol alone. At 24 h of culture, 6-bromo-2-deoxyuridine (50 μM) was added and cells were incubated an additional 48 h. Two hours before fixation colcemid (2 × 10−7 M; CIBA Pharmaceuticals, Summit, NJ) was added. The cells were collected by centrifugation, exposed to 0.075 M KCl for 8 min to spread the chromosomes and hemolyze the RBC, and fixed 3 times in methanol-acetic acid (3:1). Drops of a concentrated cell suspension were placed on microslides and air dried. The cells were stained by a modification of the fluorescence-plus-giemsa technique (21) to obtain harlequin chromosomes. The slides were immersed for 15 min in a solution of 5 μg Hoechst 33258 (Riedel-De Haen AG, Hannover, FRG)/ml in So-
GSH S-TRANSFERASE DEFICIENCY AFFECTS SCE INDUCTION

GSH S-TRANSFERASE Assay. Lymphocytes were isolated by gradient separation with Ficoll-Paque (22), counted, pelleted by centrifugation at 400 × g, and frozen at -20°C. GSH S-transferase μ was assayed by a variation of a published procedure (13). Briefly, frozen lymphocytes were sonicated. Approximately 3 × 10⁶ cells were incubated in a final volume of 100 μl containing 176 mM sodium phosphate, pH 7.2, 4 mM reduced glutathione (final concentration), and 250 μM [3H]TSO (specific activity, 1.4 Ci/mmol; Chemsyn Science Laboratories, Lenexa, KA). The reaction mixture was incubated at 37°C for 30 min. The reaction was terminated by extraction with 200 μl n-hexyl alcohol. The tritium content of the aqueous phase was determined by liquid scintillation spectrometry. Protein concentration was estimated by the modified Lowry procedure described by Peterson (23); enzyme activity was expressed as pmol conjugate formed/min/mg protein. The purity of the TSO substrate was 98.2% by thin layer chromatography. The maximum rate of nonenzymatic reaction (boiled lymphocytes) as a percentage of native activity was 24%. Variability in GSH S-transferase activity was ±5% (SE). The data presented are the means of three determinations.

Statistical Methods. To compute mean SCE/cell, 50 second-division mitotic cells were scored for each culture. Comparisons of group mean SCE/cell were made by Student's t test. Analysis of covariance for homogeneity of slopes was carried out with an established computer program (24).

RESULTS

GSH S-TRANSFERASE μ Activity in Human Mononuclear Cells. GSH S-transferase μ activity was measured in each of the 45 individuals (Table 1). Twenty-four subjects (53%) had measurable levels of the enzyme (subjects 1–24), and 21 subjects (47%) had negligible levels (less than twice the background) (subjects 25–45). The mean age of persons deficient in the isozyme was 38 ± 12 years (median, 35 years); for persons with measurable levels of GSH S-transferase μ the mean age was 39 ± 11 (median, 35 years). Linear regression analyses indicated that age had no effect on enzyme levels in either the deficient or nondeficient groups or in both groups combined. The sex of an individual had no effect on either the proportion of isozyme-positive persons or on the absolute enzyme activity measured.

Correlation of TSO-induced SCEs with Expression of GSH S-TRANSFERASE μ. Background and TSO-induced SCE frequencies were measured in each subject (Table 1). The background SCE frequencies of GSH S-transferase μ-deficient individuals did not differ from those of persons with normal isozyme levels (t = 0.399; P = 0.692). At each TSO concentration tested (50, 100, and 200 μM TSO), lymphocytes from persons with deficient isozyme activity contained increased numbers of SCEs (P < 0.001). The distributions of SCE scores for isozyme-deficient and nondeficient persons were distinct, showing no overlap at either the 100 μM or the 200 μM TSO concentration (Fig. 1).

TSO induced linear concentration-dependent increases in the SCE frequencies of lymphocyte cultures from each individual studied. To summarize the sensitivity of an individual to SCE induction with TSO, we calculated the slope of the linear regression of SCEs as a function of concentration for each person. An overall 3.7-fold variation in SCE dose-response slopes was observed (5.7–21.2 SCEs induced/cell/μM TSO × 10⁻²). The slopes of the dose-response curves for each person tested were highly reproducible, varying only by 5–10% within an individual on repeated blood sampling.

Plotting the distribution of slopes (Fig. 2) showed that, within the isozyme-deficient group, there were six individuals (subjects 25, 33, 35, 36, 38, and 44) whose slope estimates appeared to be greater than the overall group mean slope. To test whether the slopes in this subgroup were comparable to the slopes in the other isozyme-deficient individuals, we performed an analysis of covariance for homogeneity of slopes.

Analysis of covariance showed slopes for the nondeficient group to be homogeneous, but there was significant nonhomogeneity of slopes within the GSH S-transferase μ-deficient group (F = 3.631; P < 0.001). When the slope estimates for these six individuals were removed from the data set and the analysis of covariance was carried out again, the slopes for deficient individuals were homogeneous (F = 0.597; P = 0.845). Consequently, to compare the slopes of isozyme-deficient and isozyme-positive groups, the population was divided into three groups (Table 2), each of which had slope estimates judged homogeneous by analysis of covariance. The mean slope of each of the three groups differed. The mean slope of the moderately sensitive isozyme-deficient group was significantly greater than the mean slope of the resistant group with measurable GSH S-transferase μ activity (t = 11.624; P < 0.001). The mean slope of the highly sensitive isozyme-deficient group was significantly greater than that of the moderately sensitive deficient group (t = 10.400; P < 0.001). These results indicate a trimodal distribution of TSO-induced SCEs in this study group. The three types of dose-response relationships for each group are shown in Fig. 3.

To further test the relationship of enzyme activity and SCE induction, the absolute activity of GSH S-transferase was correlated with the slope of the SCE dose-response curve within the three groups and for all combinations of groups. There was no correlation of slope with isozyme activity in the nondeficient group (t = 0.455; P = 0.654) or in the deficient group (t = 0.455; P = 0.654). When the data from both groups were combined, however, there was a significant correlation of isozyme activity with SCE induction (t = -6.971; P < 0.001). Hence, the presence or absence of measurable isozyme activity had a marked effect on the sensitivity of human lymphocytes to SCE induction by TSO; variations in absolute levels of enzyme activity within either deficient or nondeficient groups had no detectable effect on SCE inducibility.

Linear correlation analysis of age and the slope of the SCE dose-response curve indicated that age did not influence the sensitivity of cells to the induction of SCEs by TSO. However, five of the six isozyme-deficient persons who were also highly sensitive to SCE induction by TSO were women, whereas based on the number of women in the study, only two would have been expected.

cis-Stilbene Oxide-induced SCEs in GSH S-TRANSFERASE μ-Deficient Lymphocytes. To test the specificity of GSH S-transferase μ deficiency on SCE induction by alkene oxide mutagens, lymphocyte cultures were established from five isozyme-deficient and five nondeficient individuals and treated with 50, 100, or 200 μM CSO, a poor substrate for GSH S-transferase μ in human lymphocytes. CSO induced SCEs in a concentration-dependent fashion in cells from each of the subjects (Fig. 4). Linear correlation coefficients were computed for each experiment. The mean slope of the CSO SCE dose-response curve in nondeficient individuals (7.8 ± 2.54 SCE/cell/μM × 10⁻²) was not significantly different from the slope for isozyme-deficient individuals (10.34 ± 1.6; t = 1.914; P = 0.092). The mean slopes for these same individuals when TSO was used as an inducer of SCE were 10.23 ± 1.07 and 17.54 ± 2.9 SCE/cell/μM TSO × 10⁻² for nondeficient and deficient subjects, respec-
Table 1 GSH-S-transferase μ activity and trans-stilbene oxide-induced sister chromatid exchanges in lymphocyte cultures from 45 individuals

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* ND, not detected, i.e., no detectable activity.

DISCUSSION

Humans are exposed to epoxides directly from environmental sources and via metabolic oxidation of exogenous and endogenous alkene and arene compounds (25–28). Many epoxides are potent mutagens and carcinogens (29–31). The detoxification of epoxides is mediated by conjugation with GSH, a reaction catalyzed by GSH transferases, or through hydrolysis by epoxide hydrolases (32, 33). In humans, the existence of a genetic deficiency in the μ-isozyme of GSH transferase provides a unique opportunity to test the relative importance of GSH conjugation in the induction of genetic damage by epoxide mutagens.

Our observation that approximately half of the test population was deficient in GSH S-transferase μ activity agrees well with previous estimates (12–17). Our data also show that enzyme-deficient individuals can be differentiated from normal individuals by analysis of TSO-induced SCEs. Relative insensitivity to TSO induction of SCEs was observed only in individuals who expressed GSH S-transferase β. Individuals who lacked this isozyme activity showed moderate or high sensitivity to SCE induction. Therefore, the failure of GSH S-transferase μ deficiency to affect CSO-induced SCEs cannot be attributed to differences in the numbers of SCEs induced by the two isomers.

These observations represent the first demonstration in a human population of a common genetic deficiency in carcinogen metabolism that is associated with increased susceptibility to mutagen-induced cytogenetic damage. The epoxide used to demonstrate this effect is a specific substrate for GSH S-transferase μ, but it has not been shown to be a constituent of cigarette smoke. In future studies of the relationship between GSH S-transferase μ deficiency and lung cancer, it will be important to identify substrates of this isozyme that are derived from exposure to cigarette smoke itself. For example, it is known that benzo(a)pyrene, which is found in cigarette smoke, can be metabolized in human cells to benzo(a)pyrene 4,5-oxide and anti-benzo(a)pyrene 7,8-diol 9,10-oxide, both substrates...
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Fig. 1. Population distribution of mean SCE/cell for lymphocyte cultures from 45 subjects. Cultures for each individual were treated with ethanol alone (A), 50 μM TSO (B), 100 μM TSO (C), or 200 μM TSO (D). ■, SCE scores for individuals with measurable GSH S-transferase μ activity; ●, SCE scores for persons deficient in GSH S-transferase μ. Note that the ordinate scale is different for each panel. Where the solid and hatched bars are at the same SCE value, the hatched bars should be read as beginning at the top of the black bars.

Fig. 2. Population distribution of linear regression slopes for SCE dose-response curves for TSO-treated lymphocyte cultures from 45 subjects. Slope estimates are based on mean SCE/cell at 0, 50, 100, or 200 μM TSO for each individual. The units for the slopes are SCEs/cell/μM x 10^-2. ■, slope estimates for individuals with measurable GSH S-transferase μ activity; ●, slope estimates for persons deficient in GSH S-transferase μ.

for GSH S-transferase μ (34, 35). Other potential substrates associated with smoking include styrene oxide (16, 36) and ethylene oxide (16, 37). Although the genotoxic effects of these and other epoxide substrates for GSH S-transferase μ would be expected to be greater in isozyme-deficient cells, other factors, including competing pathways for epoxide degradation, GSH concentration, and the subcellular compartmentalization of GSH S-transferase isozymes (38, 39), may also affect genotoxicity. Consequently, the degree of increased susceptibility to genotoxicity in GSH S-transferase μ-deficient cells will probably vary for each mutagen substrate and will have to be determined experimentally.

GSH S-transferase μ activity toward TSO is inherited as a simple autosomal dominant trait (15). Consequently, the population distribution of sensitivities to TSO-induced cytogenetic damage should be bimodal: resistant individuals with enzyme activity and sensitive individuals deficient in the enzyme. However, we found a trimodal distribution of sensitivities to TSO, including two subgroups of GSH S-transferase μ-deficient individuals, one with moderate sensitivity to TSO-induced damage and one with high sensitivity. Although the mechanisms responsible for this variation in mutagen sensitivity among isozyme-deficient groups are as yet unknown, it is possible that the highly sensitive individuals carry a second genetic deficiency that further increases susceptibility to epoxide-induced DNA damage. It has been proposed that double genetic polymorphism in drug metabolism can lead to greatly exaggerated drug toxicity (40).

Epoxide hydrolases, which catalyze the conversion of epoxides to dihydrodiols, could also modify the genotoxic effects of TSO. Although genetic deficiency in epoxide hydrolases has not been reported in humans, cytosolic epoxide hydrolase activity toward TSO varies widely in liver (41) and mononuclear cells (42) from different individuals. A microsomal epoxide hydrolase with activity toward TSO has also been described in mice (43). Methods have been developed that allow the measurement of both GSH S-transferase μ and cytosolic epoxide hydrolase sequentially, using the same cell homogenate (44). It is likely that the induction of SCEs by TSO, like epoxide-induced mutagenicity (45), is the product of several enzymes that control the concentrations of reactive metabolites capable of damaging DNA (46). A full understanding of the significance of various enzymes in the control of TSO-induced damage will likely require a correlation of various enzyme activities with measures of genotoxicity. In addition, it may also be important to consider the potential role of individual variations in DNA repair in TSO induction of SCEs.

The increased frequency of GSH S-transferase μ deficiency observed in lung cancer patients has led to the proposal that this isozyme deficiency is a genetic marker of cancer susceptibility in humans (18). As we have shown, GSH-transferase μ...
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Table 2 Induction of sister chromatid exchanges in lymphocyte cultures by trans-stilbene oxide from 24 individuals with measurable GSH-S-transferase μ activity and 21 isozyme-deficient individuals

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<th>Mean SCE/cell at indicated TSO concentration</th>
<th>Mean slope</th>
<th>Range of slopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>24</td>
<td>17.2 ± 6.1</td>
<td>8.87 ± 1.22</td>
<td>13.13 ± 1.34</td>
</tr>
<tr>
<td>Sensitive</td>
<td>21</td>
<td>1.8 ± 1.8</td>
<td>9.02 ± 1.30</td>
<td>18.87 ± 1.94</td>
</tr>
<tr>
<td>Intermediate sensitivity</td>
<td>15</td>
<td>1.6 ± 1.7</td>
<td>8.95 ± 1.02</td>
<td>18.13 ± 1.58</td>
</tr>
<tr>
<td>High sensitivity</td>
<td>6</td>
<td>2.4 ± 1.9</td>
<td>9.18 ± 1.96</td>
<td>20.73 ± 1.50</td>
</tr>
</tbody>
</table>

* The mean ± SD of the individual linear regression coefficients for each group indicated. Units for the slope estimates are SCE/cell/μM x 10⁻³.

Fig. 3. SCE dose-response curves of human lymphocyte cultures from individuals with different sensitivities to the induction of SCEs by TSO. The lower curve is a plot of mean SCE/cell for subjects with GSH S-transferase μ activity (Table 1). The middle curve illustrates moderate sensitivity to SCE induction in isozyme-deficient individuals, and the upper curve is a plot of SCE induction in highly sensitive enzyme-deficient individuals. Error bars indicate the SD of the mean SCE/cell for each group.

deficiency is also a marker of susceptibility to induced chromosome damage by a certain class of agents. The relationship of other GSH S-transferase isozymes and cancer, however, is complex. Many human tumors contain increased concentrations of various GSH S-transferase isozymes (47−54). Increased expression of GSH S-transferase by tumor cells may be a marker of acquired drug resistance or other late changes accompanying carcinogenesis. Alternatively, increased enzyme activity may play a direct role in the early events of cell transformation. Because of the genetic nature of the defect in GSH S-transferase μ deficiency and the role the isozyme plays in epoxide detoxification, the mechanism for its epidemiological association with lung cancer is thought to involve increased induction of genetic damage in enzyme-deficient cells. Our observation that GSH S-transferase μ-deficient cells are more sensitive to the induction of cytogenetic damage supports this proposed mechanism. Correlating biochemical analysis of enzyme deficiencies with indices of genotoxicity in living cells can thus provide important information concerning the significance of specific metabolic pathways for carcinogen metabolism and their potential roles in human cancer susceptibility.

Fig. 4. The induction of SCEs by CSO in human lymphocyte cultures from five individuals with measurable GSH S-transferase activity (O) and five isozyme-deficient individuals (C). The dashed and solid lines are the best-fit linear regression lines of SCE on CSO concentration for the nondeficient and deficient groups, respectively.

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REFERENCES


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