Short Communication

The Interaction between Alcohol Consumption and GSTM1 Genotype on Polycyclic Aromatic Hydrocarbon-DNA Adduct Levels in Breast Tissue

Andrew Rundle, Deliang Tang, LaVerne Mooney, Surah Grumet, and Frederica Perera

Departments of Epidemiology [A. R.] and Environmental Health Sciences [D. T., L. M., S. G., F. P.], Mailman School of Public Health, Columbia University, New York, New York 10032

Abstract

We investigated the association between alcohol consumption, GSTM1 genotype, and polycyclic aromatic hydrocarbon (PAH)-DNA adduct levels in breast tissue. Women referred for breast surgery were enrolled prior to surgery, responded to an interview, and gave a blood sample. Women diagnosed with ductal carcinoma in situ and invasive ductal or lobular cancer were defined as cases, and women with benign conditions without atypia were defined as controls. Paraffin-embedded tumor and nontumor tissue from cases and benign tissue from controls were retrieved from the pathology samples. GSTM1 genotype status was determined by PCR using WBC DNA, and PAH-DNA adduct levels were measured in breast tissue using immunohistochemistry. In tumor and nontumor tissue from cases, the GSTM1-null genotype was associated with increased adduct levels among current alcohol consumers but not among nondrinkers. In nontumor tissue, the interaction between genotype and alcohol consumption was significant (P = 0.02), but in tumor tissue, the interaction did not achieve statistical significance (P = 0.10). In benign tissue from controls, there was no association between genotype and adducts, regardless of drinking status. Among subjects with the null genotype who drank alcohol, adduct levels were significantly higher in tumor and nontumor tissue from cases than in benign tissue from controls. These results indicate the presence of a novel gene-lifestyle interaction that influences PAH-DNA adduct levels in breast tissue from cases but not controls. This apparent difference in PAH metabolism in response to alcohol may be an important clue as to how alcohol influences breast cancer risk.

Introduction

We have been investigating the genetic and lifestyle determinants of PAH-DNA adduct levels in breast tissue and the association between increased levels and breast cancer case-control status (1–3). When adduct levels in tumor tissue from cases were compared with adduct levels in benign tissue from controls, high adduct levels were associated with breast cancer case-control status (1). The GSTM1-null genotype was associated with increased adduct levels in tumor and nontumor tissue from cases, but it was not associated with adduct levels in benign tissue from controls (2). Case-control differences in adduct levels were stronger among those with the GSTM1-null genotype.

As in many other studies of breast cancer (4, 5), we found current alcohol consumption to be associated with breast cancer case-control status (3). The mechanisms through which alcohol may cause breast cancer are unknown, although several have been have proposed (4). We have hypothesized that alcohol consumption may influence breast cancer risk by inducing and suppressing genes responsible for the metabolism of xenobiotics (6, 7). Several animal and cell culture studies have demonstrated that ethanol exposure alters PAH metabolism and increases adduct formation (7–11). Recent experiments with MCF-10F breast cells have shown that cotreatment of cells with B[a]P and ethanol produced higher adduct levels than treatment with B[a]P alone (7). Ethanol treatment was shown to reduce the expression of GSTP1, and the authors attributed the increased adduct levels to ethanol-induced reductions in B[a]P metabolism by GSTP1 (7). GSTP1 and GSTM1 both detoxify PAH, creating water-soluble conjugates that are less reactive than the PAH diol-epoxide metabolites (12, 13). Thus, the GST enzymes are thought to prevent reactive xenobiotics from damaging DNA (2, 12). Here we extend our earlier findings on GSTM1 and show that the GSTM1 genotype is a stronger predictor of PAH-DNA adduct levels in subjects who were regular alcohol drinkers.

Materials and Methods

Study Population. Patient recruitment has been described previously in detail (1) and will only be described briefly here. From 1994 to 1998, women referred for breast surgery at Columbia-Presbyterian Medical Center were enrolled before surgery into a hospital-based case-control study. After informed consent had been obtained, during their preoperative tests, patients took part in a structured interview covering established reproductive breast cancer risk factors, active and passive smoking, dietary practices, other environmental and occupa-
tional exposures, and vitamin consumption. Patients whose confirmed diagnosis was of ductal carcinoma in situ or invasive ductal or invasive lobular cancer were defined as cases. Patients with rare tumors were not included because the small numbers precluded analyses by histological type. Patients diagnosed with BBD or BBD with hyperplasia were classified as controls. Because patients were enrolled before diagnosis, matching of BBD controls to cases was not possible. BBD patients whose diagnoses were other than these categories (e.g., BBD with atypia or lobular carcinoma in situ) were excluded from analysis because of their elevated risk of future breast cancer. Breast cancer patients seen at Columbia-Presbyterian Medical Center for follow-up surgery (e.g., mastectomy or re-excisions) after an initial surgical biopsy at another hospital were excluded from the study. Additional exclusion criteria included a prior history of cancer at any site except basal skin cancer, current pregnancy, recent bone fractures, or recent breastfeeding. The history of cancer at any site except basal skin cancer, current consumption status. Within the consumption status. For each tissue type (tumor, nontumor, and benign tissue), as were samples of tissue from controls, as were samples of tissue from BB samples. Sample of paraffin-embedded tumor and nontumor tissue were retrieved from pathology blocks from cases, as were samples of tissue from controls. Thus, two tissue samples, benign and nontumor, were available from cases, and one tissue sample, benign, was available from the BBD controls. A total of 119 cases and 108 BBD controls were enrolled with a response rate of 76%. Data on adduct levels, GSTM1 status, and alcohol consumption were available from 82 cases for analyses in tumor tissue, 76 cases for analyses in nontumor tissue, and 81 controls for analyses in benign tissue.

**Laboratory Methods.** To assess individual responses in tumor tissue, 76 cases for analyses in nontumor tissue, and one tissue sample, benign, was available from the BBD controls. Thus, two tissue samples, retrieved from pathology blocks from cases, as were samples of paraffin-embedded tumor and nontumor tissue were assessed for adduct levels in tumor tissue by current drinking status and GSTM1 genotype analyzed with the Cell Analysis System 200 microscope (Becton-Dickinson, San Jose, CA). Thaining the Cell Measurement software. A total of 50 cells (5 fields with 10 cells/field scored) were measured on each tissue slide. Results are reported in optical density units as described previously (15). Serial tissue slices from laboratory control breast tissue specimens previously shown to have low and high staining for adducts were used as negative and positive control samples, respectively, and were run with every batch. As an additional negative control, in each batch, a laboratory control sample was run without the primary antibody.

**Statistical Methods.** Absorbance data reflecting the extent of staining for PAH-DNA adducts was log (In) transformed for analyses to produce a more normal distribution. Results are reported as geometric means and geometric SDs (16). As part of the interview, subjects were asked to estimate the average number of drinks they consumed per week during the 12 months before the interview. Study subjects who responded that on average they drank more than 0 drinks/week were classified as nonconsumers, and subjects who responded that on average they drank more than 0 drinks/week were classified as regular current consumers of alcohol. Subjects were cross-classified into four groups based on their GSTM1 genotype status and whether they were current alcohol consumers. For each tissue type (tumor, nontumor, and benign), geometric mean adduct levels were calculated for each of the four alcohol consumption/genotype strata. t tests were used to determine whether GSTM1 status was associated with adduct levels within each stratum of alcohol consumption. Within each tissue type, linear regression analyses, with interaction terms for the joint effects of genotype and alcohol consumption, were used to determine whether the association between GSTM1 and adduct levels differed by alcohol consumption status. t tests were used to determine whether, among subjects who were GSTM1 null and drank alcohol, adduct levels were higher in cases versus controls.

**Results**

Among current alcohol consumers, the GSTM1-null genotype was significantly associated with higher adduct levels in tumor and nontumor tissue, but not in benign tissue from controls (see Table 1). Among nondrinkers, GSTM1 genotype was not associated with adduct levels in any of the tissues (see Table 1). In tumor tissue, the apparent interaction between GSTM1 status and current alcohol consumption on adduct levels approached but did not reach statistical significance (P = 0.10). However, in nontumor tissue from cases, the interaction term was significant (P = 0.02), indicating that the association between GSTM1 and adduct levels significantly differs by alcohol consumption status. Within the GSTM1-null/alcohol consumer stratum, adducts levels were significantly higher in tumor tissue.
Discussion

We report here on a gene-lifestyle interaction that influences PAH-DNA adduct levels in breast tissue from cases but not in tissue from BBD controls. This is an extension of our prior work showing that GSTM1 was associated with PAH-DNA adduct levels in breast tissue from cases but not from BBD controls (2). The presence of the interaction in cases only provides further evidence that women with breast cancer differ from women with benign conditions in some as yet unknown manner that makes GSTM1 a more important determinant of PAH-DNA adduct levels in breast tissue (2). Additionally, case-control differences in adduct levels were strongest among women who consumed alcohol and were GSTM1 null.

The analyses presented here were motivated by the recent report of breast cell culture experiments showing that ethanol increases the extent of adduct formation in cells treated with B[a]P (7). The authors attributed the increased levels of adducts to ethanol-induced reductions in the expression of GSTP1 that they observed in the cell cultures (7). GSTP1 and GSTM1 both metabolize PAH, creating water-soluble conjugates that are less reactive than the PAH diol-epoxide metabolites; however, neither gene is involved in the metabolism of ethanol (12, 13). This overlapping specificity may provide multiple layers of defense against genetic insult from PAH. Our data indicate that the GSTM1 genotype is an important predictor of PAH-DNA adduct levels in subjects who were regular alcohol consumers but not among nonconsumers, suggesting that other detoxifying pathways were compromised among alcohol consumers.

One limitation of our research is that data are not available on GSTM1 and GSTP1 expression in the tissue sections. The literature on whether alcohol alters GST expression is inconsistent (7, 8, 17–21). There appear to be organ- and species-specific differences in whether alcohol exposure inhibits or induces GST expression, and the effects appear to differ by GST subclass. A recent study in rats found that long-term ethanol treatment induced GST-µ and GST-α but not GST-π class activity in liver cells, yet a study in hamsters observed no increase in GST-mediated metabolism of B[a]P in response to ethanol treatment (8, 17). A recent study using cDNA expression arrays found that chronic ethanol feeding was associated with increased GSTM1 expression in C57BL/6J, ethanol-preferring mice but not in BALB/c, ethanol-avoiding mice (21). Our results suggest that alcohol consumption does influence PAH metabolism and that GSTM1 is not inhibited by alcohol consumption. In addition to the possibility that alcohol influences GSTP1 expression, alcohol may alter the expression of important phase 1 genes, such as CYP1B1, which activates PAH to the reactive diol-epoxide form (7). Increased availability of reactive metabolites would also be hypothesized to make GSTM1 genotype a more important determinant of adduct levels among alcohol consumers.

If alcohol consumption is indeed reducing the expression of GSTP1 in our subjects, and this accounts for the interaction between alcohol consumption and GSTM1, the presence of the interaction in cases but not controls suggests that the effect of alcohol on GSTP1 expression is stronger in cases than BBD controls. Differences in responsiveness to alcohol in terms of gene inhibition or induction may, especially for genes related to PAH metabolism, represent an important determinant of breast cancer risk. GSTP1 has a polymorphism that appears to influence adduct levels in a substrate-specific manner (22). However, given the evidence that alcohol consumption inhibits GSTP1 expression, genotype analyses may not be useful in understanding the role of GSTP1 in adduct formation.

Subjects were defined as current consumers if they reported any consumption on a regular weekly basis over the past year. Among the consumers, the average number of drinks/week was 4.82, with a range of 0.07–36 drinks/week. The interview question on current alcohol consumption asked about drinking patterns in the past year; however, many of the women who consumed alcohol reported that they had been drinking more since they had been told they needed breast surgery. Thus because our question on past year consumption may underestimate very recent consumption patterns, we did not categorize the women who reported the lowest alcohol consumption, for instance 0.07 drink/week, as nondrinkers. Because the interview was conducted before surgical biopsy, and none of the women knew whether they were cancer free, we do not expect changes in recent drinking patterns to be differential by case-BBD control status. Overall, the data suggest that these women were relatively light consumers of alcohol, and the effect appears to occur at low levels of alcohol consumption. This is consistent with the work of Barnes et al. (7), who treated MCF-10F cells with alcohol at concentrations consistent with blood alcohol levels achieved by drinking 1–3 drinks. The level of drinking seen in our study is consistent with the popularly held but unproven belief that a glass of red wine a day is protective against heart disease (23–25). Should these findings be replicated and prove to be causal, current recommendations regarding alcohol consumption may need to be reconsidered (23–25).

In noting the apparent case-control differences in the response to alcohol consumption, we hypothesize that there exists some constitutive difference between cases and controls in gene expression in response to alcohol. It is possible, however, that this differential response represents a local effect of alcohol on tumor tissue. It is possible that, in some manner, alcohol alters metabolism in tumor cells such that GSTM1 is an important determinant of adduct levels in tumor tissue, but metabolism in normal breast cells is unaffected. The observed interaction between GSTM1 and alcohol consumption in nontumor tissue from cases argues against the hypothesis that the effect is a result of alcohol influencing metabolism in tumor tissue only. However, because the nontumor tissue analyzed in this work was adjacent to the tumors in situ, the possibility of a field effect or that the tumor influenced metabolism in nearby tissues cannot be ruled out. Further work is needed to determine whether the apparent differential responses to alcohol represent a constitutive difference between cases and controls or a local effect of alcohol on metabolism in and around the tumor.

Another consideration in assessing the apparent case-control differences in adduct levels is the use of women with BBD as the control group. Although the BBD control group only included diagnoses with a low risk for future breast cancer, a concern is that the BBD controls may overly share risk factors, both measured and unmeasured, with the cases (26). To the extent that an exposure of interest is positively associated with another risk factor overrepresented in the controls, the use of BBD controls will cause risk estimates for the exposure to be attenuated to the null. However, in comparisons of odds ratios for known breast cancer risk factors calculated using a healthy control group and the BBD control group, we did not find that the use of BBD controls resulted in a consistent trend of attenuated risk estimates (3).

In conclusion, we report here on a novel gene-lifestyle interaction that links alcohol consumption with the expression of GSTM1 and GSTP1 and suggests that these gene products may influence PAH metabolism in breast tissue in a manner that makes GSTM1, rather than GSTP1, an important determinant of PAH-DNA adduct levels in breast tissue in cases.
interaction that influences PAH-DNA adduct levels in breast tissue and appears to occur in women with breast cancer but not in women with benign conditions. We suggest that cases and controls may differ in their metabolic responses to alcohol, and this may be an important clue as to how alcohol influences breast cancer risk. This work requires confirmation in a larger population and further investigations of how alcohol consumption influences xenobiotic metabolism and risk through gene induction and/or suppression.

Acknowledgments

We acknowledge the thoughtful comments of Stan Cho and Dr. Alex Halim.

References


The Interaction between Alcohol Consumption and GSTM1 Genotype on Polycyclic Aromatic Hydrocarbon-DNA Adduct Levels in Breast Tissue

Andrew Rundle, Deliang Tang, LaVerne Mooney, et al.