Levels of 5-Hydroxymethyl-2′-deoxyuridin in DNA from Blood of Women Scheduled for Breast Biopsy

Short Communication

Zora Djuric,2 Lance K. Heilbrun, Samir Lababidi, Egle Berzinkas, Michael S. Simon, and Mary A. Kosir

Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, Michigan 48201

Abstract

Systemic oxidative stress is thought to contribute to risk of various cancers, including breast cancer. DNA repair ability also has been associated with breast cancer risk. In this work, we examined levels of oxidative DNA damage as an indication of breast cancer risk in women because oxidative DNA damage levels should reflect the net balance of oxidative stress and DNA repair ability. Levels of 5-hydroxymethyl-2′-deoxyuridine, one form of oxidative DNA damage, were measured in DNA from blood of women scheduled for breast biopsy. The blood samples analyzed included women whose biopsy results indicated invasive breast cancer, high-risk lesions (atypical hyperplasia or carcinoma in situ), or benign lesions. Mean levels of 5-hydroxymethyl-2′-deoxyuridine were significantly higher in blood of women who had high risk or invasive breast lesions versus women with benign lesions. If atypical hyperplasia or carcinoma in situ are precursor lesions for breast cancer, then these results suggest that oxidative DNA damage may be involved in the cancer process before invasive cancer develops.

Introduction

Systemic oxidative stress levels, as evidenced by DNA damage and lipid oxidation products in blood and urine, appear to be associated with increased risk of various cancers, including that of the breast (1, 2). DNA repair ability in blood lymphocytes also has been shown to be related to breast cancer risk (3–6). Interestingly, individuals with BRCA1 or BRCA2 mutations also has been shown to be related to breast cancer risk (3–6). DNA repair ability in blood lymphocytes is associated with increased risk of various cancers, including breast cancer. DNA repair ability also has been associated with breast cancer risk. In this work, we examined levels of oxidative DNA damage as an indication of breast cancer risk in women because oxidative DNA damage levels should reflect the net balance of oxidative stress and DNA repair ability. Levels of 5-hydroxymethyl-2′-deoxyuridine, one form of oxidative DNA damage, were measured in DNA from blood of women scheduled for breast biopsy. The blood samples analyzed included women whose biopsy results indicated invasive breast cancer, high-risk lesions (atypical hyperplasia or carcinoma in situ), or benign lesions. Mean levels of 5-hydroxymethyl-2′-deoxyuridine were significantly higher in blood of women who had high risk or invasive breast lesions versus women with benign lesions. If atypical hyperplasia or carcinoma in situ are precursor lesions for breast cancer, then these results suggest that oxidative DNA damage may be involved in the cancer process before invasive cancer develops.

Materials and Methods

Patients. Women were recruited from the Karmanos Cancer Institute Comprehensive Breast Clinic during the period October 1994 through January 1996. A research assistant asked women who were scheduled for a biopsy whether they would fill out a questionnaire and provide a blood sample for a biomarker study. This occurred at a clinic visit that was typically several days before the biopsy. Eligibility criteria were that subjects be at least 18 years of age and had never had a previous diagnosis of cancer. During this time period, a total of 252 subjects were enrolled.

Blood Samples. Heparinized blood (10–ml) was used to prepare nuclei by the method of Ciulla et al. (11). Nuclei were frozen at −70°C in 50 mm mannitol, 1 mm EDTA, and 1% SDS until DNA could be extracted. DNA was isolated from nuclei by a modification of the procedure of Miller et al. (12). Briefly, the nuclei were treated with RNases A and proteinase K. The proteins were precipitated by the addition a one-third volume of 6 m sodium chloride, shaking, and centrifugation. One extraction with chloroform-isoamyl alcohol (48:2, v/v) and one extraction with n-butyl alcohol were used to remove residual protein. The DNA was precipitated twice and dissolved in 200 μl of water, and a UV scan was obtained.

The isolated DNA (100–150 μg) was hydrolyzed enzymatically and derivatized, and 5-OHmdU levels were determined by gas chromatography–mass spectrometry using isotopically labeled internal standards as described previously (13). The samples were derivatized with N,O-bis(trimethylsilyl)-trifluoroaceticamide containing 1% trimethylchlorosilane and acetoniatrile (2:1, v/v) by heating at 120°C for 20 min. Gas chromatographic separations were performed with a 25-m Hewlett-Packard SE54 Ultra 2 column using helium as the carrier gas.

Statistical Analyses. Simple descriptive statistics were used to summarize the baseline demographic variables and levels of 5-OHmdU, by diagnosis category. Spearman’s rank correlation

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2 To whom requests for reprints should be addressed, at Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, MI 48201.
3 The abbreviations used are: 5-OHmdU, 5-hydroxymethyl-2′-deoxyuridine; dThd, thymidine.

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was used to assess the relationship of continuous covariables and 5-OHmdU levels. Comparison of pre- and postbiopsy 5-OHmdU levels (in a small subset of the women) was performed using the nonparametric Wilcoxon signed-rank test for paired data. Simultaneous comparison of 5-OHmdU distributions by diagnosis group (and by other factors) relied on the k-sample Kruskal-Wallis rank-sum test. All pairs of diagnosis groups were then contrasted using a nonparametric multiple-comparisons procedure (14). Despite age-matching of the subjects with benign breast lesions, a significant correlation of age with levels of 5-OHmdU remained. Thus, comparison of 5-OHmdU levels by diagnosis group, after adjustment for age and smoking status, was performed via a two-way analysis of covariance model (15), with age as a continuous covariate. For this statistical modeling work, a double natural log transformation was necessary to normalize the 5-OHmdU data. The adjusted means (and confidence limits) were transformed back to the original scale of measurement to facilitate results interpretation.

Results

Blood Samples. A total of 252 subjects were enrolled. Of those, 225 were considered analysis-eligible based on whether the blood was in fact drawn, whether the biopsy was subsequently performed at our institution, and whether a definitive pathology report was available. The samples included 131 biopsies that were considered benign (adenosis, apocrine or squamous metaplasia, cysts, mild hyperplasia, duct ectasia, fibroadenoma, mastitis) and 35 biopsies that are associated with a slightly increased risk of breast cancer (moderate or florid hyperplasia, ductal papilloma with fibrovascular core and sclerosing adenosis). The high-risk biopsies included 15 with atypical hyperplasia, ductal papilloma with fibrovascular core and sclerosing adenosis, 9 with ductal carcinoma in situ, and 3 other cancers (medullary, phyllodes, and a poorly differentiated carcinoma).

The blood samples analyzed were all those available for this study from the high-risk and invasive groups: 22 of 27 subjects with biopsies in the high-risk category (2 with lobular carcinoma in situ, 7 with ductal carcinoma in situ, and 13 with atypical hyperplasia), and 23 of 29 in the invasive category (22 ductal and 1 lobular infiltrating cancer). Of the samples that were not available for this study, three did not have a tube of blood available for this assay. There were also 28 age-matched subjects from the benign category selected for analysis, but 1 of those samples yielded insufficient DNA, which left 27 for analysis. The age matching was done by first determining the age distribution, by decades, of the subjects in the high-risk and invasive categories. This turned out to be similar in each of those two groups. Subjects in the benign category were then randomly selected to match this distribution (with one extra subject for each age group), at the same time ensuring that samples were evenly distributed from the early, mid, and late enrollment periods.

Levels of 5-OHmdU. We examined the association of 5-OHmdU with the following covariates: age, race (Caucasian, African-American, Asian), body weight, smoking status (current, past, never), menopausal status (pre-, peri-, or postmenopause), first-degree family history of breast cancer or any other nonaerodigestive cancer (yes/no), and other health problems (cardiovascular/hypertension problem, diabetes, arthritis, asthma versus none of these). Some of these factors are summarized in Table 1. The only significant associations with 5-OHmdU were with age (rank correlation = 0.25; P = 0.033), and smoking status (Kruskal-Wallis test, P = 0.031). Information on use of medications, such as hormone replacement therapy, was not available and is a potential limitation of this study.

The mean levels of 5-OHmdU in blood of women with either invasive cancer or high-risk lesions were significantly higher than in women with benign lesions (Table 2). After adjustment for age and smoking status, the results were similar to the unadjusted results (see Table 2). The adjusted mean 5-OHmdU of women with benign lesions was significantly lower than that of women in either of the other two diagnosis groups. The high-risk and invasive cancer groups again were not statistically significantly different. Interestingly, the distribution of 5-OHmdU levels across quintiles was such that the women with high-risk lesions exhibited levels only in the upper three quintiles, whereas women in the other two groups were distributed across all five quintiles. The numbers of women in

### Table 1 Characteristics of the study subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Body weight (pounds)ᵃ</th>
<th>Smoking status (% smokers)ᵇ</th>
<th>Primary relatives with cancerᶜ (%</th>
<th>Race (% Caucasian)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign (n = 28)</td>
<td>55 ± 13</td>
<td>177 ± 51</td>
<td>38</td>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td>High risk (n = 22)</td>
<td>55 ± 12</td>
<td>174 ± 37</td>
<td>30</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Invasive (n = 23)</td>
<td>59 ± 13</td>
<td>162 ± 37</td>
<td>24</td>
<td>19</td>
<td>29</td>
</tr>
</tbody>
</table>

ᵃ Body weight was missing for three women. Data are means ± SD.
ᵇ Smoking status was missing for eight women.
ᶜ There were three women for whom family history of cancer was not available. Of the women who had primary relatives with breast cancer, only three had primary relatives with breast cancer in addition to other types of cancer.

### Table 2 DNA damage levels in prebiopsy blood samples from women with three different kinds of breast lesions

<table>
<thead>
<tr>
<th>Breast histology</th>
<th>Unadjusted (mean ± SD; median)</th>
<th>Adjustedᶜ (mean ± SD; 95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign (n = 28)</td>
<td>101 ± 114; 69</td>
<td>72 (58–91)</td>
</tr>
<tr>
<td>High risk (n = 22)</td>
<td>285 ± 180; 241ᵇ</td>
<td>236 (171–332)ᶜ</td>
</tr>
<tr>
<td>Invasive cancer (n = 23)</td>
<td>233 ± 224; 126ᵇ</td>
<td>137 (99–194)ᶜ</td>
</tr>
</tbody>
</table>

ᵃ Adjusted for age and smoking status by analysis of covariance.
ᵇ Differences in unadjusted DNA damage among groups were highly significant by the Kruskal-Wallis rank-sum test, P < 0.0001. The multiple-comparisons procedure of Dunn indicated that the benign group differed significantly from each of the other two groups (P < 0.05).
ᶜ Mean is significantly different from the mean of women with benign histology, after adjustment for age and smoking status.
each quintile were as follows: quintile 1 (0–60 fg of 5-OHmdU/ng of dThd), 3 with invasive cancer and 11 with benign lesions; quintile 2 (61–91 fg/ng of dThd), 5 with invasive cancer and 10 with benign lesions; quintile 3 (92–145 fg/ng of dThd), 5 with invasive cancer, 6 with high-risk lesions, and 3 with benign lesions; quintile 4 (146–308 fg/ng of dThd), 4 with invasive cancer, 9 with high-risk lesions, and 2 with benign lesions; and quintile 5 (>308 fg/ng of dThd), 6 with invasive cancer, 7 with high-risk lesions, and 2 with benign lesions.

In 13 women, a second blood sample was obtained at a follow-up clinic visit, after the results of the biopsy were known. This included 2 women with high-risk biopsies and 11 women with invasive cancer who had not begun treatment. Average time post biopsy or surgery (if surgery was also done) was 10.2 days (SD = 5.5 days; range, 3–21 days). There was no significant difference between the levels of 5-OHmdU pre and post biopsy by the Wilcoxon signed-rank test (P = 0.414; for samples obtained before biopsy, mean ± SD, 303 ± 257 fg/ng of dThd; median, 218 fg/ng of dThd; for samples obtained after biopsy, 248 ± 245 fg/ng of dThd, mean ± SD; median, 191 fg/ng of dThd). This would suggest that differential psychological factors associated with the clinic visits (i.e., anxiety that a biopsy is needed) or any effects of having had surgery or biopsy in the recent past did not appreciably affect 5-OHmdU levels.

Discussion

Oxidative DNA damage has been suggested previously to be associated with increased cancer risk (16). Levels of such damage can be envisioned to represent the net result of oxidant effects via environmental or endogenous sources and DNA repair ability. For example, family history of breast cancer in primary relatives has uniformly been shown to be associated with decreased DNA repair ability of lymphocytes (3–6). Some studies, but not all, suggest that levels of oxidative DNA damage are higher in human breast tumor tissue than in normal breast tissue (17–20). This is also evident in blood, with DNA damage are higher in human breast tumor tissue than in controls (1, 2). In the study of Frenkel et al. (2), blood was obtained 0.5–6 years before the diagnosis of breast cancer was made, which helps to implicate oxidative damage in cancer etiology. In that study, women with self-reported benign breast disease (chiefly fibrocystic disease) or a family history of breast cancer with no reported breast problems also had surgery or biopsy in the recent past did not appreciably affect 5-OHmdU levels.

References

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