Short Communication

Increased Rates of Chromosome Breakage in BRCA1 Carriers Are Normalized by Oral Selenium Supplementation

Elzbieta Kowalska,1 Steven A. Narod,2 Tomasz Huzarski,1 Stanislaw Zajaczek,1 Jowita Huzarska,1 Bohdan Gorski,1 and Jan Lubinski1

1Department of Genetics and Pathology, Hereditary Cancer Centre, Pomeranian Academy of Medicine, Szczecin, Poland and 2Centre for Research on Women’s Health, Sunnybrook and Women’s College Health Sciences Centre, Toronto, Ontario, Canada

Abstract

Women who are born with constitutional heterozygous mutations of the BRCA1 gene face greatly increased risks of breast and ovarian cancer. The product of the BRCA1 gene is involved in the repair of double-stranded DNA breaks and it is believed that increased susceptibility to DNA breakage contributes to the cancer phenotype. It is hoped therefore that preventive strategies designed to reduce chromosome damage will also reduce the rate of cancer in these women. To test for increased mutagenicity of cells from BRCA1 carriers, the frequency of chromosome breaks was measured in cultured blood lymphocytes following in vitro exposure to bleomycin in female BRCA1 carriers and was compared with noncarrier relatives. The frequency of chromosome breaks was also measured in BRCA1 carriers following oral selenium supplementation. Carriers of BRCA1 mutations showed significantly greater mean frequencies of induced chromosome breaks per cell than did healthy noncarrier relatives (0.58 versus 0.39; P < 10−5). The frequency of chromosome breaks was greatly reduced following 1 to 3 months of oral selenium supplementation (mean, 0.63 breaks per cell versus 0.40; P < 10−10). The mean level of chromosome breaks in carriers following supplementation was similar to that of the noncarrier controls (0.40 versus 0.39). Oral selenium is a good candidate for chemoprevention in women who carry a mutation in the BRCA1 gene. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1302–6)

Introduction

Women who carry a mutation of the BRCA1 gene face a lifetime risk of breast cancer of ~80% and a lifetime risk of ovarian cancer of ~40% (1). Men who carry a BRCA1 mutation seem at elevated risk for breast and prostate cancer (2). The BRCA1 gene product is involved in the maintenance of the integrity of the human genome and functions in conjunction with BRCA2 and RAD51 to repair double-stranded DNA breaks through the mechanism of homologous recombination (3). Deficiencies in the ability to repair double-stranded DNA breaks lead to an increase in the formation of gross chromosome rearrangements. Structural chromosomal aberrations seem in excess both in BRCA1-deficient mouse cells (4) and in BRCA1-deficient cancer cells (5). BRCA1 serves multiple functions, including DNA damage response (3), nucleotide excision repair (6), and protection against oxidative stress (7). It is not yet clear to what extent each of these contributes to the cancer susceptibility phenotype of BRCA1 heterozygotes. Nevertheless, it is believed that the chromosomal instability of BRCA1-deficient cells contributes to their tendency to undergo neoplastic transformation (3). Strategies to reduce the frequency of chromosome breaks, therefore, may result in a reduction in the cancer risk. Selenium has several anticancer properties, including protection against oxidative damage and enhancing nucleotide excision repair (8).

One of the most commonly used cytogenetic tests for the assessment of chromosome instability is the in vitro bleomycin assay (9). This is a simple and reproducible assay that measures induced chromosome breaks. Patient lymphocytes are cultured in the presence of bleomycin, a known mutagenic agent that typically induces double-strand breaks (similar to those induced by ionizing radiation). The mean number of chromosomal breaks per cell is then measured after bleomycin exposure. The assay has been used to study susceptibility to various types of cancer (9, 10). Using this technique, we explored the possibility that oral selenium supplementation may reduce the formation of induced chromosome breaks in female BRCA1 carriers.

Materials and Methods

Study Subjects. Female study subjects were recruited from among the attendees of a single familial cancer clinic of the Hereditary Cancer Centre of the Pomeranian Academy of Medicine in Szczecin, Poland. Women were referred to this clinic because of a family history of breast or ovarian cancer. The women who participated in this study had previously been offered and had consented to genetic testing. There were two phases to this study involving two groups of women. The first group of women was recruited for the purpose of comparing the frequencies of chromosome breaks in BRCA1 carriers and noncarriers. Case women were recruited from among those who had been found to be carriers of a deleterious mutation in the BRCA1 gene. Control subjects were recruited from among the family members of the carriers (cases) but who had been determined not to carry the deleterious mutation. It was possible to generate 26 case-control pairs. In 20 instances, the control relative was the sister of the case and in six instances, she was a more distant relative. Only healthy women were invited to participate in this study; women with a past history of breast, ovary, or other...
forms of cancer were excluded. Each woman provided a blood sample at some time between February 2001 and January 2002.

A second group of BRCA1 carriers was recruited from this clinic for the purpose of studying the effect of selenium supplementation on frequencies of chromosome breaks. Thirty-five women with BRCA1 mutations agreed to participate in this phase of the study. Blood samples were taken before the onset of selenium supplementation, and again at a time from 1 to 3 months following the start of daily selenium supplementation. Because the two studies were conducted at different times and because of the availability of suitable controls, and because of willingness to participate, there was little overlap in the study subjects between the two groups of carrier subjects. Only six carrier women participated in both phases of the study.

Bleomycin Assay. Chromosome sensitivity to bleomycin was measured according to the method of Hsu et al. (9) Bleomycin (Nippon-Kayuka, Tokyo, Japan) was added to the cell culture 5 hours before the end of the culture at a concentration of 0.03 IU/mL. Conventional harvesting and Giemsa staining were made. For each subject, 100 consecutive euploid cells in metaphase were read (cells with overlapping chromosomes were excluded). Each chromatid aberration (excluding gaps) was scored as one breakpoint and each exchange-type aberration was scored as two breakpoints. The total number of breakpoints per 100 cells was recorded for each subject. Reading of coded slides was done blindly by one of us (E.K.) For 20 randomly selected cases, the counts were determined independently by two cytogenetic technicians with good agreement.

Selenium Supplementation. An oral selenium solution was provided to the study subjects which contained 690 μg of pure selenium, in the form of sodium selenite (Na2SeO3) per mL of 70% ethanol. Subjects were requested to consume 0.2 mL of the solution twice daily. Among the BRCA1 carriers (n = 32), this level of supplementation resulted in an increase in the mean serum selenium from 56.7 ± 12.7 to 90.2 ± 17.6 ng/mL (P < 0.001). Selenium levels were not measured in the noncarrier controls.

Statistical Analysis. The mean number of breaks per cell was calculated after inspection of 100 cells. The paired t test was used to compare the pre- and post-supplementation selenium levels and numbers of chromosome breaks. The nonparametric sign test was also used to the mean number of induced chromosome breaks per cell. The mean number of induced chromosome breaks per cell was 0.63 (range, 0.42-0.81) and after selenium supplementation the mean number of chromosome breaks per cell was reduced from 0.63 to 0.40 (range, 0.27-0.60). In every case, the post-supplementation level of chromosome breaks showed a decline from the baseline level (Table 2) and the mean difference was highly significant (P < 0.001).

Discussion

In the first phase of this two-part study, we observed that the lymphocytes from heterozygous carriers of deleterious mutations of the BRCA1 gene show an elevated frequency of chromosome breaks after exposure to bleomycin in vitro compared with noncarriers. In the second part of the study, we have shown that in most cases, these elevated levels can be reduced to normal with oral selenium supplementation.

Table 1. Comparison of chromosome breaks (per cell) in BRCA1 carriers and matched controls

<table>
<thead>
<tr>
<th>Case ID</th>
<th>Case (carrier)</th>
<th>Control (noncarrier)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Chromosome breaks per cell</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>0.58</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>0.61</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>0.51</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>0.48</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>0.57</td>
</tr>
<tr>
<td>7</td>
<td>45</td>
<td>0.60</td>
</tr>
<tr>
<td>8</td>
<td>47</td>
<td>0.52</td>
</tr>
<tr>
<td>9</td>
<td>51</td>
<td>0.61</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>0.66</td>
</tr>
<tr>
<td>11</td>
<td>48</td>
<td>0.57</td>
</tr>
<tr>
<td>12</td>
<td>24</td>
<td>0.65</td>
</tr>
<tr>
<td>13</td>
<td>23</td>
<td>0.65</td>
</tr>
<tr>
<td>14</td>
<td>31</td>
<td>0.73</td>
</tr>
<tr>
<td>15</td>
<td>31</td>
<td>0.52</td>
</tr>
<tr>
<td>16</td>
<td>23</td>
<td>0.52</td>
</tr>
<tr>
<td>17</td>
<td>44</td>
<td>0.59</td>
</tr>
<tr>
<td>18</td>
<td>59</td>
<td>0.48</td>
</tr>
<tr>
<td>19</td>
<td>53</td>
<td>0.54</td>
</tr>
<tr>
<td>20</td>
<td>58</td>
<td>0.59</td>
</tr>
<tr>
<td>21</td>
<td>44</td>
<td>0.51</td>
</tr>
<tr>
<td>22</td>
<td>22</td>
<td>0.67</td>
</tr>
<tr>
<td>23</td>
<td>34</td>
<td>0.60</td>
</tr>
<tr>
<td>24</td>
<td>34</td>
<td>0.71</td>
</tr>
<tr>
<td>25</td>
<td>18</td>
<td>0.59</td>
</tr>
<tr>
<td>26</td>
<td>27</td>
<td>0.64</td>
</tr>
<tr>
<td>Mean</td>
<td>35.3</td>
<td>0.58</td>
</tr>
</tbody>
</table>
per cell was higher in 11 patients with familial breast cancer.

Small study (12), the mean number of chromosome breaks will be the subject of a future investigation. In a previous study, there was no significant correlation between the age at testing and the observed number of chromosome breaks. In cell culture are representative of the type of chromosome damage in irradiated lymphocytes compared with 7 of 74 healthy controls ($P < 10^{-4}$). In a small study, Barrios et al. (14) found the frequency of chromosomal aberrations per cell to be higher in lymphocytes taken from breast cancer patients than from healthy controls. It has been proposed that direct mutagenic effects of estrogen may contribute to breast cancer incidence, but this hypothesis has not been proven (15).

Several other studies have reported a correlation between the level of chromosome breaks and cancer susceptibility. In a cohort study of 3,182 workers exposed to mutagens, the frequency of chromosomal aberrations at baseline predicted subsequent cancer risk (16). Several case-control studies have been conducted in populations where exposure to mutagenic carcinogens is common (e.g., lung cancer and head and neck cancer; refs. 17-22); however, an important etiologic role for mutagens in breast cancer has not yet been clearly defined.

The value of selenium as a chemopreventive agent is based upon the assumption that bleomycin-induced breaks in cell culture are representative of the type of chromosome breaks that occur in vivo.

Although our sample size was small, our results were highly significant; in every case, selenium supplementation resulted in a reduced frequency of chromosome breaks.

It is likely that the observed difference between carriers and noncarriers is due to the functional consequences of the BRCA1 mutation; however, alternate explanations must be considered. The cases and controls were matched within families and neither were affected with cancer. The mean (absolute) age difference between the case and her matched control was about 6 years, but the mean ages of the groups differed by <1 year. This difference is relatively small and in our study group there was no significant correlation between the age at testing and the observed number of chromosome breaks; the number of chromosome breaks declined slightly with age ($r = -0.16$; $P = 0.35$). Previously, Gu et al. showed that neither age, sex, nor smoking status were significantly correlated with the frequency of bleomycin-induced breaks in 56 healthy individuals (11). Nor could the difference in the pre- and post-supplementation chromosome breaks levels be accounted for by variation in the assay with the date of testing. The median date of testing of the pre-supplementation testing was in October 2001. There were two phases to this study. Ideally, we would have used the same cases and controls in the two phases. However, this was impractical because the first phase was completed before the development of the protocol for the second phase and only six carriers were enrolled in both phases. We did not offer selenium supplementation to controls because we did not feel the intervention was justified in women who were not at increased risk of cancer and who showed normal levels of chromosome breakage.

In this pilot study, we did not measure the relationship between chromosome breaks and breast cancer risk in the same patients with familial breast cancer.
damage that occurs \textit{in vivo}, and that genetic instability in BRCA1 carriers is related to carcinogenesis. One of the functions of BRCA1 is to repair double-stranded DNA breaks through homologous recombination, but it is not clear if this is the critical function which, when impaired, leads to carcinogenesis. It has also recently been shown that BRCA1 up-regulates multiple genes involved in the antioxidant response and thereby helps protect cells against oxidative stress (7). Selenium is known to protect against oxidative damage, induces apoptosis, and has also been found to assist in nucleotide excision repair (8). It may be that BRCA1 carriers are particularly sensitive to oxidative stress and that this sensitivity may be mitigated by dietary selenium. We have previously evaluated the potential chemopreventive properties of selenium compounds. In a randomized trial designed to evaluate skin cancer prevention, patients who received selenium supplementation experienced declines in the rates of cancer of the prostate, colon, and lung (23). In an update of this prospective study, Duffield-Lillico et al. (24) observed a decrease in the overall cancer rate (relative risk, 0.75; 95% confidence interval, 0.58-0.97) but not in the incidence of breast cancer (relative risk, 1.82; 95% confidence interval, 0.62-6.01). In general, no consistent relationship has been seen between either dietary intake or tissue levels of selenium intake and breast cancer risk in case-control and cohort studies. For example, in the large Nurses’ Health Study, there was no correlation between toenail selenium levels and subsequent risk of breast cancer (25). However, this may not necessarily be the case for breast cancers in BRCA1 carriers. It is likely that the risk factors for breast cancer in BRCA1 carriers are different than for sporadic cancer and that DNA stability and repair is more important for hereditary breast cancer than for cancer in noncarriers. It is of interest that the other genes involved in hereditary susceptibility to breast cancer, such as BRCA2, CHEK2, p53, NBS1, ATM, and Rad51 also participate in the DNA damage response pathway (refs. 3, 26-30) and selenium may be a candidate preventive agent in these syndromes as well.

We chose to give selenium as selenium selenite (inorganic selenium). This preparation is easily dissolved, has high bioavailability, and was effective in preventing chemically induced breast cancer in mice (31). Our daily dose of supplemental selenium was 276 µg. This is slightly lower than the recommended upper dose of 400 µg recommended by the WHO in 1996 and is approximately one third of the daily mean marginal level of safe selenium intake (32). Our study was conducted in Poland, where mean serum selenium levels are lower than in other countries in Europe, the United States, and Australia. The mean level before supplementation in our study subjects was 56 ng/mL, which is lower than the level deemed to meet nutritional requirements. Selenium levels in soil and forage crops differ significantly from country to country and it may be that the results of this experiment would be less dramatic in a country where mean selenium levels are higher. Liu et al. observed that dietary selenium (as sodium selenite) profoundly inhibited rates of mammary carcinogenesis in 7,12-dimethylbenz(a)anthracene–treated mice (33). This mouse model may not adequately represent the majority of human sporadic breast cancers (where the role of mutagenic carcinogens is uncertain) but may be relevant for hereditary syndromes in which the breast cancers show genomic instability. Foray et al. (33) reported a diminished capacity of immortalized lymphocytes from BRCA1 carriers to repair double-stranded DNA breaks and an increase in the number of micronuclei. These observations were not confirmed by Nieuwenhuis et al. (34) who found that fibroblasts from BRCA1 carriers did not show a greater frequency of unrepaired double-stranded DNA breaks following exposure to radiation than fibroblasts derived from noncarrier controls. The mechanism by which selenium reduces the frequency of chromosome breaks in BRCA1 carriers is unclear, but it is likely that selenium reduces the frequency of double-stranded breaks, as opposed to correcting the capacity of the cells to repair broken strands of DNA. We did not offer selenium supplementation to healthy controls; future studies of this type may help to resolve this question.

In general, tumor cells from women with BRCA1 mutations will have lost both functional copies of the BRCA1 gene; however, the present study indicates that there may be a subclinical phenotype at the cellular level associated with the loss of a single copy of BRCA1. We did not confirm that the cells with chromosome breaks in our study have an intact BRCA1 allele; however, because of the high frequency of induced cell deaths observed in both cells in culture and in controls, this is likely to be the case. This data suggests that there may be a mutator phenotype associated with loss of a single copy of BRCA1.

Our study was small and was limited to women with BRCA1 mutations. It is important that these results be replicated before selenium is offered to high-risk patients. Ultimately, it is hoped to show a reduction in breast cancer incidence in BRCA1 carriers associated with selenium supplementation.

References
Increased Rates of Chromosome Breakage in BRCA1 Carriers Are Normalized by Oral Selenium Supplementation

Elzbieta Kowalska, Steven A. Narod, Tomasz Huzarski, et al.


**Updated version**
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/14/5/1302

**Cited articles**
This article cites 33 articles, 9 of which you can access for free at:
http://cebp.aacrjournals.org/content/14/5/1302.full#ref-list-1

**Citing articles**
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/14/5/1302.full#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.