Hypersensitivity to Mitomycin C-induced Sister Chromatid Exchange as a Biomarker of Past Exposure to Arsenic

Saou-Hsing Liou, Tyan-Luen Gu, and Chien-Jen Chen

School of Public Health, National Defense Medical Center, 18 Shi-Yuan Street, P. O. Box 90048-509, Taipei, Taiwan, 10107 [S.-H. L., T.-L. G.] and School of Public Health, Taiwan University, Taipei, Taiwan, 10018 [C.-J. C.]. Republic of China

Abstract
The objective of this study was to determine if cytogenetic markers can be used as indicators of prior exposure to arsenic compounds. Baseline sister chromatid exchange (SCE) and mitomycin C-induced (MMC) SCE were measured in four study populations recruited from a blackfoot (BF) disease endemic area, including 22 patients with cancer (CA) only, 8 patients with both BF disease and CA (BF+CA), 10 patients with BF disease only, and 26 healthy residents (HRs). Another group of 23 healthy, nonarsenic-exposed workers were recruited as external healthy controls (HCs). Characteristics of study population were collected by questionnaire, and 10 ml of venous blood were drawn for lymphocyte culture. The results showed that the frequencies of baseline SCE did not differ among the five study groups. The frequencies of ΔSCE (MMC-induced SCE minus baseline SCE) in CA only, BF disease only, and HRs, three arsenic-exposed groups, were significantly higher than in HCs. The frequency of ΔSCE in the BF+CA group was nonsignificantly higher than in HCs, probably due to small sample size. The frequencies of both baseline SCE and ΔSCE did not differ among CA only, BF disease only, BF+CA, and HR groups. The observation that baseline SCE did not increase in the arsenic-exposed populations indicates either that exposures were insufficiently high to change this marker or that lesions did not persist. The increased SCE response to MMC in arsenic-exposed populations suggests that previous arsenic exposure may result in hypersensitivity of human lymphocytes to carcinogens and/or mutagens. Both baseline SCE and ΔSCE were not different among patients with arsenic-induced diseases and healthy normal residents, indicating that hypersensitivity may have been due to previous arsenic exposure but was not associated with disease status.

Introduction
Arsenic is a well-known human carcinogen based on epidemiological evidence from occupational exposures and from community exposure to high levels of arsenic in drinking water (1-3). In addition to BF disease, a peripheral vascular disease with discoloration of the skin of extremities, follow-up studies of exposure to arsenic in drinking water in Taiwan (Republic of China) have indicated increased cancer risk of skin, lung, bladder, kidney, liver, and other internal organs (4-8). Biomarkers for monitoring previous exposure and predicting cancer risk are useful for epidemiological follow-up of cancer development in this high-risk group.

Cytogenetic markers, e.g., SCEs, have been used to monitor populations currently exposed to carcinogens (9, 10). Ongoing or recent exposure to carcinogens has been shown to induce SCEs (11-14). However, cytogenetic markers (e.g., SCEs) had a disadvantage of limited persistence. After cessation of exposure, the cytogenetic markers can diminish or disappear in 2-5 years. It has been shown that mutagen or carcinogen exposure can induce heritable susceptibility, which can be detected by mutagen challenge at a later time (15). These results suggest human exposure to carcinogens will alter cytogenetic responses of cultured lymphocytes to subsequent mutagenic challenge. The mutagenic challenge marker, such as MMC-induced SCE, may be an indicator for detection of previous exposure.

This study was designed to explore the feasibility of using genotoxic indicators of past arsenic exposure in a BF endemic area. Increased SCEs and mutagenic susceptibility to genotoxins have been hypothesized to be risk indicators of cancer (16-20). This study also investigated whether susceptibility to the induction of SCE by MMC was associated with disease status related to arsenic ingestion.

Materials and Methods
Selection of Study Population. Arsenic-exposed study populations were selected from Pu-Dai county (Tainan, Taiwan, Republic of China), which exhibits the highest incidence of BF disease and cancers in the BF endemic area. Volunteers were classified into four groups based on the existing disease status: 26 HRS (internal controls), 10 patients with BF disease only, 22 patients with CA only, and 8 patients with BF+CA. All of the BF disease patients were confirmed by the disease registry in the local health department. Seventeen of 22 patients with CA only were diagnosed by a dermatologist to have skin cancer (21). The cancer types of the other five patients included bladder (1), liver (1), stomach (1), and other (2) cancers. Six of eight patients with BF+CA were diagnosed with skin cancer. The other two patients had bladder cancer.

Received 4/19/95; revised 11/9/95; accepted 11/9/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore by hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The study was supported by a grant from National Science Council Grant NSC 81-0421-B-016-533-Z, the Republic of China.

2 To whom requests for reprints should be addressed. Phone: 886-2-3655559; Fax: 886-2-3673742.

The abbreviations used are: BF, blackfoot; SCE, sister chromatid exchange; MMC, mitomycin C; HC, healthy control; CA, cancer; HR, healthy resident.
In addition, 23 healthy workers who neither lived in the BF endemic area nor were occupationally exposed to arsenic compounds were selected as the HC. Both internal and external controls were examined by a physician to exclude the suspicious cases of BF disease and cancer. Each participant was interviewed using a formatted questionnaire to collect related variables. Blood (10 ml) was drawn from each participant.

**Measurement of Baseline and MMC-induced SCE.** After collection of specimens from both arsenic-exposed and control populations, the lymphocytes were cultured simultaneously within 12–16 h of drawing blood. The same batch of cell culture medium and chemical solutions were used in all experiments. Whole blood (0.5 ml) was cultured at 37°C in 5 ml of RPMI 1640 supplemented with 15% FCS, 1% L-glutamine, and 1% antibiotics. For MMC-induced SCE, 10 ng/ml were added at the time of culture initiation and left in contact with the cells for the entire culture period (72 h). Each culture was run in duplicate to ensure a sufficient number of mitoses for analysis (10).

Two h before harvest, colchicine (15 μg/ml) was added to block the cell in metaphase. The cell pellets were treated with 0.075 M KCl hypotonic solution and fixed with glacial acetic acid:methanol (1:3) solution. The chromosomes were stained with the fluorescence plus Giemsa method. SCEs were scored
Occupations was also not different among these four groups. One person in the CA-only group and one person in the HR ceased drinking artesian water 20 years before this study. Only tap water system was set up in late 1960, and most residents had consumed artesian water for >30 years. The duration of consumption of artesian well water was not different among the four groups residing in the BF endemic area. One-half of them drank artesian water for >30 years. The frequency of baseline SCEs/cell and LSCE was 4.63 ± 2.88 SCEs/cell. The frequency of SCEs in internal HRs was 9.40 ± 2.27, 10.04 ± 1.63, and 9.86 ± 1.54 SCEs/cell, respectively. The frequencies of SCEs/cell in the patients with CA only and in patients with BF disease only are 10.06 ± 2.13 (8) and 15.56 ± 4.97 (8), respectively. The mean baseline SCE frequencies for these five study groups are listed in Table 2. The frequency of baseline SCEs/cell, MMC-induced SCEs and baseline SCEs/cell in each study group is listed in Table 2. The frequencies of SCEs/cell in the patients with CA only and in patients with BF disease only were 10.06 ± 2.13, 10.04 ± 1.63, and 9.86 ± 1.54 SCEs/cell, respectively. The frequencies of SCEs/cell in the patients with CA only and in patients with BF disease only were 10.06 ± 2.13, 10.04 ± 1.63, and 9.86 ± 1.54 SCEs/cell, respectively. Most of them were engaged in seafood harvesting. None of them had a history of exposure to other known mutagens or carcinogens.

**Statistical Method.** The distribution of SCE- and MMC-induced SCE was expressed as mean and SD. The mean induction by MMC (ΔSCE) was calculated by subtraction of baseline SCE from the MMC-induced SCE. Comparison among groups was tested by the nonparametric Mann-Whitney method.

**Results**

**Characteristics of the Study Population.** The distribution of the characteristics of the five study populations is listed in Table 1. The distribution of gender, marital status, smoking status, quantity of cigarettes smoked, and alcohol and tea consumption was not significantly different among the study groups. No difference was found among these groups for mean age of males, but the mean age of female external HC was significantly younger than it was for the females of the other four groups residing in the BF endemic area. The education levels in the external HC group was also higher than it was in the other four groups. Single variable analysis showed that age and education level did not affect the SCE outcomes.

The duration of consumption of artesian well water was not different among the four groups residing in the BF endemic area. One-half of them drank artesian water for >30 years. The tap water system was set up in late 1960, and most residents had ceased drinking artesian water 20 years before this study. Only one person in the CA-only group and one person in the HR group are currently consuming artesian well water. These two individuals were excluded in the analysis. The distribution of occupations was also not different among these four groups.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>The means of baseline SCE, MMC-induced SCE, and ΔSCE (unit: SCE/cell) among the five study groups</th>
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<td>Groups</td>
<td>Frequencies</td>
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<td>BF + CA (n)</td>
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<tr>
<td>SCE</td>
<td>Baseline SCE</td>
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<tr>
<td>MMC-induced SCE</td>
<td>15.56 ± 4.97 (8)</td>
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<tr>
<td>ΔSCE</td>
<td>7.05 ± 3.71 (7)</td>
</tr>
</tbody>
</table>

* Exclude one individual with current use of well water.
* Exclude individuals without sufficient number of metaphases for scoring SCE.
* Significantly higher than HC (P < 0.05, Mann-Whitney test).
* ΔSCE, MMC-induced SCE-baseline SCE.
* Exclude one individual whose MMC-induced SCE is less than baseline SCE (ΔSCE is negative).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>The means of baseline SCE, MMC-induced SCE, and ΔSCE among the five study groups, stratified by smoking status</th>
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<td>Groups</td>
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<td>SCE</td>
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<td>MMC-induced SCE</td>
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<td>ΔSCE</td>
<td>Smokers</td>
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<td>Nonsmokers</td>
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* P < 0.10 (Mann-Whitney test), compared with HC.
* ΔSCE, MMC-induced SCE-baseline SCE.
* P < 0.05 (Mann-Whitney test), compared with HC.
patients with BF+CA and in HRs were not different from controls. 

\[ \Delta \text{SCE} \text{ in the arsenic-exposed groups was between 7.05 and 7.99 SCEs/cell.} \]

The net induction of SCE (\( \Delta \text{SCE} \)) in patients with CA only, in patients with BF only, and in the HR group were all significantly higher than in external HCs. The lack of significant difference in \( \Delta \text{SCE} \) in patients with BF+CA may be due to the small sample size. The results showed that the arsenic-exposed individuals were more susceptible to SCE induction induced by MMC than were healthy nonexposed controls. However, the susceptibility was not associated with disease status of the arsenic-exposed study population.

The frequencies of baseline and MMC-induced SCE stratified by smoking status, an important influencing factor of SCE, is listed in Table 3. The results showed that the frequency of baseline SCE in smokers was significantly higher than it was in nonsmokers. However, frequencies of baseline SCE in arsenic-exposed groups were also not significantly higher than in external HCs, for both smoking and nonsmoking groups. The net induction of SCEs did not differ between smokers and nonsmokers. The net induction of SCEs in arsenic-exposed groups was significantly higher than in HCs, only for nonsmoking individuals. The net induction of SCE in smoking, arsenic-exposed groups was not significantly higher than the smoking controls, with the exception of smoking patients with BF only. Statistical nonsignificance in intergroup comparisons of smokers may be due to the small numbers of smokers in each group.

Discussion

Individuals with ongoing occupational arsenic exposure and individuals ingesting arsenic through drinking water have been shown to have increased frequency of baseline SCE (11–14). A previous study of the frequency of SCE in BF patients (11) who ceased drinking artesian well water for a mean of 12 years revealed an increase of baseline SCE. An increased frequency of SCE in arsenic-treated patients has been shown to persist for >5 years (22). However, this study found that previous (<20 years) arsenic exposure in BF endemic area residents did not result in higher baseline SCE than nonexposed controls. The frequencies of SCEs in patients with BF+CA, with BF only, or with CA only were not higher than in normal HCs. This finding suggests that baseline SCE was not associated with disease status. One recent study (19) also showed that baseline SCE was not related to cancer risk.

Challenged with MMC, the lymphocytes of arsenic-exposed people showed increased SCE compared to those of nonexposed controls. Arsenite has been shown to enhance the DNA damage caused by UV light and DNA cross-linking agents in rodent and human cells (23–25). Arsenite may also exert its co-genotoxic effects by inhibiting DNA repair (26). The increased susceptibility of lymphocytes from an arsenic-exposed population indicates that arsenic exposure may result in hypersensitivity of human lymphocytes to carcinogens and/or mutagens. In addition to ongoing or recent exposures shown in other studies (16–18, 27, 28), this study revealed that previous exposures can also be detected by a mutagen challenge method. These results suggest that the susceptibility due to carcinogen exposure may persist for a long time. These results are consistent with previous findings that previous exposure to carcinogens may increase sensitivity to other carcinogens (15). However, previous studies in the BF endemic area found that lymphocytes of BF patients were not more sensitive to arsenite than were controls (11).

The induced hypersensitivity was also not associated with the existing disease status. The net induction of SCEs in patients with BF+CA, with BF disease only, or with CA only was not higher than in normal HCs. Epidemiological evidence revealed a dose-response relationship between arsenic intake and BF diseases (4). A dose-response relationship between arsenic intake and cancers was also shown in Taiwanese studies (5, 6). The incidence of cancers was associated with the existence of BF disease, even after the adjustment for arsenic intake (7, 8). Arsenic exposure results in the development of BF disease and cancers, and BF patients are predisposed to develop cancers. There seems to be a sequential changes associated with arsenic intake, BF disease, and cancer. If hypersusceptibility was associated with disease status, then the cancer patients should be more susceptible than the BF disease patients, who ought to be more sensitive than the HCs. However, the results of this study do not support this hypothesis. This finding suggests that the net induction of SCE was associated with arsenic exposure but not with disease status (29). These results are consistent with previous findings that lung cancer patients were not more sensitive to benzo(a)pyrene than normal controls (16–18).

Although MMC-induced susceptibility was shown to be associated with previous arsenic exposure in the BF endemic area, application of this indicator to other exposed populations or other chemicals requires additional investigation. In addition, whether susceptibility to mutagen challenge can be used as an indicator of cancer risk will be confirmed by a follow-up study of the relation between susceptibility and cancer risk in the BF endemic area.

Acknowledgments

The authors thank David Jacobson-Kram, Ph.D., for preparation of the manuscript.

References


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