

Decrease in Bladder Cell Micronucleus Prevalence after Intervention to Lower the Concentration of Arsenic in Drinking Water¹

Lee E. Moore, Allan H. Smith,²
Claudia Hoppenhayn-Rich, Mary Lou Biggs,
David A. Kalman, and Martyn T. Smith

School of Public Health, University of California at Berkeley, Berkeley, California 94720 [L. E. M., A. H. S., C. H.-R., M. L. B., M. T. S.]; and School of Public Health and Community Medicine, University of Washington, Seattle, Washington, 98195 [D. A. K.]

Abstract

Epidemiological studies performed in Taiwan, Argentina, and Chile suggest that ingestion of arsenic (As) may cause bladder cancer. Because of these findings, we previously investigated the relationship between As ingestion and genetic damage to the urothelium in two cross-sectional biomarker studies, one in Nevada and one in Chile. In both studies, we found that increased levels of micronucleated cells (MNCs) in exfoliated bladder cells were associated with elevated concentrations of As in drinking water, suggesting that As induces genetic damage to bladder cells. To further investigate this relationship, we conducted an intervention study in a subset of highly exposed men ($n = 34$) from the cross-sectional study in Chile. Subjects whose usual source of water contained about 600 $\mu\text{g/liter}$ As were supplied with water lower in As (45 $\mu\text{g/liter}$) for 8 weeks, allowing ample opportunity for renewal and exfoliation of bladder epithelial cells. Mean urinary As levels decreased during the intervention from 742 to 225 $\mu\text{g/liter}$. Bladder MNC prevalence also decreased from 2.63 MNCs/1000 cells preintervention to 1.79 MNCs/1000 cells postintervention ($P < 0.05$). When the analysis was limited to individuals previously having subcytotoxic urinary As levels ($<700 \mu\text{g/liter}$), the change between pre- and postintervention MNC was more pronounced: the level decreased from 3.54 to 1.47 MNCs/1000 cells, respectively ($P = 0.002$). Among smokers, MNC prevalences decreased from 4.45 MNCs/1000 cells preintervention to 1.44 MNCs/1000 cells postintervention ($P = 0.002$). Among nonsmokers, the decrease was much smaller: 2.04 MNCs/1000 cells preintervention to 1.90 MNCs/1000 cells postintervention ($P = 0.25$), suggesting that smoker's bladder cells could be more susceptible to genotoxic damage caused by As. The reduction in bladder MNC prevalence with reduction in As intake provides further evidence that As is genotoxic to bladder cells.

Introduction

The exfoliated cell MN³ assay is a useful method for measuring and quantifying recent DNA damage that results from environmental exposure (1). The use of such a biomarker of genetic damage as an outcome in an epidemiological study not only shortens the length of time between exposure and effect but is also a unique opportunity to measure the effects of exposure reduction using each person as his or her own control. To date, very few intervention studies using biomarkers have been conducted. Moreover, to our knowledge, cytogenetic intervention studies have never been used to evaluate the relationship between an environmental exposure and a genotoxic effect in humans. Examples of exposures that have been examined using the MN assay and the intervention study design include chemopreventative agents such as antioxidants (*i.e.*, vitamin A and β -carotene) retinyl palmitate (2, 3), α -tocopherol (4), antischistosomal drugs (5, 6), and genotoxic agents, such as radiation (7, 8) and formaldehyde (9, 10).

MNs are extranuclear bodies in the cytoplasm of a cell that form when acentric fragments or whole chromosomes are left behind the main nucleus at telophase. They provide a quantifiable yet nonspecific measurement of recent DNA injury that results from genotoxic exposure. An increase in the prevalence of MN in a population of cells indicates that chromosome damage has occurred as a result of an exposure that caused either a clastogenic or an aneuploidogenic effect.

Environmental exposure to In-As, a known human carcinogen, primarily occurs from contaminated drinking water sources (11). In-As is a known cause of lung cancer, via inhalation, and skin cancer, via ingestion. Its ingestion is also thought to cause more fatal internal cancers, including those of the bladder, kidney, and lung (12-16).

Exfoliated urothelial cells are epithelial cells that are sloughed from the surface of the genitourinary tract, with a turnover time of about 3 weeks. Because exfoliated epithelial cells are derived from basal cells, recent genetic damage to the basal layer of the bladder is thought to be reflected by the presence of MNs in these cells. Arsenic (As) has been shown to have both clastogenic and aneuploidogenic properties *in vitro* (17-20) and *in vivo* (8, 21).

Previously, we investigated the relationship between chronic ingestion of In-As and the prevalence of exfoliated MNCs of the bladder in two cross-sectional biomarker studies. The first, performed in a small county in Nevada, detected an exposure-dependent increase in bladder MNC frequencies with urinary As levels, suggesting that chronic ingestion of In-As may have a genotoxic effect on the bladder epithelium (22). A larger cross-sectional biomarker study conducted in Chile also

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² To whom requests for reprints should be addressed, at School of Public Health, University of California at Berkeley, 140 Warren Hall 7360, Berkeley, CA 94720. Phone: (510) 843-1736; Fax: (510) 843-5539.

³ The abbreviations used are: MN, micronucleus; In-As, inorganic As; MNC, micronucleated cell; MMA, methylarsonate; DMA, dimethylarsinate; Tot-As, In-As, MMA, and DMA; FISH, fluorescence *in situ* hybridization; PR, prevalence ratio.

revealed a higher prevalence of bladder MNCs in a high- versus low-exposure group. When the study population was divided into quintiles by urinary As levels, an exposure-dependent increase was seen at urinary As levels between 54 and 729 $\mu\text{g}/\text{liter}$, and the MNC prevalence doubled at levels as low as 54–137 $\mu\text{g}/\text{liter}$. At higher urinary As levels (between 729 and 1894 $\mu\text{g}/\text{liter}$), the prevalence of bladder MNCs returned to baseline levels, possibly due to cytostasis or cytotoxicity.

To confirm whether As ingestion was responsible for the increased prevalence of MN that we found in our previous cross-sectional studies, we conducted a prospective short-term intervention study involving 39 men from our Chilean study, who regularly drank water with 600 $\mu\text{g}/\text{liter}$ In-As. We supplied them with water that was lower in As (about 45 $\mu\text{g}/\text{liter}$) for 8 weeks, allowing ample opportunity for renewal and exfoliation of bladder epithelial cells. To determine whether a change in As exposure results in a change in the prevalence of exfoliated bladder MNCs, we used a fluorescent version of the MN assay (23, 24) to compare MNC levels before and after intervention.

Patients and Methods

Study Subjects. Participants in the intervention study were a subset of a high-exposure group from a large cross-sectional biomarker study that was performed previously in the area (21). Briefly, the cross-sectional biomarker study compared residents of San Pedro in northern Chile (population, 1600) to those of the neighboring town of Toconao (population, 360), which had water with low levels of As (about 15 $\mu\text{g}/\text{liter}$), with respect to MNC prevalences in exfoliated bladder cells, urinary As levels, and questionnaire data. Prospective participants were contacted through public announcements, meetings, and door-to-door contact. Local recruiters interviewed them to ascertain age, duration of residence, smoking status, and interest in participation. Recruitment was limited to those who were at least 18 years of age and had lived in the town for at least 3 months. All study subjects were interviewed by trained interviewers, regarding demographic characteristics, smoking and drinking habits, and medical, occupational, and residential histories. Urine and exfoliated bladder cells were collected. We included only male participants in the study because cells found in male urine are almost exclusively transitional bladder cells (25, 26). Although females exfoliate a similar number of transitional cells as males, they also exfoliate squamous cells from the bladder trigone, vulva, and lower genital tract (25–27). Because transitional cells derive from the target tissue of As-induced cancers, without the ability to differentiate squamous cells from transitional cells, any association between chronic ingestion of In-As and bladder MNCs in women would be diluted, biasing the results toward no effect.

Intervention. The intervention included 34 men, who were all participants in a previous cross-sectional study that had been conducted in the area (21). Immediately after the cross-sectional study was conducted, those who agreed to participate in the intervention phase of the study were provided with low-As water (45 $\mu\text{g}/\text{liter}$) for a 2-month period. The water, originating from the town of Calama, located 100 km from San Pedro, was delivered biweekly, directly to participants' homes, in 60-liter tanks. Each family was instructed to use only the low-As water for all drinking and food preparation purposes. During the 2-month period, local study personnel contacted families to ensure that they were not running low on supplied water.

Data Collection. Following a 2-month intervention period, urine samples were collected from all participants for exposure assessment and to obtain exfoliated bladder cells for the MN assay. Participants were also administered a questionnaire by

oral interview. Data that were collected postintervention were compared to corresponding data that were collected preintervention during the previously described cross-sectional study.

Exposure Assessment and As Speciation. To collect exfoliated bladder cells, participants were supplied with precoded polypropylene bottles and instructions for urine collection, both pre- and postintervention. In addition to dichotomous exposure status, which was determined by pre- or postintervention status, the combination of In-As and the metabolites MMA and DMA, referred to as Tot-As, was used to assess exposure. This measure is considered to be the best biomarker of recent In-As exposure. Urinary concentrations were also adjusted for creatinine; however, in the MN analyses, only unadjusted values were used because they more closely reflect the As concentrations to which the urothelial cells were actually exposed (28). The first morning urine void was collected from each subject and was analyzed for In-As, MMA, and DMA. Urine samples were analyzed for As content by hydride generation atomic absorption spectroscopy, based on Andreae's method (29, 30). In-As, MMA, and DMA were converted to their respective arsines by treatment with sodium borohydride under acidic conditions and were collected by sparging and cryogenic trapping. Following the collection of vapors, the trap was allowed to warm, and the arsine species were sequentially volatilized and detected by atomic absorption spectroscopy using a microburner combustion cell. Detection limits for In-As, MMA, and DMA were 0.5, 1.0, and 2.0 $\mu\text{g}/\text{liter}$, respectively.

MN Assay. To obtain bladder cell samples, each subject was asked to provide a total of four urine samples, two nonmorning samples on 2 consecutive days. Only the second and third urine voids of the day were used for cell isolation. The first morning urine void was not used for exfoliated cell collection because exfoliated bladder cells tend to degrade from overnight exposure to urine. Instead, this sample was used for exposure assessment purposes, as described above. Bladder cells were isolated and stored as described previously.

Cells were permeabilized, the MN assay was performed, and slides were scored as described elsewhere (23, 24). A fluorescent version of the MN assay, which uses FISH with a biotin-labeled α -satellite probe for all human centromeres (Oncor), was used. In certain urine voids from some participants, cell pellets were heavily contaminated with crystals or a sand-like sedimentation that obscured cells on the slide. In such situations, we used 0.9% NaCl washes and a Percoll gradient to separate the bladder cells from crystals without jeopardizing cell recovery.

Slides were coded and scored blindly in sets, each containing the same participant's slides, both pre- and postintervention. Only cells that were not smeared, clumped, or overlapped and that contained intact nuclei were included in the analysis, thus excluding cells that were undergoing abnormal cell division and degenerative processes, such as karyorrhexis, karyolysis, nuclear fragmentation, and pyknosis. The prevalence of MNCs was calculated, based only on the number of normal, intact exfoliated cells, using established scoring criteria. All questionable MNs were cross-checked by two observers and discussed until a consensus was reached. We also assessed the percentage of abnormal cells per person by scoring the number of cells with the degenerative processes of karyolysis and karyorrhexis in two sets of 100 cells each. If the two values differed by >15%, a third set of 100 cells was scored and incorporated into the analysis.

Compliance. To ensure compliance with the intervention protocol, attempts were made to collect urine samples from participants at two intervals during the intervention period. The first compli-

ance sample was collected about 2–3 weeks after the change in water supply, and the second was collected after an additional 2–3 weeks. Twenty-five subjects participated on the first collection visit, and 24 subjects participated on the second.

Statistical Analyses. To obtain group values, the MNC prevalence for each individual was first calculated, and then all prevalences were averaged for the group. The MNC PR of the group was calculated by dividing the MNC prevalence postintervention by that preintervention. To quantify the magnitude of change, we also calculated the average change in MNC prevalence by subtracting the preintervention prevalence from the postintervention prevalence and then averaging this difference for the group. Because the changes in bladder cell MNs were not normally distributed, statistical significance of MNC prevalence between intervention groups was assessed by the Wilcoxon sign-rank test. It was hypothesized *a priori* that a decrease in As exposure would be associated with a decrease in the prevalence of MNC, so one-tailed tests were used. Urinary As levels, the number of cells scored, and the percentage of abnormal cells scored/person were compared using Student *t* tests. All analyses were performed first for the group as a whole and then stratified by age, years of residence, ethnicity, and smoking.

We also divided the preintervention group by urinary Tot-As levels because our previous cross-sectional study revealed an unusual dose-response relationship when the group was divided into quintiles (Q1–Q5) by urinary As levels (Q1, <53.8 $\mu\text{g/liter}$; Q2, 53.9–137.3 $\mu\text{g/liter}$; Q3, 137.4–414.6 $\mu\text{g/liter}$; Q4, 414.7–728.9 $\mu\text{g/liter}$; and Q5, >728.9 $\mu\text{g/liter}$). An exposure-dependent increase was seen between Tot-As and MNC prevalence when Q1 (1.61 MNCs/1000 cells) was compared to Q2, Q3, and Q4 (3.39, 3.69, and 4.77 MNCs/1000 cells, respectively). In Q5, MNC prevalences returned to baseline levels (1.52 MNCs/1000 cells), probably due to the cytostatic or cytotoxic effects of As at such high levels. Because dividing cells are needed to produce MN after a genotoxic insult, both cytostasis and cytotoxicity could inhibit their expression. To preclude the possibility that individuals exposed to very high doses of As (urinary Tot-As > 700 $\mu\text{g/liter}$) might mask a reduction in MNs that could only be seen in those within the genotoxic or subcytotoxic range (Q2–Q4), we divided the group into two groups, those with Tot-As over 700 $\mu\text{g/liter}$ preintervention (cytotoxic range) and those with Tot-As levels below 700 $\mu\text{g/liter}$ preintervention (genotoxic range), and we analyzed each group separately.

Results

Thirty-nine men participated in the intervention study. Five subjects did not exfoliate urothelial cells, either before or after intervention, leaving 34 males to be included in the analyses. General characteristics of the study population are presented in Table 1. The average age, length of residence, years of education, and ethnicity distributions were similar to those of the high-exposure group of our previous cross-sectional study (21). Twenty-four % were current smokers, most of whom smoked five or fewer cigarettes per day (88%).

For the group as a whole, preintervention As concentrations in participants' drinking water were approximately 600 $\mu\text{g/liter}$, whereas the As concentration in the water delivered to participants' homes during the intervention period averaged about 45 $\mu\text{g/liter}$. The corresponding average urinary Tot-As concentration decreased from 742 (preintervention) to 225 (postintervention) $\mu\text{g/liter}$, with a mean decrease of 510 $\mu\text{g/liter}$. For the subset of subjects from which compliance samples were collected, the average Tot-As was 779 $\mu\text{g/liter}$ before

Table 1 Descriptive characteristics of intervention study participants (n = 34 men)

Characteristic	Mean (range)	Frequency (%)
Age (yr)	42 (20–74)	
Years of residence	19 (0.4–61)	
Years of education	7 (0–16)	
Ethnicity		
Atacameño		27 (79%)
European		2 (6%)
Aymara		2 (6%)
Other		3 (9%)
Nonsmokers		26 (76%)
Smokers (cigarettes/day)		8 (24%)
<1		3 (38%)
1–2		1 (12%)
2–5		3 (38%)
>5		1 (12%)

intervention, 252 and 196 $\mu\text{g/liter}$ during intervention, and 213 $\mu\text{g/liter}$ at the conclusion of the intervention phase. Details concerning the changes in methylation patterns during intervention are given in a separate paper (31).

For the MN assay, a total of 36,890 and 37,910 cells were scored in the pre- and postintervention samples, respectively. The prevalence of MNCs/1000 cells decreased from 2.63 MNCs/1000 cells preintervention to 1.79 MNCs/1000 cells postintervention (PR = 0.7, $P < 0.05$; Fig. 1A). There was an average decrease of 0.84 MNCs/1000 cells, with 18 of the 34 participants (53%) experiencing a decrease (average decrease = 3.16 MNCs/1000 cells), 11 (32%) showing an increase (average increase = 2.57 MNCs/1000 cells), and 5 showing the same prevalence of MNCs. The five individuals whose MNC prevalences stayed the same had no MNCs, either pre- or postintervention.

The results of the analyses for the group of individuals with urinary Tot-As concentrations within a subcytotoxic range before intervention (<700 $\mu\text{g/liter}$) are shown in Fig. 1B. The average concentration of Tot-As before and after intervention decreased from 421 to 189 $\mu\text{g/liter}$, with an average decrease in Tot-As of 232 $\mu\text{g/liter}$. The prevalence of MNCs/1000 cells decreased from 3.54 to 1.47 MNCs/1000 cells (PR = 0.4, $P = 0.002$), with an average decrease in MNCs of 2.07 MNCs/1000 cells. Thirteen of the 18 individuals (72%) had bladder MNC prevalences fall an average of 3.1 MNCs/1000 cells, three (17%) had prevalences increase an average of 1.03 MNCs/1000 cells, and two (11%) showed the same prevalences.

The results of the same analyses for individuals who began the intervention within a cytotoxic range of As exposure (>700 $\mu\text{g/liter}$) are presented in Fig. 1C. The average concentration of Tot-As, before and after intervention, decreased from 1103 to 265 $\mu\text{g/liter}$, with an average decrease of 838 $\mu\text{g/liter}$. The prevalence of MNCs/1000 cells increased from 1.60 to 2.14 MNCs/1000 cells (PR = 1.3, $P = 0.25$), with a mean increase of 0.54 MNCs/1000 cells. In 5 of 16 (31%) participants, MNC prevalences decreased an average of 3.3 MNCs/1000 cells, eight (51%) increased an average of 3.2 MNCs/1000 cells, and three (19%) did not change.

Stratified comparisons of the MNC prevalences by several variables, pre- and postintervention, are presented in Table 2. Among smokers and nonsmokers, the average changes in Tot-As were similar: –452 and –527 $\mu\text{g/liter}$, respectively. However, in smokers, the bladder MNC prevalences fell from 4.54 MNCs/1000 cells preintervention to 1.44 MNCs/1000 cells postintervention (PR = 0.3, $P = 0.002$) but did not change significantly in nonsmokers. Six of the eight smokers (75%)

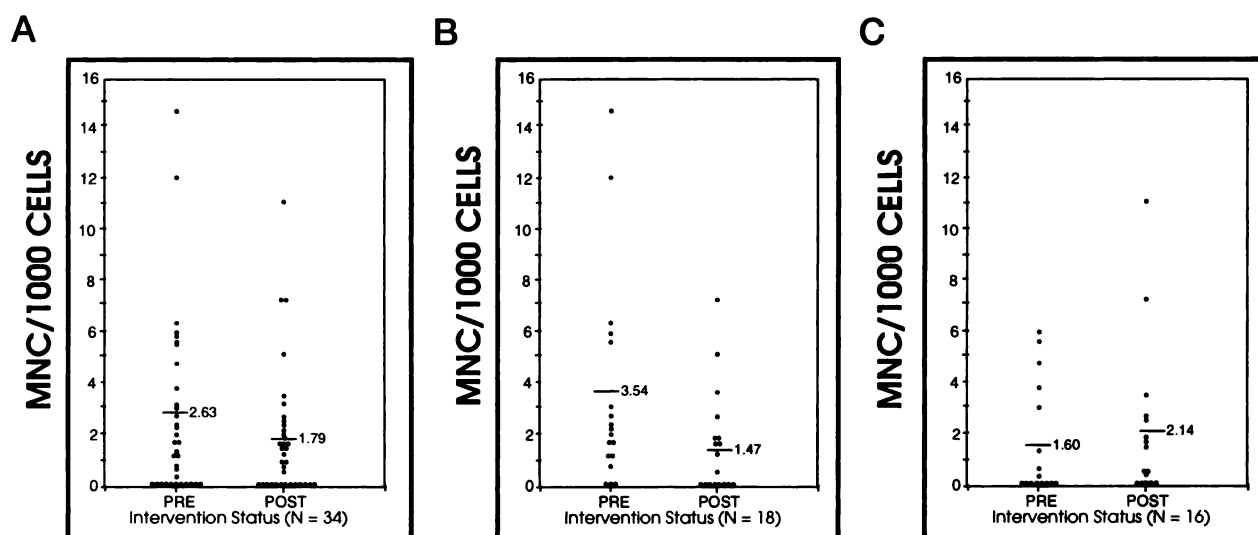


Fig. 1. Prevalence of MNCs/1000 cells before and after intervention. A, all individuals; B, individuals with urinary As < 700 µg/liter preintervention; C, individuals with urinary As > 700 µg/liter preintervention.

had MNC prevalences fall an average of 4.47 MNCs/1000 cells, and two (25%) had MNC prevalences increase an average of 1.00 MNCs/1000 cells. No such decrease was seen among nonsmokers. The average change in MNCs/1000 cells was also greater among smokers than nonsmokers: -3.1 versus -0.14 . In the comparison by ethnicity, the group was divided into Atacameños and all others combined because 79% of the population were of Atacameño origin. The average change in MNCs/1000 cells was greater in the non-Atacameño group than it was in the Atacameño group, but, after stratification by ethnicity and smoking status, the apparent decrease was primarily due to smoking. After stratification by age and length of residence, the average change in MNCs/1000 cells appeared to be greatest in the oldest group (>50) and in those who had lived in the high-exposure town for <5 years.

There was no evidence of an association between the percentage of abnormal cells, the number of cells scored per person, and the change in MNC prevalence.

Discussion

The results of this intervention study provide further evidence that As in drinking water is responsible for the increased bladder MNC prevalence that was found in our previous cross-sectional studies. Bladder cells turn over within 1–3 weeks, and MNCs do not accumulate over time. Therefore, a prospective intervention study design can be used to provide experimental causal evidence for the associations that are found in cross-sectional studies. Because each individual serves as his or her own control, problems due to confounding are largely eliminated. Causal inference in biomarker studies is thus greatly strengthened by conducting intervention trials.

The results of this study demonstrate that MNC prevalences decreased when the As concentration in drinking water was lowered. Urinary As levels dropped from 742 to 225 µg/liter As, indicating that urinary levels were still higher than those that would be expected from sole consumption of water with 40–50 µg/liter As. In unexposed populations, background levels of urinary As generally range from 4.4 to 57.2 µg/liter (32). Some reasons for the elevated levels include: participants

drank fluids or ate food prepared outside their homes; they consumed food grown in the area and with the usual farming water source (San Pedro River, containing 170 µg/liter As; and compliance was incomplete, although most study subjects reported using only the provided water at home at the time of the last interview (31). The higher level could also partly be attributed to As stores in internal tissues from previous exposure. For the group as a whole, the bladder MNC prevalence fell from 2.63 to 1.79 MNCs/1000 cells. A larger reduction was seen when individuals who may have been experiencing cytotoxic effects of As were removed from the analysis, from 3.43 to 1.47 MNCs/1000 cells. An increase was seen when MNC prevalences from individuals within the cytotoxic range of urinary As preintervention were compared, adding further weight to the hypothesis that individuals who are exposed to high levels of As may not be able to express As-induced damage in the form of MNs. We attempted to use the percentage of abnormal cells as a measure of cytotoxicity to the bladder epithelium but did not find that an increase in this measurement correlated with a decrease in the MNC prevalence. To date, a relationship between an exposure and abnormal cell prevalence has been demonstrated in buccal cells but not in bladder cells (8, 21, 33), suggesting that this method is not a sensitive indicator of cell death in a bladder cell sample. We also used hematuria as an indicator of cytotoxicity and cellular damage to the bladder mucosa, but no relationship was seen with As exposure. Here, hematuria was only seen in 1 of 34 males preintervention and 0 of 34 males postintervention, suggesting that As exposure does not cause bleeding from inadequate epithelial lining.

It is noteworthy that postintervention MNC prevalences were lower than those that would be expected from our previous cross-sectional study, in which MNC prevalences for quintiles Q1–Q5 were 1.61, 3.39, 3.69, 4.77, and 1.52 MNCs/1000 cells, respectively (Q1, <53.8 µg/liter; Q2, 53.9–137.3 µg/liter; Q3, 137.4–414.6 µg/liter; Q4, 414.7–728.9 µg/liter; and Q5, >728.9 µg/liter). According to our previous results, the expected postintervention MNC prevalence at a urinary Tot-As concentration of 225 µg/liter (Q3) would be about 2-fold higher, approximately 3.7 rather than the observed 1.79 MNCs/1000 cells (Fig. 1A). Similarly, in Fig. 1, B and C, the expected

Table 2 Prevalence of MNCs/1000 cells, pre- and postintervention, with all ethnicities combined and by various factors

Factor	Intervention status	n	Urinary Tot-As ($\mu\text{g/liter}$)	Average change in Tot-As	MNCs/1000 cells	Average change in MNCs/1000 cells	PR	P (one-tailed)
None	Pre	34	742	-517	2.63	-0.84	0.68	<0.05
	Post		225		1.79			
Smoking								
No	Pre	26	765	-527	2.04	-0.14	0.93	0.25
	Post		237		1.90			
Yes	Pre	8	668	-452	4.54	-3.10	0.32	0.002
	Post		217		1.44			
Ethnicity								
Atacameño	Pre	27	808	-560	2.14	-0.30	0.86	0.15
	Post		248		1.85			
Other	Pre	7	486	-315	4.50	-2.94	0.22	0.03
	Post		171		1.56			
Age (yr)								
<30	Pre	7	582	-303	3.63	-1.30	0.64	0.11
	Post		279		2.33			
30-50	Pre	16	939	-672	1.77	0.19	1.11	0.45
	Post		266		1.96			
>50	Pre	11	557	-404	3.24	-2.05	0.37	0.03
	Post		154		1.19			
Years of residence								
<5	Pre	4	661	-518	3.63	-2.83	0.22	0.04
	Post		143		0.80			
5-15	Pre	12	863	-657	2.70	-0.89	0.67	0.15
	Post		206		1.80			
>15	Pre	18	679	-409	2.36	-0.36	0.84	0.30
	Post		270		1.99			

MNC prevalence postintervention (Tot-As levels, 189 and 265 $\mu\text{g/liter}$) should have been greater, about 3.7 MNCs/1000 cells rather than the observed 1.47 and 2.14 MNCs/1000 cells, respectively. It is possible that individuals previously exposed to higher levels of As may be more efficient at detoxifying As by some unknown mechanism as part of an adaptive response to exposure. The major route for In-As detoxification involves methylation of As by methyltransferase, followed by elimination via urinary excretion (31, 34, 35). Two methylation steps take place producing MMA and DMA, both considered to be less toxic than In-As. Pre- and postintervention, In-As and its methylated metabolites were measured in the urine in the following approximate proportions: In-As, 19.3%; MMA, 16.7%; DMA, 64.0%; and In-As, 17.7%, MMA, PMA, 14.3%, and 68.3%, respectively. If highly exposed individuals had increased methylation capacity, one would expect the proportion of methylated As species to increase when exposure dropped. However, after intervention, the percentage of In-As did not decrease significantly, (from 19.3 to 17.7%; $P = 0.54$), suggesting that increased methylation capacity was not responsible for the lower MNC prevalences measured. A decrease was seen, however, in the MMA:DMA ratio postintervention (0.28 and 0.22, respectively; $P = 0.005$, paired t test), which could mean that participants were more efficient methylators at the second methylation step as a consequence of their previous high exposure.

With respect to the different subgroups, other factors were also associated with a greater change in MNC prevalence after intervention, including smoking (Table 2). MNC prevalences decreased substantially among smokers, but there was little change among nonsmokers. It is possible that the increased MNC prevalence found in smokers is due to chance because of the small number of very light smokers (88% smoked <5 cigarettes per day). Alternatively, it is also possible that bladder cells of smokers may be more susceptible to genotoxic damage caused by As, due to competition between As and the chemicals

that are found in cigarette smoke for substrates that are involved in As detoxification, such as glutathione or the methyltransferases (15, 36, 37). If so, one would expect to see a relationship between MNC prevalence and methylation capacity, which was not observed in this study. It has also been proposed that As inhibits DNA repair enzymes, which would be necessary to repair genetic damage caused by tobacco carcinogens (38). Cells treated with As *in vitro* have shown enhanced sensitivity to X-ray-mediated death and retarded repair of X-ray-induced single-strand breaks of DNA (39). Li and Rossman (38) have shown that arsenite inhibits DNA ligase II, which is required in DNA excision repair. Arsenic is highly reactive with closely spaced vicinal dithiol groups in proteins (40, 41). The number of proteins containing vicinal dithiols is relatively small, but this feature is common among DNA-binding proteins, transcription factors, and DNA repair proteins.

In our previous cross-sectional study, we were able to determine that chromosome breakage was the major cause of MN formation in As-exposed bladder cells. We used a fluorescent version of the exfoliated bladder cell MN assay, using FISH with a centromeric probe to identify the presence (MN+) or absence (MN-) of whole chromosomes within MN, thereby determining the mechanism of As-induced genotoxicity *in vivo*. Here, preintervention samples had almost three times as many unscorable MNs as postintervention, making it impossible to compare the mechanism of genotoxic damage between groups. This disproportionality of unscorable cells in the highly exposed groups was seen to some extent in studies of both radiation and As-exposed individuals when interphase cytogenetics with centromeric probes were used. In these studies, it was inferred that probe penetration to the nucleus may have been impeded by thickening of the cell membrane, as a protective response to a genotoxic or cytotoxic exposure. For this reason, we chose not to present the centromeric probe data.

In summary, we have shown genetic damage in the form of

bladder cell MNs to decrease after changing from a high to a lower concentration of As in drinking water, from 2.63 and 1.79 MNCs/1000 cells, respectively. The greatest change was seen when only individuals at a subcytotoxic urinary As level ($<700 \mu\text{g/liter}$) were included in the analysis, from 3.54 and 1.47 MNCs/1000 cells. The prevalence of MNCs from very highly exposed individuals (urinary Tot-As $> 700 \mu\text{g/liter}$) did not decrease, possibly due to the fact that their cells were experiencing cytotoxicity or cytostasis. Both factors would make cells less likely to express the genetic damage that they have incurred in the form of MNs. In addition, MNC prevalences decreased only in smokers, suggesting that their bladder cells could be more susceptible to genotoxic damage caused by As, supporting the suggestion of synergy between smoking and As exposure.

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