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Chromosome aberrations in cultured human lymphocytes exposed to trivalent and pentavalent arsenic

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NORDENSON I, SWEINS A, BECKMAN L. Chromosome aberrations in cultured human lymphocytes exposed to trivalent and pentavalent arsenic. Scand j work environ health 7 (1981) 277-281. Cultured human lymphocytes were exposed to trivalent (NaAsO₂) and pentavalent (Na₂HAsO₄) arsenic in concentrations comparable to the arsenic levels found in the urine of copper smelter workers. Significantly increased frequencies of chromosome aberrations (gaps, chromatid breaks, chromatid exchanges and chromosome breaks) were found after exposure to trivalent but not pentavalent arsenic. This effect was not found when nonstimulated (G₀) lymphocytes were exposed to trivalent arsenic and then cultured. The rate of sister chromatid exchanges was also found to be increased after exposure to trivalent arsenic. The results suggest that trivalent arsenic is more genotoxic than pentavalent arsenic and that arsenic exerts its effect mainly during cell division.

Key terms: smelter workers, sister chromatid exchanges, urinary arsenic.

Arsenic has been reported to be carcinogenic, teratogenic, and mutagenic (1, 5). Occupational and environmental exposure to arsenic has attracted increasing interest in recent years (4). The prospect of an increased usage of coal as an energy source, eg, in coal-fired power plants, gives further emphasis to the importance of studying the genotoxic effects of arsenic.

Trivalent arsenic is more toxic than pentavalent arsenic and the major part of ingested or inhaled inorganic arsenic is biotransformed and excreted in the urine as dimethylarsinic acid and methylarsonic acid (3, 17).

Increased frequencies of chromosome aberrations have been observed in lymphocytes of individuals exposed to arsenic, eg, wine-growers using arsenic-containing pesticides (13, 15), patients given arsenic for therapeutic reasons (9, 13), and copper smelter workers (7). Burgdorff et al (2) reported an increased rate of sister chromatid exchanges (SCEs) in lymphocytes from arsenic treated patients, whereas Nordenson et al (9) found no such increase.

In cultured cells arsenic has been reported to cause mitotic arrest and chromosome damage (10, 11, 13, 14). In a recent study Nakamuro & Sayato (6) showed that the chromosome-breaking effect in cultured lymphocytes and fibroblasts was significantly higher with trivalent than with pentavalent arsenic. Zanzoni & Jung (18) found an increased rate of SCEs in human lymphocytes treated in vitro with sodium arsenate.

In this paper we report the results of studies on chromosome aberrations and SCEs in cultured human lymphocytes exposed to trivalent and pentavalent arsenic.

Material and methods

The experiments were performed on lymphocytes from three different healthy individuals. Lymphocyte culturing, chromosome preparation, and the scoring of chromosome aberrations were done according to previously described methods (8).
Sodium arsenite (NaAsO₂) or sodium arsenate (Na₂HAsO₄ × 7H₂O) (Merck) was added to the cultures 24 h after the start. The arsenic compounds were dissolved in 0.9% sodium chloride, and four different doses of the compounds were tested (tables 1 & 2). The cells were exposed for 48 h before the harvesting. Cultures without the addition of arsenic served as controls.

In order to study the effects of arsenic on cells in the G₀ phase, the cells were exposed to sodium arsenite for 24 h, washed twice in culture medium, and then grown in fresh medium supplemented with phytohemagglutinin for another 48 h.

The method used for the studies of SCEs was essentially according to Perry & Wolff (12). The final concentration of bromodeoxouridine was 100 µM. Sodium arsenite was added 24 h after the start, and the cultures were harvested 48 h later. In the scoring of the SCEs, the exchanges in the centromere regions were included in the total frequencies.

**Results**

The mitotic activity was found to be decreased in lymphocyte cultures exposed to arsenic, especially NaAsO₂. As a result of this cytotoxic effect it was sometimes difficult to find the preferred number of 100 intact cells per experiment.

Table 1 shows the numbers of cells and chromosome aberrations in duplicate experiments with NaAsO₂ and Na₂HAsO₄ and in controls. There was no heterogeneity between cells from different individuals. The results of the duplicate experiments were essentially the same. Significant differences (on the 5% level) were found concerning gaps. The frequency of gaps was higher in experiment 1 at the dose

| Dose                  | Number of cells | Aberrations                  |
|-----------------------|-----------------|------------------------------|
|                       |                 | Gaps                        |
| Sodium arsenite       |                 | Chromatid breaks            |
| 77 · 10⁻⁸M            |                 | Chromosome breaks           |
| Experiment 1          | 100             | 18                          |
| Experiment 2          | 100             | 6                           |
| 150 · 10⁻⁸M           |                 | 43                          |
| Experiment 1          | 100             | 28                          |
| Experiment 2          | 90              | 8                           |
| 230 · 10⁻⁸M           |                 | 74                          |
| Experiment 1          | 92              | 91                          |
| Experiment 2          | 83              | 22                          |
| 310 · 10⁻⁸M           |                 | 63                          |
| Experiment 1          | 50              | 50                          |
| Experiment 2          | 27              | 19                          |
| Sodium arsenate       |                 | 1                           |
| 77 · 10⁻⁸M            |                 | 0                           |
| Experiment 1          | 100             | 2                           |
| Experiment 2          | 100             | 3                           |
| 150 · 10⁻⁸M           |                 | 8                           |
| Experiment 1          | 82              | 3                           |
| Experiment 2          | 150             | 4                           |
| 230 · 10⁻⁸M           |                 | 1                           |
| Experiment 1          | 100             | 2                           |
| Experiment 2          | 100             | 1                           |
| 310 · 10⁻⁸M           |                 | 0                           |
| Experiment 1          | 100             | 0                           |
| Experiment 2          | 100             | 2                           |
| Control               | 500             | 9                           |
|                       |                 | 2                           |
|                       |                 | 3                           |
|                       |                 | 14                          |
77 • 10^{-8}M, but higher in experiment 2 at the doses 230 • 10^{-8}M and 310 • 10^{-8}M. Those differences were apparently non-systematic and rather small compared to the differences between doses, and therefore the results of experiments 1 and 2 were combined.

Table 2 shows aberrations per cell after exposure to NaAsO_2 and Na_2HAsO_4. Increased frequencies of aberrations were found only in cultures exposed to trivalent arsenic (NaAsO_3). Gaps and chromatid breaks showed a significant and dose-dependent increase. There was also a significant increase in chromosome breaks, but this increase was apparently not dose-related. On the contrary there was a tendency towards a negative correlation between arsenic dose and chromosome breaks.

Chromatid exchanges are a very rare type of aberration. Yet there were 19 such aberrations in 642 cells exposed to trivalent arsenic as compared to none in 832 cells exposed to pentavalent arsenic and 500 control cells.

No increased frequency of aberrations was found when nonstimulated (G_0) lymphocytes were exposed to 390 • 10^{-8}M NaAsO_4. In 390 cells exposed in G_0 only seven gaps, two chromatid breaks, and one chromosome type aberration were found.

Exposure of lymphocytes to 390 • 10^{-8}M NaAsO_4 for 24 h caused an increase in the SCE rate (table 3). The mean per cell increased by 50 %, and the difference between the distributions of SCEs in arsenic-treated cells and controls was highly significant (χ^2 = 70.2, 3 df, p < 0.001).

**Discussion**

In this study we found a distinct difference between trivalent and pentavalent arsenic with respect to the clastogenic effect in vitro. Nakamuro & Sayato (6) also found that trivalent arsenic was more clastogenic. Petres et al (13, 14, 15), who studied only pentavalent arsenic (Na_2HAsO_4) in relatively high doses, found a clastogenic effect for this compound. With the

| Table 2. Chromosome aberrations per cell in cultured lymphocytes exposed to trivalent and pentavalent arsenic. Significance of difference from preceding dose step or control. |
|---|---|---|---|---|
| Dose | Aberrations per cell | | | |
| | Gaps | Chromatid breaks | Chromosome breaks | All |
| **Sodium arsenite** | | | | |
| 77 • 10^{-8}M | 0.120 *** | 0.015 | 0.045 | 0.180 |
| 150 • 10^{-8}M | 0.374 *** | 0.111 *** | 0.032 | 0.516 |
| 230 • 10^{-8}M | 0.943 *** | 0.211 * | 0.034 | 1.189 |
| 310 • 10^{-8}M | 1.468 *** | 0.364 * | 0.013 | 1.844 |
| **Sodium arsenate** | | | | |
| 77 • 10^{-8}M | 0.035 | 0.000 | 0.000 | 0.035 |
| 150 • 10^{-8}M | 0.022 | 0.017 | 0.004 | 0.043 |
| 230 • 10^{-8}M | 0.010 | 0.005 | 0.005 | 0.020 |
| 310 • 10^{-8}M | 0.010 | 0.005 | 0.000 | 0.015 |
| **Control** | | | | |
| | 0.018 | 0.004 | 0.006 | 0.028 |

* p < 0.05, *** p < 0.001, determined by the chi-square test.

| Table 3. Sister chromatid exchanges (SCEs) in lymphocytes exposed to trivalent arsenic and in controls. |
|---|---|---|---|---|
| Dose | Distribution of SCEs | Mean | SD | Number of cells |
| | 0–5 | 6–10 | 11–15 | >15 | |
| **Sodium arsenite (390 • 10^{-8}M)** | | | | | |
| Control | 0 | 24 | 37 | 13 | 12.47 | 3.63 | 74 |
|Control | 7 | 91 | 10 | 0 | 8.16 | 1.86 | 108 |

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doses of pentavalent arsenic used in our experiments no significant increase of chromosome aberrations was found. It seems likely, however, that there is a clastogenic effect also of pentavalent arsenic, but in considerably higher doses than with trivalent arsenic. With respect to the types of aberrations found in the experiments with arsenical compounds, there are both differences and similarities between our investigation and that by Nakamuro & Sayato. A similarity is that in both investigations a relatively high frequency of chromatid exchanges was found.

Differences between the two investigations occurred with respect to the distributions of gaps, chromatid breaks, and chromosome breaks. Thus Nakamuro & Sayato recorded no chromosome breaks, and they found fewer gaps in comparison with our study. These differences may be due to variations between laboratories with respect to the routines for classifying and recording aberrations.

In our study there was an almost linear increase of gaps and chromatid breaks with (trivalent) arsenic dose. This observation is in agreement with the findings of Nakamuro & Sayato. Chromosome breaks showed no such dose-dependence. There was, on the contrary, a tendency towards a decrease of chromosome breaks at higher doses. An explanation for this lack of dose-dependence may be that the cytotoxic effect slows down the rate of cell proliferation and also kills a large fraction of cells with chromosome damage. Since chromosome type aberrations can be observed only in the second cell division in cells exposed to a mutagen during S or G2 phases, higher doses of arsenic may cause a marked decrease of cells in second cell division and thereby decrease the possibilities to observe chromosome breaks.

The mode of action of arsenic is not known. In Escherichia coli arsenic has been shown to inhibit deoxyribonucleic acid (DNA) repair (16), and in human lymphocytes arsenic has been found to inhibit the incorporation of nucleotides into DNA and ribonucleic acid and to block the cells in the S and G2 phases (13). We found no increase in chromosome damage after exposure of lymphocytes to arsenic in the G0 phase. Thus arsenic seems to exert its effect mainly during cell division. Arsenic may not be directly clastogenic per se, but could nevertheless have a genotoxic effect, e.g., by interfering with the DNA repair or by interacting with different enzyme systems. Trivalent arsenicals are sulfhydryl reagents, which inhibit a large number of thiol-dependent enzyme systems, while pentavalent arsenicals have a lower affinity for hydroxy and thiol groups and inhibit fewer enzyme systems (5).

Our results concerning the effect of arsenic on the rate of SCEs should be looked upon as preliminary until confirmed in further experimental studies. The information on the relationship between in vivo exposure to arsenic and SCEs is scanty, and the results from the only two studies known to us (2, 9) were contradictory. The study by Zanzoni & Jung (18) on the effect of in vitro exposure to arsenic is difficult to evaluate. The authors claim to have studied inorganic trivalent arsenic, but the chemical formula contradicts this statement.

Our experiments indicate that trivalent arsenic in the doses used produces gaps, breaks and SCEs. The arsenic concentrations in the culture medium were of the same order of magnitude as the arsenic concentrations in the urine of copper smelter workers. In 33 smelter workers examined by us the mean level of urinary arsenic was 306 \( \mu g/l \) (408 \( \cdot 10^{-8} M \)) with a range of 110—1,080 \( \mu g/l \) (147—1,440 \( \cdot 10^{-8} M \)). The doses used in our experiments varied between 60 and 390 \( \mu g/l \) (77 \( \cdot 10^{-8} \) and 390 \( \cdot 10^{-8} M \), respectively). The concentrations of arsenic in urine and in cell cultures are, however, not comparable with respect to their toxicity since the major part of ingested or inhaled inorganic arsenic appears to be biotransformed and excreted as methylated species (3, 17). In preliminary experiments with dimethylarsinic acid \( (\text{CH}_3\text{H}_2\text{AsO}_3) \) in the same dose range as in the experiments with sodium arsenite, we have found no clastogenic effect.

Thus our results indicate that the genotoxic effect of occupational arsenic exposure may be ascribed mainly to the fraction of trivalent arsenic that is not detoxified by methylation in the body.

A better understanding of arsenic genotoxicity would require further studies of arsenic metabolism, including individual
variability and also the interaction of arsenic with other toxic substances. Smelter workers, eg, may be exposed to arsenic, as well as sulfur dioxide and lead.

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