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Gene-mutation induction by arsenic compounds in the mouse lymphoma assay

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Abstract

Arsenic compounds are generally considered as poor inducers of gene mutations. To investigate the mutagenicity of several arsenic compounds at the thymidine kinase (*Tk*) gene, a reporter gene for mutation induction, we used the mouse lymphoma assay (MLA). This test is widely applied and detects a broad spectrum of mutational events, from point mutations to chromosome alterations. The selected arsenic compounds were two inorganic (sodium arsenite and arsenic trioxide) and four organic compounds (monomethylarsonic acid, dimethylarsinic acid, tetraphenylarsenium and arsenobetaine). The results show that sodium arsenite, arsenic trioxide, monomethylarsonic acid and dimethylarsinic acid are mutagenic, showing a clear dose–response pattern. On the other hand, tetraphenylarsenium and arsenobetaine are not mutagenic. Inorganic arsenic compounds are the more potent agents producing significant effects in the micromolar range, while the mutagenic organic arsenic compounds induce similar effects but in the millimolar range.

Keywords: *Tk*; Mouse lymphoma assay; Mutant frequency; Gene-mutation; Arsenic compounds

1. Introduction

Arsenic is an important environmental toxicant that is widespread in water, soil and air, from both natural and man-made sources. It is naturally occurring in the drinking water in certain regions and therefore, large groups of people are exposed to concentrations much higher than those considered safe, which poses an important health concern. According to epidemiological evidence, arsenic has been classified by the International Agency for Research on Cancer (IARC) [1,2] as a human carcinogen, and chronic exposure to this metalloid has been associated with different types of cancer in skin, lung, bladder and liver [3]. Nevertheless, in spite of the large amount of literature dealing with both the genotoxic and carcinogenic effects of arsenic, until now it remains unclear how arsenic causes cancer and what types of arsenic compound are most likely associated with carcinogenicity.

Arsenic can exist in different oxidative states and in several inorganic and organic forms, characterizing its toxic and genotoxic properties. Arsenate and arsenite are the most known inorganic forms and mammals are able to metabolize these chemicals to monomethylarsonic and dimethylarsinic acids. Additional sources of arsenic

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exposure are the organic forms contained in seafood, such as arsenobetaine and arsenosugars [4].

Due to the carcinogenic risk of arsenic, many studies have been carried out to determine its genotoxic potential, but no general agreement has been reached about its mode of action. In bacteria arsenic is not mutagenic, although the results obtained in cytogenetic studies mainly indicate that arsenic is a clear clastogenic agent, as reviewed by Basu et al. [5]. Thus, the ability of arsenic compounds to induce gene mutations is under discussion.

The mouse lymphoma assay (MLA) using the thymidine kinase (*Tk*) gene is the most widely used of the various *in vitro* mammalian cell gene-mutation assays [6]. This assay detects a wide spectrum of genetic damage, including both gene and chromosomal mutations. Due to the autosomal localization of the *Tk* gene, the MLA detects not only intragenic events, mainly point mutations, but also loss of heterozygosity (LOH). This LOH can result from entire *Tk* gene loss leading to karyotypically visible deletions and rearrangements of the Tk^{+}-bearing chromosome [7]. These features make the MLA particularly useful for evaluating the ability of chemicals to induce a wide variety of mutational events [8,9].

To add more information on the mutagenic action of arsenic compounds, in the present work we show the results obtained in a study on mutation induction by six different arsenic compounds, comprising both inorganic and organic forms, by using the microwell MLA.

2. Materials and methods

2.1. Chemicals

Sodium arsenite, arsenic trioxide, monomethylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}), arsenobetaine and tetraphenylarsenium chloride were obtained from Sigma (Steinheim, Germany). The solutions were prepared just prior to each experiment, using an appropriate solvent, and diluted as appropriate for treatment of cells in suspension. Methyl methanesulfonate (MMS, Sigma) was used as a positive control.

RPMI 1640 medium, horse serum, L-glutamine, penicillin, streptomycin, sodium pyruvate and amphotericin B were purchased from PAA Laboratories (Pasching, Austria). Trifluorothymidine, thymidine, hypoxantine, methotrexate and glycine were purchased from Sigma.

2.2. Cells

L5178Y/$Tk^{+/-}$-3.7.2C mouse lymphoma cells were utilized for the mutation assay. They were kindly provided by Dr. Olivier Gillardeux (Sanofi-Synthélabo, Paris, France) and cultured to prepare master stocks, which were maintained in liquid nitrogen at a density of 2×10^6 cells/mL, in culture medium containing 5% dimethyl sulfoxide (DMSO). They were confirmed as free of mycoplasma by PCR.

Cells were cultured in suspension in RPMI 1640 medium supplemented with 10% heat-inactivated horse serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate and 2.5 μg/mL amphotericin B. The serum concentration was lowered during the treatment to 5% and raised to 20% when cells were dispensed into microwells. The cultures were routinely diluted at 2×10^5 cells/mL each day to prevent overgrowth (>10^6 cells/mL). Cell density was determined with a haemocytometer.

2.3. Cell cleansing

To prepare working stocks for gene-mutation experiments, cultures were purged of excess $Tk^{-/-}$ mutants by culturing in THMG medium for 24 h. This medium contains thymidine (9 μg/mL), hypoxantine (15 μg/mL), methotrexate (0.3 μg/mL) and glycine (22.5 μg/mL). After that, cells were transferred to THG medium (without methotrexate) for 2 days. The purged cultures were checked for low background $Tk^{-/-}$ mutants and stored in liquid nitrogen.

2.4. Gene-mutation assay

Preliminary experiments were conducted to determine solubility and cytotoxicity of the test chemicals. Cytotoxicity was determined by the relative total growth (RTG) following 4-h treatments at concentrations up to 10 mM (arsenobetaine was also treated for 24 h, without metabolic activation) [10]. The RTG measurement takes into account cell loss after treatment, reduction in growth rate over the expression period, and any reduction in cloning efficiency on the day of selection for mutants [11]. The recommended highest concentration was one with an RTG of 10–20%. There was no perceived need to test concentrations >10 mM.

Each main experiment consisted of one negative control, one positive control and at least four concentrations of each test compound. A total of 10^7 cells per culture were grown in R10 media (10% horse serum) and placed in a series of sterile centrifuge tubes. Solvent, test chemical or positive control was added. Cells were incubated with gentle shaking in an incubator at 37 °C for 4 or 24 h. After exposure, cultures were washed and diluted to 2×10^5 cells/mL. Cells were transferred to culture flasks, counted daily and sub-cultured to 2×10^5 cells/mL for a period of 2 days to allow expression of induced mutations (Tk-deficient phenotype). Then the cells were seeded in duplicate plates per culture to calculate viability and mutation frequency (MF) on day 2.

Cell-count data were used, in conjunction with viability data on day 2 to generate the RTG values corrected for post-treatment toxicity, which provides an indication of post-treatment toxicity in comparison with the vehicle controls. The RTG values were used to decide on the acceptability of the toxicity at each dose level.