Arsenic Modulates Cell Proliferation Pathways in MCF-7 Breast Cancer Cells

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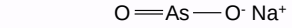
ABSTRACT

Arsenic is a major contaminant in drinking water that is associated with various cancers, skin lesions, peripheral vascular disease and hypertension. The main sources of arsenic in drinking water are erosion of natural deposits; runoff from orchards, runoff from glass and electronics production wastes. Arsenic accumulates in the skin. A well-established exposure-response relationship exists between arsenic level of drinking water and various pathological conditions. Arsenic is a well-established human carcinogen, however, the exact mechanism by which it causes cancer is not known. Most of the effects of arsenic on human diseases have been established on the basis of epidemiologic studies, which have shown a significant association between the consumption of arsenic through drinking water and cancers of the skin, lung, bladder, liver, and kidney, neurologic disease, cardiovascular disease and other nonmalignant diseases. We analyzed the effects of Arsenic on MCF-7 breast cancer cells using Sodium Arsenite. At low concentrations, Sodium Arsenite increased the proliferation of MCF-7 cells. However, at high concentrations, it inhibited the cell proliferation and induced apoptosis. Activation of p53 and its target p21 protein was observed at high concentrations. Sodium Arsenite also activated extracellular signal-regulated kinases (ERK) pathway in MCF-7 cells. Cell signaling pathways modulated by Arsenic in MCF-7 breast cancer cells are discussed.

BACKGROUND

Arsenic is a natural element that is found in the environment. Arsenic has chemical properties that include inorganic and organic compounds. The inorganic compounds have been linked to cancer and other health problems. The form used in our research is sodium arsenite. Sodium Arsenite is the trivalent form of arsenic which is the most deadly and passes more rapidly through the tissues.



OBJECTIVES

The objective of this study was to determine the effects of Sodium Arsenite on MCF-7 breast cancer cells.

MATERIALS AND METHODS

Cell Culture and Reagents: MCF-7 cells were grown as monolayer in Dulbecco's modified Eagle medium (DMEM) (Invitrogen) supplemented with 5% heat-inactivated fetal bovine serum and 25 µg/ml gentamicin. The cells were grown and maintained in 75-cm2 tissue culture flasks in a humidified atmosphere of 5% CO2 and 95% air at 37°C. VES was obtained commercially from the Sigma Chemical Company.

Cell Viability Assay: The cell viability and proliferation of MCF-7 breast cancer cell lines were determined using a cell viability detection kit (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate, WST-1) according to the manufacturer's instructions (Roche Applied Science, Indianapolis, IN).

DAPI Staining: MCF-7 cells were grown on cover slips in 6-well plate and treated as indicated. The cells were fixed in methanol and stained with 4'-6-Diamidino-2-phenylindole (*DAPI*) nuclear stain to visualize the apoptotic nuclei by fluorescent microscopy.

Western Blotting: Expression of various signaling proteins was determined by Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Blotting.

RESULTS

1. Cell Culture, Reagents and Materials

2. Effects of Sodium Arsenite on the proliferation of MCF-7 cells

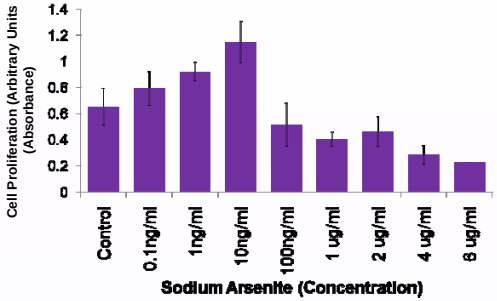


Figure 2. MCF-7 breast cancer cell lines were treated with various concentrations (0.1ng/mL-6ug/mL) of sodium arsenite for 48h. Cell proliferation was determined by WST-1 assay as described in Materials and Methods. Sodium Arsenite increased the proliferation at low concentrations where as higher concentrations of sodium arsenite inhibited the growth of MCF-7 cells.

3. Sodium Arsenite causes apoptotic phenotype in MCF-7 cells

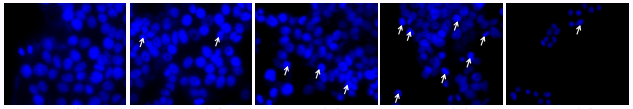


Figure 3. MCF-7 breast cancer cell lines were plated on coverslips in a 6-well plate. After 24h, the cells were treated with indicated concentrations (0.1ng/mL-100 ng/mL) of sodium arsenite for 48h, stained with DAPI and visualized under fluorescent microscope. Apoptotic nuclei with distinct nuclear fragmentation (arrow) were observed in treated cells.

4. Sodium Arsenite activates ERK Signaling in MCF-7 Cells

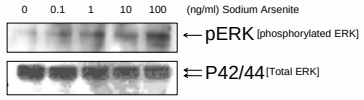


Figure 4. MCF-7 breast cancer cells were treated with various indicated concentrations (0.1ng/mL-100 ng/mL) of Sodium Arsenite for 24h. Expression of phosphorylated ERK (activated ERK) and total ERK was determined by Western Blotting.

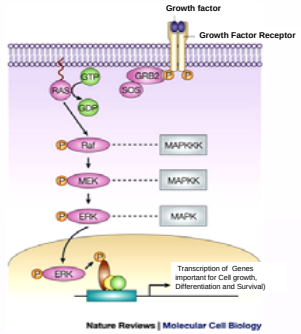


Figure 5. The ERK/MAPK Cascade (From Nature Reviews/Molecular Cell Biology).

5. Sodium Arsenite upregulates p53 signaling at higher concentrations in MCF-7 cells

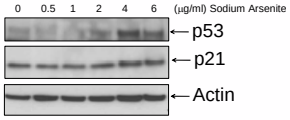


Figure 6. MCF-7 breast cancer cells were treated with indicated concentrations of sodium arsenite for 24h. Expression of p53, p21 and actin was determined by Western Blotting. Actin was used as loading control.

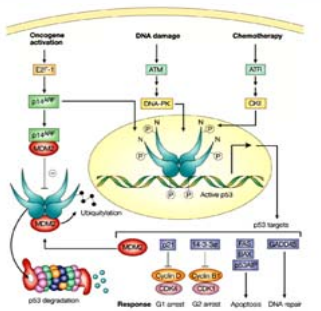


Figure 7. The p53 signaling: DNA damage and chemotherapeutic agents increase p53 levels and activate the transcription of p53 target genes. p21 and 14-3-3 promote growth arrest at the G1 and G2 DNA-damage checkpoints by inhibiting cyclin-dependent protein kinase (CDK) activity; FAS, BAX and p53AIP promote apoptosis if repair is not possible; and GADD45 promotes DNA repair [From Nature Reviews/Cancer].

CONCLUSIONS

- Sodium Arsenite at higher concentrations inhibits the proliferation of MCF-7 breast cancer cells.
- Arsenic exposure causes apoptotic phenotype in MCF-7 cells.
- Low doses of Sodium Arsenite activates ERK signaling pathway.
- Higher concentrations of Sodium arsenite activates p53 signaling in MCF-7 cells.

ACKNOWLEDGEMENTS

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