Arsenic Compounds in Carcinogenesis: Cytotoxic Testing by Liver Stem Cells in Culture

Shen Cherng[1], Hongbao Ma[2], Jinlian Tsai[3]

1. *Graduate Institute of Electrical Engineering, Chengshiu University, Niausong, Kaohsiung, Taiwan 830, ROC*
2. *School of Medicine, Michigan State University East Lansing, MI 48823, USA*
3. *Graduate Institute of Occupational Safety and Health, Kaohsiung Medical University, Kaohsiung City, Taiwan 801, ROC*

Abstract: Much of the work conducted on adult stem cells has focused on the application for carcinogenesis. By using stem cell model and gap junctional intracellular communication (GJIC) assay for studying the role of arsenic compounds in carcinogenesis including the cytotoxicity comparison of arsenic (Ⅲ) oxide, arsenic (V) oxide, dimethyl arsenic acid and disodium methyl arsenate are demonstrated in this article. From cell surviving curve of human liver stem cell (HL1), rat epithelial cell (WB) and liver cancer cell line (Malhava cells) in culture, the doses and time period of the arsenic exposure for 50% cell surviving were obtained. Conclusively arsenite and arsenate significantly affected GJIC within the cells in 50% cell surviving dose dependent inhibition. [Life Science Journal. 2006;3(3): 45-48] (ISSN: 1097-8135).

Keywords: arsenate; carcinogenesis; cytotoxicity; gap junctional intracellular communication; stem cell

Abbreviations: BPE: bovine pituitary extract; DMA: dimethylarsinic acid; EGF: epidermal growth factor; FBS: fetal bovine serum; GJIC: gap junctional intracellular communication; MMA: monomethylarsonic acid; PKC: protein kinase C; SL/DT: scrape-loading/dye-transfer; TMAO: trimethylarsine oxide; WB: WB-F344 rat liver epithelial cells

1 Introduction

Arsenic is a known human carcinogen and being initiated different tumours in many sites of the body organs, such as skin, lung, liver, urinary bladder, prostate, and many others[1]. The adverse effects of arsenic are dependent on its chemical form and metabolism. Inorganic arsenicals were basically more acutely toxic than organic species since the methylation of inorganic arsenic was involved in the detoxification process[2]. However, more evidences indicate that the trivalent organic arsenicals being as metabolic products of inorganic arsenic can be more toxic than the parent compound[3,4]. It is well known that As(V) can be first reduced to As(Ⅲ) and As(Ⅲ) being produced by this reduction or from direct ingestion can be methylated primarily to pentavalent organic arsenicals including monomethylarsonic acid [MMA(V)] and dimethylarsinic acid DMA(V). MMA and DMA are the predominant metabolites of inorganic arsenic. However, DMA may be further methylated to. The forms of arsenic being exposed either directly or via metabolism may complicate toxic and carcinogenic mechanisms of action. Drinking ground water was reported being arsenic contaminated by electroplating industry severely in regions of west-south seashore of Taiwan fifteen years ago. At that time, people exposed into drinking water in high concentration of arsenic (~300 ppb) being affected to lung cancer probability was four times, bladder cancer probability was eight times, skin cancer probability was twenty times, prostate cancer probability was three times more than in low concentration (~0.1 ppb)[5]. However, no publication expresses that any cell or animal model can successfully propose the mechanism of arsenic being initiated cancers.

In a cell, six connexin 43 subunits oligomerze in the Golgi apparatus into a connexon, called hemi channel and be transported to plasma membrane of the cell. Before pairing process, hemi channels are closed to avoid leakage of cellular contents and entry of extra-cellular materials. During the pairing of connexons and aggregation into plaques at the plasma membrane, connexin 43 is phosphorylated at least twice and connexons are attracted to those located on the adjacent cells. Two connexons join in an end-to-end manner to form a complete channel.

The channel aggregate into large gap junction plaques open to connect two cells for cell-to-cell communication and is called gap junctional intracellular communication (GJIC)[6], which can be modulated by environmental factors, such as effects of arsenic compounds. Since the function of the GJIC, cultured cells coupled together *in vitro*. The scrape loading dye transfer technique can identify the GJIC modulation by observing the diffusive range of the fluorescence[7]. The varied diffuse range of Lucifer yellow fluorescence expresses the cellular response under the exposure of arsenic toxic compounds. Since GJIC is affiliated with many pathological endpoints, GJIC modulation can be a good factor to evaluate the cellular response to the reaction of chemical toxicities. In this article, a liver stem cell model is proposed to investigate the order of cell toxicity of arsenic compounds by the Lucefer yellow dye mobility of the GJIC within the cells in a concentration and time dependent manner.

2 Materials and Methods

2.1 Reagents

Keratinocyte serum-free medium, Dulbecco's modified Eagle medium, modified Eagle's minimum essential medium, recombinant human epidermal growth factor (EGF), bovine pituitary extract (BPE), fetal bovine serum (FBS), penicillin, streptomycin, trypsin-EDTA and Trizol reagent were purchased from Invitrogen (GIBCO-Invitrogen Corporation, Carlsbad, CA, USA). Arsenic (Ⅲ) oxide, arsenic (V) oxide, dimethylarsenic acid, disodium methyl arsenate, N-acetyl-L-cysteine, DMSO, L-ascorbic acid 2-phosphate and nicotinamide were obtained from Sigma Chemical Co. (St Louis, MO, USA). Anti-THY1.1, AFP, albumin, and Oct4 monoclonal antibodies were purchased from Santa Cruz (Santa Cruz, CA, USA).

2.2 Cell culture

The medium used to develop the putative human liver stem/progenitor cell cultures (HL-1) is a modified MCDB 153 (Keratinocyte-SFM, GIBCO - Invitrogen Corporation, Carlsbad, CA, USA) supplemented with N-acetyl-L-cysteine (NAC) (2 mM) and L-ascorbic acid 2-phosphate (Asc 2P) (0.2 mM) (referred to as K-NAC medium). WB-F344 rat liver epithelial cells (WB), originally isolated in the laboratory of Joe W. Grisham, National Cancer Institute, Bethesda, MD, USA, were kindly provided by Chia-Cheng Chang (Michigan State University, East Lansing, MI, USA). Cells were grown in modified Eagle's minimum essential medium (Formula No. 03-5045EF, Gibco, Rockville, MD, USA), supplemented with 5% FBS. The hepatoma cells (Malhava) were grown in Dulbecco's modified Eagle medium supplemented with 10% FBS. All cell cultures were incubated at 37 ℃ in incubators supplied with humidified air and 5% CO_2.

2.3 Treatment of cells with arsenic compounds

HL-1, WB and Malhava cells were grown to approximately 80% confluence and then treated with arsenic (Ⅲ) oxide, arsenic (V) oxide, DMA and MMA for one day. The culture medium was changed before treatment with arsenic compounds. Arsenic compounds were dissolved in distilled water and then applied to the cells at carious concentrations in medium. The control cells were either not treated with any arsenic chemicals.

2.4 GJIC assay

GJIC was measured using scrape-loading/dye-transfer (SL/DT)[8]. The image pro plus software (Media Cybernetics, Georgia, USA) was used for scanning the size of the fluorescence area along the scrape line on monolayers to quantitate the levels of GJIC.

2.5 Statistical analysis

All data were presented as the mean group value ± standard error of the mean (SEM). The data were analyzed using one-way analysis of variance (ANOVA). Significant differences between control and arsenic compounds treatment were evaluated using Dunnett's method. The level of statistical significance was set at $P < 0.05$.

3 Results

3.1 Effects of arsenic exposure to cell toxicity

Tests of different concentrations of the 24 hours inorganic arsenic exposure to three different liver cells, HL-1 liver stem cell, WB cell line and Malhava cell line appear the results being depicted in Figure 1. Cell toxicity is to be in the order of As(Ⅲ) > As(V) > MMA > DMA for all cell lines and arsenic-dose dependent. In the arsenic (Ⅲ) concentration of 5 ppb, no WB cells can be found in surviving but 50% HL-1 cells was survived. In comparison, the cell toxicity of As(Ⅲ) is forty times more than As(V) and two hundred times more than DMA or MMA. The cell line came from different sources has different sensitivity to the arsenic toxicity. Normal liver cell (WB cell line) can only be survived less in 50% under concentration of As(Ⅲ) at 1.25 ppb. However, under the same dosage, 83% of the HL-1 liver stem cell can be survived and no effects to the cells of Malhava cell line.

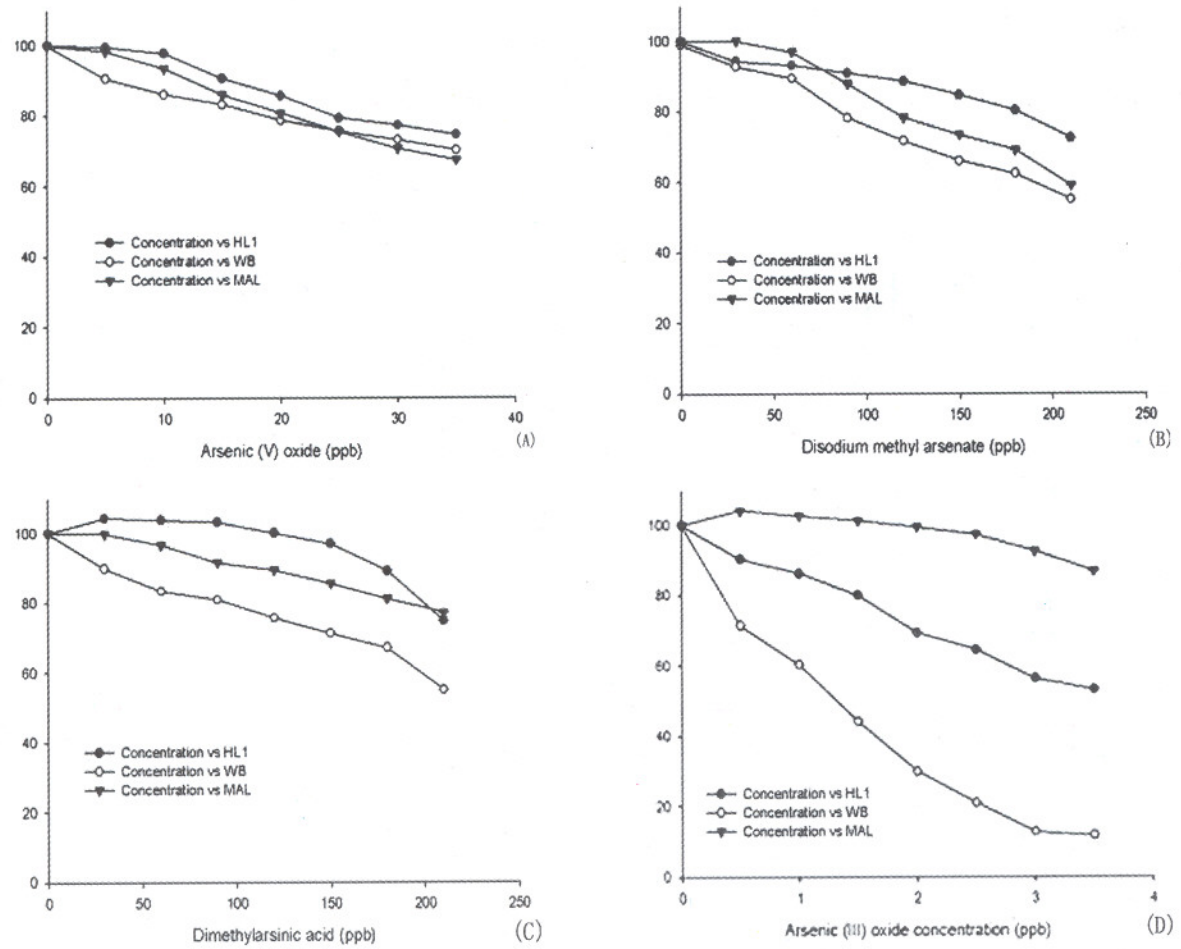

Figure 1. Inhibition of the proliferation of HL1, WB and Malhare cells by arsenic compounds. The cells were treated with various concentrations of arsenic compounds for 24 h. (A) arsenic (V) oxide, (B) disodium acid-treated oxide, (C) methyl arsenate dimethylarsenic, (D) arsenic (Ⅲ), and untreated HL1, WB and Malhava cells from quaternary determinations.

3.2 Effects of arsenic exposure to cell lines by expression of GJIC

Under the arsenic As(Ⅲ) exposure of 60 hours at 25 μg/ml, the GJIC within WB cells was completely inhibited. In Figure 2, it depicts the statistic difference($P<0.05$) of the GJIC inhabitation among As(Ⅲ) arsenic exposure at concentration of 50, 100 and 200 μg/L for cell toxic test of WB cells being exposed 24 hours. The arsenic As(Ⅲ)-dose dependent correlation is thus can be expressed *vs*. the inhabitation of GJIC being exposed to 12, 24 and 60 hours. The arsenic As(V)-dose dependent GJIC correlation also is depicted under the concentration of 5, 10, 30 and 50 μg/ml for WB cell being exposed to 10 hours. Under the treatments repeated for DMA and MMA in contrary, same GJIC inhabitation response required dosage is about 500 to 700 times more than As(Ⅲ).

4 Discussion

The homeostasis is mediated by cell to cell GJIC being associated with cell differentiation, proliferation and apoptosis[8]. Thus, the factors affected connexin gene, such as the mutant, reduced expression, degrading, changing of the transcription of connexin protein, can alter the GJIC from normal to block process and create the cancer promotion phase of carcinogenesis within the cells. In addition, gene mutant and epigenetic events must be happened in a multi-stage and multi-mechanism process in carcinogenesis. The metabolism of the arsenic compounds is in liver for the mammals. The catabolism of As(V) to As(Ⅲ) will continue being demethylated to MMA and then to DMAA. This pathway is not reversible and poisoned to the organs. The observation of the inhibition of the GJIC of the WB cells reflected the effect of consequence of cell toxicity of the arsenic exposure at dose and

time dependent manner. The mediated connexin protein has no doubt played an important role in carcinogenesis[9] under the arsenic exposure. The early research of the peroxisome proliferator activated receptor (PPAR) revealed the interaction of peroxisome proliferators being mediated by inhibition of PPAR under As(Ⅲ) exposure[10]. However, the connection of PPAR and GJIC is still unknown. No published papers or reports suggested the mechanism or model for the study. Deng demonstrated As(Ⅲ) and As(V) can inhibit the GJIC within skin fiberblast cells through the interaction of increasing of protein kinase C (PKC)[11]. Tsuchiya reported that both As(Ⅲ) and As(V) can inhibit the GJIC of the cells of V79 in dose dependent manner[12]. The toxicity concentration of As(Ⅲ) is about 10 times more than As(V) exposure. However, in our experiments, the DMA and MMA are not very dose sensitive to the inhibition of GJIC within WB cells.

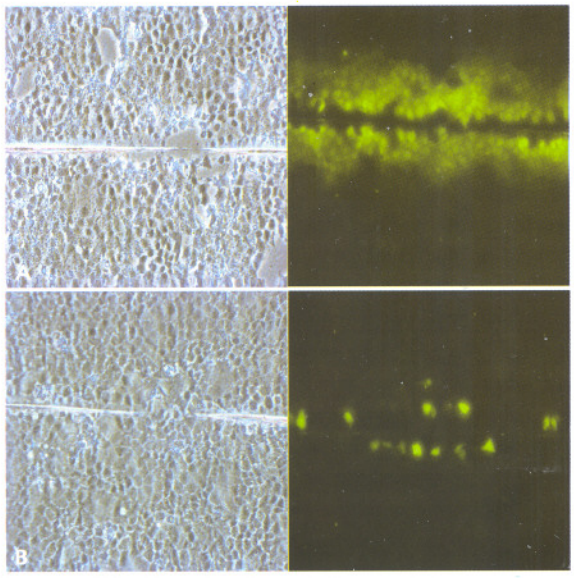

Figure 2. Gap junctional intercellular communication in WB cells as measured by the SL/DT technique. Cells were untreated (A) and 25 μg/L arsenic (Ⅲ) oxide (B) for 60 hours.

5 Conclusion

GJIC assay revealed WB cells being cancerized after enough time and dose arsenic exposure. This report depicted again that arsenic compound must be the carcinogen in carcinogenesis. Based upon the basic theory of the GJIC, the possible mechanism in arsenic carcinogenesis is that arsenic compound blocks the connexin gene expression and its phosphorylation to inhibit the GJIC of the normal cells. Further and advance study of this mechanism will be investigated later.

Correspondence to:
Shen Cherng, P.E., Ph.D., M.D.
Associate Professor
Graduate Institute of Electrical Engineering
Chengshiu University
Niausong, Kaohsuing, Taiwan, 833, ROC
Telephone: 86-7732-0489
Email: cherng@msu.edu

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