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CELL-CYCLE DEPENDENCE OF THE CYTOTOXICITY AND CLASTOGENICITY OF SODIUM ARSENITE IN CHINESE HAMSTER OVARY CELLS[1]

TE-CHANG LEE, KARL C. C. LEE, CHENG CHANG
and WOON-LONG JWO

Institute of Zoology, Academia Sinica,
Taipei, Taiwan 115, Republic of China

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Te-Chang Lee, Karl C. C. Lee, Cheng Chang and Woon-Long Jwo (1986) Cell-cycle dependence of the cytotoxicity and clastogenicity of sodium arsenite in Chinese hamster ovary cells. *Bull. Inst. Zool., Academia Sinica* **25**(1): 81-87. The cell-cycle time of the replated shake-off mitotic cells was determined to be 16 h, with an G1 of 6 h, an S of 6 h and G2 plus mitosis of 4 h. Cells at early G1 were the most sensitive to the killing and mitotic inhibition of sodium arsenite. Whereas, the highest clastogenicity of sodium arsenite was detected at G1/S border. Sodium arsenite may be classified as an S-dependent clastogen, because the aberrations indcued were mainly chromatid breaks and chromatid exchanges, when the cells were treated with sodium arsenite in G1 and early S phases.

Arsenic, one of the most common and important trace elements in our environment, has been well known in its acute and chronic toxicity, clastogenicity, teratogenicity and carcinogenicity (Squibb and Fowler, 1983). There is a possibility that the fossil-fuel energy will be utilized in the next several decades. Thus, the conversion process of fossil fuel will result in massive mobilization of arsenic (Piver, 1983; Woolson, 1983). However, our present knowledge concerning biochemical mechanisms of arsenic toxicity in mammals is far from complete. The position of a cell in its life cycle influences the lethal effect of genotoxic agent (Sinclair, 1968; Clarkson, 1978; Riddle and Hsie, 1978; Burki *et al.*, 1980; Goth-Goldstein *et al.*, 1984; Wiezorek, 1984; Enninga *et al.*, 1985). As a prerequisite for understanding the cellular response to the insult of arsenic, we have studied the cytotoxic and clastogenic effects of sodium arsenite (a trivalent arsenic) in different phases of the cell cycle in Chinese hamster ovary (CHO) cells.

MATERIALS AND METHODS

Cell line and cell culture

Chemicals for cell culture were obtained from GIBCO (Grand Island, NY). Sodium arsenite was purchased from Merck (Darmstadt., F. R. G.). CHO cells were kindly provided by Dr. S. Soukup (Children's Hospital Research Foundation, Cincinnati, OH), and maintained in McCoy's 5a medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics as described previously (Lee *et al.*, 1985).

Synchronizaton

Synchronous cell populations were obtained by selective mitotic detachment as described in Crespi and Thilly (1982). Briefly, 3×10^6 cells were inoculated in a 80 cm^2 flask and grown overnight. Colcemid was added to a final concentration of 0.037 μg/ml and

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the culture was incubated at 37°C for 1.5 h. The cultural medium with detached cell was discarded, since the mitotic index was only about 85%. The flasks were refed with fresh medium containing colcemid and incubated for another 1.5 h. Mitotic cells (about 6×10^5 cells from each flask) were shaken off and collected by centrifugation. The mitotic index in the second harvest, used for the experiments, was above 98%.

Determination of cell cycle phases

Synchronized mitotic cells (5×10^4) were plated in 24-well plates with Thermanox tissue culture cover slips (15 mm in diameter). Every 2 h the cells were trypsinized and counted on a hemacytometer. An aliquot of suspension was fixd in methanol-acetic acid (3:1), dropped on a slide, and stained with 3% Giemsa solution. The number of mitotic cells was counter under a microscope. The phase of DNA synthesis was determined by the incorporation of [^{3}H]-thymidine (80.2 Ci/mM obtained from New England Nuclear, Boston, MA). At the time indicated, [^{3}H]-thymidine (1 μCi/ml) was added and incubated for 30 min at 37°C. The cover slips were then removed, and washed three times with cold phosphate-buffered solution, twice with cold 5% trichloroacetic acid, and twice with 95% ethanol. After drying in air, the cover slips were placed in 10 ml scintillation fluid (4 g PPO and 0.1 g POPOP in 1000 ml toluene) and counted in a Beckman LS-8000 liquid scintillation counter. Each determination was the average of three replicates.

Cytotoxicity

The cytotoxic effect of sodium arsenite was determined by colony formation. Synchronized mitotic cells (3×10^5) were plated in a 60-mm petri dish. The treatment of sodium arsenite was performed at sequential intervals of 4 h as indicated. At the end of the treatment, the cells were trypsinized and counted. An aliquot of 200 cells was replated in a 60-mm petri dish (3 dishes for each treatment) and incubated for 7 days, then stained with Giemsa solution. The colony number was scored under a dissected microscope. The relative survival was calculated by dividing the colony forming efficiency of treated cells by that of untreated cells.

Analysis of chromosome aberrations and mitotic index

The synchronous cells (3×10^5) were plated in a 60-mm petri dish and treated with sodium arsenite for 4 h at sequential intervals as indicated. The treated cells were then washed with Hank's blanced salts solution and incubated at 37°C with fresh medium. Metaphases were harvested at 20 h after plating of synchronized mitotic cells, and colcemid (0.2 μg/ml) was added 2 h before harvest. All the cells were trypsinzed, treated with hypotonic solution (0.5% KCl for 6 min) and fixed with methanol-acetic acid (3:1) solution. An aliquot of cell suspension was then dropped on a clean slide. The air-dried slides were stained with 3% Giemsa in Sorenson's buffer (pH 6.8) for 10 min and coded for the examination of chromosome aberrations and mitotic index. Chromosome aberrations were identified by following the criteria described by Buckton and Evans (1973) and 100 metaphases were examined for each treatment.

RESULTS

The cell-cycle time of the replated shake-off mitotic cells was estimated to be 16 h (Fig. 1). The cell-cycle phases were determined: an G1 of 6 h, an S phase of 6 h, and an G2 plus mitosis of 4 h. This result was similar to that reported by Crespi and Thilly (1982).

To study the cytotoxic and clastogenic effects of sodium arsenite in different cell-cycle phases, the cultures were exposed to chemical for 4 h at sequential intervals after plating of shake-off mitotic cells. According to Fig. 1, stage A (0 to 4 h) represents early G1 phase, stage B (4 to 8 h) late G1 and early S phase, stage C (8 to 12 h) S phase,

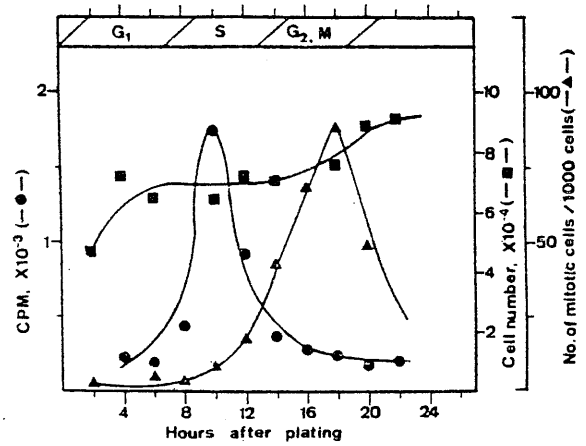

Fig. 1. Cell cycle progression in CHO cells. Synchronous mitotic cells obtained by selective detachment were plated at the density of 5×10^4 cells per well. Cell number, [^{3}H] thymidine incorporation and mitotic index were determined at 2-h intervals according to the procedures described in MATERIALS AND METHODS. ■, cell number; ●, [^{3}H] thymidine incorporation (cpm); and ▲, mitotic index.

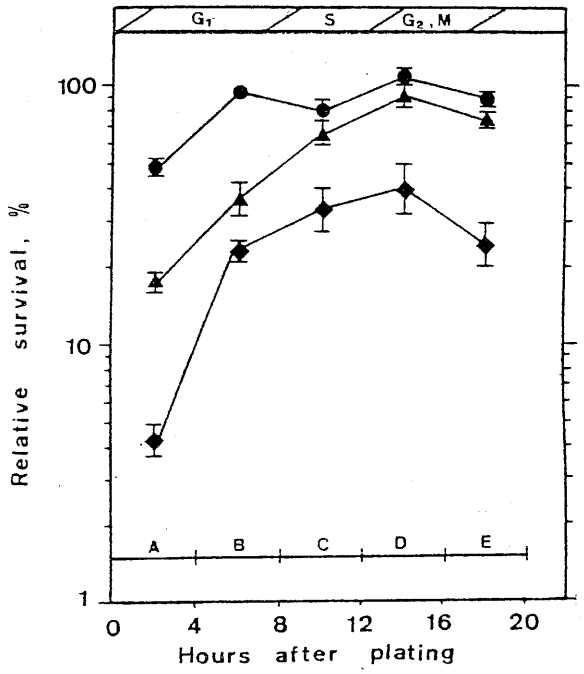

Fig. 2. Cell cycle variation of sodium arsenite-induced killing in CHO cells. See MATERIALS AND METHODS for detail. The capital letters indicate the intervals of treatment of sodium arsenite, and bars represent 1 SD. ●, 20 μM; ▲, 40 μM; ■, 60 μM.

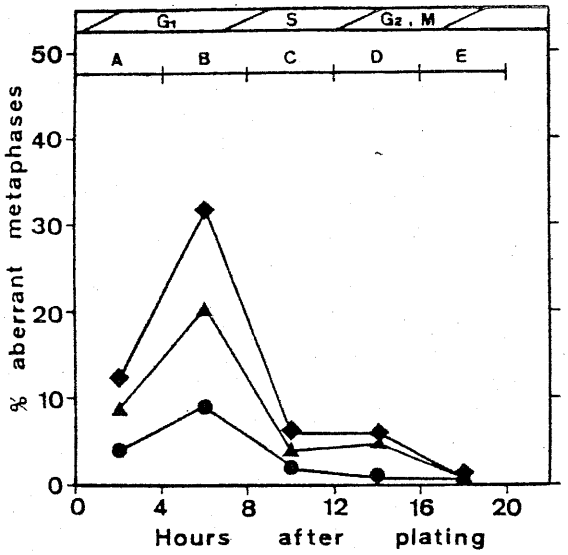

Fig. 3. Cell cycle variation of sodium arsenite-induced chromosome aberrations in CHO cells. See MATERIALS AND METHODS for detail, and Fig. 2 for symbols.

TABLE 1

Types of chromosome aberrations induced by sodium arsenite (AS) in synchronous CHO cells

Treatment		Incidences per 100 metaphases				
		Chromatid types		Chromosome types		Shattered
Stage (h)	AS (μM)	Breaks	Exchanges	Breaks	Exchanges	metaphases
A	0	0	0	0	0	0
(0-4)	20	3	2	0	0	0
	40	7	3	0	0	0
	60	19	5	2	0	0
B	0	0	0	0	0	0
(4-8)	20	6	3	0	0	0
	40	24	16	2	2	0
	60	28	24	6	0	1
C	0	0	0	0	0	0
(8-12)	20	0	0	2	0	0
	40	2	3	0	0	0
	60	1	3	2	0	0
D	0	0	0	0	0	0
(12-16)	20	0	1	0	0	0
	40	2	4	0	0	0
	60	3	3	1	0	0
E	0	0	0	0	0	0
(16-20)	20	0	0	1	0	0
	40	1	0	0	0	0
	60	0	0	0	1	0

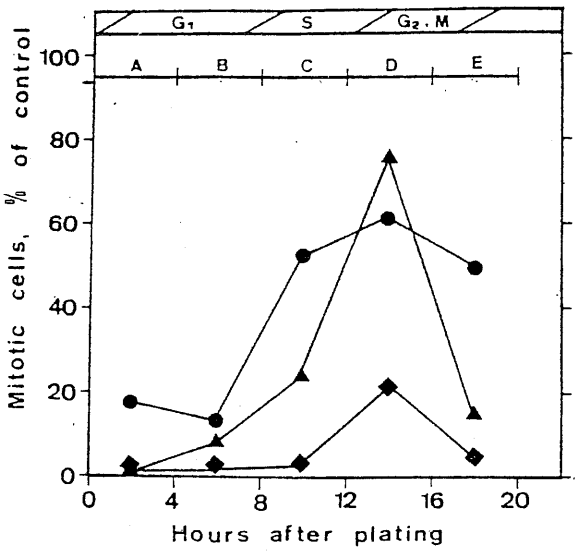

Fig. 4. Cell cycle variation of sodium arsenite-induced mitotic inhibition in CHO cells. See MATERIALS AND METHODS for detail, and Fig. 2 for symbols.

stage D (12 to 16 h) late S and early G2 phase, and stage E (16 to 20 h) late G2 phase and mitosis. Cell survival and induction of chromosome aberrations changed appreciably with a 4-h treatment of sodium arsenite a different stage in the cell cycle (Figs. 2 and 3). Cells were most sensitive to the killing of sodium arsenite at early G1 (stage A). Survival increased to near control levels in late S and early G2 (stage D). However, a slightly decline was observed when cells were treated at late G2 and mitosis (stage E). On the other hand, a significant increase of chromosome aberrations was observed when the treatment of sodium arsenite was performed during the late G1 and early S (stage B). Chromatid breaks and chromatid exchanges were the main aberration types (Table 1). The highest inhibition on mitosis was observed when sodium arsenite was given in the G1 phase (Fig. 4, stage A and B).

DISCUSSION

Our results are consistent with the reports that sodium arsenite is cytotoxic, clastogenic and mitosis-poisonous (Zanzoni and Jung, 1980; Larramendy *et al.*, 1981; Nakamuro and Sayato, 1981; Lee *et al.*, 1985). The mos important mechanism by which trivalent arsenicals exert their toxic effect is through their interaction with sulfhydryl groups in active tissue (Squibb and Fowler, 1983). The cellular ATP level is significantly decreased through the inhibitory effect on oxidative phosphorylation in mitochondria. The blocking of sulfhydryl groups and reducing the cellular ATP level may also directly or indirectly interfere with DNA synthesis and hence result in chromosomal aberrations (Paton and Allison, 1972; Petres *et al.*, 1977; Gurley *et al.*, 1980; Dresler and Lieberman, 1983). Alternatively, arsenic may replace phosphorus in the phosphate group of DNA, and form a weak bond in DNA (Sibatani, 1959).

Our results show that cells are most sensitive to sodium arsenite at early G1 in terms of cell killing and mitotic inhibition. The coincidence of these two phenomena suggests a common sensitive point in G1 (Rothstein, 1982). On the other hand, early G1 was the most sensitive stage to the cytotoxic effect, whereas the G1/S border was most sensitive to the clastogenic effect of sodium arsenite. These results indicate that

sodium arsenite may kill a cell by ways other than chromosomal damages. Since the most sensitive stage of the clastogenic effect of sodium arsenite was located at G1/S border, we suggest that sodium arsenite may interfere the initiation of DNA replication and hence results in chromosome aberrations. Because the major types of chromosome aberrations were chromatid breaks and chromatid exchanges, we also suggest that sodium arsenite is an S-dependent clastogen. This implys that sodium arsenite predominantly induces single-strand breaks in DNA and these damages have to pass DNA replication before they can be recognized as chromatid aberrations.

Although arsenic by itself is not or only a very weak mutagen in *E. coli*, barley and mammalian cells (Rossman *et al.*, 1980; Oberly *et al.*, 1982; Thengane, 1984; Lee *et al.*, 1985), arsenic has been demonstrated to enhance tumor induction in rats (Shirachi *et al.*, 1983), to potentiate genotoxic effects of a variety of mutagens and/or carcinogens in vitro (Taylor *et al.*, 1984; Lee *et al.*, 1985; Jan *et al.*, 1985). Arsenic can apparently affect cancer development through several indirect or direct mechanisms (Leonard and Lawerys, 1980; Nielsen *et al.*, 1983). Unfortunately arsenic is ubiquitous in our environment. Therefore, further investigation is needed to understand the biological consequences of arsenic contamination.

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中國倉鼠卵巢細胞的細胞週期與亞砷酸鈉的細胞毒殺及染色體損害效應

李德章　李俊傑　張　誠　卓文隆

利用搖脫法收集同步化的中國倉鼠卵巢細胞，研究細胞週期和亞砷酸鈉的細胞生物學效應。細胞週期長約16小時，包括複製前期（G1）6小時、DNA 複製期（S）6小時及複製後期加分裂期（G2+M）4小時。G1早期的細胞對於亞砷酸鈉的細胞毒性及抑制分裂作用最敏感。於G1後期及S前期，亞砷酸鈉對染色體損害力最強，產生的異常染色體多屬染色分體斷裂及染色分體互換。因此亞砷酸鈉可歸屬於核酸複製相關的染色體損害物質。