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# CELL-CYCLE DEPENDENCE OF THE COCLASTOGENICITY OF SODIUM ARSENITE[1]

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**Hai-Mei Huang, Chian-Li Peng, Te-Chang Lee and Kuo-Yan Jan** (1989) Cell cycle dependence of the coclastogenicity of sodium arsenite. *Bull. Inst. Zool., Academia Sinica* **28**(1): 49-53. Previous study has shown that the potentiation of sodium arsenite (SA) on the ethyl methanesulfonate (EMS) clastogenicity is only detected when SA is given immediately after EMS treatment. In this report further evidence are presented to show that the processing pathways of different DNA lesions to chromosome aberrations are cell-cycle dependent, and SA may act on some of these pathways to exert its coclastogenicity. The duration from G1 to early S is an important period for SA to potentiate the chromosomal aberrations, particularly chromatid exchanges, induced by methyl methanesulfonate (MMS), mitomycin C (MMC) and cis-platin (PT).

**Key words:** Alkylating agents, Arsenite, Chromosome aberrations, Coclastogenicity, DNA-crosslinking agents.

Arsenite is known to induce chromosome aberrations in mammalian cells in culture (Larramendy *et al*, 1981; Nakamuro and Sayato, 1981; Lee *et al*, 1986a). Arsenite may be classified as an S-dependent clastogen because few chromosomal aberrations are induced when the treatment is performed after DNA replication and mainly chromatid-type aberrations are observed when the treatment is performed before DNA replication (Lee *et al*, 1986a).

In addition to clastogenicity, arsenite has also been shown to have coclastogenicity. Posttreatment with sodium arsenite (SA) synergistically increases the chromatid breaks and chromatid exchanges induced by many S-dependent clastogens (Lee *et al*, 1985; Lee *et al*, 1986b; Jan *et al*, 1987). The SA coclastogenicity is associated with the progression of cell cycle (Huang *et al*, 1987). The potentiation of SA on ethyl methanesulfonate (EMS) clastogenicity is only detected when SA is given immediately after EMS treatment (Jan *et al*, 1986). These results suggest that the SA coclastogenicity may be cell stage specific. In this report we have presented further evidence to show that the duration from G1 to early-S phase is an important period for SA to

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potentiate the chromosome aberrations induced by S-dependent clastogens.

## MATERIALS AND METHODS

### Materials

Chemicals for cell culture were obtained from Gibco. Methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS) were purchased from Sigma Chemical Co.; dimethyl sulfoxide (DMSO) and sodium arsenite (SA) from Merck; cis-platin (PT) from Bristol-Meyers, and mitomycin C (MMC) from Kyowa Hakko Kogyo Co. MMS was pre-dissolved in phosphate-buffered saline; EMS in dimethyl sulfoxide; MMC, PT, and SA in double-distilled water. They were then diluted with medium immediately before use.

### Cell culture

A subclone of Chinese hamster ovary (CHO) cells, originally obtained from Dr. S. Wolff (University of California, San Francisco), was used throughout the experiments. The cells were cultured in McCoy's 5a medium supplemented with 15% heat-inactivated fetal calf serum, 0.02% sodium bicarbonate, 100 units/ml penicillin, 100 $\mu$g/ml streptomycin, and 0.03% L-glutamine. The cultures were maintained in a humidified incubator at 37°C with 5% $CO_2$ in air.

### Chromosome aberrations

Logarithmically growing CHO cells were plated at the density of $3\times10^5$ cells/60 mm petri dish and then incubated for 18 h. They were then treated with MMS, EMS, MMC, or PT for 2 h, washed twice with Hanks' balanced salt solution, and reincubated in fresh medium. To test the cell-cycle effect of SA coclastogenicity, a treatment with SA (10 $\mu$M) for 6 h was given at different periods during the 18 h incubation time after removing clastogens, i. e., 0-6 h (stage I), 6-12 h (stage II), and 12-18 h (stage III). Colcemid 0.2 $\mu$g/ml was added for the last 2 h of incubation and mitotic cells were harvested by shake-off method. The method for analysis of chromosomal aberrations has been described previously (Lee *et al*, 1985).

## RESULTS

The effects of SA on the chromosome aberrations induced by MMS, EMS, MMC, or PT were shown in Fig. 1. Only the incidences of aberrant metaphases, chromatid breaks, and chromatid exchanges were presented because chromatid breaks and chromatid exchanges were the main aberrations induced by these clastogens and enhanced by posttreatment with SA.

Posttreatment with SA in stage I, was the most prominent time period for potentiating the chromosome aberratons induced by the four clastogens reported here. The incidences of both chromatid breaks and chromatid exchanges were synergistically increased. However, the increase of chromatid exchanges was far more greater than that of chromatid breaks.

Posttreatment with SA in stage II also increased the chromatid breaks and chromatid exchanges induced by MMC and PT, but had no effect on the chromatid aberrations induced by MMS and EMS. Posttreatment with SA in stage III increased the chromatid breaks induced by PT, but had no effect on the chromatid aberrations induced by MMS, EMS, or MMC.

## DISCUSSION

In provious study (Jan *et al*, 1986) the cell cycle time of this cell line has been determined to be about 12 h. By using the bromodeoxyuridine mediated sister-chromatid differential staining technique,

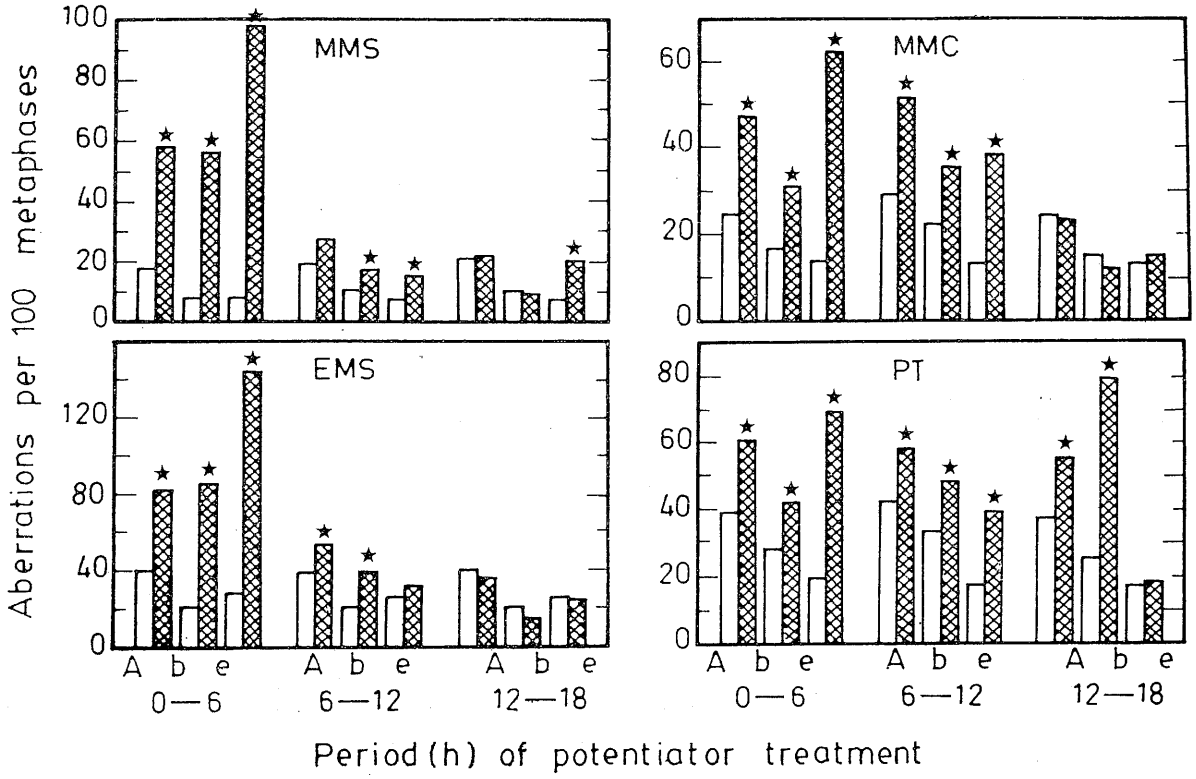


Fig. 1. Effect of posttreatment with sodium arsenite on the chromosomal aberrations induced by methyl methanesulfonate, ethyl methanesulfonate, mitomycin C, and cis-platin. Log-phase CHO cells were treated with MMS 0.4 mM, EMS 15 mM, MMC 0.6 $\mu$M, or PT 4 $\mu$M for 2 h and then sodium arsenite 10 $\mu$M for 6 h at the period indicated. The metaphases were harvested at 18 h after the removal of clastogens. A: aberrent metaphases; b: chromatid breaks; e: chromatid exchanges; □: additive value of two single treatments; ▨: value of combined treatment; ☆: value of combined treatment that significantly greater ($p<0.05$) than expected from purely additive effects. Average of two to three independent experiments.

the metaphase cells harvested after 2 h treatment with 5 mM EMS followed by incubation in 10 $\mu$M SA for another 18 h were all in their first cell division, and the coclastogenicity of SA was more obvious in metaphase cells harvested 18 h after EMS was removed than at earlier time interval. Therefore in this report, the chromosomal aberrations were analyzed at 18 h after the removal of clastogens. Thus stage I may represent G1 and early S phase, stage II may represent early and middle S phase, and stage III may represent late S and G2 phase. However, the exact cell stage has not been determined.

Regardless different clastogens used, a large increase of chromatid exchanges was observed when SA was given in stage I; a small increase was observed when SA was given in stage II and no increase was observed when SA was given in stage III. These results suggest that passing through S phase seems to be an important step for the formation of chromatid exchange after mutagen treatment (Natarajan, *et al.*, 1982). Thus, SA may enhance the incidence of chromatid exchange by promoting misreplication no matter what types of DNA lesions.

On the other hand, the potentiation of chromatid breaks by SA followed a

more complicated pattern. The potentiation patterns of chromatid breaks induced by monofunctional alkylating agents, MMS and EMS were parallel to those of chromatid exchange, i. e. high in stage I, low in stage II, and no potentiation in stage III. Potentiation of chromatid breaks induced by MMC was obvious when SA was given in stages I and II, but not in stage III. In contrast, potentiation of chromatid breaks induced by PT gradually increased from stage I to stage III. These results imply that DNA-DNA crosslinking induced by MMC and PT seems to have a longer half life as compared to the simple alkylation adducts. Therefore, a large porportion of SA potentiable lesions remained untill stage III. Since the formation of chromatid exchanges have to pass through S phase, all SA potentiable lesions are transformed into chromatid breaks. Therefore, the maximal potentiation of chromatid breaks induced by PT was observed when SA was given in stage III. In fact, the potentiation of PT-induced aberrant metaphases by SA at different stage is almost the same.

The present results indicate that the potentiation effect of SA on the induction of chromosome aberrations was dependent on the time period of SA treatment. The SA potentiable lesions induced by monofunctional alkylating agents exist only in stage I. The SA potentiable lesions induced by MMC, a bifunctional alkylating agent are present in stages I and II, and that induced by PT are present in stages I, II, and III. It can be seen from Fig. 1 that the presence of SA in stage I potentiated the chromosomal aberrations induced by all four clastogens studied. Using the same protocol, similar results were also obtained in cells treated with ultraviolet light and 4-nitroquinoline 1-oxide (data not shown). Thus, the period from G1 to early S seems to be an important time period for the SA coclastogenicity with the S-dependent clastogens, and for the formation of chromatid exchanges. Interestingly, the G1/S border is also found to be the most sensitive stage to the clastogenic effect of arsenite (Lee *et al*, 1986). Whether the clastogenicity of SA is related to its coclastogenicity remains to be determined.

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# 亞砷酸鈉協力破壞染色體的作用與細胞週期內特定時段之相關性

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先前的研究結果顯示：細胞經甲烷磺酸乙酯作用後，必需立刻加入亞砷酸鈉才能檢測出協力破壞染色體的作用。本研究結果指出：不同型式的 DNA 傷害演變成染色體異常的各種步驟與細胞週期有關。亞砷酸鈉可能作用在這些步驟上而產生協力破壞染色體的現象。從 G1 到 S 早期這段時間爲亞砷酸鈉協力作用以及染色分體互換形成的重要時段。