*Bull. Inst. Zool., Academia Sinica* **31**(4): 270-275 (1992)

# EFFECTS OF TREATMENT WITH ARSENITE ON THE CYTOTOXICITY OF R-RAY-IRRADIATED CHINESE HAMSTER OVARY CELLS AND HUMAN FIBROBLASTS

H. HUANG, S.H. TYAN and H.J. HUANG

*Institute of Radiation Biology National Tsing-Hua University Hsinchu, Taiwan 30043, R.O.C.*

(Accepted February 10, 1992)

**H. Huang, S.H. Tyan and H.J. Huang** (1991) Effects of treatment with arsenite on the cytotoxicity of r-ray-irradiated Chinese hamster ovary cells and human fibroblasts. Bull. Inst. Zool., Academia Sinica 31(4): 270-275. Pretreatment with arsenite for 12 h enhanced the cytotoxicity of r-ray-irradiated Chinese hamster ovary (CHO-K1) cells and human fibroblasts (HF). However, treatment with arsenite increased cellular glutathione in CHO-K1 cells, but not in HF. Post-treatment with arsenite for 12 h had no effect on the r-ray-irradiated CHO-K1 cells, and only slightly enhanced the cytotoxicity of r-ray-irradiated HF. These results are similar to the effects of pre- and post-treatment with arsenite on the cytotoxicity of bleomycin - a radiomimetic drug which also breaks cellular DNA by means of a free radical mechanism and induces both single-strand and double-strand DNA breaks.

**Key words:** Cytotoxicity, Radiation, Arsenite

For some time arsenic - a human carcinogen - has been known to be genotoxic. Recently arsenite - a trivalent form of arsenic - has been shown to have a modulating effect on the genetic toxicity of many mutagens. Post-treatment with arsenite has been shown to enhance the cytotoxicity of ultraviolet light (Lee *et al.*, 1985; Okui and Fujiwara, 1986), cis-diaminedichloroplatinum (II) (Lee *et al.*, 1986a), methyl methanesulfonate (Lee *et al.*, 1986b), and ethyl methanesulfonate (Jan *et al.*, 1990), but not the cytotoxicity of r-rays (Huang *et al.*, 1989), or bleomycin (Jan *et al.*, 1990). On the other hand, pretreatment with arsenite has been shown to enhance the cytotoxicity of bleomycin (Jan *et al.*, 1990), but not that of methyl methanesulfonate (Lee *et al.*, 1986b). Inasmuch as bleomycin breaks cellular DNA strands through free radicals that mimic the effects of ionizing radiation, and since both bleomycin and ionizing radiation induce both singlestrand and double-strand DNA breaks, experiments were conducted to see if similar modulating effects of arsenite on r-ray-irradiated cells could be observed. This information may help in understanding the types of damage that can be influenced by arsenite.

## MATERIALS AND METHODS

### Cell cultures

Chemicals for cell cultures were obtained from GIBCO (Grand Island, NY). Sodium arsenite (SA) was purchased from Merck (Darmstadt, F.R.G). Chinese hamster ovary (CHO-K1) cells and human fibroblasts (HF)

were provided by Drs. T.C. Lee and C.L. Yang, respectively. These cells were maintained in McCoy's 5A or Dulbecco's modified Eagle media supplemented with 10% heatinactivated fetal calf serum, 100 unit/ml penicillin, 100 ug/ml streptomycin, and 0.03% L-glutamine (Lee, *et al.*, 1985). Both the CHO-K1 cells and HF were incubated in a water saturated atmosphere containing 5 and 10% CO2 at 37°C. Stock cultures were maintained by routine subculture procedures every 2-4 days, with an initial density of $2\times10^6$ cells in 75-T flasks.

### Cell treatment

Log cells were prepared one day prior to each experiment. Some cultures were pretreated with SA for 12 h. Gamma-irradiation was carried out with a Cs-137 source generating 0.6 Gy/min of irradiation. Cells were kept on ice five minutes before and during irradiation. After SA pretreatment and gamma irradiation, cells were released and replated for clonogenic formation assay as described below (Huang, *et al.*, 1989). Irradiated cells which underwent SA post-treatment were incubated in SA-containing medium five minutes before (to avoid fast repair following irradiation) and 12 h after irradiation. Cells that did not undergo SA post-treatment were replated either immediately or following the 12 h incubation period after irradiation.

### Colony forming ability

The ability of cells to form colonies following various treatment protocols was determined as follows: cells were harvested with trypsin, then seeded at densities of 200-200,000 cells/60 mm tissue culture dish. CHO-K1 cells were grown for seven days; HF were grown for fourteen days with one medium change on day 6. Dishes were then stained with 1% crystal violet in 30% ethanol, after which the colonies were counted. Untreated CHO-K1 cells and HF exhibited clone plating efficiencies of approximately 85 and 50%, respectively.

### Glutathione (GSH) assay

GSH levels were determined according to the method described by Cohn and Lyle (1966). Cells were harvested with trypsin/EDTA, then resuspended in 10 ml of fresh medium. Cells then spun in a centrifuge at 1000 xg for 10 mins. Pellets were washed twice with 1 ml Puck's saline A buffer (0.04% KCl, 0.8% NaCl, 0.035% $NaHCO_3$ and 0.1% glucose), then resuspended in 0.2 ml of cold distilled water. Fifty ul of 25% metaphosphoric acid ($HPO_3$) was added to denature the protein. After 12,000 xg centrifugation for 15 min at 4°C, 40 ul of clear supernatant was thoroughly mixed with 2 ml distilled water, 0.5 ml of 0.1 M potassium phosphate buffer (pH 8.0), and 0.1 ml of 0.1% (w/v) OPT (0.1% o-phthalaldehyde in methanol). This mixture was kept at room temperature for 15 to 20 mins. Fluorescence excited at 350 nm and emitted at 420 nm was measured with a Hitachi F-4000 fluorescence spectrophotometer. GSH concentration were calculated from a standard GSH-OPT fluorescence curve.

## RESULTS AND DISCUSSION

The D levels of r-rays in the CHO-K1 cells and HF were 3 and 2 Gy°, respectively. Thus, CHO-K1 cells were 1.5 times more resistant to r-rays and 10 times more resistant to SA than HF. In our experiments, the cellular GSH contents for the HF and CHO-K1 cells were 0.39±0.07 and 1.02±0.27 nmole/$10^6$ cells respectively (Table 1). In Lee *et al.* (1989), the cellular GSH contents for HF and CHO cells (which were not the K1 strain) were 0.169±0.028 and 1.283±0.269, respectively. The ratio of GSH content in HF to that in CHO cells was

Table 1. GSH ratio in Chinese hamster ovary (CHO-K1) cells and human fibroblasts (HF) with or without pretreatment with 10 uM (CHO-K1) or 1uM (HF) SA for 12 h, irradiated or non-irradiated with 6 Gy r-rays, and incubated in SA-free medium 0 or 4 h.

| Treatment | GSH ratio[b] | |
|---|---|---|
| | CHO-K1 | HF |
| Control | 1 | 1 |
| SA (0 h)[a] | 3.54±0.11 | 1.09±0.16 |
| SA (4 h)[a] | 3.45±0.51 | 0.98±0.13 |
| r-ray (0 h) | 0.93±0.07 | 1.11±0.20 |
| r-ray (4 h) | 0.80±0.17 | 1.02±0.10 |
| SA[a] r-ray (0 h) | 3.32±0.36 | 1.36±0.21 |
| SA[a] + r-ray (4 h) | 2.10±0.61 | 1.38±0.17 |

a: with 12 h sodium arsenite pretreatment
b: ratio = treated cells/control cells
The GSH levels in untreated cells were 1.02±0.27 and 0.39±0.07 nmole/$10^6$ cells for CHO-K1 and HF, respectively.

about 1:2.6 in our experiments, and 1:7.6 in Lee *et al.* (1989). The higher cellular GSH content may be due to a higher resistance to the cell killing capabilities of SA and r-rays when comparing CHO-K1 cells with HF.

Pretreatment with SA for 12 h enhanced the cytotoxicity of r-ray-irradiated CHO-K1 (Fig. 1A) and HF (Fig. 2A). Post-treatment with SA for 12 h had no effect on the r-ray-irradiated CHO-K1 cells (Fig. 1B), and only slightly enhanced the cytotoxicity of r-ray-irradiated HF (Fig. 2B). Since r-rays may damage cells by generating free radicals, and since the cellular GSH may serve as a radical scavenger, it is possible that SA treatment may decrease cellular GSH content, making the cells more susceptible to radiation damage. Surprisingly, a 12-h treatment with SA increased cellular GSH in CHO-K1 but not in HF (Table 1). Therefore, the en-

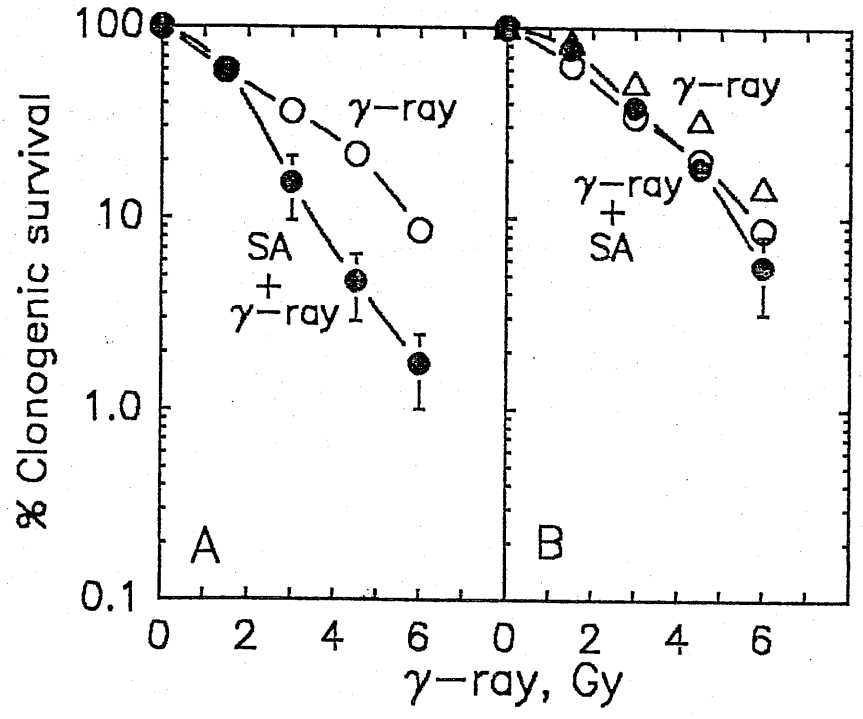


Fig. 1. Effects of (A) pretreatment or (B) post-treatment with 10 uM sodium arsenite for 12 h on the clonogenic survival of r-ray-irradiated CHO-K1 cells. Each point is the average of nine dishes from three separate experiments.
(o) replated immediately after irradiation
( ) replated 12 h (in normal medium) after irradiation

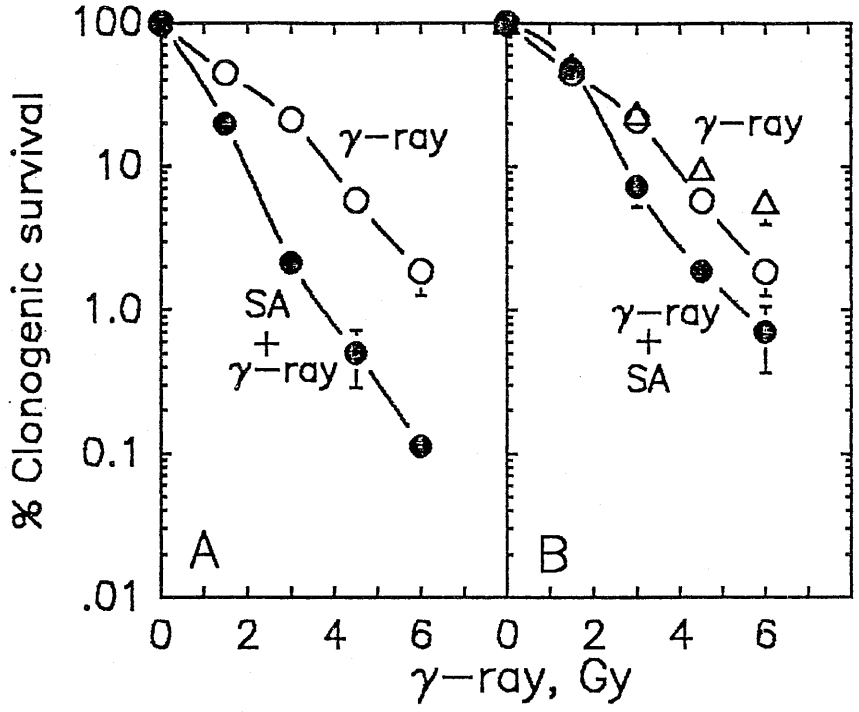


Fig. 2. Effects of (A) pretreatment or (B) post-treatment with 1 uM sodium arsenite for 12 h on the clonogenic survival of r-ray-irradiated human fibroblasts. Each point is the average of six dishes from two separate experiments.
(o) replated immediately after irradiation
( ) replated 12 h (in normal medium) after irradiation

hancing effects of SA pretreatment on the cytotoxicity of r-rays does not seem to be due to the decrease of cellular GSH content, even though SA may bind the cellular thiol groups (Fluharty and Sanadi, 1961; Jennette *et al.*, 1981).

Our results, together with those reported by Jan *et al.* (1990), indicate that pretreatment of CHO-K1 cells with SA enhances the cytotoxicity of r-rays and bleomycin, but that post-treatment with SA has no apparent effect on the cytotoxicity of r-rays and bleomycin. These observations are in contrast to the effect of SA on the cytotoxicity of methyl methanesulfonate, in which pretreatment with SA has no effect, but post-treatment with SA enhances the cytotoxicity of methyl methanesulfonate (Lee *et al.*, 1986b). Bleomycin and r-rays are known to damage cells through a free-radical mechanism, and to induce both single-strand and double-strand DNA breaks (Saito and Andoh, 1973). Since SA increased the cellular GSH content of CHO-K1 cells, it is unlikely that SA pretreatment would enhance free-radical damage in comparison with the HF data. Thus, a simple explanation may be that SA inhibits those enzymes responsible for the repair breaks in DNA strands. Much of the ionizing radiation- or bleomycine-damaged DNA is repaired during the first half hour following the damage (Moore and Little, 1985), whereas the repair of methyl methanesulfonated-damaged DNA appears to take several hours.

Recently, SA treatment has been found to induce heme oxygenase mRNA in HF and CHO cells (Keyse and Tyrrell, 1989; Apple-

gate *et al.*, 1991). It is possible that only heme oxygenase is involved in the SA detoxification of HF, whereas both heme oxygenase and GSH are involved in the SA detoxification of CHO cells. Thus, treatment with SA then r-rays or r-rays then SA induced higher cytotoxicity in HF than in CHO-K1 cells.

**Acknowledgements:** This study was supported by grant NSC-79-0203-07 from the National Science Council of the Republic of China.

## REFERENCES

Applegate, L.A., P. Luscher and R.M. Tyrrel (1991) Induction of heme oxygenase: A general response to oxidant stress in cultured mammalian cells. *Cancer Res.* **51**: 974-978.

Cohn, V.H. and J. Lyle (1966) A fluorometric assay for glutathione. *Anal. Biochem.* **14**: 434-440.

Fluharty, A.L. and D.R. Sanadi (1961) On the mechanism of oxidative phosphorylation: II. Effect of arsenite alone and in combination with 2,3-dimercaptopropanol. *J. Biochem. Chem.* **236**: 2772-2778.

Huang, H., T.C. Lee and K.Y. Jan (1989) Effects of post-treatment with arsenite on genetic toxicity of ultraviolet light and ionizing radiation. *Nucl. Sci. J.* **26**(6): 97-502.

Jan, K.Y., Y.C. Lin, I.C. Ho, S.L. Kao and T.C. Lee (1990) Effects of sodium arsenite on the cytotoxicity of bleomycin. *Toxicology Letters* **51**: 81-90.

Jennette, K.W. (1981) The role of metals in carcinogenesis: Biochemistry and metabolism. *Environ. Health. Perspect.* **40**: 233-252.

Keyse, S.M. and R.M. Tyrrell (1989) Heme oxygenase is the major 32-KDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. *Pro. Natl. Acad. Sci. USA* **86**: 99-103.

Lee, T.C., R.Y. Huang and K.Y. Jan (1985) Sodium arsenite enhances the cytotoxicity, clastogenicity, and 6-thioguanine-resistant mutagenicity of ultraviolet light in Chinese hamster ovary cells. *Mutation Res.* **148**: 83-89.

Lee, T.C., K.C. Lee, Y.J. Tzeng, R.Y. Huang and K.Y. Jan (1986a) Sodium arsenite potentiates the clastogenicity and mutagenicity of DNA crosslinking agents. *Environ. Mutagenesis* **8**: 119-128.

Lee, T.C., S. Wuu-Wang, R.Y. Huang, K.C.C. Lee and K.Y. Jan (1986b) Differential effects of pre-and post-treatment of sodium arsenite on the genotoxicity of methyl methanesulfonate in Chinese hamster ovary cells. *Cancer Res.* **46**: 1854-1857.

Lee, T.C., J.C. Ko and K.Y. Jan (1989) Differential cytotoxicity of sodium arsenite in human fibroblasts and Chinese hamster ovary cells. *Toxicology* **56**: 289-299.

Moore, C.W. and J.B. Little (1985) Rapid and slow DNA rejoining in nondividing human diploid fibroblasts treated with bleomycin and ionizing radiation. *Cancer Res.* **45**: 1982-1986.

Okui, T. and Y. Fujiwara (1986) Inhibition of human excision DNA repair by inorganic arsenic and the comutagenic effect in V79 Chinese hamster cells. *Mutat. Res.* **12**: 69-76.

Saito, M. and T. Andoh (1973) Breakage of a DNA-protein complex induced by bleomycin and its repair in cultured mouse fibroblasts. *Cancer Res.* **33**: 1696-1700.

# 砷化物對輻射線處理之中國倉鼠細胞與人類表皮纖維細胞協力毒性之研究

黃海美　田雪慧　黃惠貞

三價砷化物前處理12小時，會協力增加中國倉鼠卵巢細胞以及人類表皮纖維細胞被加瑪射線照射所引起的細胞毒性。此砷化物處理，使前者的麩胱甘太增加，對後者的麩胱甘太則無影響。輻射線照射細胞後，若再以三價砷化物處理12小時，並不改變中國倉鼠卵巢細胞之細胞毒性;但些微地增加人類表皮纖維細胞之細胞毒性。這些結果與砷化物前或後處理細胞對撲類惡抗癌劑之細胞毒性協力影響相類似。撲類惡抗癌劑被認為是一種擬輻射作用的藥劑，經由自由基作用機轉而打斷了細胞內去氧核醣核酸分子，造成其單股以及雙股斷裂。

*Bull. Inst. Zool., Academia Sinica* **31**(4): 276-289 (1992)

# DYNAMIC CHANGES IN THE LOCALIZATION AND QUANTITY OF HEAT SHOCK PROTEINS IN A CULTURED FIN CELL LINE OF COLOR CARP *CYPRINUS CARPIO*

CHEN-CHUN KU, SHIU-NAN CHEN
and GUANG-HSIUNG KOU

*Department of Zoology, National Taiwan University, Taipei, Taiwan 10764, Republic of China*

(Accepted February 12, 1992)

**Chen-Chun Ku, Shiu-Nan Chen and Guang-Hsiung Kou** (1992) Dynamic changes in the localization and quantity of heat shock protein in a cultured fin cell line of color carp *Cyprinus carpio*. *Bull. Inst. Zool., Academia Sinica* **31**(4): 276-289. Immunoblotting and indirect immunofluorescence were used to study the dynamic changes in quantity and intracellular distribution of major heat shock proteins *hsp*87, 70, 33, and 27 in cultured fin cells of color carp, *Cyprinus carpio* (CCF), subjected to heat shock at 40°C and allowed to recover at 31°C.

*Hsp*87 was enhanced immediately following heat shock; however, cellular *hsp*87 did not increase noticeable until 12 h following heat shock. Through indirect immunofluorescence, *hsp*87 was observed localizing in the cytoplasm before and after heat shock. During heat shock, significant amounts of *hsp*70 were immediately synthesized and moved from the cytoplasm to both the nucleolus and the nucleus; it then "overflowed" into the perinuclear region. Intracellular levels of *hsp*33 increased significantly (as quickly as *hsp*70) following heat shock, but disappeared completely 12-24 h later. Furthermore, during heat shock *hsp*33 appeared first at the nucleus; it was then found exclusively in the cytoplasm before disappearing. *Hsp*27 was detected in nostressed cells; after reaching maximum level 6 h following heat shock,a steady-state level was maintained for the remainder of the experimental period. Also by indirect immunofluorescence, *hsp*27 was detected in the nuclei of about half of the tested unstressed CCF cells. During heat shock, cells with nuclear staining appeared diminished, and little or no fluorescence was observed in the CCF cells. Subsequently, filament staining within the perinucleus began to increase, then intense *hsp*27 cytoplasmic staining appeared.

CCF cells were heated to 40°C for 10 h, then cooled to 31°C. Indirect immunofluorescence showed *hsp*87, 70, and 33 levels returned to preheat-shock levels 36 h after recovery. However, intense *hsp*27 cytoplasmic staining remained even after the 36-h recovery period. These results suggest heat shock proteins are degraded in the cytoplasm of *C. carpio*.

**Key words:** Heat shock proteins, localization, fish cell line

Heat shock proteins are produced when a cell is stressed by various environmental insults including high temperatures, heavy metals, amino acid analogues, and exposure to a variety of chemical compounds (Lee and Hahn, 1988). Although these factors do not affect various species equally, the heat shock