Environmental Mutagens & Carcinogens 15-2 : 94-99

Low-dose of Ultraviolet radiation-, Ethyl methanesulfonate- or Bleomycin-Induced Adaptive Response in Chinese hamster ovary Cells

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(Received July 26, 1995)

ABSTRACT : **The adaptive response and cross-adaptive response to sister chromatid exchanges (SCEs) and DNA single-strand breaks (SSBs) in Chinese hamster ovary (CHO)-K_1 cells treated with ultraviolet radiation (UV), ethyl methanesulfonate (EMS), or bleomycin (BLM) were investigated. Two assays were used in this study ; SCEs and alkaline elution. The pretreatment with low conditioning dose of 2 mM EMS or 1 J/m^2 UV decreased the yield of SCEs induced by subsequent treatment with 8 mM EMS, 5 J/m^2 UV or 5 μg/ml BLM. And the pretreatment with low conditioning dose of 1 μg/ml BLM decreased the yield of SCEs induced by subsequent treatment with 5 μg/ml BLM or 5 J/m^2 UV. The rejoining of DNA SSBs in cells subsequently treated with 2 J/m^2 UV, 50 mM EMS or 400 μg/ml BLM is higher than that only treated with 2 J/m^2 UV, 50 mM EMS or 400 μg/ml BLM. These results suggest that there are the adaptive response and cross-adaptive response to SCEs, and is the adaptive response to the rejoining of DNA SSBs in CHO cells.**

Keywords : Adaptive response, SCEs, DNA single-strand breaks, CHO cells.

INTRODUCTION

Samson and Cairns (1977) first demonstrated that *Escherichia coli* exhibit an `adaptive response' to alkylating agents via the induction of the DNA repair system. The day after, many researches of the adaptive response have been reported in prokaryotic cells (Hadden *et al.*, 1983; Volkert, 1988) and eukaryotic cells (Bosi and Olivieri, 1989; Shadley and Dai, 1992; Moon *et al.*, 1993). Furthermore, the existence of adaptive response in mammalian cells has been reported for various alkylating agents (Samson and Schwartz, 1980; Kaina, 1982), and in response to ionizing radiation or tritiated thymidine (Wiencke *et al.*, 1986; Fan *et al.*, 1990). And also, the phenomenon of adaptive response was assayed by the induction of chromosome aberrations (Shadley and Dai, 1992), SCEs (Ikushima, 1989), micronuclei (Dominguez *et al.*, 1993), and on cell survival (Moon *et al.*, 1993).

On the other hand, Vijayalaxmi and Burkart (1989) reported that the yield of chromosomal aberrations induced by a subsequent treatment of X-ray was significantly reduced in human peripheral blood lymphocytes cultured in the presence of low concentrations of bleomycin. A similar response subsequently reported by Nunoshiba *et al.* (1991) was cross-adaptive response, defined as the reduction of the effects of an agent by pretreament with another agent, in *E. coli*. Therefore, the purpose of this study is to determine wheather there is adaptive response or cross-adaptive response to SCEs and the rejoining of DNA SSBs in CHO cells treated with various mutagens.

MATERIALS AND METHODS

Cell Culture

Chinese hamster ovary (CHO)-K_1 cells were used throughout this investigation. Monolayer cultures of this cell line were grown at 37°C in humidified 5% CO_2 incubator us-

This study was supported by the Korea Science and Engineering Foundation (No. 951-0508-038-1), 1995.

ing Eagle's minimum essential medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10 % newborn calf serum and 50 μg/ml gentamycin.

UV-Irradiation

Cells were cultured for more than 24 hours in culture dishes prior to UV-irradiation, and then the growth medium was removed from the cultures and the cells were washed twice with phosphate buffered saline (PBS). Cells were then exposed to various doses of 254 nm UV from mercury germicidal lamps at an incident dose rate of 1 J/m^2/sec. Dose rate was determined by UVX digital radiometer No. A 030848 (San Gabrial, CA 911778 USA). The fresh medium was added immediately after irradiation.

Chemical Treatments

Ethyl methanesulfonate (EMS, Tokyo Kasei Co., Tokyo, Japan) or bleomycin (BLM, Nihon Kayaku, Japan) was dissolved in the serum-free medium prior to use and exposed to cells at 37°C for desired time.

Inhibitor Treatments

Metabolic inhibitor of DNA synthesis, 1-β-D-arabinofuranosyl cytosine and hydroxyurea, were dissolved in the growth medium at final concentration of 10 μM and 2 mM, respectively. The treatment with inhibitor was performed immediately after exposure to UV-radiation.

Sister Chromatid Exchanges (SCEs) Experiments

Differential staining of chromatid was done according to the technique of Perry and Wolff (1974) with slight modifications. The cells were treated with chemicals for desired time. To produce harlequin chromosomes in which the sister chromatids were stained differentially, the cells were grown for 2 rounds of replication in the presence of 20 μM 5-bromodeoxyuridine (BrdU, Sigma). Cultures containing BrdU were grown in the dark to avoid photolysis of BrdU substituted DNA. Chromosome preparation were made by air-drying technique. The slides were stained with Hoechst 33258 (0.5 μg/ml in Sörensen buffer) for 15 minutes and exposed to light 9 hours and stained with 8% Giemsa (Gurr's R66, pH 6.8) for 20 minutes.

Alkaline Elution Experiments

Cells were labeled with 0.2 μCi/ml of ^{3}H-thymidine (specific activity, 85.6 Ci/mmol, Dupont, USA) for 24 hours and then exposed to chemicals. The cells harvested with cold PBS-Merchant solution (150 mM NaCl, 4.28 mM K_2HPO_4, 0.71 mM KH_2PO_4), and filtered onto 2 μm pore size polycarbonate filter (Nuclepore Co., Pleasanton, U.S.A.), and lysed with lysing solution (2 % SDS, 0.1 M Glycine, 0.025 M Na_2 EDTA, pH 10.0). Cells were eluted in the dark with eluting solution (30 mM tetrapropylammonium hydroxide, 0.02 M EDTA, 1 % SDS, pH 12.1) at a flow rate of 0.035 ml/min. Fractions were collected to 90 minutes interval. The radioactivity remaining on filter after 9 hours elution were plotted.

RESULTS

Sister Chromatid Exchanges (SCEs)

Table 1 shows that pretreatment with conditioning dose of 1 J/m^2 UV decreases the yield of SCEs induced by subsequent treatment with 5 J/m^2 UV, 8 mM EMS or 5 μg/ml BLM, respectively. This result shows that the yields of SCEs in the groups (U1, U2, U3) are significantly lower than the expected values.

Table 2 shows that pretreatment with conditioning dose of 2 mM EMS decreases the yield of SCEs induced by subsequent treatment with 8 mM EMS, 5 J/m^2 UV or 5 μg/ml BLM, respectively. The result shows that the yields of SCEs in the groups (E1, E2, E3) are significantly lower than the expected values.

Table 3 shows that pretreatment conditioning dose of 1 μg/ml BLM decreases the yield of SCEs induced by subsequent treatment with 5 μg/ml BLM or 5 J/m^2 UV, respectively. This result shows that the yields of SCEs in the groups (B1, B2) are significantly lower than the expected values. But pretreatment with 1 μg/ml BLM increases the yield of SCEs induced by subsequent treatment with 8 mM EMS, and thus, the yield of SCEs in the group (B3) is higher than the expected values. It is different from others'. The overall results show that there are

Table 1. The effect of pretreatment with conditioning dose (1 J/m^2) of UV on the yield of SCEs induced by subsequent treatment with 5 J/m^2 UV, 8 mM EMS or 5 μg/ml BLM

Exp. group	Conditioning treatment	Incubation time	Subsequent treatment	No. of cell	Expected values	SCEs/cell (Mean ± S.E.)
C1	none		none	150		9.97±0.73
C2	UV		none	150		15.43±0.74
C3	UV	4 hours	none	150		12.43±0.70
C4	none		UV	150		27.22±1.07
C5	none		EMS	150		54.02±1.37
C6	none		BLM	150		11.08±0.40
U1	UV	4 hours	UV	50	29.34	26.12±1.18*
U2	UV	4 hours	EMS	50	56.14	53.96±1.15*
U3	UV	4 hours	BLM	50	13.20	11.42±0.47*

The expected values in the adapted group were obtained by c3+(c4, c5 or c6)-c1. c1, c2, c3, c4, c5 and c6 are the yields of SCEs in the groups, C1, C2, C3, C4, C5 and C6, respectively. *p<0.05.

Table 2. The effect of pretreatment with conditioning dose (2 mM) of EMS on the yield of SCEs induced by subsequent treatment with 8 mM EMS, 5 J/m^2 UV or 5 μg/ml BLM

Exp. group	Conditioning treatment	Incubation time	Subsequent treatment	No. of cell	Expected values	SCEs/cell (Mean±S.E.)
C1	none		none	150		8.11±0.44
C2	EMS		none	150		24.75±1.05
C3	EMS	4 hours	none	150		21.78±0.94
C4	none		EMS	150		53.21±1.85
C5	none		UV	150		30.44±1.29
C6	none		BLM	100		10.52±0.49
E1	EMS	4 hours	EMS	50	66.88	58.66±2.01*
E2	EMS	4 hours	UV	50	44.11	27.04±1.18*
E3	EMS	4 hours	BLM	50	24.19	20.22±0.69*

*p<0.05.

Table 3. The effect of pretreatment with conditioning dose (1 μg/ml) of BLM on the yield of SCEs induced by subsequent treatment with 5 μg/ml BLM, 5 J/m^2 UV or 8 mM EMS

Exp. group	Conditioning treatment	Incubation time	Subsequent treatment	No. of cell	Expected values	SCEs/cell (Mean±S.E.)
C1	none		none	150		7.18±0.39
C2	BLM		none	150		9.69±0.52
C3	BLM	4 hours	none	150		7.86±0.46
C4	none		BLM	150		10.36±0.46
C5	none		UV	50		27.11±0.93
C6	none		EMS	50		50.16±2.46
B1	BLM	4 hours	BLM	50	11.04	8.90±0.52*
B2	BLM	4 hours	UV	50	27.79	25.98±0.71*
B3	BLM	4 hours	EMS	50	50.84	60.96±1.56

*p<0.05.

the adaptive response and cross-adaptive response in the groups of conditioning treatment with EMS, UV or BLM, but the B3 group is not shown the cross-adaptive response.

DNA single-strand breaks (SSBs)

This study was undertaken to determine the adaptive response to the rejoining of DNA SSBs in CHO cells treated with UV, EMS or BLM. The quantitation of DNA SSBs was based on the relative percentage of ^{3}H-thymidine of DNA remaining on filter after 9 hours elution.

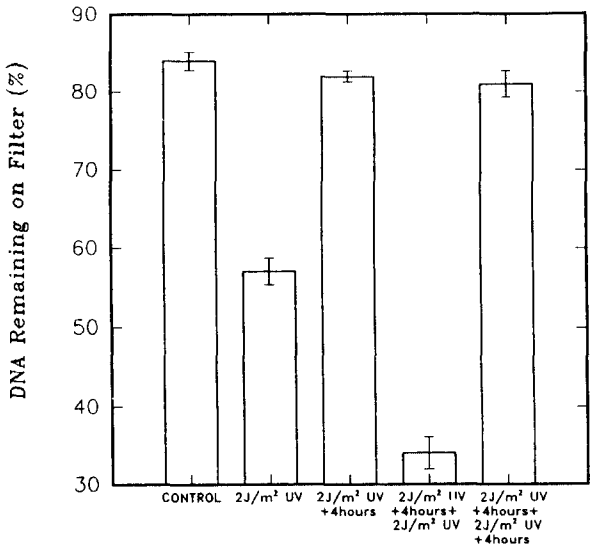

Fig. 1. The effect of pretreatment with conditioning dose of 2 J/m^2 UV on DNA single-strand breaks induced by 2 J/m^2 UV in CHO cells. DNA single-strand breaks were measured by the percentage of DNA remaining on filter at 9 hour elution.

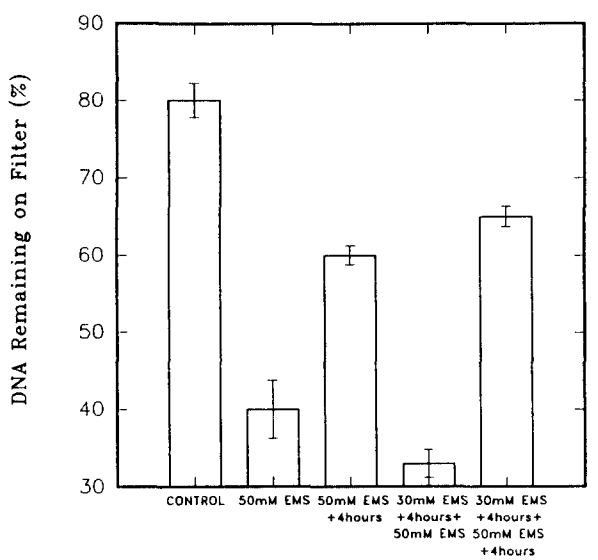

Fig. 2. The effect of pretreatment with conditioning dose of 30 mM EMS on DNA single-strand breaks induced by 50 mM EMS for 2 hours in CHO cells.

Fig. 1 shows the effect of pretreatment with conditioning dose of 2 J/m^2 UV on the rejoining of DNA SSBs induced by subsequent treatment with 2 J/m^2 UV. Compairing adaptive group (the rejoining of DNA SSBs pretreated with conditioning dose of mutagen) with non-adaptive group (the rejoining of DNA SSBs only treated with mutagen), the former is higher than the latter.

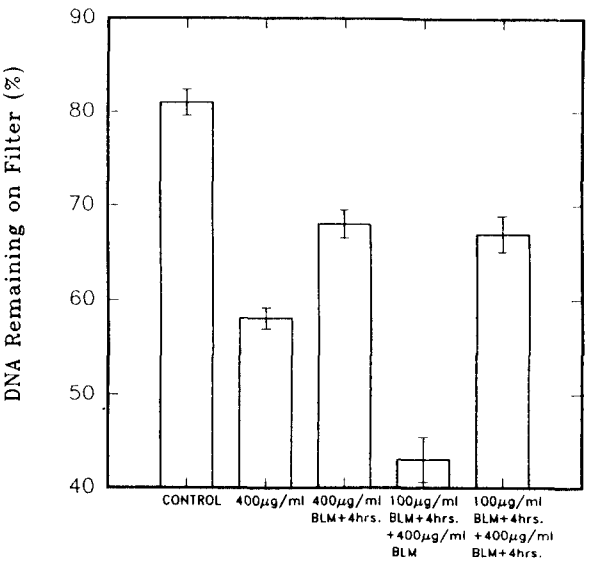

Fig. 3. The effect of pretreatment with conditionig dose of 100 μg/ml BLM on DNA single-strand breaks induced by 400 μg/ml BLM for 2 hours in CHO cells.

Fig. 2 shows the effect of pretreatment with conditioning dose of 30 mM EMS on the rejoining of DNA SSBs induced by subsequent treatment with 50 mM EMS. Compairing adaptive group with non-adaptive group, the rejoining of DNA SSBs of the former is higher than that of the latter.

Fig. 3 shows the effect of pretreatment with conditioning dose of 100 μg/ml BLM on the rejoining of DNA SSBs induced by subsequent treatment with 400 μg/ml BLM. Compairing adaptive group with non-adaptive group, the rejoining of DNA SSBs of the former is higher than that of the latter.

These results show that there is the adaptive response to the rejoining of DNA SSBs in CHO cells pretreated with low conditioning dose of UV, EMS or BLM.

DISCUSSION

Samson and Schwartz (1980) reported that CHO and GM637 cells pretreated with 0.01 μg/ml N-methyl-N'-nitro-nitrosoguanidine (MNNG) for 48 h and 72 h, respectively, were very resistant to the SCEs-inducing effects of 0.2 μg/ml MNNG. The resistance to SCEs induction decayed when pretreatment was stopped. They suggest that this resistance seems to be specific for alkylation damage because 0.01 μg/ml

MNNG-pretreated cells are not resistant to the induction of SCEs by UV (0-2s, fluence rate 12 erg $mm^{-2}s^{-1}$) light. Ikushima (1989) showed that pretreatment with low doses of β-rays from incorporated tritiated thymidine or of Co-60 γ-rays (1 or 5 cGy) rendered actively growing Chinese hamster V79 cells more resistant to the induction of micronuclei or SCEs by subsequent high dose of γ-rays (1 Gy). And also, human peripheral lymphocytes cultured in the low concentrations of BLM (0.01- 0.1 μg/ml) for 48 h and then treated with a high concentration (1.5 μg/ml) of the same agent or with 1.5 Gy X-rays, became significantly less sensitive to the induction of chromosomal damage than those which did not receive the pretreatment with BLM (Vijayalaxmi & Burkart, 1989). In the present studies, the pretreatment with conditioning dose of 1 J/m^2 UV or 2 mM EMS decreased the yield of SCEs in CHO cells subsequently treated with 5 J/m^2 UV, 8 mM EMS or 5 μg/ml BLM. And the pretreatment with conditioning dose of 1 μg/ml BLM decreased the yield of SCEs in CHO cells subsequently treated with 5 J/m^2 UV or 5 μg/ml BLM, but increased the yield of SCEs in the cells subsequently treated with 8 mM EMS.

At the molecular level, adaptation results from an increased expression of at least 4 genes, *ada*, *alkA*, *alk*B and *aid*B (Volkert, 1988). Among these genes, the ada gene product, O^6-methylguanine-DNA methyltransferase (MGMT), plays a key role in this process (Lindahl and Sedgwick, 1988). The activated MGMT binds to a conserved sequence, the ada box, found associated with the regulatory regions of two genes, *ada* itself, and *alk*A, the gene encoding the inducible 3-mthyladenine DNA glycosylase II enzyme. And the *alk*A glycosylase is responsible for the removal of several different alkylation lesions from DNA. Two other genes, of unknown funtion, are also part of the adaptive response (Baker *et al.*, 1992). On the other hand, Moon *et al.* (1993) reported that pretreatment with 2 J/m^2 UV or 30 mM EMS increased DNA SSBs induced by 100 mM EMS, and also pretreatment with 100 μg/ml BLM increased DNA SSBs induced by 400 μg/ml BLM. But they failed to observe an adaptive response to DNA SSBs in CHO cells, because they did not investigate the rejoining of DNA SSBs according to repair time. In our results, the adaptive groups (pretreated with conditioning dose of 2 J/m^2 UV, 30 mM EMS or 100 μg/ml BLM) are more effective to the rejoining of DNA SSBs than non-adaptive groups (only treated with 2 J/m^2 UV, 50 mM EMS or 400 μg/ml BLM).

Considering above others' and our results obtained, it is suggest that there are the adaptive response and cross-adaptive response to SCEs, and is the adaptive response to the rejoining of DNA SSBs in CHO cells. To elucidate the detailed molecular mechanism of adaptive response and/or cross-adaptive response, further studies are necessary.

REFERENCE

1. Baker, S.M., G.P. Margison, and P. Strike, (1992): Inducible alkyltransferase DNA repair proteins in the filamentous fungus *Aspergillus nidulans, Nucleic Acid Res.* **20-4:** 645-651.
2. Bosi, A. and G. Olivieri, (1989): Variability of the adaptive response to ionizing radiations in humans, *Mutat. Res.* **211:** 13-17.
3. Dominguez, I., N. Panneerselvam, P. Escalza, A.T. Natarajan, and F. Cortes, (1993): Adaptive response to radiation damage in human lymphocytes in conditioned with hydrogen peroxide as measured by the cytokinesis-block micronucleus technique, *Mutat. Res.* **301:** 135-141.
4. Fan, S., G. Vijayalaxmi, G. Mindek, and W. Burkart, (1990): Adaptive response to two doses of X-rays in human blood lymphocytes, *Mutat. Res.* **243:** 53-56.
5. Hadden, C.T., R.S. Foote, and S. Mitra, (1983): Adaptive response of *Bacillus subtilis* to N-methyl-N'-nitro-N-nitrosoguanidine, *J. of Bacteriol.* **153-2:** 756-762.
6. Ikushima, T., (1989): Radio-adaptive response: characterization of a cytogenetic repair induced by low-level ionizing radiation in cultured Chinese hamster cells, *Mut. Res.* **227:** 241-246.
7. Kaina, B., (1982): Enhanced survival and reduced mutation and aberration frequencies in V79 Chinese hamster cells pre-exposed to low levels of methylating agents, *Mutat. Res.* **93:** 195-211.
8. Lindahl, T., B. Sedgwick, M. Sekiguchi, and Y. Nakabeppu, (1988): Regulation and expression of the adaptive response to alkylating agents, *Ann. Rev. Biochem.* **57:** 133-157.
9. Moon, Y.S., E.Y. Kim, and K.I. Um, (1993): Effect of pretreatment with various mutagens on cell survival and DNA repair in Chinese hamster ovary cells, *Environ. Mutagens & Carcinogens* **13-2:** 83-91.
10. Nunoshiba, T., M. Hashimoto, and H. Nishioka, (1991): Cross-adaptive response in *Escherichia coli* caused by pretreatment with H_2O_2 against form aldehyde and other aldehyde compounds, *Mutat. Res. DNA Repair.* **255:** 265-271.
11. Perry, P. and S. Wolff, (1974): New Giemsa method for differential staining of sister chromatids, *Nature (London)*

251: 156-158.
12. Samson, L. and J. Cairns, (1977): A new pathway for DNA repair in *Escherichia coli, Nature* **267:** 281-283.
13. Samson, L. and J.L. Schwartz, (1980): Evidence for an adaptive DNA repair pathway in CHO and human skin fibroblast cell lines, *Nature* **287:** 861-863.
14. Shadley, J.D. and G. Dai, (1992): Cytogenetic and survival adaptive responses in G1 phase human lymphocytes, *Mutat. Res.* **265:** 273-281.
15. Vijayalaxmi and W. Burkart, (1989): Resistance and cross-resistance to chromosome damage in human blood lymphocytes adapted to bleomycin, *Mutat. Res.* **211:** 1-5.
16. Volkert, M.R., (1988): Adaptive response of *Escherichia coli* to alkylating damage, *Environ. Mol. Mutagen* **11:** 241-255.
17. Wiencke, J.K., V. Afzal, G. Olivieri, and S. Wolff, (1986): Evidence that the [^{3}H]thymidine-induced adaptive response of human lymphocytes to subsequent doses of X-rays involves the induction of a chromosomal repair mechanism, *Mutagenesis* **1:** 375-380.

Chinese hamster 난소세포에서 저농도 자외선과 Ethyl methanesulfonate 및 Bleomycin에 의한 적응반응

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적 요

Chinese hamster 난소세포에 자외선과 Ethyl methanesulfonate (EMS) 및 Bleomycin (BLM)을 처리하여, 적응반응 또는 교차적응반응을 자매염색분체교환법과 알카리 유출법으로 조사하였다. **2 mM EMS와 1 J/m² 자외선**의 전처리는 **8 mM EMS와 5 J/m² 자외선** 및 **5 μg/ml BLM**의 후처리에 의해 유발되는 자매염색분체교환율을 감소시켰고, **1 μg/ml BLM**의 전처리는 **5 μg/ml BLM**과 **5 J/m²** 자외선의 후처리에 의해 유발되는 자매염색분체교환율을 감소시켰다. **2 J/m²** 자외선과 **50 mM EMS** 및 **400 μg/ml BLM**을 후처리한 세포의 **DNA** 단사절단의 회복율은 **2 J/m² 자외선**과 **50 mM EMS** 및 **400 μg/ml BLM**을 단독처리한 세포의 **DNA** 단사절단 회복율보다 훨씬 크다. 이상의 결과로 보면, **CHO**세포에서 자매염색분체교환에 대해서는 적응반응과 교차적응반응이 존재하고, **DNA** 단사절단의 회복에서는 적응반응이 존재하는 것으로 추측된다.