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Effects of Ethyl methanesulfonate and Ultraviolet light on Induction of the Adaptive Response in Chinese Hamster Ovary and Sarcoma 180 Cells

Gyoo Cheon Kim, Dong Wook Lee, Eun Joo Shin and Kyung Il Um

Department of Biology, Dong-A University, Pusan 604-714, Korea

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ABSTRACT : **This study was performed by the sister chromatid exchanges (SCEs) and micronuclei (MN) assays to investigate the adaptive response to ultraviolet light (UV) or ethyl methanesulfonate (EMS) in Chinese hamster ovary (CHO) and Sarcoma 180 (S180) cells. The pretreatment with 1 J/m^2 UV or 2 mM EMS decreased the frequency of SCEs induced by the treatment with 5 J/m^2 UV or 8 mM EMS in CHO cells. The pretreatment with UV (1 or 2 J/m^2) or EMS (1, 2 or 3 mM) did not affect the SCEs induced by the treatment with 7 J/m^2 UV or 10 mM EMS in S180 cells. On the other hand, the pretreatment with 1 J/m^2 UV or 2 mM EMS decreased the frequency of MN induced by the treatment with 5 J/m^2 UV or 8 mM EMS in CHO cells. The pretreatment with UV (1 or 2 J/m^2) or EMS (1, 2 or 3 mM) did not affect the frequency of MN induced by the treatment with 7 J/m^2 UV or 10 mM EMS in S180 cells. It is suggested that there are adaptive responses at the level of chromosome and micronuclei to UV and EMS in CHO cells.**

Key words : Adaptive response, Chinese hamster ovary and Sarcoma 180 cells, Sister chromatid exchanges, Micronuclei.

The exposure to various DNA damaging stresses like mutagenic and clastogenic agents is known to induce an adaptive response in pro- and eukaryotic cells (Samson and Cairns, 1977; Samson and Schwartz, 1980; Shadley and wolff, 1987; Wolff *et al.*, 1990). The adaptive response is a phenomenon by which cells given a sublethal exposure to low doses of DNA-damaging agents develop an increased resistance to a subsequent exposure to a higher dose (Farooqi and Kesavan, 1993). And this phenomenon has been found to be dependent upon the adapting dose, dose rate and expression time (Azzam *et al.*, 1994).

Since the first report by Samson and Cairns (1977), an adaptive response to various mutagens has been observed in human lymphocytes, and cells of several mouse tissues (Wolff *et al.*, 1990; Wojcik and Tuschl, 1990). Most studies of adaptive response have been performed in normal cells, while the adaptive response in cancer cells has been reported rarely. Because the development of a cancer occurs as the result of several mutations in the cell, cancer cells are likely to have deficiency in expression of several enzymes (Goodman, 1994). Especially, adaptive response to alkylating agents involves the activated synthesis of O^6-alkylguanine-DNA alkyltransferase (AGAT) (Lindahl *et al.*, 1988). And a considerable number of human tumor cell lines have been found to lack AGAT activity (Pegg, 1990). Therefore, the purpose of this study is to elucidate the existence of adaptive response to ultraviolet light (UV) and ethyl methanesulfonate (EMS) in Sarcoma 180 (S 180) and Chinese hamster ovary (CHO) cells by cytogenetic means, sister chromatid exchanges (SCEs) and micronuclei (MN) analysis.

MATERIALS AND METHODS

Cell Culture

Chinese hamster ovary (CHO)-K_1 and Sarcoma 180 (S180)

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cells were used throughout this investigation. Monolayer cultures of CHO and suspension cultures of S180 cells were grown at 37°C in humidified 5% CO_2 incubator using Eagle's minimum essential medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% newborn calf serum and gentamycin (50 μg/ml).

UV Irradiation

Cells were cultured for more than 24 hours in culture dishes prior to UV irradiation, and the growth medium was removed from the cultures and then 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 Gabriel, CA 911778 U.S.A.). The fresh medium was added immediately after the irradiation.

EMS Treatment

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

Sister Chromatid Exchanges (SCEs) Experiments

Differential staining of chromatid was performed according to the technique of Perry and Wolff (1974) with slight modification. The cells were exposed to UV or EMS for desired time. To produce harlequin chromosomes, the cells were grown for 2 rounds of replication in the presence of 20 μM 5-bromodeoxyuridine (BrdU, Sigma). The cultures containing BrdU were grown in the dark to avoid photolysis of BrdU substituted DNA. Chromosome preparations were made by air-drying technique. The slides were stained with Hoechst 33258 (0.5 μg/ml in Söerenssen buffer) for 15 minutes and exposed to light for 5 hours and stained with 8 % Giemsa (Gurr's R66, pH 6.8) for 20 minutes.

Micronuclei (MN) Experiments

MN analysis was done by cytokinesis-block method. A cytokinesis-block method which results in the formation of binucleated cells was perfomed according to the technique of Fenech and Morley (1985) with a slight modification. Cytochalasin B (Cyt B, Sigma) was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1.0 mg/ml, and stored at -65°C. The stock solution was diluted with PBS and added to the cell cultures at a final concentration of 6.0 μg/ml after UV or EMS exposure.

RESULTS

The results of the adaptive response by UV are represented in Table 1. The frequence of SCEs (1.38±0.06) in CHO cells exposed to low dose of UV (1 J/m^2) and subsequently exposed to challenging dose (5 J/m^2) is lower than expected value (1.57) (Table 1A). In S180 cells that are pre-exposed to two low doses of UV (1 or 2 J/m^2) and subsequently exposed to challenging dose of UV (7 J/m^2), there is no significant difference between the observed and expected values (Table 1B). Table 2A shows that the frequency of SCEs (3.12±0.10) in CHO cells pretreated with low dose of EMS (2 mM) and subsequently treated with challenging dose of EMS (8 mM) is lower than expected value (3.69). Whereas, there is no induction of adaptive response in S180 cells by low doses of EMS

Table 1. The effect of pre-exposure to low dose of UV (1 or 2 J/m^2) on the frequency of SCE induced by subsequent exposure to challenging dose of UV (5 or 7 J/m^2) in CHO(A) and S180(B) cells

	low dose exposure (J/m^2)	Incubation time (hour)	challenging dose exposure (J/m^2)	SCE/chromosome	
				observed values (Mean±S.E.)	expected values
(A)	none		none	0.46±0.02	
	1	4	none	0.69±0.02	
	none		5	1.34±0.05	
	1	4	5	1.38±0.06*	1.57
(B)	none		none	0.22±0.01	
	1	4	none	0.30±0.02	
	2	4	none	0.38±0.02	
	none		7	0.64±0.02	
	1	4	7	0.70±0.03	0.72
	2	4	7	0.78±0.03	0.80

The expected values were obtained by calculating the values of low dose exposure and 4 hours incubation plus the value of challenging dose exposure minus the control value.
*$p<0.05$

(Table 2B).

The production of MN has been used to assess chromosomal damage in cultured mammalian cells. Table 3A shows that the frequency of MN (250±2.91) in CHO cells that are pre-exposed to low dose of UV (1 J/m^2) and subsequently exposed to challenging dose (5 J/m^2) is lower than expected value (341). Table 3B shows that pre-exposure to low doses of UV (1 or 2 J/m^2) does not affect the frequency of MN in S180 cells subsequently challenged with 7 J/m^2 UV, and thus, there is no significant difference between the observed and expected values. Table 4A shows that the frequency of MN (287±2.78) in CHO cells pretreated with low dose of EMS (2 mM) and subsequently treated with challenging dose of EMS (8 mM) is lower than expected value (392). Table 4B represents that the pretreatment with low doses of EMS (1, 2 or 3 mM) does not affect the frequency of MN in S180 cells subsequently challenged with 10 mM EMS, and thus, there is no significant difference between the observed and expected values. The above results show that there is the adaptive response to SCE and MN in CHO cells, but not in S180 cells.

Table 2. The effect of pretreatment with low dose of EMS (1, 2 or 3 mM) on the frequency of SCE induced by subsequent treatment with challenging dose of EMS (8 or 10 mM) in CHO(A) and S180(B) cells

	low dose pretreatment (mM)	Incubation time (hour)	challenging dose treatment (mM)	SCEs/chromosome	
				observed values (Mean±S.E.)	expected values
(A)	none		none	0.48±0.01	
	2	4	none	1.20±0.03	
	none		8	2.97±0.09	
	2		8	3.12±0.10*	3.69
(B)	none		none	0.22±0.01	
	1	4	none	0.28±0.01	
	2	4	none	0.31±0.01	
	3	4	none	0.36±0.02	
	none		10	0.88±0.02	
	1	4	10	0.96±0.02	0.94
	2	4	10	1.01±0.03	0.97
	3	4	10	1.03±0.02	1.02

* P<0.05

Table 3. The effect of pre-exposure to low dose of UV (1 or 2 J/m^2) on the frequency of MN induced by subsequent exposure to challenging dose of UV (5 or 7 J/m^2) in CHO(A) and S 180(B) cells

	low dose exposure (J/m^2)	Incubation time (hour)	challenging dose exposure (J/m^2)	MN/1000 cells	
				observed values (Mean±S.E.)	expected values
(A)	none		none	68±0.51	
	1	4	none	106±0.65	
	none		5	303±1.10	
	1	4	5	250±2.91*	341
(B)	none		none	82±0.52	
	1	4	none	115±0.71	
	2	4	none	166±1.02	
	none		7	389±2.36	
	1	4	7	457±2.81	422
	2	4	7	494±3.00	473

*p<0.05

DISCUSSION

The adaptive response to radiation or alkylating agents have been demonstrated in a wide range of *in vitro* (Shadley and Wolff, 1987; Ikushima, 1989) and *in vivo* (Cai and Liu, 1990; Liu *et al.*, 1992). Pretreatment with low doses of β-rays (0.37-1. 85 kBq/ml) rendered actively growing Chinese hamster V79 cells more resistant to the induction of MN or SCEs by a subsequent high dose of γ-rays (1 Gy), but the cells pre-exposed to low dose of γ-ray (5 cGy) were not shown cross-resistance to challenge dose of EMS (100 μg/ml) for SCEs induction (Ikushima, 1989). Wojcik and Tuschl (1990) have reported con-

Table 4. The effect of pretreatment with low dose of EMS (1, 2 or 3 mM) on the frequency of MN induced by subsequent treatment with challenging dose of EMS (8 or 10 mM) in CHO(A) and S180(B) cells

	low dose pretreatment (mM)	Incubation time (hour)	challenging dose treatment (mM)	MN/1000 cells	
				observed values (Mean±S.E.)	expected values
(A)	none		none	65±0.53	
	2	4	none	124±0.68	
	none		8	333±1.05	
	2		8	287±2.78*	392
(B)	none		none	80±0.48	
	1	4	none	97±0.58	
	2	4	none	128±0.78	
	3	4	none	175±1.06	
	none		10	394±2.40	
	1	4	10	426±2.58	411
	2	4	10	457±2.76	442
	3	4	10	533±3.24	489

*p<0.05

comitant reduction in SCE frequencies in mice pre-exposed to low doses of ionizing radiations. Recently, Farooqi and Kesavan (1993) have reported that the pretreatment with low doses of γ-rays (0.025 and 0.05 Gy) increases the resistance of bone marrow cells of mice to challenging dose (1 Gy) of same radiation by analyzing MN. In this study, the SCEs and MN induced by 5 J/m^2 UV or 8 mM EMS were decreased by the pretreatment with 1 J/m^2 UV or 2 mM EMS in CHO cells. Considering our results obtained, these results were generally consistent with others' reports.

On the other hand, O^6-alkylguanine is the major lethal, mutagenic and carcinogenic lesion in DNA induced by alkylating agents (Isowa *et al.*, 1991). O^6-alkylguanine is repaired by the protein AGAT in normal human cell lines, but is not repaired in certain human tumor lines (Rasouli *et al.*, 1994). And AGAT activity is undetectable or very low in human tumor cell strains (Day *et al.*, 1987). Comparing with repair-proficient cell lines and repair-deficient tumor lines, repair-deficient cell lines are hypersensitive in the production of SCE, mutations and lethality by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Rasouli *et al.*, 1994). The present results showed that there was no statistically significant difference between the observed and the expected values in S180 cells. Thus, these results suggest that there is not adaptive response to EMS or UV for MN and SCEs induction in S180 cells.

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Chinese hamster 난소세포와 Sarcoma 180 세포에서 적응반응 유도에 대한 Ethyl methanesulfonate와 자외선의 효과

김규천 · 이동욱 · 신은주 · 엄경일
동아대학교 자연과학대학 생물학과

적 요

자매염색분체교환 (SCE)법과 소핵 (MN)법을 사용하여 **Chinese hamster** 난소 (CHO) 세포와 **Sarcoma 180 (S 180)** 세포에서 자외선과 **Ethyl methanesulfonate (EMS)**에 대한 적응반응을 조사하였다. **CHO** 세포에서는 **1 J/m²**의 가외선 또는 **2 mM EMS**의 전처리가 **5 J/m²** 자외선 또는 **8 mM EMS**에 의해 유발되는 **SCE**율을 감소시켰다. **S180** 세포에서는 **1**과 **2 J/m²** 자외선, 또는 **EMS (1, 2, 3 mM)**의 전처리가 **7 J/m²** 자외선 또는 **10 mM EMS**에 의해 유발되는 **SCE**에 영향을 주지 않았다. 한편, **CHO** 세포에서 **1 J/m²**의 자외선 또는 **2 mM EMS**의 전처리는 **5 J/m²** 자외선 또는 **8 mM EMS**에 의해 유발되는 소핵율을 감소시켰다. 그러나 **S180** 세포에서는 **1**과 **2 J/m²** 자외선, 또는 **EMS (1, 2, 3 mM)**의 전처리가 **7 J/m²** 자외선 또는 **10 mM EMS**에 의해 유발되는 소핵율에 영향을 주지 않았다. 이상의 결과로 보면, **CHO** 세포에서는 자외선과 **EMS**에 대한 염색체 및 소핵 수준에서의 적응반응이 존재하는 것으로 추측된다.