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Effects of Inhibitors on Cross-Adaptive Response to Ultraviolet Radiation or Ethyl methanesulfonate in Chinese Hamster Ovary Cells

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ABSTRACT : **This study was performed by the sister chromatid exchanges (SCEs) to investigate the effects of Aphidicolin (APC) or 2,4-dinitrophenol (DNP) on cross-adaptive response to ultraviolet radiation (UV) or ethyl methanesulfonate (EMS) in Chinese hamster ovary (CHO) cells. The pretreatment with 1 J/m^2 UV decreased the yield of SCEs induced by subsequent treatment with 8 mM EMS in CHO cells. And the treatment with 10 μg/ml APC or 50 μM DNP during incubation after pretreatment with 1 J/m^2 UV increased the yield of SCEs induced by 8 mM EMS. The pretreatment with 2 mM EMS decreased the yield of SCEs induced by subsequent treatment with 5 J/m^2 UV. The treatment with 10 μg/ml APC during incubation after 2 mM EMS increased the yield of SCEs induced by 5 J/m^2 UV. These results suggest that APC and DNP inhibit cross-adaptive response to pretreatment with UV and subsequent treatment with EMS, and also cross-adaptive response to pretreatment with EMS and subsequent treatment with UV is inhibited by APC in CHO cells.**

Key words : Cross-adaptive response, SCEs, Aphidicolin, 2,4-dinitrophenol, CHO cells.

Samson and Cairns (1977) first reported that the adaptive response is an inducible form of DNA repair acting on alkylation damage, and that exposure of *Escherichia coli* cells to a low level of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) results in induction of the adaptive response which renders bacteria more resistant to the killing and the mutagenic effects of the same agents. There were other reports that showed the existence of adaptive response for various mutagens such as, hydrogen peroxide (H_2O_2) (Winquist *et al.*, 1984), *N*-methyl-*N*-nitrosourea, methyl methanesulfonate and methyl iodide (Takahashi *et al.*, 1991), in prokaryotes. And cross-adaptive response to H_2O_2 and aldehyde compounds was reported (Nunoshiba *et al.*, 1991). Also, in mammalian cells, the phenomenon of adaptive response was reported for various alkylating agents (Samson and Schwartz, 1980; Lee *et al.*, 1995), ionizing radiation or tritiated thymidine (Ikushima, 1989), bleomycin (Vijayalaxmi and Burkart, 1989; Lee *et al*, 1995), radon (Wolff *et al.*, 1991) and ionizing radiation (Shadley and Dai, 1992).

On the other hand, the repair of DNA alkylation damage and UV-induced DNA lesions reduces cell killing and the induction of mutations and chromosome damage, and SCEs was strongly induced by various alkylating agents and UV (Kato, 1974). The specific DNA polymerases α inhibitor such as Aphidicolin (APC) enhances clastogen-induced SCE frequencies (Ikegami *et al.*, 1978). And ATP is required for specific incision of UV-damaged DNA in permeable cells (Dresler and Lieberman, 1983). 2,4-dinitrophenol (DNP) depleted of intercellular ATP has also been used to seek correlations between DNA damage and killing (Shibuya *et al.*, 1991). Also DNA damage induced by alkylation agents could give rise to SCEs is made from a consideration of the relation of SCEs to mutation (Wolff, 1978). And pyrimidine(6-4)pyrimidone lesions, long-term replication blocks, should play a major role in UV-induced SCE induction (Taft *et al.*, 1991).

Therefore, the purpose of this study is to elucidate the effects of APC and DNP on cross-adaptive response to UV or EMS in Chinese hamster ovary (CHO) cells.

MATERIAL AND METHODS

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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 using 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. Cells were then exposed to various doses of 254 nm UV from mercury germicidal lamps at an incident dose rate of 1 J/m²/sec. Dose rate was determined by UVX digital radiometer No. A 030848 (San Gabriel, CA 911778 USA). The fresh medium was added immediately after irradiation.

Chemical Treatments

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

Inhibitor Treatments

Aphidicolin (APC, Sigma chemical Co., USA), inhibitor of DNA polymerase α, was dissolved in dimethyl sulfoxide, and 2, 4-dinitrophenol (DNP, Sigma chemical Co., USA) was dissolved in the glucose-free Hanks solution, pH was adjusted to 6.8~7.0 with 7.5% $NaHCO_3$.

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.

RESULTS

Table 1 shows that the effect of 10 μg/ml APC on the yield of SCEs induced by pretreatment with 1 J/m² UV and subsequent treatment with 8 mM EMS. Treatment with 10 μg/ml APC during 4 hour incubation increases the yield of SCEs induced by subsequent treatment with 8 mM EMS. This result

Table 1. The effect of 10 μg/ml APC on the yield of SCEs induced by pretreatment with 1 J/m² UV and subsequent treatment with 8 mM EMS

Group	Pretreatment	Inhibitor treatment	Subsequent teatment	No. of Cells	Expected value	SCEs/cell (Mean±S.E.)
C1	none	none	none	50		8.2±0.43
	none	APC	none	50		9.0±0.82
	UV	none	none	50		15.5±0.64
C2	none	none	EMS	50		42.4±1.36
C3	UV	none[a]	none	50		12.7±0.87
C4	UV	APC[b]	none	50		15.7±0.69
	UV	none[a]	EMS	50	46.9	33.9±1.08*
	UV	APC[b]	EMS	50	49.9	38.6±1.80*

*$p<0.05$

[a]4 hour incubation

[b]treatment with APC during 4 hour incubation.

Expected values in the adapted group were obtained by (c3 or c4)+c2 -cl. cl, c2, c3 and c4 are the yield of SCEs in the groups, C1, C2, C3 and C4.

shows that APC inhibits cross-adaptive response induced by pretreatment with UV.

The effect of 50 μM DNP on the yield of SCEs induced by pretreatment with 1 J/m^2 UV and subsequent treatment with 8 mM EMS is shown in table 2. Treatment with 50 μM DNP during incubation increases the yields of SCEs induced by subsequent treatment with 8 mM EMS. The result shows that DNP inhibits cross-adaptive response induced by pretreatment with UV.

Table 3 shows that the effect of 10 μg/ml APC on the yield of SCEs induced by pretreatment with 2 mM EMS and subsequent treatment with 5 J/m^2 UV. Treatment with 10 μg/ml APC during incubation increases the yields of SCEs induced by subsequent treatment with 5 J/m^2 UV. This result shows that APC inhibits cross-adaptive response induced by pretreatment with EMS.

The effect of 50 μM DNP on the yield of SCEs induced by pretreatment with 2 mM EMS and subsequent treatment with 5 J/m^2 UV is shown in table 4. Treatment with 50 μM DNP during incubation does not significantly increase the yield of SCEs induced by subsequent treatment with 5 J/m^2 UV. The result

Table 2. The effect of 50 μM DNP on the yield of SCEs induced by pretreatment with 1 J/m^2 UV and subsequent treatment with 8 mM EMS

Group	Pretreatment	Inhibitor treatment	Subsequent treatment	No. of Cells	Expected value	SCEs/cell (Mean±S.E.)
C1	none	none	none	50		8.2±0.43
	none	DNP	none	50		9.3±0.54
	UV	none	none	50		15.5±0.64
C2	none	none	EMS	50		42.4±1.36
C3	UV	none[a]	none	50		12.7±0.87
C4	UV	DNP[b]	none	50		14.0±0.64
	UV	none[a]	EMS	50	46.9	33.9±1.08*
	UV	DNP[b]	EMS	50	48.2	37.9±1.05*

Table 3. The effect of 10 μg/ml APC on the yield of SCEs induced by pretreatment with 2 mM EMS and subsequent treatment with 5 J/m^2 UV

Group	Pretreatment	Inhibitor treatment	Subsequent treatment	No. of Cells	Expected value	SCEs/cell (Mean±S.E.)
C1	none	none	none	50		10.9±0.61
	none	APC	none	50		13.3±0.52
	EMS	none	none	50		26.4±0.73
C2	none	none	UV	50		38.8±1.23
C3	EMS	none[a]	none	50		21.3±0.92
C4	EMS	APC[b]	none	50		26.8±1.05
	EMS	none[a]	UV	50	49.2	37.2±1.92*
	EMS	APC[b]	UV	50	54.7	43.0±1.55*

Table 4. The effect of 50 μM DNP on the yield of SCEs induced by pretreatment with 2 mM EMS and subsequent treatment with 5 J/m^2 UV

Group	Pretreatment	Inhibitor treatment	Subsequent treatment	No. of Cells	Expected value	SCEs/cell (Mean±S.E.)
C1	none	none	none	50		10.9±0.61
	none	DNP	none	50		12.8±0.54
	EMS	none	none	50		26.4±0.73
C2	none	none	UV	50		38.8±1.23
C3	EMS	none[a]	none	50		21.3±0.92
C4	EMS	DNP[b]	none	50		21.9±0.77
	EMS	none[a]	UV	50	49.2	37.2±1.92*
	EMS	DNP[b]	UV	50	49.8	37.4±1.46*

shows that DNP does not inhibit cross-adaptive response induced by pretreatment with EMS.

DISCUSSION

Nunoshiba *et al.* (1991) demonstrated cross-adaptive response that pretreatment with sublethal dose (60 μM) H_2O_2 for 30 min made *E. coli* WP2 cells resistant to the killing effects of formaldehyde, glutaraldehyde, glyoxal, methyl glyoxal and chloroacetaldehyde. Ikushima (1989) reported that pretreatment with low doses of β-rays (0.11-10 cGy) from incorporated tritiated thymidine (3.7×10^{-3} kBq/ml) 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 a subsequent high dose of γ-rays (1 Gy). The yield of chromosome aberrations was significantly decreased in human peripheral blood lymphocytes cultured in the presence of low concentrations of bleomycin (BLM, 0.01-0.1 μg/ml) for 48 h and then treated with a high concentration (1.5 μg/ml) of BLM or with 1.5 Gy X-rays (Vijayalaxmi and Burkart, 1989). And in human lymphocytes low doses of X-rays (2 cGy) can decrease the number of chromatid deletions induced by subsequent exposure to radon (15.3 cGy) (Wolff *et al.*, 1991).

Ikegami *et al.* (1978) reported that Aphidicolin enhanced clastogen-induced SCEs frequency. Taft *et al.* (1991) reported that for SCE induction, pyrimidine(6-4)pyrimidone lesions appeared to play a predominant role in AA8, UV61 and UV5 cells treated with 0, 1, 2, 2.5 and 5 J/m^2 UV. And, in mammalian cells, ATP is required for specific incision of UV-damaged DNA for some obligatory step preceding incision in the excision repair pathway (Dresler and Liberman, 1983).

On the other hand, human lymphocytes treated with [^{3}H]thymidine (0.01 μCi/ml, [^{3}H]dThd) became refractory to the induction of chromosomal aberrations by subsequent dose of X-rays (1.5 Gy). And this adaptive response to [^{3}H]dThd did not occur in the presence of 0.2 mM 3-aminobenzamide (3-AB), inhibitor of the synthesis of poly(ADP-ribose) (Wiencke, 1987). In the present studies, pretreatment with 1 J/m^2 UV decreased the yield of SCEs induced by subsequent treatment with 8 mM EMS. This cross-adaptive response was inhibited by treatment with 10 μg/ml APC or 50 μM DNP during incubation. Also pretreatment with 2 mM EMS decreased the yield of SCEs induced by subsequent treatment with 5 J/m^2 UV. This cross-adaptive response was inhibited by treatment with 10 μg/ml APC, but was not inhibited by 50 μM DNP.

Considering above other's and our results obtained, it is suggested that polymerase α or ATP is involved in cross-adaptive response to SCEs induced by UV or EMS in CHO cells. However, to elucidate the detailed molecular mechanism of cross-adaptive response, further studies will be necessary.

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Chinese hamster 난소세포에서 자외선과 Ethyl methanesulfonate에 의한 교차적응반응에 대한 저해제의 영향

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적 요

Chinese hamster 난소세포에서 **자외선**과 **Ethyl methanesulfonate (EMS)**에 처리하여 유도된 교차적응반응에 대해 저해제인 **Aphidicolin (APC)**과 **2,4-dinitrophenol (DNP)**의 영향을 자매염색분체교환법으로 조사하였다. **1 J/m² 자외선**을 전처리한 후 **4**시간의 회복시간동안 **10 μg/ml APC**와 **50 μM DNP**를 처리하고 **8 mM EMS**를 처리하면, 저해제를 처리하지 않은 실험군에 비해 자매염색분체교환율이 증가함을 보였다. 또한 **2 mM EMS**을 전처리한 후 **4**시간의 회복시간동안 **10 μg/ml APC**와 **50 μM DNP**를 처리하고 **5 J/m² 자외선**를 처리하면, **APC**를 처리한 실험군은 저해제를 처리하지 않은 실험군에 비해 자매염색분체교환율이 증가함을 보였다. 이상의 결과로 보면 **CHO**세포에서 **APC**와 **DNP**는 **자외선**을 전처리하고 **EMS**를 후처리한 교차적응반응을 저해하고, **EMS**를 전처리하고 자외선을 후처리한 교차적응반응은 **APC**에 의해서만 저해된 것으로 추측된다.