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Effect of Low Dose Mutagens on Adaptive Response and Plasma Membrane Glycoconjugates in Sarcoma 180 Cells

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Mannose
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The present investigation was performed to elucidate the effect of pretreatment with low dose ultraviolet radiation (UV) and ethyl methanesulfonate (EMS) on cell survival by trypan blue dye exclusion method and plasma membrane glycoconjugates by lectin-cytochemistry in sarcoma 180 (S180) cells. Pretreatment with 2 J/m^2 UV or 2 mM EMS increased the percentage of survival of cells subsequently treated with high dose UV (10 or 20 J/m^2) or EMS (10 or 20 mM), respectively. Staining intensity of concanavalin A (Con A) of the cells pretreated with 2 J/m^2 UV or 2 mM EMS and subsequently treated with 10 or 20 mM EMS was stronger than that of the cells treated with 10 or 20 mM EMS. These results suggest that there is an adaptive response on cell survival to EMS or UV in S180 cells. And the results show a change in mannose-containing glycoconjugates of plasma membrane in S180 cells pretreated with EMS or UV and subsequently treated with EMS.

Cells have capacity to protect themselves from toxic substances, and the studies on adaptive response and multidrug resistance (MDR) have uncovered many of these abilities (Ford and Hait, 1990). Adaptive response, such as inducible DNA repair mechanism, caused by exposure to a very low level of an alkylating agent induces resistance to cell killing that is caused by subsequent exposure to the same or different chemical in bacteria (Samson and Cairns, 1977) and mammalian cells (Samson and Schwartz, 1980). Quantitative determination of survival response in cultured mammalian cells should provide essential chemotherapeutic information (Terasima et al., 1972). A cytogenetic adaptive response could lead to increase in cell survival (Shadley and Dai, 1992). Also, in mammalian cells, the phenomena of adaptive response on cell survival were reported for alkylating agents, ultraviolet (UV) radiation (Moon et al., 1993), and ionizing radiation (Shadley and Dai, 1993).

Drug resistance, termed MDR, is a phenomenon where mammalian tumor cell lines resistant to a single drug show cross resistances drug compounds without structural and functional similarities due to enhanced outward transport of drugs mediated by plasma membrane glycoprotein, P-glycoprotein (P-gp), "drug transport pump" (Juliano and Ling, 1976). The expression of a high-molecular weight P-gp has been shown to he correlated with multidrug resistance (Bradley et al., 1988). This resistance is due to decreased accumulation of drugs in cells caused by an energy-dependent drug transport protein (Gottesman and Pastan, 1993). The acquired resistance to alkylating agent is related to glycoconjugates of plasma membrane involved in multidrug resistance or adaptive response in HeLa cells (Lee et al., 1998). On the other hand, exposure to UV also triggers a transcriptional induction response known as the UV response. It is believed that the mammalian UV response serves a protective function (Holbrook and Fornace, 1991). The UV response is initiated at or near the plasma membrane rather than in the nucleus (Devary et al., 1992). Therefore, the purpose of this study was to investigate the acquired resistance and change of glycoconjugates of plasma membrane to UV or ethyl methanesulfonate (EMS) in sarcoma 180 (S180) cells.

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Materials and Methods

Cell culture

S180 cells were used throughout this investigation. Suspension cultures of these cell lines were grown at 37℃ in humidified 5% CO_2 incubator using Eagle's minimum essential medium (MEM, GIBCO) supplemented with 10% newborn calf serum and gentamycin (50 μg/ml).

UV irradiation

Cells were cultured in culture dishes for more than 24 h prior to treatment with UV, and then the growth medium was removed from the culture. The cells were washed twice with phosphate buffered saline (PBS). Cells were then exposed to various doses of 254 nm UV from mercury germicidal lamp at an incident dose rate of 1 J/m^2/sec. The dose rate was determined by UVX digital radiometer No. A030848. Fresh medium was added immediately after the irradiation.

EMS treatment

EMS (Sigma) was dissolved in a serum-free medium prior to use and applied to cells at 37℃ for desired time.

Procedure for adaptation

S180 cells were grown in MEM at 37℃ for 24 h. Cells were collected by centrifugation, and then resuspended in equal volume of PBS. After cells were treated with low dose UV for 2 sec or EMS for 2 h, they were incubated for 2 d in fresh medium. The cells were subsequently treated with high dose UV (for 10 or 20 sec) or EMS for 2 h. After the treatments, cell survival and lectin cytochemistry were performed.

Measurement of cell survival

Cell survival test was performed according to Perera et al. (1986) with minor modifications. For trypan blue dye exclusion methods, S180 cells which were in logarithmic growth phase were used. The cells were exposed to the mutagens for desired time, and then washed three times with PBS. Flasks containing the treated cultures and the untreated controls (each containing 20 ml of cell suspension) were placed in humidified 5% CO_2 incubators at 37℃. An aliquot of cells was added to an equal volume of 0.4% trypan blue, and mixed. The number of cells were counted twice using hemocytometer at daily intervals for 7 d.

Lectin cytochemistry

The change of glycoconjugates on plasma membrane was detected by lectin cytochemistry according to the technique of Lundh et al. (1989) with minor modifications. Glycoconjugates on plasma membrane were detected with Vectastatin ABC kit (Vector Laboratories) using 0.05% diaminobenzidine tetrahydrochloride (Sigma) as the chromogen, and all biotinylated lectins were obtained from Vector Laboratories. The lectin conjugates were diluted in tris-buffered saline (TBS, pH 7.4) containing 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$. Exponentially growing cells were seeded onto coverglass, which was placed in petridishes. The cells were treated with mutagens for desired time, washed three times with PBS, and fixed in fresh cold acetone at -20℃. The cells were then washed with PBS. Endogenous peroxidase activity was extinguished with a solution of 0.3% H_2O_2 in methanol for 30 min at room temperature, followed by PBS washing. To prevent non-specific protein binding, 5% normal rabbit serum and 2% bovine serum albumin in PBS were applied for 20 min at room temperature. The biotinylated lectin was applied to the cells for overnight at 4℃, and then washed three times with PBS. To demonstrate the biotinylated lectins, an avidin-biotin horseradish peroxidase complex was applied for 1 h at room temperature, and then washed three times with PBS. The peroxidase activity was visualized using a solution of 1% H_2O_2 and 10% diaminobenzidine in TBS.

Results

UV and EMS Effects of pretreatment with on survival of S180 cells

Effect of pretreatment with 2 J/m^2 UV or 2 mM EMS on the survival of cells treated with UV (10 or 20 J/m^2) is shown in Fig. 1 and 2. When the cells were incubated for 2 d after treatment with 2 J/m^2 UV or 2 mM EMS, percentage of cell survival increased up to the control level. Percentage of survival of cells pretreated with 2 J/m^2 UV or 2 mM EMS and subsequently treated with UV (10 or 20 J/m^2) was higher than the expected value. Effect of pretreatment with 2 mM EMS or 2 J/m^2 UV on the survival of cells treated with EMS (10 or 20 mM) is shown in Fig. 3 and 4. When the cells were incubated for 2 d after treatment with 2 mM EMS or 2 J/m^2 UV, percentage of cell survival increased up to the control level. The percentage of survival of cells pretreated with 2 mM EMS or 2 J/m^2 UV and subsequently treated with EMS (10 or 20 mM) was higher than the expected value. These results showed that

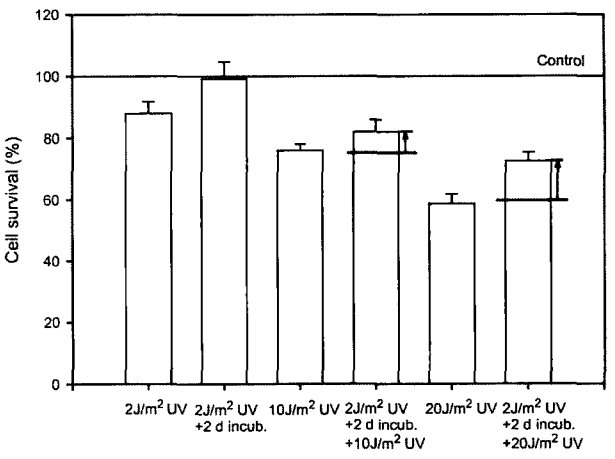

Fig. 1. Effect of pretreatment with 2 J/m^2 UV on the survival of S180 cells subsequently treated with 10 or 20 J/m^2 UV. Error bars indicate standard error of the mean. Solid line is expected value which was obtained by calculating the value of treatment with low dose mutagens following 2 d incubation plus that of treatment with high dose mutagens minus the control value.

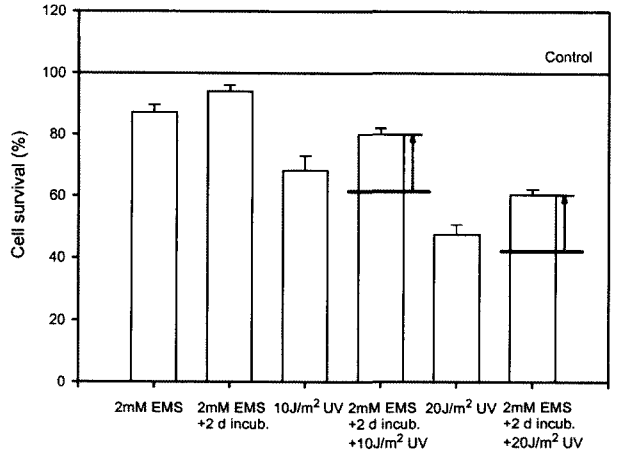

Fig. 2. Effect of pretreatment with 2 mM EMS on the survival of S180 cells subsequently treated with 10 or 20 J/m^2 UV.

there is an adaptive response on cell survival to UV or EMS.

Effects of pretreatment with UV and EMS on glycoconjugates of plasma membrane in S180 cells

The origins, abbreviations and sugar specificities of the biotinylated lectins used are shown in Table 1. Table 2 and 3 show the patterns of lectin-binding in the cells pretreated with 2 J/m^2 UV or 2 mM EMS and subsequently treated with 10 or 20 J/m^2 UV. Staining intensity for all lectins of the cells pretreated with 2 J/m^2 UV or 2 mM EMS and subsequently treated with 10 or 20 J/m^2 UV was similar to that of the cells treated with 10 or 20 J/m^2 UV. The patterns of lectin-binding in the cells pretreated with 2 mM EMS or 2 J/m^2 UV and subsequently treated with 10 or 20 mM EMS are shown in Table 4 and 5. The staining intensity of ConA of cells pretreated with 2 mM EMS or 2 J/m^2 UV and subsequently treated with 10 or 20 mM EMS was stronger than that of the cells treated with 10 or 20 mM EMS.

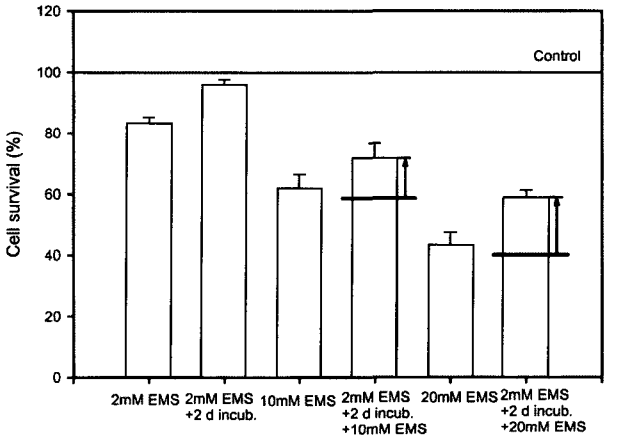

Fig. 3. Effect of pretreatment with 2 mM EMS on the survival of S180 cells subsequently treated with 10 or 20 mM EMS.

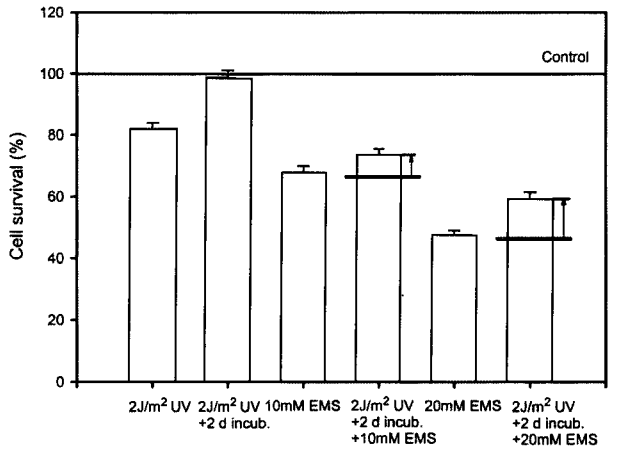

Fig. 4. Effect of pretreatment with 2 J/m^2 UV on the survival of S180 cells subsequently treated with 10 or 20 mM EMS.

The results showed increment of glycoconjugates containing mannose of plasma membrane in S180 cells.

Discussion

Chronic treatment of non-toxic levels (0.5-1.0 μg/ml) of N-methyl-N'-nitro-nitrosoguanidine to CHO cells renders the cells resistant to killing (Samson and Schwartz, 1980). Recently, Moon et al. (1993) reported that the survival of cells treated with various doses of UV (2, 5 or 10 J/m^2) or EMS (1, 5 or 30 mM) after pretreatment with 2 J/m^2 UV was higher than that of cells treated with various doses of UV (2, 5 or 10 J/m^2) or EMS (1, 5 or 30 mM). They suggested the existence of an adaptive response in the survival of CHO cells. Shadley and Dai (1993) also reported that the ratio of %NA cells/%S (the percent nonaberrant cells/percent survival) is significantly lower in the 5+100 cGy X-ray treated cells compared to the 100 cGy X-ray alone treated. Thus, a large fraction of the increase in survival was due to a decrease in lethal damage in cytologically nonaberrant cells. But detailed mechanism of adaptive response to each different mutagen has not been well understood.

Table 1. Origin, abbreviation, and sugar specificity of the biotinylated lectins used

Lectin origin	Abbreviation	Lectin concentration (μg/ml)	Sugar specificity
Triticum vulgaris	WGA	10	GlcNAc > Neu-5-Ac
Triticum vulgaris	sWGA	10	GlcNAc
Canavalia ensiformis	ConA	10	α-D-Man
Dolichos biflorus	DBA	10	GalNAc-α-1,3-GalNAc
Arachis hypogaea	PNA	20	galactosyl-β-1,3-GalNAc
Glycine max	SBA	20	D-GalNAc
Griffonia simplicifolia	GSLI	20	α-D-GalNAc
Ulex europaeus-I	UEAI	20	α-L-Fucose

WGA; wheat germ agglutinin, sWGA; succinylated wheat germ agglutinin, ConA; concanavalin A, DBA; *Dolichos biflorus* agglutinin, PNA; peanut agglutinin, SBA; soybean agglutinin, GSLI; *Griffonia simplicifolia* lectin I, UEAI; *Ulex europaeus* agglutinin I, GlcNAc; N-acetyl-D-glucosamine, Man; mannose, GalNAc; N-acetyl-D-galactosamine, Neu-5-Ac; N-acetyl neuraminic acid or sialic acid.

Table 2. Lectin-binding pattern in S180 cells pretreated with 2 J/m^2 UV and subsequently treated with 10 or 20 J/m^2 UV

Lectin	Control	2 J/m^2 UV	2 J/m^2 UV + 2 d incub.	10 J/m^2 UV	2 J/m^2 UV + 2 d incub. + 10 J/m^2 UV	20 J/m^2 UV	2 J/m^2 UV + 2 d incub. + 20 J/m^2 UV
WGA	++	++	++	++	++	++	++
sWGA	++	++	++	++	++	++	++
ConA	++	++	++	++	++	++	++
DBA	+	+	+	+	+	+	+
PNA	+	+	+	+	+	+	+
SBA	+	+	+	+	+	+	+
GSLI	++	++	++	++	++	++	++
UEAI	+	+	+	+	+	+	+

Staining intensities are given in arbitary units; +, weak staining; ++, moderate staining; +++, strong staining.

Table 3. Lectin-binding pattern in S180 cells pretreated with 2 mM EMS and subsequently treated with 10 or 20 J/m^2 UV

Lectin	Control	2 mM EMS	2 mM EMS + 2 d incub.	10 J/m^2 UV	2 mM EMS + 2 d incub. + 10 J/m^2 UV	20 J/m^2 UV	2 mM EMS + 2 d incub. + 20 J/m^2 UV
WGA	++	++	++	++	++	++	++
sWGA	++	++	++	++	++	++	+
ConA	++	++	++	++	++	++	++
DBA	+	+	+	+	+	+	+
PNA	+	+	+	+	+	+	+
SBA	+	+	+	+	+	+	+
GSLI	++	++	++	++	++	++	++
UEAI	+	+	+	+	+	+	+

In the present study, pretreatment with low dose UV or EMS increased the percentage of survival of the cells subsequently treated with high dose UV or EMS, respectively. Thus, the present results are generally consistent with the others' reports.

Overexpression of the Mr 170 kDa P-gp drug ATP-driven efflux pump called P-170 is responsible for the resistance of many multidrug resistant cell lines and some human tumors (Cole et al., 1994) to various hydrophobic neutral (Gottesman and Pastan, 1993) or positively charged cytotoxic drugs (Ford and Hait, 1990). Devary et al. (1992) reported that the UV response in HeLa S_3 cells is initiated at or near the plasma membrane, involving transcription factor AP-1, composed of Jun and Fos proteins, rather than in the nucleus. Transcription of both c-*jun* and c-*fos* is rapidly induced by exposure to 40 J/m^2 UV-C. Bhushan et al. (1992) suggested that a *fos*/*jun* transcriptional control element may participate in the regulation of P-gp expression. In the previous study, we suggested that acquired resistance on the survival of cells may be related to the change in glycoconjugates containing sialic

Table 4. Lectin-binding pattern in S180 cells pretreated with 2 mM EMS and subsequently treated with 10 and 20 mM EMS

Lectin	Control	2 mM EMS	2 mM EMS + 2 d incub.	10 mM EMS	2 mM EMS + 2 d incub. + 10 mM EMS	20 mM EMS	2 mM EMS + 2 d incub. + 20 mM EMS
WGA	++	++	++	++	++	++	++
sWGA	++	++	++	++	++	++	++
ConA	++	++	++	++	+++	++	+++
DBA	++	++	++	++	++	++	++
PNA	+	+	+	+	+	+	+
SBA	+	+	+	+	+	+	+
GSLI	++	++	++	++	++	++	++
UEAI	+	+	+	+	+	+	+

Table 5. Lectin-binding pattern in S180 cells pretreated with 2 J/m^2 UV and subsequently treated with 10 or 20 mM EMS

Lectin	Control	2 J/m^2 UV	2 J/m^2 UV + 2 d incub.	10 mM EMS	2 J/m^2 UV + 2 d incub. + 10 mM EMS	20 mM EMS	2 J/m^2 UV + 2 d incub. + 20 mM EMS
WGA	++	++	++	++	++	++	++
sWGA	++	++	++	++	++	++	++
ConA	++	++	++	++	+++	++	+++
DBA	++	++	++	++	++	++	++
PNA	+	+	+	+	+	+	+
SBA	+	+	+	+	+	+	+
GSLI	++	++	++	++	++	++	++
UEAI	+	+	+	+	+	+	+

acid of plasma membrane in HeLa cells pretreated with 1 mM EMS and subsequently treated with 10 mM EMS (Lee et al., 1998). In the present study, staining intensity of ConA of the cells pretreated with low dose of EMS or UV and subsequently treated with high doses of EMS was stronger than that of the cells treated with high dose of EMS, respectively. Thus, there is a change in glycoconjugates containing mannose of plasma membrane in S180 cells subsequently treated with EMS. The results show that the change to mutagens is different according to the cell line. Considering others' and our results, it is suggested that there is an adaptive response on cell survival to EMS or UV, and that ConA-binding glycoconjugates of plasma membrane may be related to the increment of survival in S180 cells subsequently treated with EMS. To elucidate the detailed molecular mechanisms of adaptive response, further studies are necessary.

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