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Inducible protective processes in animal systems: IV. Adaptation of mouse bone marrow cells to a low dose of ethyl methanesulfonate

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Ethyl methanesulfonate (EMS), a monofunctional alkylating agent, was used in the present investigations to investigate the induction of adaptive response (inducible protective processes) in mitotic cells of Swiss albino mouse. When a low (conditioning) dose of 80 mg/kg body wt was challenged with a subsequent high (challenging) dose of 240 mg/kg body wt, after different time lags, the yield of chromosomal aberrations in bone marrow cells was found to be significantly reduced compared with that of the challenge dose. It appears, therefore, that a low dose of EMS offered resistance to the mitotic cells against further clastogenic effect of any challenge dose of EMS employed. It is clear from the results that the phenomenon of adaptive response can also be encountered in mammalian *in vivo* systems.

Introduction

A search for the existence of an adaptive DNA repair pathway in eukaryotic cells is gaining more importance after the discovery of an adaptive response in *Escherichia coli* with low levels of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (Samson and Cairns, 1977). A considerable amount of work has been carried out in *in vitro* mammalian cells to assess the inducibility of the repair system by low levels of alkylating and non-alkylating agents (*cf.* Frosina and Abbondondolo, 1985). Chronic treatment of Chinese hamster ovary cells and SV40 transformed human skin fibroblasts (GM637) cells with non-toxic levels of MNNG renders resistance to the induction of sister chromatid exchange by further treatment with high toxic levels of MNNG (Samson and Schwartz, 1980). Olivieri *et al.* (1984) showed that pre-exposure of human blood lymphocytes to low levels of radioactive thymidine led to a significant reduction in chromatid aberrations induced by subsequent challenge with a high dose of X-rays. Similar results have been shown by other workers using X-rays in human lymphocytes (Shadley and Wolff, 1987; Sankaranarayanan, 1989). Human peripheral blood lymphocytes cultured in the presence of low concentrations of bleomycin (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 compared with cultures without bleomycin pre-treatment (Vijayalaxmi and Burkart, 1989). Furthermore, in plants, a more or less similar pattern of inducible repair has been described by Rieger and co-workers (Rieger and Michaelis, 1986; Rieger *et al.*, 1984, 1985) using *Vicia faba* root tip meristems with various alkylating and non-alkylating agents. They termed this phenomenon 'clastogenic adaptation'. However, a need to evaluate inducible repair in *in vivo* animal system is essential as the human race is continuously exposed to non-toxic levels of hazardous agents both from the environment and from the diet. Moreover, reports in this direction are very meagre and, hence, we designed experiments to investigate the induction of an adapative response in a mouse *in vivo* system using a monofunctional alkylating agent, i.e. ethyl methanesulfonate (EMS).

Materials and methods

Male swiss albino mice, 6–8 weeks old and weighing 25–30 g, were used in all the experiments. The monofunctional alkylating agent EMS (CAS 62-50-0) obtained from Sigma (St Louis, MO) served as the clastogenic agent in the present study. The bone marrow cytogenetic assay was employed to detect the clastogenic effect of the chemical in mitotic chromosomes. The required doses of EMS were obtained by dissolving the chemical in 0.7% NaCl solution. Fresh chemical solution was prepared every time. In all experiments, 0.5 ml of the test solution was injected intraperitoneally into the animals. A low (conditioning) dose of 80 mg/kg body wt (~33% of the LD_{50}) and a higher (challenge) dose of 240 mg/kg body wt (10 mg less than the LD_{50}) of EMS were selected following the dose–effect relationships (unpublished data). A single conditioning and challenging dose was given to animals with time lags of 2, 5 and 10 h between the two doses. Three to four animals were used at each dose point. After the challenge treatment, recovery times (RTs) of 24, 48 and 72 h were employed, and animals were sacrificed by cervical dislocation. Note that 0.5 ml of 0.05% colchicine (a mitotic arrester) was injected into the animals 90 min before sacrifice. The routine 'air-dry' technique of Evans *et al.* (1964) was followed for the preparation of slides. Air dried slides were stained in Giemsa. Coded slides were screened for the presence of chromosomal aberrations, i.e. achromatic lesions, chromatid breaks, chromosomal breaks, dicentrics, rings, chromatid exchanges, deletions, triradials, minutes and RB' complexes. From each sample, ~500 well spread metaphase plates were scored. RB' complexes and achromatic lesions were not included in the total number of breaks. The results were subjected to statistical analysis by employing the one-tailed Student's *t*-test.

Results and discussion

Pooled data from a minimum of two experiments are presented in Tables I–III. The conditioning and challenging doses of EMS induced exclusively chromatid-type aberrations, which included mostly chromatid breaks and exchanges, and these are statistically significant compared with controls in the bone marrow cells of the mouse at different recovery times of 24, 48 and 72 h. Dicentrics were not observed. Similar types of induction of chromatid aberrations by EMS have been reported by many workers in different test systems (*cf.* Vogel and Natarajan, 1982). The challenging dose of EMS could induce the highest number of chromatid-type aberrations compared with controls and conditioning dose (Tables I-III). Such dose-dependent induction of chromosome aberrations by EMS has been demonstrated previously by workers (Cattanach, 1968; Matter and Grauwiler, 1974; Henry *et al.*, 1980).

Earlier experiments to investigate the adaptive response in grasshopper, *Poecilocerus pictus*, by the authors with a time lag of 2 h between conditioning and challenging revealed the protective function of the low dose of EMS (Riaz Mahmood and Vasudev, 1990). In a similar situation, contrary results were obtained in mouse bone marrow cells using a 2 h time lag—the adaptive dose did not offer any protection to chromosomes (Riaz Mahmood and Vasudev, 1991). The data were thought to mean that in insect systems a 2 h time period is sufficient between conditioning and challenging doses to express the inducible protective repair process, whereas in mammalian system this period is not sufficient and the amount of repair enzymes induced

Table I. Percentage of chromosome aberrations observed after conditioning, challenging and combined treatments of EMS in mouse bone marrow cells (challenge treatment of EMS was given 2, 5 or 10 h after the conditioning dose of EMS and animals were sacrificed after 24 h RT)

Treatment (mg/kg body wt)	No. of replicates	No. of cells scored	Aberrant metaphases (%)										Aberrations (%)	Total No. of breaks	No. of breaks per cell
			Al	B′	B″	RB′	RB′B″	Dic	ID	rings	minutes	RB′ complex			
Control	2	1015	0.19	0.49	—	—	—	—	—	—	—	—	0.68	5	0.005
80 (conditioning)	2	902	0.44	6.09	0.11	—	—	—	0.22	—	—	—	6.86	61[a]	0.06
240 (challenging)	2	1100	0.72	30.27	0.36	11.0	0.81	—	0.54	0.09	5.18	0.27	49.27	681[a]	0.61
80−5hTL−240	2	1080	0.09	28.79	0.55	10.09	1.57	—	0.27	—	3.42	0.27	45.00	635[b]	0.58
80−10hTL−240	2	1029	—	16.32	0.38	2.33	0.19	—	0.19	—	4.17	0.09	23.71	277[b]	0.26

Pooled data of two independent experiments;
h, hours; TL, time lag; Al, Achromatic lesion; B′, chromatid break; B″, isochromatid break; RB′, chromatid translocation; RB′B″, triradials, Dic, dicentrics; ID, intrachromatid deletion; Acromatic lesions and RB′ complexes were not included in the total number of breaks.
[a]Significant compared to controls ($P < 0.05$).
[b]Significant compared to challenge dose ($P < 0.05$).

Table II. Percentage of chromosome aberrations observed after conditioning, challenging and combined treatments of EMS in mouse bone marrow cells (challenge treatment of EMS was given 2, 5 or 10 h after the conditioning dose of EMS and animals were sacrificed after 48 h RT)

Treatment (mg/kg body wt)	No. of replicates	No. of cells scored	Aberrant metaphases (%)										Aberrations (%)	Total No. of breaks	No. of breaks per cell
			Al	B′	B″	RB′	RB′B″	Dic	ID	rings	minutes	RB′ complex			
Control	2	995	—	0.40	—	—	—	—	—	—	0.10	—	0.50	5	0.005
80 (conditioning)	2	806	—	4.71	0.12	—	—	—	0.12	—	—	—	4.96	43[a]	0.05
240 (challenging)	2	1025	0.09	32.68	0.58	11.60	0.78	—	0.48	0.09	11.80	—	58.14	742[a]	0.72
80−5hTL−240	2	962	0.10	8.00	—	1.45	—	—	0.10	0.10	4.98	0.20	14.96	157[b]	0.16
80−10hTL−240	2	1051	—	7.70	0.09	0.28	—	—	0.38	—	2.66	0.19	11.32	125[b]	0.11

Notes as Table I.

Table III. Percentage of chromosome aberrations observed after conditioning, challenging and combined treatments of EMS in mouse bone marrow cells (challenge treatment of EMS was given 2, 5 or 10 h after the conditioning dose of EMS and animals were sacrificed after 72 h RT)

Treatment (mg/kg body wt)	No. of replicates	No. of cells scored	Aberrant metaphases (%)										Aberrations (%)	Total No. of breaks	No. of breaks per cell
			Al	B′	B″	RB′	RB′B″	Dic	ID	rings	minutes	RB′ complex			
Control	2	1031	0.09	0.38	—	—	—	—	—	—	—	—	0.48	4	0.003
80 (conditioning)	2	1027	0.09	4.28	—	0.29	—	—	—	—	—	—	4.67	50[a]	0.04
240 (challenging)	2	1013	—	10.76	—	0.98	—	—	—	—	0.78	—	12.43	138[a]	0.13
80−5hTL−240	2	1040	—	7.98	0.09	1.25	—	—	0.09	—	—	—	9.43	113[a]	0.11
80−10hTL−240	2	1060	—	9.05	0.28	—	—	—	0.09	—	0.18	—	9.70	117[b]	0.10

Notes as Table I.

may be insufficient to repair the lesions produced by the challenge dose. Interesting results were obtained when further experiments were conducted to investigate the same processes. There was a decline in the total number of breaks observed after combined treatment with a 5 h time lag compared with the amount of breaks produced by the challenge dose alone at all recovery times analysed (Figure 1). A maximum reduction of 78.8% in the total number of breaks was noticed at 48 h RT (from 742 to 157, Table II). Thus, it can be assumed that the increase in the time lag from 2 to 5 h might have accumulated sufficient repair enzymes to initiate repair activity, resulting in the reduction in the yield of chromatid aberrations.

It is very interesting that when animals had been pretreated with a low dose of EMS and challenged with a high dose after 10 h a significant reduction in chromatid aberrations resulted compared with a 5 h time lag. Thus the reduction in the number of aberrations is a consequence of the high activity of repair enzyme in the cells that were adapted for the 10 h time lag. It is pertinent to mention here that a time span of 30 min is sufficient for the induction of the 'highly protective' function against a challenge dose of EMS in *P.pictus* (Riaz Mahmood and Vasudev, 1992).

Whatever the consequence of adapted cells in insect and mammalian systems, the results point to an adaptive response,

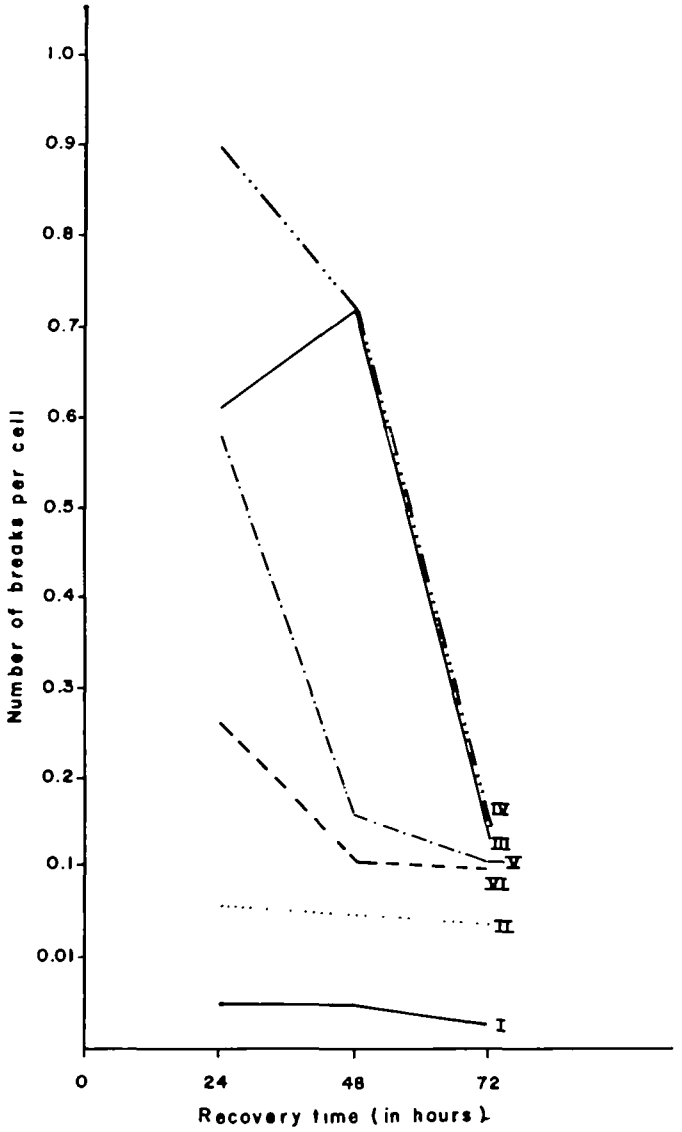

Fig. 1. Reduction in the yield of chromatid breaks by treatment with a low dose of EMS prior to treatment with a high dose. Curve I: controls (0.7% NaCl); curve II: 80 mg/kg body wt EMS (conditioning); curve III: 240 mg/kg body wt EMS (challenging); curve IV: 80−2hTL−240 (data from Riaz Mahmood and Vasudev, 1991); curve V: 80−5hTL−240; curve VI: 80−10hTL−240. See notes to Table I.

as has already been shown by *in vitro* studies by several authors (Olivieri *et al.*, 1984; Sanderson and Moreley, 1986; Wolff *et al.*, 1988; Vijayalaxmi and Burkart, 1989; Sankaranarayanan *et al.*, 1989).

Shadley *et al.* (1987) suggested that the response ceases after the third mitosis of adapted cells, due to a dilution of the repair system as the cells divide over subsequent cell cycles. Similarly, in the present studies at 72 h RT, i.e. in third and subsequent mitoses, the reduction in frequency of chromatid aberrations is very minimal (Table III). Another point to note is that the lowest aberration frequency was observed at 72 h RT compared with 24 and 48 h RTs (Tables I−III). This may be due to the presence of third and subsequent mitoses after the treatment. This agrees with earlier reports where it has been amply proved that the decrease in aberration frequency with increasing culture time reflects a mechanism of mitotic selection of aberration-bearing cells (Obe and Beek, 1982). The results of the present investigations, together with previous studies of Tuschl *et al.* (1980) Wojcik and Tuschl (1990), Rieger *et al.* (1984) and Youngblom *et al.*, (1989), indicate that the factors involved in the adaptive response may be very complex in eukaryotic systems.

Studies on plants and human lymphocytes *in vitro* have clearly revealed that clastogenic adaptation depends on unimpaired protein synthesis (Rieger *et al.*, 1984; Youngblom *et al.*, 1989) and on the metabolic state of the cells (Nicoloff *et al.*, 1985). These results have been taken by these authors to be indicative of inducible protective functions (possible repair activities). Even though the adaptive repair system in bacteria is well known, the situation as to the existence of such a mechanism in mammalian cells is not yet clear. Furthermore, underlying mechanisms of clastogenic adaptation in mammalian *in vivo* systems are presently unknown.

In conclusion, it is clear from the results that *in vivo* pretreatment of mouse cells with a low dose of EMS causes an increase in the cellular repair capacity in an ascending order up to 10 h and manifests itself as lower chromatid aberrations compared with non-adapted mice. Evidence is presented to show that phenomenon of adaptive response can also be encountered in *in vivo* mammalian systems.

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