Inducible protective processes in animal systems: VIII. Enhancement of adaptive response by nicotinamide

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The molecular mechanism of the adaptive response or inducible DNA repair process has not been clearly demonstrated in eukaryotic systems. The involvement of poly(ADP-ribose) polymerase (PARP), a DNA repair enzyme has been reported in the adaptive response (Shadley and Wolff, 1987; Wiencke, 1987). Hence, the present studies were undertaken to understand the role of PARP in ethyl methanesulfonate (EMS)-induced adaptive response in mouse bone marrow cells by employing the inhibitor of this enzyme, nicotinamide. Inter-, pre- and post-treatments of nicotinamide with EMS were made. The results have revealed that there is a reduction in the frequencies of chromosomal aberrations compared with combined or challenge treatment at the different recovery times tested. These results are discussed with reference to the enhancement of the adaptive response by nicotinamide in mouse bone marrow cells.

Introduction

The adaptive DNA repair process or inducible DNA repair pathway is a novel type of repair pathway among the several repair mechanisms known to date, wherein the cells pre-exposed to a low dose of a clastogen are more resistant to the damaging effects of a challenge dose of the same agent. This phenomenon, termed 'the adaptive response', was first demonstrated by Samson and Cairns (1977) in *Escherichia coli*. Extensive reports are available on the existence of the adaptive response in prokaryotes and in *in vitro* eukaryotes using physical agents like X-rays, γ-rays (Olivieri *et al.*, 1984; Shadely and Wolff, 1987; Sankaranarayanan *et al.*, 1989; Liazen Zhang, 1995; Ikushima *et al.*, 1996; Wolff, 1996; Lankinen and Vilpo, 1997; Nikolai *et al.*, 1998) and chemicals such as, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), methylnitrosourea (MNU), ethylnitrosourea (ENU) and mitomycin C (Samson and Schwartz, 1980; Kaina, 1982; Olivieri and Bosi, 1990; Mudrigal-Bujaidar *et al.*, 1994; Kleczkowska and Althaus, 1996; Nikolova and Huttner, 1996).The same phenomenon was reported in the cells of higher plants by using alkylating and non-alkylating agents (Rieger *et al.*, 1982, 1990; Baranczewski *et al.*, 1997). We demonstrated the existence of adaptive response in grasshopper and mouse *in vivo* (Riaz Mahmood and Vasudev, 1990–1993; Riaz Mahmood *et al.*, 1996; Vasudev *et al.*, 1997) and also *in vitro* in human lymphocytes (Harish *et al.*, 1998). Although there are considerable data on the adaptive response, the molecular mechanism remains unclear. Different repair enzymes are implicated in this repair pathway. Poly(ADP-ribose) poly-

merase (PARP) is one such repair enzyme that has been reported to participate in DNA repair processes. PARP is a nuclear enzyme activated by DNA strand breaks induced by alkylating agents or X-rays (Cleaver *et al.*, 1983; Chatterjee and Berger, 1994; Kleczkowska and Althaus, 1996). PARP upon activation catalyses of poly(ADP-ribosyl)ation of the various nuclear proteins and also that of PARP by utilizing NAD+ as substrate. Furthermore, the adaptive response was prevented *in vitro*, when inhibitors of PARP were administered 2 h after the adaptive treatment (Wiencke *et al.*, 1986; Shadely and Wolff, 1987; Wiencke, 1987). These reports suggest the involvement of PARP in the adaptive response. Hence, in the present investigations, an attempt has been made using *in vivo* mouse bone marrow cells to understand the role of the PARP in ethyl methanesulfonate (EMS)-induced adaptive response. Nicotinamide, as an inhibitor of PARP (Purnell and Whish, 1980), has enhanced the EMS-induced adaptive response. The involvement of PARP in the adaptive response and the above results are discussed in this paper.

Materials and methods

Animals

Male Swiss albino mice, 6–8 weeks old and weighing 25–30 g were used in the present studies.

Chemicals

The monofunctional alkylating agent, EMS (CAS-62-50-0) and the nicotinamide (N, CAS-98-92-0) were obtained from Sigma Chemical Company (USA). The EMS and nicotinamide were dissolved in 0.7% NaCl and distilled water, respectively, to obtain required concentrations. 0.5 ml of the fixed concentration was injected intraperitoneally. Freshly prepared chemical solutions were used. Two doses of EMS, 80 (conditioning, L) and 240 mg/kg body weight (challenge, H) were selected from the earlier experiments (Riaz Mahmood and Vasudev, 1993). Nicotinamide concentrations ranging from 5 to 50 mM/kg body weight were employed in the initial experiments to evaluate the toxicity (Table I). The results indicated that the lowest dose of 5 mM produces least toxicity when administered with combined treatments and at the same time the mitotic index was equivalent to controls when compared with higher doses. Hence, the dose of 5 mM/kg body weight was selected.

Treatment schedule

EMS combined treatment

This was again selected from the previous experiments of Riaz Mahmood and Vasudev (1993), who have shown that the 8 h time lag (TL) between the conditioning and challenging treatment offered maximum protection with respect to the chromosomal aberrations in mouse bone marrow cells; thus exhibiting a peak of repair activity compared with other TLs. Therefore, the 8 h TL was selected for the present studies.

Nicotinamide inter-treatment

Nicotinamide inter-treatment was made during the period between the conditioning and challenging doses. As the 8 h TL was found to be at peak action or repair (Riaz Mahmood and Vasudev, 1993), this TL was used. Nicotinamide was injected 2 or 4 h after the conditioning dose. Then after 6 or 4 h they were challenged with the challenge dose of EMS.

Nicotinamide pretreatment

In this treatment schedule, animals received nicotinamide, 4 or 6 h prior to the conditioning dose of EMS and 8 h later they were challenged with the EMS high dose.

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Table I. Toxicity of nicotinamide to mouse

Nicotinamide (mM/kg body weight)	% Lethality	% Mitotic index	% Lethality when given with combined treatment (L+N+H)	% Mitotic index when given with combined treatment
50	90	1.0	100	0.5
25	20	2.0	50	1.5
15	10	2.5	25	2.0
10	Nil	2.5	Nil	2.0
7.5	Nil	3.8	Nil	3.0
5	Nil	6.7	Nil	6.5

Table II. Frequency of chromosomal aberrations observed after inter-, pre- and post-treatment with nicotinamide in EMS treated mouse bone marrow cells at 24 h recovery time

Treatments	Series no.	No. aberrant cells	B′	B″	RB′	RB′B″	ID	Rings	Minutes	Total no. breaks	Breaks/cell
Control	1 A	8	8	–	–	–	–	–	–	8	0.01 ± 0.01
	B	6	6	–	–	–	–	–	–	6	0.01 ± 0.01
Mean ± SE			7 ± 0.3	–	–	–	–	–	–	7 ± 0.3	0.01 ± 0.01
Nicotinamide	2 A	10	9	–	–	–	–	–	1	10	0.2 ± 0.01
(N)	B	9	9	–	–	–	–	–	–	9	0.01 ± 0.01
Mean ± SE			9 ± 0.0	–	–	–	–	–	0.5 ± 0.1	9.5 ± 0.3	0.02 ± 0.01
EMS-low	3 A	60	48	9	1	–	–	–	13	81	0.14
(L)	B	55	42	5	1	–	–	–	16	70	0.12
Mean ± SE			45 ± 2.1	7.0 ± 1.4	1 ± 0.0	–	–	–	14.5 ± 1.0	75.5 ± 3.9	0.13 ± 0.01[a]
EMS–high	4 A	342	236	30	34	3	3	–	78	457	0.76
(H)	B	360	244	28	27	1	5	1	85	454	0.75
Mean ± SE			240 ± 2.8	29 ± 0.7	30.5 ± 2.4	2 ± 0.7	4 ± 0.7	0.5 ± 0.3	81.5 ± 2.4	455.5 ± 1.0	0.76 ± 0.01[a]
Combined	5 A	230	165	24	17	0	4	2	34	293	0.49
L–8 h–H	B	242	173	17	13	1	3	0	45	287	0.48
Mean ± SE			169 ± 2.8	20.5 ± 2.4	15 ± 1.4	0.5 ± 0.3	3.5 ± 0.3	1 ± 0.3	39.5 ± 3.9	290 ± 2.1	0.49 ± 0.01[b]
Inter-treatment	6 A	165	122	7	10	–	7	1	47	219	0.36
L–2 h–N–6 h–H	B	153	108	9	12	–	5	1	38	200	0.35
Mean ± SE			115 ± 4.9	8 ± 0.7	11 ± 0.7	–	6 ± 0.7	1 ± 0.0	42.5 ± 3.1	209.5 ± 6.4	0.35 ± 0.01[c]
L–4 h–N–4 h–H	7 A	156	99	4	10	–	3	–	40	173	0.29
	B	148	108	5	9	–	4	–	39	183	0.31
Mean ± SE			103.5 ± 3.1	4.5 ± 0.3	9.5 ± 0.3	–	3.5 ± 0.3	–	39.5 ± 0.3	178 ± 3.5	0.30 ± 0.01[c]
Pretreatment	8 A	170	141	6	5	–	–	1	42	207	0.35
N–6 h–L–8 h–H	B	162	130	6	8	–	–	2	49	211	0.35
Mean ± SE			35.5 ± 3.9	6 ± 0.0	6.5 ± 1.1	–	–	1.5 ± 0.3	45.5 ± 2.8	209 ± 1.4	0.35 ± 0.00[c]
N–4 h–L–8 h–H	9 A	143	122	6	5	–	1	1	37	185	0.31
	B	155	134	5	2	–	0	0	33	181	0.30
Mean ± SE			128 ± 4.2	5.5 ± 0.3	3.5 ± 1.0	–	0.5 ± 0.3	0.5 ± 0.3	35 ± 1.4	183 ± 1.4	0.31 ± 0.01[c]
Post-treatment	10 A	215	149	22	16	–	3	–	47	279	0.47
L–8 h–H–6 h–N	B	228	161	14	15	–	3	–	43	268	0.45
Mean ± SE			155 ± 4.2	18 ± 2.8	15.5 ± 0.3	–	3 ± 0.0	–	45 ± 1.4	273.5 ± 3.9	0.46 ± 0.01[b]
L–8 h–H–12 h–N	11 A	162	120	8	5	–	2	–	48	198	0.33
	B	148	131	4	4	–	2	–	35	192	0.32
Mean ± SE			125.5 ± 3.9	6 ± 1.4	4.5 ± 0.3	–	2 ± 0.0	–	41.5 ± 4.6	195 ± 2.1	0.33 ± 0.01[c]
L–8 h–H–18 h–N	12 A	128	123	3	3	–	2	–	27	166	0.28
	B	136	108	4	2	–	1	–	38	160	0.27
Mean ± SE			115.5 ± 5.3	3.5 ± 0.3	2.5 ± 0.3	–	1.5 ± 0.3	–	32.5 ± 3.9	163 ± 2.1	0.27 ± 0.01[c]

Data of two independent experiments; 600 cells were scored per experiment; h, hours, B′, chromatid break; B″, isochromatid break; RB′, chromatid exchanges; RB′B″, triradials; ID, intrachromatid deletion;
[a]Significant compared with controls ($P < 0.05$).
[b]Significant compared with challenge dose ($P < 0.05$).
[c]Significant compared with combined treatment ($P < 0.05$).

Nicotinamide post-treatment
Nicotinamide was given 6, 12 or 18 h after the combined treatment of EMS.

Slide preparation and chromosome analysis
Animals were killed by cervical dislocation at 24, 48 or 72 h recovery times (RTs) after the challenge dose. 0.5 ml of the 0.05% colchicine was injected into the animals 90 min prior to sacrifice. After the animals had been killed, the bone marrow was processed and slides were prepared by the routine air-dry technique (Evans *et al.*, 1964). In brief, the femur bones were dissected out and cleaned. Then the bone marrow was flushed into 0.56% potassium chloride (hypotonic) solution with the help of 26 gauge needle attached to a 2 ml syringe. The suspension was incubated at 37°C for 30 min. After incubation, the cell suspension was centrifuged at 800 r.p.m. for 7 min. The supernatant was discarded. Then the fixative, methanol/acetic acid (3:1 v/v), was added to the pellet and mixed well. This suspension was centrifuged after 10 min. After fixing the cells three times, the pellet was resuspended in

Table III. Frequency of chromosomal aberrations observed after inter-, pre-, and post-treatment with nicotinamide in EMS-treated mouse bone marrow cells at 48 h recovery time

Treatments	Series no.	No. aberrant cells	Chromosomal aberrations		Total no. breaks	Breaks/cell
			No. chromatid aberrations	No. chromosome aberrations		
Control	1 A	8	8	–	8	0.01
	B	10	10	–	10	0.02
Mean ± SE			9 ± 0.7		9 ± 0.7	0.02 ± 0.01
Nicotinamide	2 A	14	14	–	14	0.02
(N)	B	16	16		16	0.03
Mean ± SE			15 ± 0.7		15 ± 0.7	0.02 ± 0.01
EMS-low	3 A	64	70	5	80	0.13
(L)	B	76	75	2	79	0.13
Mean ± SE			72.5 ± 1.8	3.5 ± 1.1	79.5 ± 0.3	0.13 ± 0.00[a]
EMS-high	4 A	325	364	26	418	0.69
(H)	B	337	374	33	436	0.72
Mean ± SE			366 ± 1.4	29.5 ± 2.5	427 ± 6.3	0.70 ± 0.01[a]
Combined treatment	5 A	183	237	20	277	0.46
L–8 h–H	B	208	250	15	280	0.47
Mean ± SE			243.5 ± 4.6	17.5 ± 1.8	278.5 ± 1.1	0.46 ± 0.01[b]
Inter-treatment	6 A	120	136	13	162	0.27
L–2 h–N–6 h–H	B	115	122	11	144	0.24
Mean ± SE			129 ± 4.9	12 ± 0.3	153 ± 6.3	0.25 ± 0.01[c]
L–4 h–N–4 h–H	7 A	102	130	13	156	0.26
	B	96	121	17	155	0.26
Mean ± SE			125.5 ± 3.2	15 ± 1.4	155.5 ± 0.3	0.26 ± 0.00[c]
Pretreatment	8 A	124	148	14	176	0.30
N–6 h–L–8 h–H	B	138	167	12	191	0.31
Mean ± SE			157.5 ± 6.7	13 ± 0.7	183.5 ± 5.3	0.31 ± 0.01[c]
N–4 h–L–8 h–H	9 A	83	104	11	126	0.21
	B	76	87	10	107	0.17
Mean ± SE			95.5 ± 6.0	10.5 ± 0.3	116.5 ± 6.7	0.19 ± 0.01[c]
Post-treatment	10 A	168	216	19	254	0.42
L–8 h–H–6 h–N	B	184	226	15	256	0.42
Mean ± SE			221 ± 3.5	17 ± 1.4	255 ± 0.7	0.42 ± 0.00[b]
L–8 h–H–12 h–N	11 A	89	138	18	174	0.29
	B	100	124	19	162	0.27
Mean ± SE			131 ± 4.9	18.5 ± 0.3	168 ± 4.2	0.28 ± 0.01[c]
L–8 h–H–18 h–N	12 A	69	101	10	121	0.20
	B	81	110	13	136	0.22
Mean ± SE			105.5 ± 3.2	11.5 ± 1.0	128.5 ± 5.3	0.21 ± 0.01[c]

[a,b,c]As in Table II.

a 0.5 ml fixative. This suspension was dropped onto clean, non-greasy and pre-chilled slides and air dried. Coded Giemsa-stained slides were screened for the presence of chromosome aberrations, i.e. chromatid breaks, exchanges, intrachromatid deletions, triradials and minutes. A minimum of two experiments were conducted using three animals in each sample. The results were subjected to statistical analysis by employing the one tailed Student's t-test.

Results

The data obtained from a minimum of two independent experiments are given in Tables II–IV and their mean values are also incorporated. There were no significant variations in the frequencies of chromosomal aberrations between the two experiments ($P > 0.05$). Chromosomal aberrations were induced after conditioning, challenging and combined treatments of EMS after 24, 48 and 72 h RTs and their frequencies are presented in Tables II–IV. These results show that the EMS induced a very high frequency of chromatid breaks, exchanges, intrachromatid deletions and minutes at all RTs. The minutes are of chromatid-type not chromosomal-type, i.e. double minutes. The conditioning dose induced 13–15% aberrations, whereas the challenging dose produced 70–75% aberrations, which is significantly higher compared with controls (Tables II–IV and Figures 1–3). The combined treatment (conditioning-

8 h TL-challenging) yielded significantly less frequencies of chromosomal aberrations compared with the respective challenge treatments at different RTs tested. These observations are compatible with the earlier experiments of the authors (Riaz Mahmood and Vasudev, 1990–1993; Riaz Mahmood *et al.*, 1996; Vasudev *et al.*, 1997). The results of inter- and pretreatments of nicotinamide have revealed a significant reduction in the frequency of chromosomal aberrations ($P < 0.05$) compared with the combined or challenge dose (Tables II–IV and Figures 1 and 2). Nicotinamide alone induced insignificant chromosomal aberration frequencies as compared with controls. On the other hand, in the post-treatment at all time pauses, the aberration frequency was significantly reduced ($P < 0.05$) compared with the challenge dose, the frequency of aberrations was almost equal to the combined treatment at the 6 h time-pause. However, it is interesting to note that, at 12 or 18 h post-treatment, the reduction of chromosomal aberrations is significant compared with the combined treatment (Tables II–IV and Figure 3). Similarly, in accordance with the reduction of the frequency of chromosomal aberrations, the aberrant cell frequency was also reduced in the combined and other treatment schedules (Tables II–IV). Mitotic indices

Table IV. Frequency of chromosomal aberrations observed after inter-, pre- and post-treatment with nicotinamide in EMS-treated mouse bone marrow cells at 72 h recovery time

Treatments	Series no.	No. aberrant cells	Chromosomal aberrations		Total no. breaks	Breaks/cell
			No. chromatid aberrations	No. chromosome aberrations		
Control	1 A	9	9	–	9	0.01
	B	7	7	–	7	0.01
Mean ± SE			8 ± 0.7		8 ± 0.7	0.01 ± 0.00
Nicotinamide	2 A	10	10	–	10	0.02
(N)	B	12	14	–	14	0.02
Mean ± SE			12 ± 1.4		12 ± 1.4	0.02 ± 0.01
EMS-low	3 A	58	58	9	76	0.13
(L)	B	67	65	8	81	0.14
Mean ± SE			61.5 ± 2.5	8.5 ± 0.3	78.5 ± 1.8	0.13 ± 0.01[a]
EMS-high	4 A	206	290	26	342	0.56
(H)	B	190	305	21	347	0.57
Mean ± SE			297.5 ± 5.3	23.5 ± 1.8	344.5 ± 1.8	0.57 ± 0.01[a]
Combined treatment	5 A	160	156	28	212	0.36
L–8 h–H	B	158	170	22	214	0.37
Mean ± SE			163 ± 4.9	25 ± 2.1	213 ± 0.7	0.36 ± 0.01[b]
Inter-treatment	6 A	89	91	17	125	0.21
L–2 h–N–6 h–H	B	72	93	11	115	0.19
Mean ± SE			92 ± 0.7	14 ± 2.1	120 ± 3.5	0.20 ± 0.01[c]
L–4 h–N–4 h–H	7 A	73	80	10	100	0.17
	B	84	92	8	108	0.18
Mean ± SE			86 ± 4.2	9 ± 0.7	104 ± 2.8	0.17 ± 0.00[c]
Pretreatment	8 A	94	120	10	140	0.23
N–6 h–L–8 h–H	B	85	105	10	125	0.21
Mean ± SE			112.5 ± 5.3	10 ± 0.0	132.5 ± 5.3	0.22 ± 0.01[c]
N–4 h–L–8 h–H	9 A	60	83	7	97	0.16
	B	54	70	17	104	0.17
Mean ± SE			76.5 ± 4.6	12 ± 3.5	100.5 ± 1.8	0.16 ± 0.01[c]
Post-treatment	10 A	171	190	20	230	0.38
L–8 h–H–6 h–N	B	158	183	17	216	0.36
Mean ± SE			186.5 ± 2.48	18.5 ± 1.1	223 ± 4.9	0.37 ± 0.01[b]
L–8 h–H–12 h–N	11 A	95	128	9	146	0.24
	B	110	110	12	134	0.22
Mean ± SE			119 ± 6.3	10.5 ± 1.1	140 ± 2.8	0.23 ± 0.01[c]
L–8 h–H–18 h–N	12 A	63	68	8	84	0.14
	B	71	80	12	104	0.17
Mean ± SE			74 ± 4.2	10 ± 1.4	94 ± 6.0	0.16 ± 0.01[c]

[a,b,c]As in Table II.
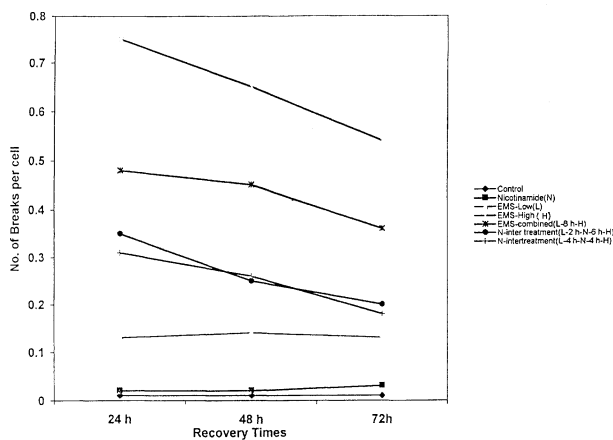
Fig. 1. Reduction in the yield of chromosomal aberrations by inter-treatment of nicotinamide EMS adapted mouse bone marrow cells.
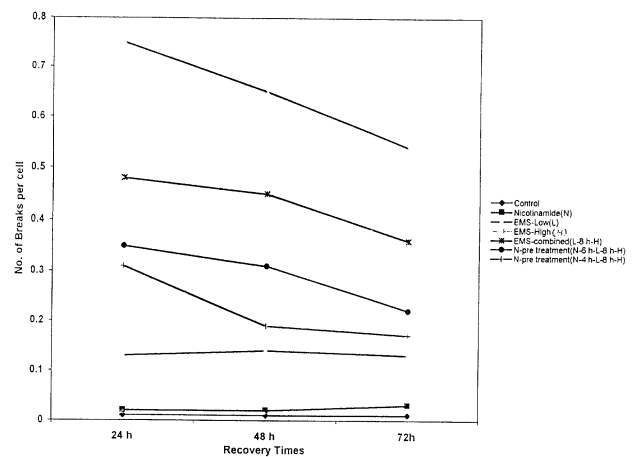
Fig. 2. Reduction in the yield of chromosomal aberrations by pre-treatment of nicotinamide EMS adapted mouse bone marrow cells.

recorded show that the pre-, post- and inter-treatments of nicotinamide to the EMS-treated cells did not prolong the cell cycle (Table V).

Discussion

The results of our present investigations clearly demonstrated the induction of chromosomal aberrations by EMS, which are
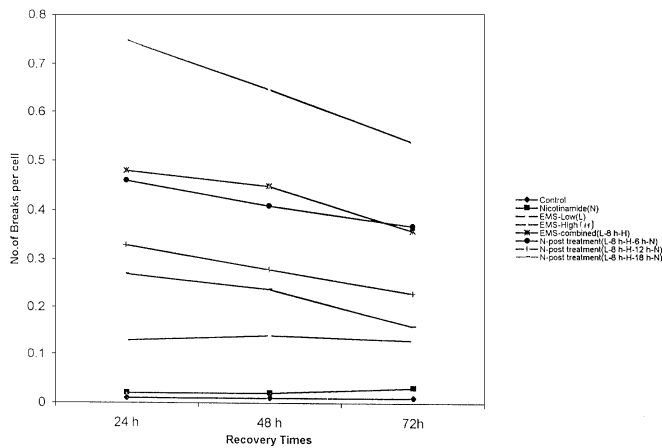
Fig. 3. Reduction in the yield of chromosomal aberrations by post-treatment of nicotinamide EMS adapted mouse bone marrow cells.

Table V. Mitotic index in the bone marrow cells of the control and treated mice at different recovery times

Treatments	Percentage of mitotic index		
	24 h	48 h	72 h
Control	7.1	6.8	6.5
Nicotinamide (N)	7.1	6.6	6.7
EMS-low (L)	6.5	6.8	6.8
EMS-high (H)	3.8	3.9	4.2
Combined treatment			
L–8 h–H	4.6	4.9	5.1
Inter-treatments			
L–2 h–N–6 h–H	6.5	6.8	6.6
L–4 h–N–4 h–H	6.8	6.9	7.1
Pretreatments			
N–6 h–L–8 h–H	7.1	6.9	6.7
N–4 h–L–8 h–H	7.2	6.8	7.1
Post-treatments			
L–8 h–H–6 h–N	5.4	5.1	4.9
L–8 h–H–12 h–N	6.9	7.2	7.2
L–8 h–H–18 h–N	7.3	7.1	6.6

Data derived from 5000 cells scored for each treatment.

mainly the chromatid-type of aberrations produced in the bone marrow cells of the mouse. This is in line with earlier observations, wherein the mutagenic and clastogenic effects of EMS were observed (Riaz Mahmood and Vasudev, 1990–1994; Riaz Mahmood *et al.,* 1996; compare with Vogel and Natarajan, 1982). Present results also point to the presence of adaptive response induced by EMS (Tables II–IV and Figures 1–3), which is consistent with the previous reports on adaptive response induced by chemicals (Samson and Schwartz, 1980; Kaina, 1982; Olivieri and Bosi, 1990; Riaz Mahmood and Vasudev, 1990–1993; Mudrigal-Bujaidar *et al.*, 1994; Kleczkowska and Althaus, 1996; Nikolova and Huttner, 1996; Riaz Mahmood *et al.*, 1996; Vasudev *et al.*, 1997; Harish *et al.*, 1998, 2000). Results obtained in the present investigations with 8 h TL between the conditioning and challenging were similar to the earlier observations of Riaz Mahmood and Vasudev (1993).

Wiencke (1987) who worked on the influence of PARP inhibitors on the adaptive response in *in vitro* human lymphocytes proposed that 'ADPRT, itself and not other metabolic processes affected by inhibitors of this enzyme, plays an important role in the adaptive response'. Keeping this in mind,

the present investigations were undertaken using nicotinamide as an inhibitor of PARP. The results of inter-treatment of nicotinamide (L–2 h–N–6 h–H and L–4 h–N–4 h–H) have revealed that the frequency of chromosomal aberrations has been significantly reduced compared with the combined treatment (Tables II–IV and Figure 1; $P < 0.05$). This indicates that the nicotinamide potentiates the EMS-induced adaptive response in the mouse bone marrow cells. In the post-treatment, as there is a significant reduction in the frequency of chromosomal aberrations at 12 or 18 h, it is proposed that nicotinamide protects the genetic system after 6 h of challenge treatment. This long duration in the activity of nicotinamide is because of the fact that the high dose of the mutagen might have disturbed the genetic machinery to release the required enzyme(s) and to repair the damage. The pre- and inter-treatment results show similarities in the way of reduction of chromosome aberrations. This may be due to the same amount of enzyme(s) released by the two different treatment schedules. This needs to be analyzed further. From the pretreatment experiments, the authors are of the opinion that nicotinamide acts as a cross-adapter by reducing the frequency of chromosomal aberrations compared with the combined treatment (Tables II–IV and Figure 2). These results are similar to that observed in the *in vivo* system of *Poecilocerus pictus*, an insect system, where the authors have demonstrated the potentiation of adaptive response by nicotinamide (Vasudev *et al.*, 1999; Guruprasad *et al.*, 2000). To our knowledge, these are the first reports in this direction in *in vivo* animal systems. Similarly, in the *in vitro* system of human lymphocytes, Wiencke (1987) has reported the enhancement of X-ray-induced adaptive response by nicotinamide. From the above data, it is clear that nicotinamide, an inhibitor of PARP has enhanced the adaptive response. In other words, it can be opined that PARP is not involved in the adaptive response. Furthermore, there are reports to show that the cell extracts depleted of PARP (Rhun *et al.*, 1998) or PARP knockout mice (Wang *et al.*, 1995) have the potency in DNA repair. Absence of PARP did not prevent DNA repair in *in vitro* cells (Ding *et al.*, 1992). In support of these observations, Melissa *et al.* (1998) have demonstrated that there is a synthesis of poly(ADP-ribose) polymers in PARP$^{-/-}$ cells in a damage-dependent manner. This indicates the involvement of different mechanism(s) for the synthesis of poly(ADP-ribose) polymers in DNA repair. Caria *et al.* (1997) have demonstrated that there is an alternative repair pathway in the absence of PARP in *in vitro* human lymphocytes of the Down syndromes. Contrary to these, inhibitors of PARP increased the incidence of chromosomal aberrations, SCEs (Wiencke *et al.*, 1986; Catena *et al.*, 1994; Kupper *et al.*, 1995; Schreiber *et al.*, 1995) and suppressed the adaptive response when applied during 2 h after the adaptive treatment (Wiencke *et al.*, 1986; Shadley and Wolff, 1987). PARP involvement in the DNA repair process was also reported in various cell types (Park and Kim, 1983; Cleaver *et al.*, 1985; Cleaver and Morgan, 1991; Shall, 1994).

In the present studies, cytotoxicity of chemicals has been analyzed using the mitotic index of treated cells. The results of the mitotic index have shown that nicotinamide has no effect on the cell cycle and in turn enhance the mitotic divisions (Table V). This may be due to the action of nicotinamide in preserving NAD$^+$ levels in the cells. In line with this, the nicotinamide and other inhibitors of PARP are reported to prevent depletion of NAD$^+$ (de Murcia and Menisser de Murcia, 1994; Lindahl *et al.*, 1995) and protect the cells from

Table VI. Comparative data showing reduction in the yield of chromosomal aberrations after inter-, pre- and post-treatment of nicotinamide in EMS-treated mouse bone marrow cells at all recovery times.

Recovery times (h)	Frequency of chromosomal aberrations (no. breaks/cell)			
	Combined treatments	Pretreatments	Inter-treatments	Post-treatments
24	0.49 ± 0.01 (5)	0.35 ± 0.00 (8)	0.35 ± 0.01 (6)	0.46 ± 0.01 (10)
		0.31 ± 0.01 (9)	0.30 ± 0.01 (7)	0.33 ± 0.01 (11)
				0.27 ± 0.01 (12)
48	0.46 ± 0.01 (5)	0.31 ± 0.01 (8)	0.25 ± 0.01 (6)	0.42 ± 0.00 (10)
		0.19 ± 0.01 (9)	0.26 ± 0.00 (7)	0.28 ± 0.01 (11)
				0.21 ± 0.01 (12)
72	0.36 ± 0.01 (5)	0.22 ± 0.01 (8)	0.20 ± 0.01 (6)	0.37 ± 0.01 (10)
		0.16 ± 0.01 (9)	0.17 ± 0.00 (7)	0.23 ± 0.01(11)
				0.16 ± 0.01 (12)

Series numbers in parentheses: for details refer to Table II.

cytotoxic effects of various chemicals and drugs (Cosi *et al.*, 1994, 1996; Cosi and Marien, 1998; Chatterjee *et al.*, 1999; Kolb and Burkart, 1999).

In conclusion, our results have clearly indicated the potentiation of the EMS-induced adaptive response by nicotinamide in the mouse bone marrow cells (Table VI). With the data available it is not possible to highlight the actual role of PARP in the adaptive response. Further studies are required to decipher the molecular mechanism of adaptive response and the involvement of PARP in adaptive response.

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