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Variability in cytogenetic adaptive response of cultured human lymphocytes to mitomycin C, bleomycin, quinacrine dihydrochloride, Co^{60} γ-rays and hyperthermia

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Adaptive response (AR) is a well-documented phenomenon by which cells or organisms exposed to low dose of a genotoxicant become less sensitive to subsequent high-dose exposure to the same or another genotoxicant. AR, if induced can modify the efficacy leading to drug or radio-resistance, during anti-neoplastic drug or radiation treatment. Contradictions exist in AR induction by different genotoxicants with respect to the biomarkers, time schedules, and inter-individual variability, reflecting the complexity of AR in eukaryotic cells. In order to further ascertain these factors, AR induced by anti-neoplastic agents mitomycin C (MMC), bleomycin (BLM) and chemosterilant quinacrine dihydrochloride was examined in different donors and time schedules using cytogenetic biomarkers chromosome aberrations, sister chromatid exchanges and micronuclei (MN). BLM- and hyperthermia (HT)-induced cross-resistance to gamma rays and MMC/BLM, respectively, was also studied. Difference between MMC- and BLM-induced protective effects in biomarkers examined in the same donors was noticed. Adaptation to BLM and HT showed cross-resistance to chromosome damage induction by gamma rays and BLM/MMC, respectively. Cell cycle analysis indicated that adaptation is not caused by change in the rate of cell proliferation after challenge dose. MN as a chromosomal biomarker in large-scale population studies on AR is advocated, based on similar AR induced in all donors by MMC/BLM and rapid assessment in binucleated cells. Influence of certain genotypes on chromosomal biomarkers used in AR studies and role of AR in radiation and chemotherapy need to be further deciphered.

Introduction

The adaptive response (AR) is a well-documented phenomenon by which cells or organisms, exposed to low doses of a genotoxicant become less sensitive to a subsequent high-dose exposure to the same or another genotoxicant. AR has been assessed using cytogenetic biomarkers [chromosome aberration (CA), micronuclei (MN), sister chromatid exchange (SCE)] and probably involves different mechanisms, including an enhanced DNA repair process via signal transduction (1–4). AR as a whole may be an important general biological mechanism to maintain genetic integrity and prevent carcinogenic initiation of cells. AR induced when exposure is spread over a period of time is relevant in understanding the risk associated with environmental and occupational radiation exposures (5). When treatment with anti-neoplastic drugs is pursued over a long period, depending on the doses employed, AR, if induced in the cells and tissues involved, can modify the efficacy of the treatment leading to drug or radio-resistance. Since DNA repair processes are temperature dependent, hyperthermia (HT) can modulate sensitivity of normal and cancer cells including the drug-resistant ones to anti-cancer drugs (6). Contradictions exist in AR induction by different genotoxicants with respect to the biomarkers, time schedules and inter-individual variability reflecting the complexity of AR in eukaryotic cells. In order to further ascertain these factors, AR induced by anti-neoplastic agents mitomycin C (MMC), bleomycin (BLM) and chemosterilant quinacrine dihydrochloride (QDH) as well as BLM- and HT-induced cross-resistance to Co^{60} γ-rays (Cγ) and MMC, BLM, respectively, were examined in different donors and time schedules. Cytotoxicity of bifuncional alkylating agents like MMC that are proliferation dependent but cell cycle phase non-specific correlates very closely with inter-strand cross-linkage of DNA. MMC is known to be a potent inducer of SCE, but do not produce chromosome-type aberrations in the first metaphases after exposure. BLM is considered to be a radiomimetic chemical because like ionizing radiation, it is clastogenic at all stages of the cell cycle, and its clastogenicity does not depend on passage of the chemical lesions through replication. BLM is an effective inducer of CA producing both chromosome- and chromatid-type aberrations and MN, but a poor inducer of SCE in human lymphocytes. MMC- and BLM- induced AR was assessed in the same donors by SCE, MN and CA as biomarkers.

Materials and methods

The AR, if any, induced in human lymphocytes *in vitro* was analysed (1) in six donors (male, age 24–33 years), by an adaptive dose (AD) of MMC 0.001 μg/ml or BLM 0.001 units/ml, at 26 h, subsequent exposure to challenge dose (CD) of MMC 0.1 μg/ml or BLM 0.01 units/ml at 48 h, using three different cytogenetic biomarkers (SCE and MN for MMC, CA and MN for BLM); (2) in two donors (male 24 years, female 25 years), after an AD of BLM at 48 h and CD at 69 h; (3) in three donors (one female 25 years, two males 24–33 years) by an AD of QDH 0.006 μg/ml at 24 or 48 h followed by CD of 0.6 μg/ml at 48 or 69 h on CA and MN; (4) in two donors (male, 25–30 years), by an AD of BLM at 24 h and CD of 1 Gy Cγ or BLM at 48 h on CA and MN; (5) in two donors (female 25 years, male 33 years), HT pretreatment at 26 h (41°C, 1 h) and CD of BLM or MMC at 48 h on MN. MMC, bleomycin sulfate and QDH (Sigma, St Louis, MO, USA) stock solutions prepared in sterile triple distilled water were used in these experiments. The details of the protocol followed, agents used, time schemes employed and biomarkers analysed are summarized in Table I.

Lymphocyte cultures, chromosome preparations, CBMN assay

Heparinized blood samples were obtained with informed consent and according to institutional procedures from healthy donors (all non-smokers), without any

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Table I. Details of the protocol followed: adaptive and challenging agents, dose, treatment schedule and biomarkers analysed

Donors	Adaptive agent: dose	Challenging agent: dose	Treatment schedule		Time between AD & CD	Biomarkers
			AD	CD		
6	MMC: 0.001 μg/ml	MMC: 0.1 μg/ml	26	48	22 h	SCE & MN
6	BLM: 0.001 units/ml	BLM: 0.01 units/ml	26	48	22 h	CA & MN
2	BLM: 0.001 units/ml	BLM: 0.01 units/ml	48	69	21 h	CA & MN
2	BLM: 0.001 units/ml	BLM: 0.01 units/ml	24	48	24 h	CA & MN
3	QDH: 0.006 μg/ml	QDH: 0.6 μg/ml	24	48	24 h	CA & MN
3	QDH: 0.006 μg/ml	QDH: 0.6 μg/ml	48	69	21 h	CA & MN
2	BLM: 0.001 units/ml	Cγ: 1 Gy	24	48	24 h	CA & MN
2	HT: 41°C, 1 h	MMC: 0.1 μg/ml	26	48	22 h	MN
2	HT: 41°C, 1 h	BLM: 0.01 units/ml	26	48	22 h	MN

MMC, mitomycin C; BLM, bleomycin; QDH, quinacrine dihydrocloride; HT, hyperthermia.

Table II. AR induced by MMC

Donor	C		AD		CD		O		E	I
	SCE	Range	SCE[a,**]	range	SCE	range	SCE[b,c,**]	Range	SCE	
1	3.92	0–12	7.10	2–13	27.96	19–37	20.32	10–29	31.14	34.75
2	5.38	0–13	7.82	3–16	26.66	15–33	18.56	11–27	29.10	36.22
3	3.60	0–10	9.84	5–16	58.52	32–93	48.58	18–90	64.76	24.98
4	6.70	0–17	10.66	3–21	38.46	11–68	29.36	9–50	42.42	30.79
5	4.24	0–09	9.70	4–16	56.74	30–90	44.00	10–30	62.20	29.26
6	4.56	0–10	10.76	5–12	37.40	23–55	28.49	15–58	43.60	34.66
Donor	C		AD		CD		O		E	I
	BNMN	MN	BNMN	MN[a,*]	BNMN	MN	BNMN	MN[b,c,**]	MN	
1	13	14	18	19	61	67	30	32	72	55.56
2	8	9	20	22	39	47	26	29	60	51.66
3	7	7	14	16	38	43	24	25	52	51.92
4	6	6	12	12	40	46	24	24	52	53.85
5	8	9	14	14	52	58	22	24	63	61.9
6	6	6	11	11	34	38	16	20	43	53.49

SCE and MN frequencies observed in human lymphocyte cultures.
C, control; O, observed = AD + CD; E, expected = AD + CD − C; I, inhibition (%); SCE, 50 second-division metaphases; MN, 1000 binucleated cells scored per donor.
Compared to control, increase in SCE ($P < 0.001$) and MN ($P < 0.05$) after adaptive treatment.
Statistically significant ($P < 0.001$) AR in both SCE and MN as biomarkers.
Paired *t*-test.
[a]Compared to control.
[b]Compared to CD.
[c]Compared to E.
*$P < 0.05$; **$P < 0.001$.

history of smoking, tobacco chewing and alcohol consumption and not taking any drugs for medical or other reasons. Phytohaemagglutinin (PHA) stimulated whole blood cultures [Ham's F10 medium, 200 mM L-glutamine, 10% foetal bovine serum, 0.2 ml reconstituted PHA (Sigma), 0.3 ml whole blood per 5 ml of culture volume, no antibiotics], with or without BrdU (10.0 μg/ml, 5-bromo-2′-deoxiuridine, Sigma), for CA, SCE and cytochalasin B (Cyt-B, Sigma) blocked micronuclei (CBMN) analysis were set up (72 h—CA, SCE, MN), following routine procedures (7). Cultures for chromosome analysis were treated with demecolcine (Sigma, 0.02 μg/ml), 3 h before harvest. Air-dried preparations of hypotonically treated (0.075 M KCl), 3:1 methanol–acetic acid (Merck, Mumbai, India)-fixed lymphocytes were made using routine techniques for chromosome analysis and stained with Giemsa (Merck). In the CBMN assay, cytochalasin B, 6 μg/ml, was added to the cultures at 24-h post-initiation as described earlier and harvested at 72 h. Following 0.8% cold KCl treatment (5 min), standard fixation including 1% formaldehyde in the second fixative and slide preparation, cells were stained with Giemsa (Merck).

Fluorescence plus Giemsa staining for SCE and cell cycle analysis

Differential sister-chromatid staining is usually used in cytogenetics for SCE analysis in second-division metaphases. Lymphocytes cultured in the presence of BrdU were stained by a modified fluorescence plus Giemsa (FPG) staining to obtain harlequin chromosomes. Chromosome preparations aged 2 days were stained in Hoechst 33258 (Sigma) 100 μg/ml in distilled water for 20 min. After rinsing in tap water, the slides were mounted in Sorensen's buffer (M/15, pH 8.0 adjusted with 5% NaOH) under a coverslip and exposed to 360-nm light from a Black ray lamp (distance 2 cm 20 J/m^2/sec) for 12 min on a slide warmer at 60°C. Finally, slides rinsed in ice-cold Sorensen's buffer pH 6.8 followed by tap water were stained in 4% Giemsa (Merck) in Sorensen's buffer pH 6.8. Optimum staining time varied between slide and stain batches, but was of the order of about 8 min. Thus, differential staining of chromatids obtained by FPG staining, after BrdU incorporation in PHA-stimulated human lymphocyte cultures, allowed unequivocal identification of metaphase cells which had replicated for one, two and three or more cell cycles (8).

Lymphocyte proliferation kinetics

BrdU labelling of chromosomal DNA also facilitated the analysis of lymphocyte proliferation kinetics. Harlequin staining of BrdU-substituted chromatids by the FPG staining allowed identification of chromosomes in the first/second/third and subsequent divisions. First-division cells—46 chromosomes with both sister chromatids stained uniformly darkly. Second-division cells—46 harlequin chromosomes with one chromatid darkly stained and its sister chromatid lightly stained. Third-division cells—some harlequin

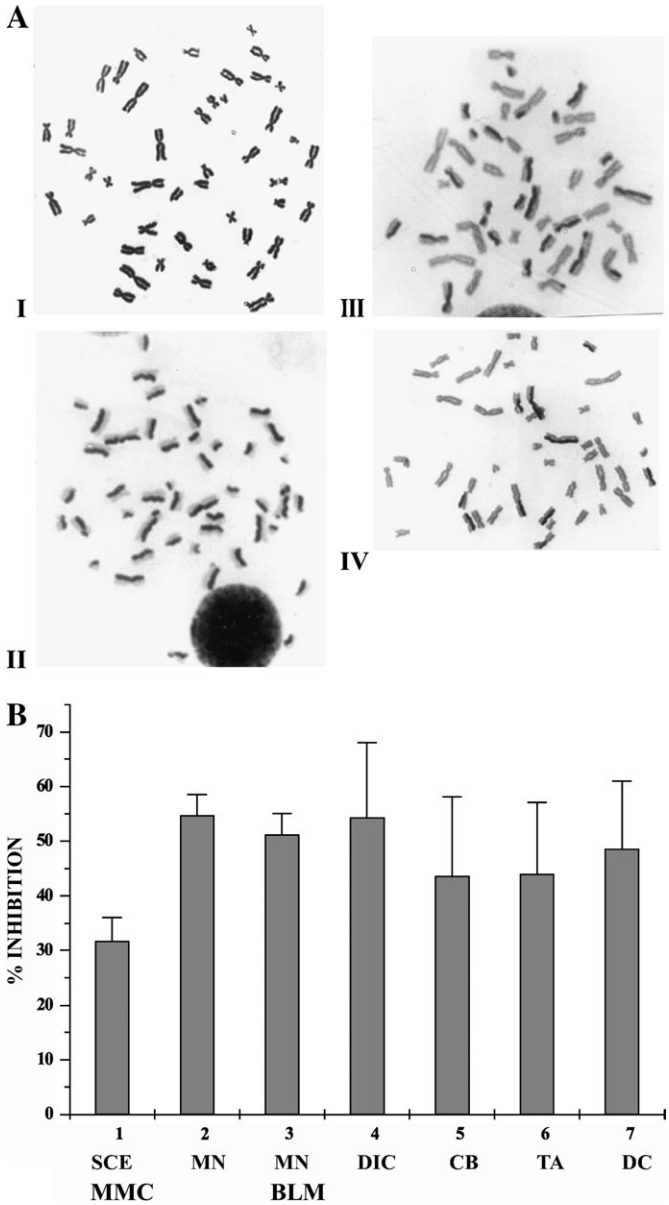

Fig. 1. (**A**) First- to Fourth-division metaphases. The differential staining pattern of sister chromatids yields information on the number of times a cell has divided in culture. First-division cells—46 chromosomes with both sister chromatids stained uniformly darkly. Second-division cells—46 harlequin chromosomes with one chromatid darkly stained and its sister chromatid lightly stained. Third-division cells—some harlequin chromosomes (15–30 chromosomes) and other chromosomes with both sister chromatids stained uniformly lightly. Fourth-division cells—some chromosomes show the light and dark pattern (7–13 chromosomes), and the rest with both sister chromatids stained uniformly lightly. (**B**) Induction of an AR by MMC and BLM used as adaptive and CDs in the 6 donors (% inhibition) in the endpoints studied. MMC—SCE, MN; BLM—MN, DIC, CB, TA, DC.

chromosomes (15–30 chromosomes) and other chromosomes with both sister chromatids stained uniformly lightly. Fourth-division cells—some chromosomes show the light and dark pattern (7–13 chromosomes), and the rest with both sister chromatids stained uniformly lightly.

Cytogenetic analysis

First-division metaphases with 46 centromeres were used in the evaluation of coded slides by established criteria for CA analysis. After BLM treatment, the abnormalities considered were dicentrics (DIC), excess acentric fragments (EA), centric rings (CR), marker chromosomes (M), chromatid breaks (CB), chromatid exchanges (CE) and chromatid gaps (CG). DIC, EA, CR, M, CB and

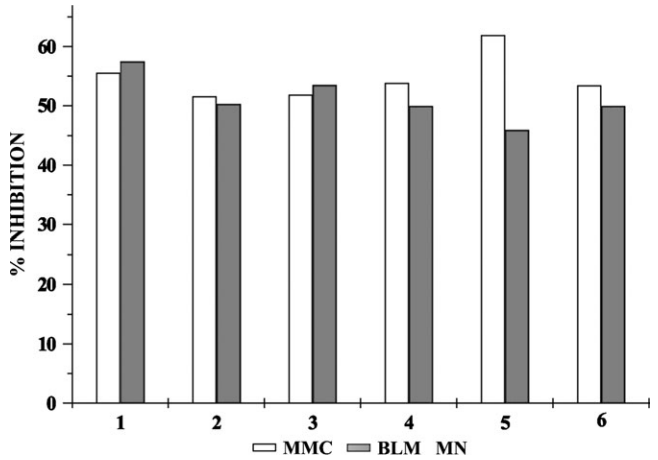

Fig. 2. Comparison of AR induced by MMC and BLM with MN as biomarker (% inhibition) in the six donors studied.

CE were included in determining the total number of damaged cells and total aberrations. For CA analysis, 100 lymphocyte metaphases in each sample were analysed. The SCE were analysed in 50 second-division metaphases, presented as frequency of SCE per cell. The proliferation rate index (PRI) was evaluated in 200 metaphases by scoring the number of cells in the first, second, third or subsequent divisions in the FPG-stained slides. The total number of micronucleated cells (MNBN) and total number of MN were determined in 1000 binucleated cells with well-preserved cytoplasm according to established criteria. Nuclear division index (NDI) was determined by scoring the number of mono-, bi-, tri-, tetra- and more (polynucleate) cells in 1000 viable cells as previously described. The data were analysed statistically with Student's *t*-test.

Results

With AD, a clear reduction was observed both in SCE and MN frequencies relative to the expected MMC-induced chromosome damage (Table II, $P < 0.001$). In case of MMC-induced AR, MN was found to be a sensitive endpoint than SCE. Adaptive treatment with MMC resulted in a higher inhibition in induced MN (range 52–62%) compared to SCE frequencies (range 25–36%) by CD of MMC (Figures 1 and 2). The PRI and NDI data (Table IV) indicated that AR is not related to modulation of the cell cycle kinetics. The cells underwent an average of two replication for all treatments as indicated by PRI values in six donors, and compared to controls, cell division delays were not observed (Table IV). Compared to controls, lymphocyte cultures for MN analysis treated with CD (alone) and adaptive treatment followed by CD showed some delay in cell cycle progression as seen by reduced NDI values ($P < 0.05$). Increased NDI values, compared to CD, after adaptive treatment followed by CD exposures indicated a reversal of cell division delays caused by CD of MMC ($P < 0.05$). It is important to recognize that many genotoxic compounds affect the ability of mammalian cells to traverse the cell cycle. The kinetics of the cell population may influence the expression of the biomarkers studied. Lymphocyte proliferation kinetics has been used by genetic toxicologists mainly to avoid evaluation of genotoxicity at cytotoxic concentrations of chemicals.

AD of BLM caused an inhibition of 30–67, 46–58% in DIC and MN frequencies induced by CD (Table III, $P < 0.05$, $P < 0.001$). In case of BLM, percentage inhibition in induced MN and DIC frequencies was found to be similar (51.22 $\pm$ 3.92, range 46–58%—MN frequency; 54.33 $\pm$ 13.71, range 30–67%—DIC frequency; Figure 3). Lower NDI values in

Table III. AR induced by BLM

DIC							CB					
Donor	C	AD	CD	O[b,c,*]	E	I	C	AD	CD	O[b,c,*]	E	I
1	0	0	10	7	10	30	2	78	112	126	188	32.97
2	0	1	8	3	9	67	2	58	110	102	166	38.55
3	0	0	7	3	7	57	2	50	131	91	179	49.16
4	0	0	2	1	2	50	6	50	164	66	208	68.26
5	0	0	3	1	3	67	2	22	102	66	122	45.9
6	0	0	11	5	11	55	2	29	111	101	138	26.81
TA							DC					
Donor	C	AD	CD	O[b,c,*]	E	I	C	AD	CD	O[b,c,*]	E	I
1	2	96	158	140	252	44.44	2	40	51	36	89	59.55
2	0	72	138	125	210	40.48	1	28	45	36	72	50.00
3	2	63	159	113	220	48.64	2	27	31	24	56	57.14
4	0	59	186	81	245	66.94	4	31	54	35	81	56.79
5	2	29	105	87	132	34.09	2	16	37	37	51	27.45
6	1	32	151	128	182	29.67	1	20	62	48	81	40.74

Donor	C		AD		CD		O		E	I
	BNMN	MN	BNMN	MN[a,*]	BNMN	MN	BNMN	MN[b,c**]	MN	
1	13	14	42	46	81	114	48	62	146	57.53
2	8	9	46	63	79	109	57	81	163	50.31
3	7	7	45	45	83	117	51	72	155	53.54
4	6	6	29	32	58	86	44	56	112	50.00
5	8	9	50	55	110	139	92	100	185	45.95
6	6	6	48	60	112	146	80	100	200	50.00

CA and MN frequencies observed in human lymphocyte cultures.
C, control; O, observed = AD + CD; E, expected = AD + CD − C; I, inhibition (%); CA, 100 metaphases; MN, 1000 binucleated cells scored per donor.
Compared to control, increase in only MN ($P < 0.05$) after adaptive treatment.
Statistically significant ($P < 0.001$) AR in MN as biomarker. $P < 0.05$ in case of CA.
Paired *t*-test.
[a]Compared to control.
[b]Compared to CD.
[c]Compared to E.
$*P < 0.05$; $**P < 0.001$.

adaptive (alone), challenge (alone) and adaptive treatment followed by CD exposures, as compared to controls, indicated delay in the cell cycle progression ($P < 0.001$). However, further slowing of the cell cycle in adapted cells after CD exposure was not observed (Table IV, $P < 0.05$, compared to CD). Inhibition in induced CB frequencies ranged from 27 to 69%, mean 43.61 ± 14.59 (Figure 3). A clear reduction was also observed in CB, TA and DC frequencies relative to the expected BLM-induced chromosome damage (Table III, $P < 0.05$). CE observed with BLM treatment were recorded and included in TA and DC analysis. A few heavily damaged cells defined as with ≥ 30 breaks per cell representing a cytogenetic endpoint of DNA damage reminiscent of apoptosis were also seen with BLM treatment. This was not included in TA or DC analysis. Spontaneous chromatid breakage was comparable in all study subjects. The range was between 0.02 and 0.06 CB per cell. DIC were not observed in 100 metaphases analysed each.

MN as an end point showed similar inhibition both in case of MMC and BLM (54.73 ± 3.79; 51.22 ± 3.92; Figures 1 and 2). Inter-individual variability in AR was evident and more pronounced in case of DIC and CB frequencies (Table V). Difference between the protective effects induced by BLM and MMC in the lymphocytes of the same donors was evident (Table V, Figures 2 and 4). In case of MMC, donors 3 and 5 showed a 2-fold difference in inhibition between the two endpoints MN and SCE. Donor 5 showed maximum inhibition (62%) in MMC-induced MN and minimum inhibition (46%) in BLM-induced MN (Figure 2). Donor 5 also showed maximum inhibition in BLM-induced DIC. The DIC frequencies showed 67% inhibition, where as damaged cells showed only 27% inhibition. (Table V, Figure 4). Donor 2 showed maximum inhibition in MMC-induced SCE and BLM-induced DIC and minimum inhibition in MMC-induced MN. Again, maximum inhibition in BLM-induced CB and TA was seen in donor 4 and minimum inhibition was seen in donor 6. Donor 1 showed maximum inhibition in BLM-induced MN and damaged cell frequencies.

BLM- and HT-adapted lymphocytes showed cross-resistance to the induction of chromosome damage by Cγ and induction of MN by BLM and MMC, respectively. BLM adaptive treatment resulted in significant reductions in gamma ray-induced CA. BLM showed 55–59, 62–63% decrease in DIC, MN frequencies and 42% increase in acentric fragments in one donor, induced by CD of Cγ (Table VII, Figure 5B). HT caused 50–66, 53–54% decrease in MN frequencies induced by MMC and BLM, respectively (Table VI, Figure 5C). The BLM AD delivered at 48 h resulted in significant AR and caused 55–57, 76–84% decrease in CB and MN frequencies (Table VIII, $P < 0.05$, Figure 5A). AR was

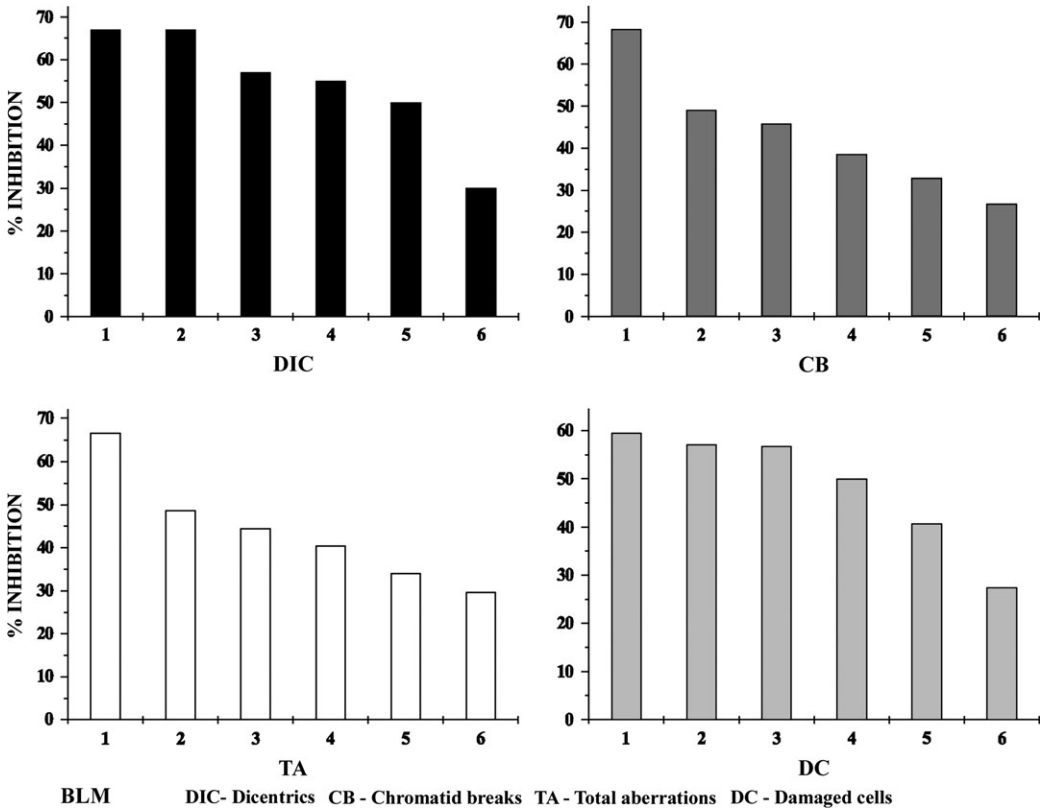

Fig. 3. Comparison of induced cytogenetic adaptation by BLM in DIC, CB, total aberrations and damaged cells as end points in the six donors studied.

not detected in CA assay with QDH treatment, where as one donor showed only 20% inhibition in MN frequencies under the 24- to 48-h treatment schedule.

Discussion

In the present study, the adaptation and cross-adaptation induced by low doses of MMC, BLM, QDH and HT treatment were examined in human blood lymphocytes. Our results suggest that in spite of the heterogeneity shown, low-dose exposures to MMC and BLM reduced chromosome damage on subsequent exposure to a high dose. Adaptation caused by low dose of BLM also protected against ionizing radiation-induced chromosome damage. The resistance and cross-resistance may be due to the induction of the same or similar mechanism involved in the repair of DNA strand breaks. Mild HT elicited an AR on subsequent high-dose exposure to MMC or BLM. The results indicate that cytogenetic AR is not a general phenomenon with respect to exposure conditions or biomarkers and AR confers resistance to the induction of chromosomal damage by the same or similar DNA-damaging agents.

Cytokinesis block micronucleus assay as an important adjunct in large-scale population studies on AR was earlier suggested (9). In the present study, MMC- and BLM-induced AR with MN as an endpoint had shown similar response in all the six donors. MN as a chromosomal biomarker in large-scale population studies on AR is advocated, based on similar AR induced in all donors by MMC/BLM and rapid assessment of MN in binucleated cells.

Cross-resistance has been demonstrated earlier for ionizing radiation, radiomimetic agents and alkylating drugs (10,11). Human blood lymphocytes adapted to low-dose ionizing radiation (LDR) showed a decrease in the frequency of chromatid and isochromatid breaks induced by a subsequent high dose of BLM *in vitro* (12). BLM-induced AR protected against subsequent IR treatment as well (10). *In vivo* AR induced in children exposed as a consequence of he Chernobyl accident, as well as AR induced by occupational exposure to very low doses of ionizing radiation, have been described to protect against a CD of BLM *in vitro* (13,14). LDR-induced AR made cells more resistant not only to radiation but also to hydrogen peroxide and anti-cancer drugs. Similarly, AR induced by low-dose anti-cancer drugs, H_2O_2 and HT protected against radiation-induced damage (15,16).

Few studies have examined the induction of an adaptative response by HT against chemical mutagens in mammalian cells. Heat treatment of tumours either alone or in combination with anti-cancer drugs and radiation is a widely accepted procedure in fighting cancer. Since DNA repair processes are temperature dependent, HT can modulate the action of many anti-cancer drugs. Heat radiosensitization, though poorly understood, is believed to be caused by an inhibition in the repair of radiation-induced DNA damage (17). The cause may be direct heat inactivation of repair enzymes or heat-induced protein denaturation and precipitation onto nuclear chromatin structures, creating a barrier preventing repair enzymes from reaching the damage sites (17). HT chemosensitization may occur by an increased permeability or decreased repair (18). It was suggested that heat increases the probability of double strand breaks (DSB) being incorrectly rejoined but it is not likely to interfere with one DSB repair pathway in particular (19). HT may significantly affect the kinetics of DNA repair in

Table IV. AR—MMC/BLM lymphocyte proliferation kinetics

MMC				
Donor	C PRI	AD PRI[a,*]	CD PRI[a,**]	O PRI[a,b,*]
1	2.32	2.16	2.30	2.20
2	2.20	2.20	1.76	2.24
3	2.41	2.32	1.93	1.96
4	2.25	2.15	2.21	2.05
5	2.36	2.64	2.10	2.28
6	2.22	2.13	1.76	2.23
	NDI	NDI[a,*]	NDI[a,**]	NDI[a,b,**]
1	1.95	1.92	1.78	1.82
2	1.99	1.96	1.90	1.93
3	1.87	1.73	1.67	1.83
4	1.83	1.86	1.75	1.85
5	1.92	1.89	1.85	1.87
6	2.10	2.05	1.95	2.01
BLM				
Donor	NDI	NDI[a,***]	NDI[a,***]	NDI[a,b,**,***]
1	1.95	1.88	1.69	1.75
2	1.99	1.84	1.76	1.79
3	1.87	1.76	1.64	1.69
4	1.83	1.73	1.62	1.71
5	1.92	1.82	1.71	1.80
6	2.10	1.98	1.84	1.90

C, control; AD, adaptive dose alone; CD, challenge dose alone; O, observed = AD + CD; PRI, 200 cells; NDI, 1000 cells scored per donor.
MMC, challenge treatment showed decreased PRI compared to control.
Decreased NDI observed after challenge, adaptive + challenge treatment, compared to control.
BLM, significant decrease in NDI after adaptive, challenge, adaptive + challenge treatment, compared to control.
MMC and BLM showed increased NDI after adaptive + challenge treatment compared to challenge treatment alone.
Paired *t*-test.
[a]Compared to control.
[b]Compared to CD.
*Not significant; significant **$P < 0.05$; ***$P < 0.001$.

Table V. AR by MMC and BLM in six donors

Donor	MMC		BLM				
	SCE	MN	MN	DIC	CB	TA	DC
1	34.75	55.56	57.53	30.00	32.97	44.44	59.55
2	36.22	51.66	50.31	67.00	38.55	40.48	50.00
3	24.98	51.92	53.54	57.00	49.16	48.64	57.14
4	30.79	53.85	50.00	50.00	68.26	66.69	56.79
5	29.26	61.90	45.95	67.00	45.90	34.09	27.45
6	34.66	53.49	50.00	55.00	26.81	29.67	40.74

Endpoints studied: SCE, MN, DIC, CB, TA and damaged cells (% inhibition).
MMC, statistically significant ($P < 0.001$) AR in both SCE and MN as biomarkers.
BLM, statistically significant ($P < 0.001$) AR in MN as biomarker. $P < 0.05$ in case of CA.

drug-treated cells and the magnitude of the effect may be different in normal and cancer cells (6). The lymphocytes incubated at 37°C needed about 60 min to remove completely the damage to their DNA, whereas at 41°C the time required for complete repair was reduced to 30 min (6). Use of the DNA repair-deficient mutants may clarify this issue to some extent.

Mild HT was shown to induce adaptation to cytogenetic damage caused by subsequent X-irradiation (15). The authors suggested that low-dose irradiation and HT may share a common mechanism of induction of adaptation to chromosome damage. Heat shock-induced tolerance to heat-induced apoptosis and sensitization followed by protection of human lymphocytes *in vitro* against radiation-induced apoptosis was also reported (20). In a study on the induction of chromosome damage in Chinese hamster ovary cells by the interaction of HT and metabolic inhibitors (actinomycin D or cyclohexamide),

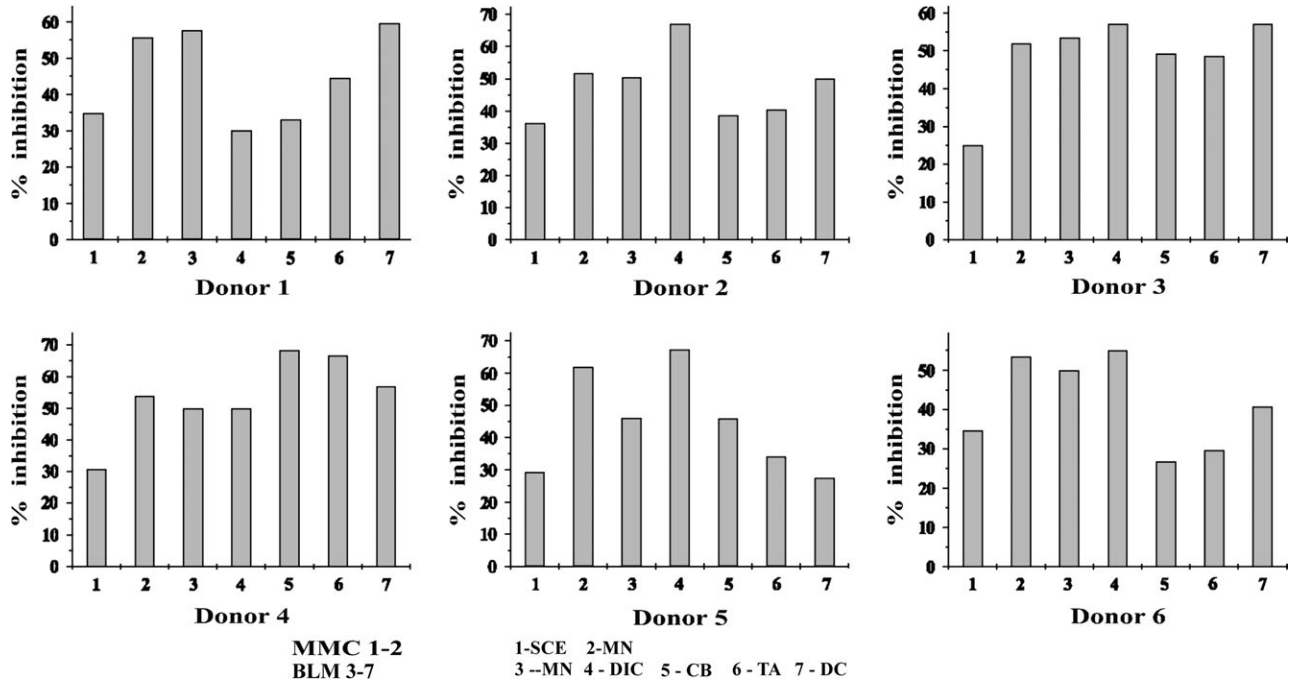

Fig. 4. Comparison of the AR by MMC and BLM in six donors.

Table VI. HT-induced cross-resistance to MMC and BLM

Donor	C			AD			CD			O			E	I[a,*]
	BNMN	MN	NDI	BNMN	MN	NDI	BNMN	MN	NDI	BNMN	MN	NDI		MN
HT/MMC														
1	2	2	2.23	18	8	1.94	30	32	1.91	19	19	1.90	38	50.0
2	8	8	1.98	12	12	1.84	45	49	1.79	18	18	1.65	53	66.0
HT/BLM														
1	2	2	2.23	8	8	1.94	70	73	1.89	32	37	1.92	79	53.2
2	8	8	1.98	12	12	1.84	63	81	1.72	30	39	1.75	85	54.1

MN frequencies observed in human lymphocyte cultures.
C, control; O, observed = AD + CD; E, expected = AD + CD – C; I, inhibition (%); DIC, 100 metaphases; MN, 1000 binucleated cells; NDI, 1000 cells scored per donor.
t-test.
[a]Compared to six donors (Tables II and III MMC, BLM—MN % inhibition).
*Not significant.

Table VII. BLM-induced cross-resistance to gamma rays

Donor	C			AD			CD			O			E	I[a,**]
	BNMN	MN	NDI	BNMN	MN	NDI	BNMN	MN	NDI	BNMN	MN	NDI		MN
BLM/γ-ray														
1	9	9	1.87	50	53	1.85	133	167	1.77	50	80	1.80	211	62.1
2	8	9	2.04	30	42	2.01	98	129	1.80	40	60	1.87	162	63.0
Donor	C	AD	CD	O	E	I[a,*]								
DIC														
1	0	1	8	4	9	55.6								
2	0	2	15	7	17	58.8								

MN frequencies and DIC observed in human lymphocyte cultures.
C, control; O, observed = AD + CD; E, expected = AD + CD – C; I, inhibition (%); DIC, 100 metaphases; MN, 1000 binucleated cells; NDI, 1000 cells scored per donor.
t-test.
[a]Compared to two donors (Tables VI and VII, BLM 24–48 h).
*Not significant; significant**$P < 0.05$.

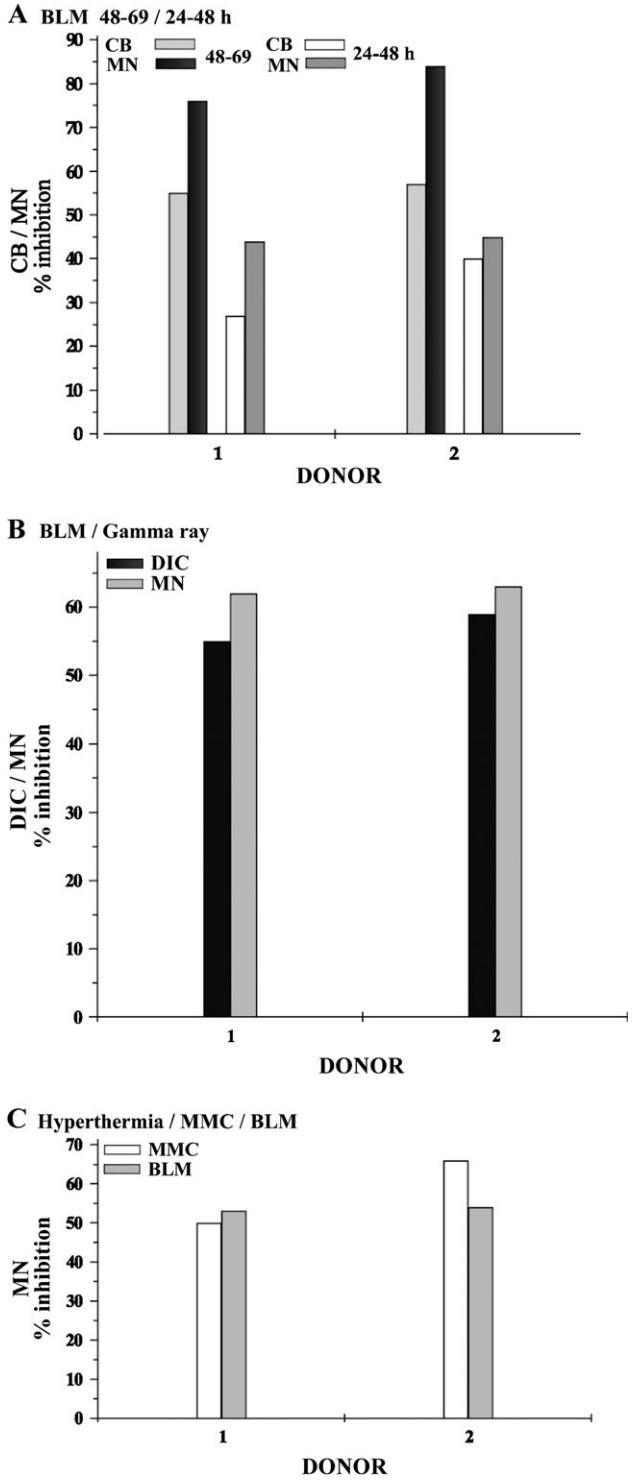

Fig. 5. Comparison of the induced AR in two donors. (**A**) effect of different time schedule for BLM adaptive and challenge exposures. (**B**) BLM as adaptive and gamma rays as challenge exposures. (**C**) HT as adaptive and MMC/BLM as challenge treatments.

actinomycin D potentiated and cyclohexamide reduced the heat shock-induced chromosome damage resulting in significant non-additive increase in cell survival following heat shock (21). Altered cell cycle progression or changes in cell cycle phase distribution during HT had been implicated in the protective effects.

Extensive variability in AR has been reported in human cell lines, including inter-individual variability in humans (22–25). Intrinsic individual variability in DNA repair system or the influence or a number of extrinsic features may play a role. Unknown transient physiological conditions of the donor or variations in G2 cell progression have been implicated in the observed remarkable inter-individual difference in AR (26). The widely reported heterogeneity, in part, may be genetically controlled (9,25). Dizygotic twins showed greater variability in AR compared to monozygotic twins implicating genetic constitution as a source of variability (27). There is conclusive evidence now for the influence of certain genotypes on cytogenetic biomarkers. Many studies have pointed out the interaction between CA and MN frequencies and a selected number of genetic polymorphisms involved in metabolic activation/deactivation or DNA repair (28–36). A recent study to assess the influence of genetic polymorphisms in GSTM1 and GSTT1 genes on MN frequencies in human populations found that GSTT1 null genotype was associated with a significantly lower level of micronucleated cells in the population and the protective effect was reversed in older age classes in occupationally exposed subjects in the population (37). A number of genetic polymorphisms of xenobiotic metabolism and genome integrity (DNA repair, folate metabolism) may affect the baseline or induced level of cytogenetic bimarkers, especially CA in human lymphocytes (38). Folate status has an important impact on chromosomal stability and is an important modifying factor of cellular sensitivity to radiation-induced damage (39).

The particular gene combination an individual has with regard to absorption, metabolism of chemical mutagens, DNA repair, cell death (apoptosis/necrosis), cell cycle control and immune response can affect an individual's response to physical or chemical stress (40). A correlation between the sensitivity or resistance of tumour cell lines and their BLM hydrolase activity has also been found (41). Whether AD of BLM stimulates BLM hydrolase activity which could counteract to some extent the CD effect is not worked out. It was evident that p53 tumour suppressor plays a key role in the radio AR which may channel the DSB repair into legitimate DNA end-joining pathway instead of an illegitimate rejoining leading to CA formation or apoptosis (42). Their observations indicated that AR and apoptotic cell death constitute a complimentary defense system via life-or-death decisions. After an *in vitro* CD of MMC, a transient adaptation to radiation observed in ^{131}I-treated non-familial thyroid cancer patients (43) further strengthened the need for long-term cytogenetic evaluation of the AR. Long-term follow-up studies on a large number AR negative donors as well as donors who show synergistic effect are suggested for the elucidation of the mechanisms of AR (44). The influence of certain genotypes on chromosomal biomarkers used in AR studies and the role of AR in radiation and chemotherapy need to be further deciphered.

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Table VIII. BLM-induced AR in the 48- to 69-h and 24- to 48-h time schemes

BLM/BLM	Donor	CB					
		C	AD	CD	O	E	I[a,b,*,**]
48–69	1	2	67	101	74	166	55.42
	2	1	46	93	59	138	57.25
24–48	1	2	26	113	100	137	27.01
	2	1	22	100	72	121	40.49

Micronuclei

	Donor	C		AD			CD			O			E		I[a, b, *,**]
		BNMN	MN	NDI	BNMN	MN	NDI	BNMN	MN	NDI	BNMN	MN	NDI	MN	
48–69	1	2	2	1.98	11	11	1.89	20	25	1.79	8	8	1.93	34	76.47
	2	5	5	2.05	14	14	1.97	34	40	1.88	8	8	1.99	49	83.67
24–48	1	2	2	1.87	50	50	1.85	90	132	1.62	75	100	1.64	180	44.4
	2	5	5	2.04	30	42	2.01	89	128	1.75	72	90	1.79	165	45.5

CB and MN frequencies observed in human lymphocyte cultures.
C, control; O, observed = AD + CD; E, expected = AD + CD − C; I, inhibition (%); CB, 100 metaphases; MN, 1000 binucleated cells; NDI, 1000 cells scored per donor.
t-test.
[a]Compared to six donors (Table III, BLM, % inhibition).
[b]Paired *t*-test. Compared to two donors, BLM 24–48 h.
*Not significant; significant $^{**}P < 0.05$.

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