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Modulation of the clastogenic activity of γ-irradiation in buthionine sulphoximine-mediated glutathione depleted mammalian cells

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Abstract.
Purpose: Chromosome aberrations (CA) were used as an end-point to investigate the effect of buthionine sulphoximine (BSO), a potent glutathione-depleting agent, on the radiosensitivity of mammalian cells. The aim was to obtain information about the role of glutathione (GSH) in physicochemical and biochemical processes in irradiated cells.
Materials and methods: CA were scored from first cycle metaphases in irradiated BSO-pretreated and untreated samples. BSO exposure was for 10 h in mouse bone marrow cells *in vivo* and 5 h for human lymphocytes *in vitro*. In further experiments fresh blood was irradiated on ice and immediately after irradiation GSH/GSH-ester was added.
Results: In both the systems BSO-treated samples showed higher sensitivity to radiation than BSO untreated samples. The frequency of all types of CA increased except exchange aberrations. GSH/GSH-ester treatment given after irradiating the cells at 4°C reduced the frequency of deletions and increased the frequency of exchange aberrations.
Conclusions: Data indicate that BSO-mediated GSH depletion increased radiation-induced chromosome aberrations, apart from exchange aberrations. This could be due to reduction in the free-radical scavenging effect of GSH, a failure in rejoining of DNA double-strand breaks, or induction of apoptosis.

1. Introduction

Endogenous thiols, especially the tripeptide reduced glutathione (GSH), have long been thought to affect the sensitivity of cells to irradiation (Meister 1983). Variations in non-protein thiol levels, and GSH in particular, are observed throughout the cell cycle (Sinclair 1969) and the radiobiological K value is related to cellular thiol levels (Cullen *et al.* 1980, Solen *et al.* 1989). It has been shown that exogenous addition of GSH could effectively reduce chromosome aberrations (CA) produced by X-rays in different systems (Chaudhury and Langendroff 1968) including the muntjac lymphocyte culture (Chatterjee and Jacob-Raman 1986).

Buthionine sulphoximine (BSO) specifically depletes the endogenous GSH level by inhibiting the enzyme γ-glutamylcysteine synthetase and increases cellular radiosensitivity. GSH is an important factor in the radiosensitization of hypoxic cells and it has been described as a chemical repair agent in mammalian cells (Midander 1982, Clark *et al.* 1984, Revesz *et al.* 1984). Observations made under oxic conditions (Koch *et al.* 1984, Shrieve *et al.* 1985) can be attributed to thiol scavenging of OH radicals (Held *et al.* 1981). In Chinese hamster V79 cells sensitization was observed with respect to both cell survival and DNA double-strand (ds) break induction in BSO-treated cells after irradiation under both oxic and hypoxic conditions (Prise *et al.* 1992).

Evidence already exists that GSH may not be an efficient protector of DNA due to its −1 net charge, which, on the basis of counter-ion condensation and co-ion depletion phenomena, may exclude it from close association with DNA (Fahey *et al.* 1991). On the other hand, it has been proposed that GSH within the cell nucleus and in particular when close to DNA is important in determining cellular radiosensitivity (Edgren 1987, Prise *et al.* 1992). There is also evidence that suggests that DNA-bound proteins may have a much more effective role in protecting DNA, in comparison with soluble compounds (Ljungman *et al.* 1991). Again Prise *et al.* (1992) and Frankenberg-Schwager (1990) demonstrated that there is a residual chemical repair capacity in eukaryotic cells that is not dependent on GSH. This suggests that other reducing agents, such as protein thiols, may have an important role to play in ionizing radiation-induced free-radical reactions which occur in the DNA of intact cells. Radiation-induced DNA lesions have been shown to be repaired rapidly by various mechanisms, some of which are error-free or error-prone (Teoule 1987, Hanawalt 1994). It has been demonstrated that the rejoining of radiation-induced single-strand breaks (ssb) under oxic conditions differs from that involved in the rejoining of hypoxically induced ssb and is clearly dependent upon GSH (Edgren *et al.* 1981). This interpretation is important, since the role of GSH in DNA synthesis

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under certain conditions (Holmgren 1979) and as a cofactor in enzymatic repair processes in the cell (Xue *et al.* 1988) has already been evident. Little is known about the importance of GSH in these processes.

Many studies suggest that DNA dsb induced by ionizing radiation are critical lesions which, if un- or misrepaired, can cause CA and cell death, as well as mutations and cell transformation (Frankenberg-Schwager 1990, Iliakis 1991). It has also been shown that depletion of endogenous GSH by BSO reduces the frequency of CA produced by radiomimetic chemical bleomycin in human lymphocytes (Chattopadhyay *et al.* 1997) and increases the frequency of CA induced by arecoline (Deb and Chatterjee 1998) and mitomycin C (Dev-Giri and Chatterjee 1998) in mouse bone marrow cells. It has been observed that there was a significant reduction in the frequency of micronuclei induced by 1 Gy γ-rays after BSO treatment in polychromatic erythrocytes of mouse bone marrow cells although the GSH levels after 2 h BSO (20 mg kg^{-1}) treatment were unaltered (Sarma *et al.* 1996). However, no study has been carried out to see the effect of endogenous GSH depletion on induction of CA by ionizing radiation in mammalian cells.

The present study has been performed to investigate the role of endogenous GSH in the induction of CA by radiation both in mouse bone marrow cells *in vivo* and human peripheral blood lymphocytes *in vitro*. Both systems are well established and suitable for the assessment of cytogenetic effects (Adler 1984). An attempt has also been made to see the role of endogenous GSH on repair/misrepair processes.

2. Materials and methods

2.1. *Chemicals*

DL-Buthionine-*S*,*R*-sulphoximine (BSO), 5,5′-dithiobis(2-nitro benzoic acid) (DTNB), reduced glutathione (GSH), GSH-ethyl ester, Hoechst 33258, 5-bromodeoxyuridine (BrdU), GSH reductase and NADPH were purchased from Sigma Chemical Co. (St Louis, MO, USA). The culture medium RPMI 1640, antibiotic penicillin and streptomycin and mitogen phytohaemagglutin (PHA) were obtained from Gibco (New York, USA). Foetal calf serum was used from Biological Industries Ltd (Israel). Giemsa stain was obtained from BDH Chemicals Ltd (Poole, UK). Other chemicals used in this study were of analytical grade.

2.2. *BSO treatment and* in vivo *irradiation*

Male Swiss-albino mice, aged 2–3 months and weighing ~25–30 g, were maintained in the laboratory in community cages in a controlled-temperature room (20 ± 2°C), with controlled lighting (12 h light/12 h dark). Standard mouse diet (NMC Oil Mills Ltd, Pune, India) and water *ad libitum* were used in all experiments.

BSO 200 mg kg^{-1} was prepared in phosphate buffer solution (pH 7.4) and injected intraperitoneally 10 h prior to irradiation. An equal volume of PBS was injected into the control (BSO untreated) mouse. Mice were kept in well-ventilated two-tier acrylic cylinders and placed in a ^{60}Co γ-chamber for whole-body exposure. The radiation doses used were 2 and 3 Gy (dose-rate 14.4 Gy min^{-1} as determined by Fricke dosimetry). Cells were fixed at 13 h after irradiation, preceded by 2 h colchicine (15 mg kg^{-1}) treatment.

2.3. *Preparation of metaphases*

Animals were killed by cervical dislocation. The femurs were dissected out and the bone marrow cells (BMC) were obtained by injecting 2 ml 0.075 M KCl prewarmed to 37°C. Cells were treated in hypotonic solution for 15 min and fixed in acetic acid and methanol (1:3). Slides were prepared by the flame drying method, stained in 5% Giemsa for 5 min and mounted in synthetic mountant.

2.4. *BSO treatment and* in vitro *irradiation*

Heparinized peripheral blood from five healthy male donors was used immediately after venipuncture. An aliquot (1 ml) of whole blood for each dose of γ-rays was taken in a sterilized small flat-bottom 25-ml glass beaker. BSO 5 mM was added and kept at 37°C for 5 h before exposure either to 1 or 2 Gy γ-irradiation (dose-rate 12.39 Gy min^{-1}; first experiment) or 2 and 3 Gy at 8.19 Gy min^{-1} (second experiment) at room temperature. The two experiments were performed at different periods. Blood samples were kept at 37°C for 1 h after irradiation to allow cellular repair before setting up the cultures.

A small amount of fresh blood was kept for 40 min at 4°C before irradiation on ice. Either GSH (20 mM) or GSH-ester (20 mM) was added to 1 ml aliquots of blood within 1 min of irradiation, mixed well and kept for 3 h at 4°C, to allow elevation in endogenous GSH level before the culture was set up.

2.5. *Culture procedure and cell fixation*

Cultures were set up in RPMI 1640 with antibiotics (200 IU penicillin and 100 μg streptomycin per culture) supplemented with 10% heat-inactivated foetal calf serum. Lymphocytes were stimulated with PHA.

5-Bromodeoxyuridine (6 μg ml^{-1}) was added to each culture when set up. All cultures were incubated at 37°C and were harvested at 48 h. Colcemid was added at a concentration of 0.01 μg ml^{-1} for the last 3 h of culture. Hypotonic treatment was done for 18 min and cells were fixed in acetic acid and methanol (1:3) and slides prepared. All experiments were repeated a minimum of two times.

2.6. *Differential staining for sister chromatids*

The method of Goto *et al.* (1975) was followed. Slides were treated for 10 min with Hoechst 33258 (50 μg ml^{-1}) at room temperature in the dark, rinsed in distilled water, mounted in 2× SSC (NaCl-Na citrate, pH 6.8) and kept in sunlight for 30–40 min, depending on the intensity of sunlight. After rinsing in distilled water, slides were stained in 2% Giemsa for 3–4 min, air-dried and mounted in synthetic mountant.

2.7. *Determination of GSH-level in mouse bone marrow cells*

The level of GSH in BMC, with or without BSO treatment, was estimated by the method of Akerboom and Sies (1981). Freshly collected BMC were flushed into ice-cold 0.1 M phosphate-buffered saline solution (pH 7.4) and the volume was made up to 1 ml. Cells were counted in a haemocytometer and lysed by alternate freezing and thawing three times at 10°C and room temperature, each for 10 min. The lysed cell suspension was centrifuged in a Beckman model J2-HS centrifuge (10 000 rpm) for 5 min at 4°C. The supernatant was de-proteinized using 100 μl ice-cold 10% 5-sulphosalicylic acid with intermittent shaking. The tubes were kept on ice for 10–15 min and the acid precipitable proteins were removed by centrifuging at 10 000 rpm at 4°C for 15 min. The supernatant was immediately used for GSH estimation. 50 μl sample suspension was added into 1 ml buffer (0.1 M EDTA phosphate buffer, pH 7.0). Then 50 μl NADPH (4 mg ml^{-1}), 20 μl DTNB (1.5 mg ml^{-1}) and 20 μl GSH reductase (6 units ml^{-1}) were added. The contents were mixed and the optical density of the samples was measured continuously for 5 min at 412 nm by UV-visible spectrophotometer (Beckman model DU-640), relative to a blank without cell extract. A standard curve was prepared from a stock solution of 10 mM GSH (3.1 mg ml^{-1}) in 5% 5-SSA diluted to 1–50 nmol.

2.8. *Scoring and statistical analysis*

Slides were random coded. CA were scored in four categories in mouse BMC: exchanges (all interchanges involving two or more different chromosomes); sister chromatid unions (SCU, intra-arm intrachanges between lesions within a chromosome); isochromatid breaks and simple chromatid breaks. Aberrations scored in human peripheral blood lymphocytes (PBL) were from first cycle metaphases and were: exchanges including dicentrics and rings (with or without fragments); deletions (both terminal and interstitial) and chromatid breaks. Translocations were not scored. The statistical significance of the difference between the control and treated groups for the frequency of aberrant metaphases was evaluated using a 2 × 2 contingency χ^2-test and for different types of aberrations a simple χ^2-test was used. The difference of GSH level between BSO treated and untreated groups was evaluated using a Student's *t*-test.

3. Results

3.1. *Effect of BSO on* in vivo*-induced aberrations*

Gamma-ray-induced CA in BSO-treated and untreated mouse BMC are shown in table 1. Isochromatid and chromatid breaks were the most frequent type of aberration and increased most consistently in presence of BSO. The degree of enhancement was higher in case of chromatid breaks (from 2.00 to 3.20 per cell) than isochromatid breaks (from 0.30 to 0.37 per cell). After BSO pretreatment the frequency of aberrant metaphases was increased from 63 to 77% by 2 Gy, and 76 to 88% by 3 Gy respectively. The frequency of exchange aberrations (including SCU) was not enhanced in the BSO-treated samples although there was a marked reduction in their frequency induced by 2 Gy (0.15–0.06 per cell). The effect of BSO itself on the formation of spontaneous CA was studied in the unirradiated mouse. A significant increase in the frequency of aberrant metaphases and chromatid breaks was observed with 200 mg kg^{-1} treatment.

3.2. *Effect of BSO on* in vitro*-induced aberrations*

The induction of aberrations by radiation was clearly dose-dependant but the frequency of chromatid breaks was very low at all radiation doses. A significant increase in the induction of deletions and aberrant metaphases was observed in all BSO-treated samples at all radiation doses. Interestingly, the frequency of exchanges did not increase at all in BSO-treated samples: rather there was a tendency for these to decrease in most cases. A significant reduction in the frequency of exchanges was observed in BSO-treated samples with 3 Gy (from 0.51 to 0.32

Table 1. Effect of BSO treatment on chromosome aberrations induced by γ-irradiation in mouse bone marrow cells.

Dose (Gy)	BSO ($mg\,kg^{-1}$)	Aberrant metaphases (%)	Total metaphase	Aberrations/cell ± SEM			
				Exchange	Isochromatid break	Chromatid break	SCU
0.0‡	0	2	462	0.00	0.00	0.03	0.00
	200	9	345	0.00	0.00	0.11	0.00
2.0	0	60	127	0.12	0.30	2.08	0.08
		65	115	0.18	0.18	1.90	0.04
		57	105	0.20	0.23	1.73	0.05
		69	196	0.10	0.43	2.32	0.06
		63 ± 2.33	543	0.15 ± 0.02	0.30 ± 0.03	2.00 ± 0.1	0.06 ± 0.01
	200	78	93	0.07	0.52	2.94	0.05
		59	107	0.04	0.30	2.06	0.00
		90	87	0.05	0.33	3.88	0.01
		81	101	0.09	0.36	3.93	0.01
		77 ± 6.52†	388	0.06 ± 0.01*	0.37 ± 0.05*	3.20 ± 0.44**	0.02 ± 0.01
3.0	0	73	111	0.28	0.82	3.11	0.18
		71	108	0.24	0.43	2.86	0.16
		83	121	0.39	0.56	3.46	0.14
		79	111	0.42	0.36	3.29	0.09
		76 ± 2.75	451	0.33 ± 0.04	0.54 ± 0.10	3.18 ± 0.12	0.14 ± 0.02
	200	90	114	0.32	0.69	5.21	0.11
		92	126	0.43	0.98	5.38	0.08
		83	91	0.26	0.91	4.81	0.06
		88 ± 2.72†	331	0.33 ± 0.05	0.86 ± 0.09**	5.13 ± 0.17**	0.08 ± 0.01

† $p < 0.001$ 2×2 contingency χ^2-test; * $p < 0.05$, ** $p < 0.001$ χ^2-test at d.f. = 2. ‡ Pooled data, a minimum of two mice were used.

per cell in sample 4; 0.42 to 0.32 per cell in sample 5; table 2).

The effect of 5 mM BSO by itself on the spontaneous CA was also studied and no significant effect was observed.

3.3. *GSH or GSH-ester post-treatment*

These data are also presented in table 2. Irradiated samples at 4°C were studied as positive controls for the GSH or GSH-ester post-treated samples. Lymphocytes irradiated at 4°C showed a higher frequency of deletions (except in sample 5 where the frequency was 0.47 and 0.46 per cell with 3 Gy exposure at room temperature and at 4°C respectively) and lower frequency of exchanges. Addition of GSH or GSH-ester after radiation significantly reduced the frequency of deletions and also increased the frequency of exchanges with respect to its positive control. A tendency of reduction in the frequency of aberrant metaphases in GSH or GSH-ester post-treated samples was also observed.

The effect of GSH and GSH-ester alone in lymphocytes were also studied and the data indicate that there was no induction of any aberrations in this cell system.

3.4. *Level of reduced GSH*

Levels of reduced GSH in mouse BMC with or without BSO are shown in figure 1. GSH concentrations in BMC ranged from 12.5 to 40.0 nmol 10^{-6} cells with an average of 24.88 ± 9.2 nmol in normal mouse marrow. This GSH concentration was depleted to 54% of the control after 10-h treatment with BSO. The statistical difference between the mean GSH concentration of these two groups was significant.

4. Discussion

BSO has been used to evaluate the effect of GSH depletion on radiosensitization of mouse BMC *in vivo* and human PBL *in vitro*. To score CA, it is advisable to use the BudR differential staining method and to determine most suitable harvesting time to have an optimum number of first-cycle metaphases (M1; Bianchi *et al.* 1982). For this reason, the present *in vivo* experiment was carried out by fixing the cells at 13 h after irradiation since the unirradiated mouse shows a very high frequency of M1 (96.5%) at a 13 h fixation (data not shown).

The results indicate that the presence of BSO

Table 2. Effect of radiation with or without BSO and radiation at 4°C with or without GSH (20 mM) and GSH-ester (20 mM) post-treatment on chromosome aberrations in human peripheral blood lymphocytes.

Experimental condition	Aberrant metaphases (%)	Total metaphases	Aberrations/cell			Donor no.
			Exchange	Deletion	Chromatid break	
First set						
Untreated	2	101	0	0.01	0.01	1
Untreated (4°C)	3	98	0	0.01	0.01	
BSO (5 mM)	3	113	0	0.01	0.02	
GSH	3	111	0	0.01	0.02	
1 Gy	37	96	0.10	0.45	0.01	
BSO+ 1 Gy	61††	102	0.09	0.74**	0.04	
2 Gy	77	109	0.20	0.74	0.05	
BSO+ 2 Gy	91††	106	0.18	1.98**	0.20*	
2 Gy (4°C)	74	92	0.11	0.99	0.08	
2 Gy (4°C)+ GSH	66	127	0.27*	0.59**	0.02	
Untreated	2	102	0	0.01	0.01	2
BSO (5 mM)	2	110	0	0.01	0.02	
GSH	1	110	0	0.01	0.02	
1 Gy	41	103	0.10	0.39	0.02	
BSO+ 1 Gy	68††	111	0.06	0.69**	0.06	
2 Gy	69	91	0.15	0.70	0.05	
BSO+ 2 Gy	92††	93	0.15	1.79**	0.16*	
2 Gy (4°C)	68	101	0.10	0.91	0.07	
2 Gy (4°C)+ GSH	65	129	0.30*	0.52**	0.06	
2 Gy	77	93	0.19	0.78	0.04	3
2 Gy (4°C)	60	96	0.06	0.86	0.06	
2 Gy (4°C)+ GSH	61	92	0.25*	0.51**	0.04	
Second set						
GSH-ester	3	125	0	0.01	0.01	4
3 Gy	53	108	0.51	0.67	0.05	
BSO+ 3 Gy	78††	105	0.32*	1.14**	0.09	
3 Gy (4°C)	70	100	0.30	0.91	0.19	
3 Gy (4°C)+ GSH	68	156	0.53*	0.36**	0.08	
3 Gy (4°C)+ GSH-ester	61	167	0.41	0.42**	0.04*	
GSH-ester	3	101	0	0.01	0.01	5
2 Gy	53	209	0.14	0.51	0.04	
BSO+ 2 Gy	71††	229	0.07	0.79**	0.06	
3 Gy	64	188	0.42	0.47	0.04	
BSO+ 3 Gy	75†	116	0.32*	1.14**	0.09	
3 Gy (4°C)	45	116	0.26	0.46	0.01	
3 Gy (4°C)+ GSH	40	143	0.32	0.12**	0.01	
3 Gy (4°C)+ GSH-ester	40	176	0.35	0.16**	0.06	

†$p < 0.05$; ††$p < 0.001$ 2×2 contingency χ^2-test; *$p < 0.05$, **$p < 0.001$ χ^2-test at d.f. = 2 compared with respective positive control.

increased cellular radiosensitivity in both cell systems. Mice treated with BSO exhibit a rapid decline in GSH in kidney, liver, pancreas and muscle and, after prolonged treatment, also showed a reduced concentration of GSH in other tissues (Griffith and Meister 1979). The reduced-GSH estimations in this study indicate that a 10 h incubation with BSO ($200\ mg\ kg^{-1}$) could deplete GSH level significantly with respect to controls. It has been demonstrated that following a single dose of $556\ mg\ kg^{-1}$ BSO, the GSH concentrations of various normal tissues were depleted in a time-dependent manner (Lee *et al.* 1987). These authors observed that the intermediate rates of depletion in the bone marrow with a nadir at 8–12 h and the GSH content of the BMC following depletion was 17% of the initial level. Therefore, in the present *in vivo* study an incubation period with BSO of 10 h was chosen. In the case of the *in vitro* study the freshly drawn blood was incubated with BSO for 5 h since in cultured cells > 75% depletion was achieved within 4–5 h by 500 μm to 10 mM BSO (Shrieve *et al.* 1985, Edgren and Revesz 1987). In

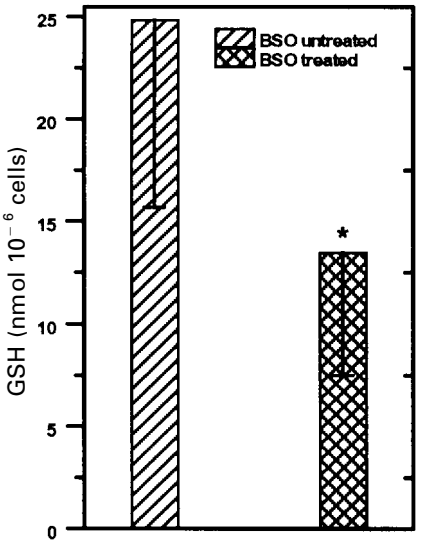

Figure 1. Levels of GSH in mouse bone marrow cells 10 h after a single treatment of BSO. Values are mean ± SEM of groups of eight mice per dose point. *$p < 0.05$ as compared with respective control group.

this study the concentration of BSO used was 5 mM since significant sensitization by BSO was observed at this higher concentration with respect to radiation-induced CA.

The BSO-mediated increase in the frequency of radiation-induced chromatid and isochromatid breaks in mouse BMC and deletions in human PBL in this study are not in agreement with the reported failure to increase radiosensitivity with respect to DNA ssb in BSO-treated, GSH-depleted cells under aerobic condition (Edgren *et al.* 1985) and also in aerated Chinese hamster lung and A549 human lung carcinoma cells (Bump *et al.* 1982, Clark *et al.* 1984). However, equal sensitization of aerated and hypoxic cells using similar *in vitro* systems has been reported (Mitchell *et al.* 1983, Koch *et al.* 1984). Depletion of intracellular GSH by BSO sensitized V79-379A cells to X-irradiation at all oxygen tensions has been demonstrated by Shrieve *et al.* (1985). Therefore, reports are available both in favour and against the view that BSO treatment increases the radiosensitivity of aerated cells and the present study also clearly indicates that depletion of GSH by BSO sensitized the cells to radiation with respect to CA.

It has been thought that the protection of DNA in cells is due to scavenging of hydroxyl radicals by soluble intracellular compounds (Revesz 1985). However, it has also been stated that both the structural arrangement of the chromatin and the presence of DNA-bound proteins offer a far more efficient protection against radiation-induced DNA strand breaks than intracellular scavengers of hydroxyl radicals (Ward 1994, Nygren *et al.* 1995). It has been claimed that at least two peptides, one being GSH, are bound to the nucleoproteins, as mixed disulphides (Modig 1973). Moreover, aminothiol radioprotectors in general have been reported to bind with DNA and slow strand separation for replication (Brown 1976). Such inhibition of DNA synthesis could induce cell cycle delay as well as SCE (Schneider *et al.* 1978). It has been shown that reduced GSH could also induce both cell cycle delay (Chatterjee and Jacob-Raman 1986, Chatterjee *et al.* 1995) and SCE (Speit *et al.* 1980, Chatterjee *et al.* 1995). It may be presumed, therefore, that a portion of cellular GSH binds on DNA or DNA-bound proteins and provides a shielding effect against radiation. Thus BSO-mediated GSH depletion could reduce the shielding effect and enhance DNA strand break induction probably by hydroxyl radicals because increase in damage by removing natural protection system is largely due to the effect of hydroxyl radical (Ljunman *et al.* 1991, Nygren *et al.* 1995).

The presently observed increase in frequency of radiation-induced CA in BSO-treated cells could also be a manifestation of apoptosis since these cells do respond in this way to irradiation. It has been demonstrated that human PBL show morphological characteristics of apoptosis following irradiation with γ-rays doses ranging from 0.1 to 5 Gy (Vral *et al.* 1998). It has been shown that the fraction of cells with aberrations will increase when the apoptotic response has been counteracted by the addition of phytohaemagglutinin (PHA) in irradiated G_0 human lymphocytes and suppression of the apoptotic process by the post-irradiation addition of PHA could be seen as late as 48 h after irradiation (Harms-Ringdahl *et al.* 1996). GSH may also have a role in modulating the mode of cell death following toxic injury (Fernandes and Cotter 1994). The role of GSH in modulating the cytotoxicity of platinum complexes by affecting DNA repair, apoptosis and free radical scavenging has also been demonstrated (Pendyala *et al.* 1997). Therefore, the present increase in the frequency of CA could be at the expense of the genomic fidelity of the population and, if this is so, then radiation-induced apoptosis induction in BSO-treated cells could be a factor in carcinogenesis. It has already been shown that tumours were sensitized to radio- and chemotherapeutic modalities when the cellular GSH content was decreased by BSO (Hamilton *et al.* 1985, Meister 1991).

The interesting observation in this study is the failure of the frequency of exchange aberrations in BSO-pretreated cells to increase in spite of significant enhancement of chromatid and deletion-type aberra-

tions. An exchange aberration is thought to arise as a consequence of illegitimate reunion ('misrejoining') of free ends from different DNA dsb (Cornforth and Bedford 1993). In an attempt to clarify the possible role of GSH in biochemical repair processes, the extent of rejoining of radiation-induced ssb was determined up to 1 h after exposure (Edgren *et al.* 1981, Revesz *et al.* 1984) and it was found that the repair system involved in the rejoining of oxically induced ssb differed from that involved in the rejoining of hypoxically induced ssb and was clearly dependent upon GSH. Therefore, the failure to observe either restitution or illegitimate reunion soon after dsb induction by radiation could lead to increase in the frequency of deletion and chromatid aberrations in BSO-pretreated cells.

This assumption is further strengthened by the observation of an increased frequency of exchange aberrations and decreased frequency of deletions in GSH/GSH-ester post-treated human PBL irradiated at 4°C. The rationale for such post-treatment to irradiated cells at 4°C is based on the premise that an increased endogenous GSH level could act on radiation-induced unrepaired DNA lesions (due to 4°C incubation) after changing the temperature from 4 to 37°C. Therefore, GSH/GSH-ester was added soon after irradiation and kept for 3 h at 4°C. GSH-ester is readily transported into cells and converted to GSH increasing the level of GSH within 3–4 h (Wellner *et al.* 1974). Our observation of an increase in the frequency of exchange aberrations and a decrease in deletions could be due to enhancement in rejoining (both restitution and illegitimate reunion) of radiation-induced DNA dsb under the influence of increased endogenous GSH. It has been demonstrated that mammalian cells require the enzymatic machinery for joining non-homologous DNA ends (Thode *et al.* 1990). Moreover, with *in vitro* assay by utilizing either 'naked' agarose-embedded cellular DNA (Cheong *et al.* 1996) or DNA organized in chromatin as found in the cell nucleus (Ganguly and Iliakis 1995), it has been shown that efficient rejoining of radiation-induced dsb require activities present in cell extracts. From the present result it seems that endogenous GSH could be one of the important components of the enzymatic machinery that is needed for dsb joining. This consideration finds further support in the report where inhibition of unscheduled DNA synthesis in BSO-treated ovarian carcinoma cell line and replenishment of GSH in BSO-treated cells with GSH monoethyl ester resulted in a complete recovery of DNA repair activity (Lai *et al.* 1989).

Revesz *et al.* (1994) investigated the selective toxicity of BSO to melanoma cells both *in vivo* and *in vitro*. It has also been reported earlier that extremely low concentration of BSO ($4\,mg\,kg^{-1}$)-induced sister chromatid exchanges significantly in mouse BMC without inducing any CA (Chatterjee *et al.* 1995). Therefore, present induction of CA by a higher concentration of BSO in the mouse could be attributed to better depletion of endogenous GSH levels and this could be an indication of an important protective role of endogenous GSH in the cell against peroxides and free radicals which are formed by normal metabolic pathways (Meister 1983). However, the failure of BSO (5 mM) to induce CA in human lymphocytes is surprising. It could be that the generation of free radicals might be negligible in non-cycling lymphocytes (before PHA addition) and thus depletion of cellular GSH could not increase the free-radical-induced spontaneous CA frequency.

In conclusion, the results indicate that BSO depletion of GSH leads to an increase in radiation-induced CA, apart from exchange aberrations, and this could be due to reduction of DNA shielding effect, failure in rejoining of DNA dsb free ends, and apoptosis.

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