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Influence of glutathione on the induction of chromosome aberrations, delay in cell cycle kinetics and cell cycle regulator proteins in irradiated mouse bone marrow cells

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Abstract

Purpose: Reduced glutathione (GSH) is an endogenous thiol and has long been thought to affect the sensitivity of cells to radiation. The aim was to see the influence of GSH on: (i) the production of all types of radiation-induced chromosome aberrations (CA), and (ii) the radiation-induced delay in cell cycle and the levels of cell cycle regulator proteins.

Materials and Methods: Cell cycle kinetics were determined by scoring the mitotic index (MI). CA and MI were scored in γ-irradiated buthionine sulfoximine (BSO) (10 h) or GSH (1 h) pretreated and untreated mouse bone marrow cells (BMC). The expression of p53 and p21 proteins after 2 and 6 h of irradiation and for the B-cell lymphoma 2 (Bcl-2) associated X-protein (Bax) after 24 h of irradiation with or without BSO or GSH treatment was analyzed by immunoblotting.

Results: Radiation delays mouse BMC in their passage through the cell cycle and induces CA. Exogenous addition of GSH protected CA uniformly at lower doses of radiation but differentially at higher doses, whereas GSH-depletion by BSO increased the frequency of radiation-induced CA. Both GSH and BSO-pretreated cells reduced the delay in cell kinetics after irradiation. Levels of both p53 and p21 were enhanced after irradiation to BSO-pretreated cells. However, in GSH-pretreated cells the level of these proteins was reduced.

Conclusion: Data indicate that the induction of CA and delay in cell kinetics by radiation may not always be interlinked and that the level of endogenous GSH exerts its influence on these parameters. Both GSH and BSO pretreatment reduce delays in cell kinetics of irradiated cells which may die apoptotically, since they have either a higher frequency of exchange aberrations or CA, respectively.

Keywords: *Glutathione, cell cycle, p53, mouse bone marrow*

Introduction

Aminothiols represent the most important group of radioprotective compounds. Reduced glutathione (GSH) is an endogenous thiol and has long been thought to affect the sensitivity of cells to radiation (Meister 1983). The working mechanism can partly be explained as a scavenging process of radicals induced in water and partly as a chemical participation in biochemical repair processes of injured DNA (Clark et al. 1984, Revesz et al. 1984, Chattopadhyay et al. 1999, Dutta et al. 2005). It has been shown that exogenous addition of GSH can effectively reduce radiation-induced micronuclei (Mazur 2000) and chromosome aberrations (CA) in different systems (Chatterjee & Jacob-Raman 1986). Reduction of short-term radiation lethality by chemical protectors alone or in a combined treatment was studied in adult male mice and it was observed that cysteine, GSH and mercaptoproprionylglycine (MPG) were less efficient radioprotectors than 2-(3-aminopropylamino)ethylsulfanyl phosphoric acid (WR-2721) (Maisin et al. 1993). However, cellular radiosensitivity is increased under the influence of buthionine sulfoximine (BSO), which specifically depletes the endogenous GSH level by inhibiting the enzyme γ-glutamylcysteine synthetase (Meister 1983, Yi et al. 1994). It has already been shown that depletion of endogenous GSH by BSO increases the frequency of CA that are induced by arecoline (Deb & Chatterjee 1998) and mitomycinC (Dev Giri & Chatterjee 1998) in mouse bone marrow cells (BMC).

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Earlier studies indicated that certain thiol compounds could remove radiation-induced cell-cycle delays measured by changes in the mitotic index in CHO cultures (Yu & Sinclair 1970, Kawasaki 1977). It was demonstrated previously that by employing fluorescence plus Giemsa staining techniques that GSH pretreatment reduced the 2 Gy-induced cell cycle delay and CA significantly but was unable to do so consistently at higher doses of radiation in muntjac lymphocytes (Chatterjee & Jacob-Raman 1986). The primary subcellular target responsible for radiation-induced delay in cell proliferation still remains undetermined. Earlier reports indicate that damage to the cellular and nuclear envelope (Myers 1970, King et al. 2004), cell division related proteins (Warenius et al. 2000), cAMP and cGMP metabolism (Fukumoto et al. 1999) and DNA (Lucke-Huhle et al. 1979, Lebedeva and Akhmamef'era 1996) contribute to the induction of radiation-induced cell cycle delays. Recently, it was shown in human lymphocytes that exogenous GSH removed the radiation-induced delay more convincingly for early first-division cells than late first-division cells and the delay-reducing effect of GSH did not correspond to a reduction in the frequency of CA (Ray & Chatterjee 2006). Therefore, the present study was carried out to investigate the role of GSH, either added exogenously or depleted by BSO, in the induction of cell cycle delays and CA by radiation in mouse BMC. In addition, the role of the cell cycle regulator proteins and the B-cell lymphoma 2 (Bcl-2) associated X protein (Bax) on the induction of cell cycle delays after irradiation was investigated and the influence of GSH on these proteins.

Materials and methods

Chemicals

DL-Buthionine-S,R-sulphoximine (BSO), Glutathione (GSH; L-γ-glutamyl-L-cysteinyl-glycine), Nonidet P-40, sodium dodecylsulphate, aprotinin, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), GSH-reductase and NADPH were obtained from Sigma Chemical Company (St Louis, MO, USA). Giemsa stain was obtained from BDH chemicals Ltd. (Dorset, UK), Primary Antibodies p53-Abs (DO7 + Bp53-12), p21 Ab-11, Bax Ab-5 and β-actin (anti-actin ACTN05) were obtained from Neomarker (Fremont, CA, USA). Secondary antibody rabbit antimouse IgG-Alkaline phosphatase conjugate and substrate for alkaline phosphatase, Bromo-4-chloro-3-indolyl Phosphate/Nitro Blue Tetrazolium (BCIP/NBT) were obtained from Bangalore Genie (Bangalore, India). Other chemicals used in the study were of analytical grade from reputable manufacturers.

Determination of GSH level in mouse BMC

Male Swiss-albino mice, aged 2–3 months and weighing 25–30 gm, were maintained in the laboratory in community cages in controlled-temperature room ($20 \pm 2°C$), 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. The rules of the Institutional Animal Care and Use Committee were strictly followed during the whole experiment and steps were taken to protect the welfare of the experimental animals.

The level of GSH, with or without BSO or GSH treatment, was estimated by the method of Akerboom and Sies (1981). BSO 4, 200 and 600 mg kg^{-1} were prepared in phosphate buffer solution (PBS, pH 7.4) and injected intraperitoneally. Total GSH was measured after 10 h of BSO treatment and in one case after 20 h of BSO treatment. In treatments with GSH, the dose of 400 mg kg^{-1} body weight for 10 h was used. An equal volume of PBS was injected into the control (BSO and GSH untreated) mice.

Freshly collected mouse BMC were flashed into ice-cold 0.1 M PBS and the volume was made up to 1 ml. Cells were counted in a haemocytometer and processed for determination of total GSH level as described earlier (Chattopadhyay et al. 1999). In brief, after deproteinization by 10% ice-cold 5-sulfosalicylic acid a 50 μl sample suspension was taken and added to 1 ml buffer (0.1 M ethylenediaminetetraacetic acid (EDTA) phosphate buffer, pH 7.0). Then 50 μl nicotinamide adenine dinucleotide phosphate reduced form (NADPH) (4 mg/ml), 20 μl DTNB (1.5 mg ml^{-1}) and 20 μl GSH reductase (6 units ml^{-1}) were added and the optical density of the samples was measured at 412 nm using a UV-visible spectrophotometer (Beckman model DU-640; Miami, Florida, USA). A standard curve was prepared from a stock solution of 10 mM GSH in 5% 5-sulfosalicylic acid (SSA) diluted to 1–50 nmol.

Treatment of mice and preparation of metaphases

For all experimental conditions either BSO or GSH was given prior to radiation exposure. BSO and GSH were dissolved in PBS just before treatment. BSO (200 mg kg^{-1}) was injected intraperitoneally 10 h prior to irradiation, whereas GSH alone, was administered 1 h prior to irradiation at the dose of 400 mg kg^{-1} body weight of mouse except in one set of mice where the dose of GSH was 700 mg kg^{-1}. An equal volume of PBS was injected into the control (BSO and GSH untreated) mice. At each data point 6 mice were used except in one case 4 mice were used.

Mice were kept in well ventilated two-tier acrylic cylinders and placed in a ^{60}Co Gamma chamber for whole-body exposure. The radiation doses used were 2, 4 and 5 Gy (dose-rate 7.06 Gy min^{-1} as determined by Fricke dosimetry). Cells were fixed at 13 h after radiation exposure, each preceded by 3 h colchicine (15 mg kg^{-1} body weight) treatment.

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

Western blot analysis

The expression of p53 and p21 proteins was analyzed by immunoblotting 2 and 6 h after irradiation (5 Gy) and for Bax 24 h after irradiation with or without BSO or GSH. Mouse BMC were isolated by flushing and washed with PBS. After centrifugation the pellet was processed for protein extraction. Cells were lysed 2, 6 and 24 h after irradiation in radioimmunoprecipitation buffer (0.1% sodium dodecyl sulphate (SDS), 2 mM EDTA, 1% NP-40, 1% sodium deoxycholate, 50 mM sodium fluoride and 100 U/ml aprotinin). After 30 min of incubation on ice, the cell lysates were centrifuged for 15 min at 4°C and the amount of protein was determined using the bicinchonic acid protein assay (Smith et al. 1985). Equal amount of protein (40 μg) from each sample were loaded in each well, and equal loading was verified by immunoblotting with actin antibodies (anti-actin ACTN05; Neo-Markers). Electrophoresis was performed in 12% polyacrylamide separating gel and 5% stacking gel. Proteins were transferred to a 0.45 μm nitrocellulose membrane (Sigma) following the standard protocols. The membranes were probed with a 1:1000 dilution of a mouse monoclonal antibody against p53 Ab-8, anti-p21 Ab-6 and anti-Bax Ab-5 (Neo-Markers). Blots were washed 3 times for 10 min each in TBST buffer pH 7.6 (1 M Tris Cl, 5 M NaCl and 0.05% Tween 20) and incubated with secondary antibody (alkaline-phosphatase conjugated anti-mouse IgG, 1:2000; Bangalore Genei) for 1 h at room temperature. After extensive washing, the blot was immersed in 4 ml substrate solution of BCIP/NBT (Bangalore Genei). Sufficient staining was obtained within 15 min. The whole experiment was repeated twice. The intensity of the protein bands were scanned for densitometry by Kodak Molecular Imaging Software (Rochester, NY, USA).

Scoring and statistical analysis

Slides were randomly coded. Cell cycle kinetics were determined by scoring of the mitotic index (MI), MI% = Number of metaphases/Total number of cells × 100. CA were scored in four categories in mouse BMC: (i) Exchanges (all interchanges involving two or more different chromosomes); (ii) sister chromatid unions (SCU, intra-arm interchanges between lesions within a chromosome); (iii) isochromatid breaks, and (iv) simple chromatid breaks. 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 group was evaluated using Student's *t*-test.

Results

Level of reduced glutathione in mouse BMC

The level of reduced GSH in mouse BMC with or without BSO is shown in Table I. GSH concentration in BMC ranged from 14–22 nmol per 10^{-6} cells with an average of 17 ± 0.7 nmol per 10^{-6} cells. This GSH concentration was depleted by 18%, 35% and 77% after 10 h treatment with 4, 200 and 600 mg kg^{-1} BSO, respectively in comparison to control. When BSO (200 mg kg^{-1}) was given for 20 h the GSH concentration was depleted by 24% of the control. The statistical difference between the mean GSH concentration of 200 and 600 mg kg^{-1} of BSO with respect to untreated control was significant. The concentration of reduced GSH was increased to 18% of the control after 10 h treatment with 400 mg kg^{-1} GSH.

Table I. Levels of glutathione in mouse BMC after a single treatment of BSO or GSH alone.

Treatment	Hours of incubation	No. of mice	Range of total GSH (nmol/10^6 cells)	Mean ± SEM (reduction%)
0		9	14–22	17 ± 0.7
BSO				
4 mg	10	4	11–17	14 ± 1.3 (−18)
200 mg	10	5	9–13	11 ± 1.0* (−35)
200 mg	20	5	8–16	13 ± 2.0* (−24)
600 mg	10	6	2–5	4 ± 1.0* (−77)
GSH				
400 mg	10	4	13–25	20 ± 2.2 (+18)

*$p < 0.05$ a Student's *t*-test compared to the control value.

Effect of BSO and GSH on delay in cell cycle after irradiation

The γ-ray induced cell cycle delays in BSO and GSH-treated and untreated mouse BMC are shown in Figure 1. A dose-dependent reduction of MI after irradiation was observed (2 with 2 Gy and 0.5 with 5 Gy). Presence of GSH before irradiation increased the MI significantly. However, the addition of GSH alone in unirradiated samples reduced the frequency of MI. A higher concentration of GSH (700 mg kg^{-1}) pretreatment increased the MI more than what it was in the case of 400 mg kg^{-1} pretreatment (star symbol in the Figure 1). Irradiation of BSO-treated cells improved the MI compared to cells exposed to higher radiation doses.

Effect of BSO and GSH on CA induced by radiation

Radiation-induced CA were studied as positive control to GSH + γ-ray and BSO + γ-ray treated samples, and the data are presented in Figures 2a–2d. Aberrant metaphases and all the aberrations were observed to be increased after irradiation in a dose-dependent manner. Pretreatment with GSH before radiation significantly reduced the frequency of CA, however, the frequency of aberrant metaphases was not reduced at higher doses of radiation. GSH (400 mg kg^{-1}) pretreatment protected all types of CA induced by 2 Gy; however, it failed to protect exchanges at higher radiation doses. Interestingly, pretreatment with higher concentration of GSH (700 mg kg^{-1}) failed to protect aberrations induced by 4 Gy and the frequency of exchanges was found to increase significantly. The frequency of CA increased significantly when radiation was given to BSO-treated cells, however the frequency of exchange aberrations was reduced in the presence of BSO.

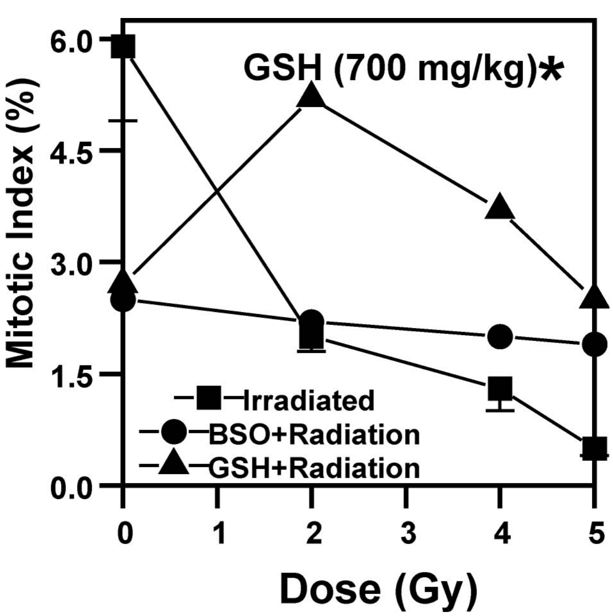

Figure 1. The effect of γ-radiation with or without BSO (200 mg kg^{-1}) or GSH (400 mg kg^{-1}) pretreatment on the percentage of mitotic index in mouse BMCs. BSO was given 10 h or GSH was given 1 h prior to irradiation. The star symbol shows data point from the results obtained with higher doses of GSH. Data are the means ± standard errors of the mean.

Western blot analysis

Representative results of the Western blot analysis are illustrated in Figures 3a and 3b and the densitometric intensity of bands were presented as histograms showing the relative induction of p53 (Figure 3c), p21 (Figure 3d) and Bax (Figure 3e) proteins. β-actin was included as an internal indicator in all analyses to control for potential discrepancy in sample loading. The levels of p53 and p21 proteins were increased in samples after 2 and 6 h post-irradiation compared to the unirradiated control. However, irradiation to BSO-treated cells caused a 28-fold induction in the level of p53 as compared to radiation alone after 6 h of irradiation, whereas for p21 the level was a 9-fold higher in BSO-treated cells after 2 h of radiation. Interestingly, both p53 and p21 proteins after radiation of GSH-pretreated cells showed reduced levels in comparison to the cells exposed to radiation alone.

The expression of Bax increased 24 h after radiation when compared to the unirradiated sample and its level increased further when radiation was given either to BSO-treated or GSH-treated cells (Figures 3b and 3e).

Discussion

BSO and GSH have been used to evaluate the effect of endogenous GSH levels on radiosensitization of mouse BMC *in vivo*. Data shown here indicate radiation causes delay in cell passage through the cell cycle and induces CA. GSH pretreatment reduced both the radiation-induced delay in cell cycle and the frequency of CA. However, in BSO-treated samples the frequency of radiation-induced CA was found to increase though the extent of delay of cells in the cell cycle was reduced.

In the present study, an incubation period with BSO (200 mg kg^{-1}) for 10 h showed 35% reduction in the level of endogenous GSH with respect to untreated control cells. The 10 h incubation period was chosen in this study since the intermediate rate of depletion of GSH by BSO in the mouse BMC has a nadir at 8–12 h as was demonstrated by Lee et al. (1987). However, it was observed that the level of GSH was slightly more when BSO was given for 20 h. Such a small decrease in the level of GSH could be due to recovery of endogenous GSH, which

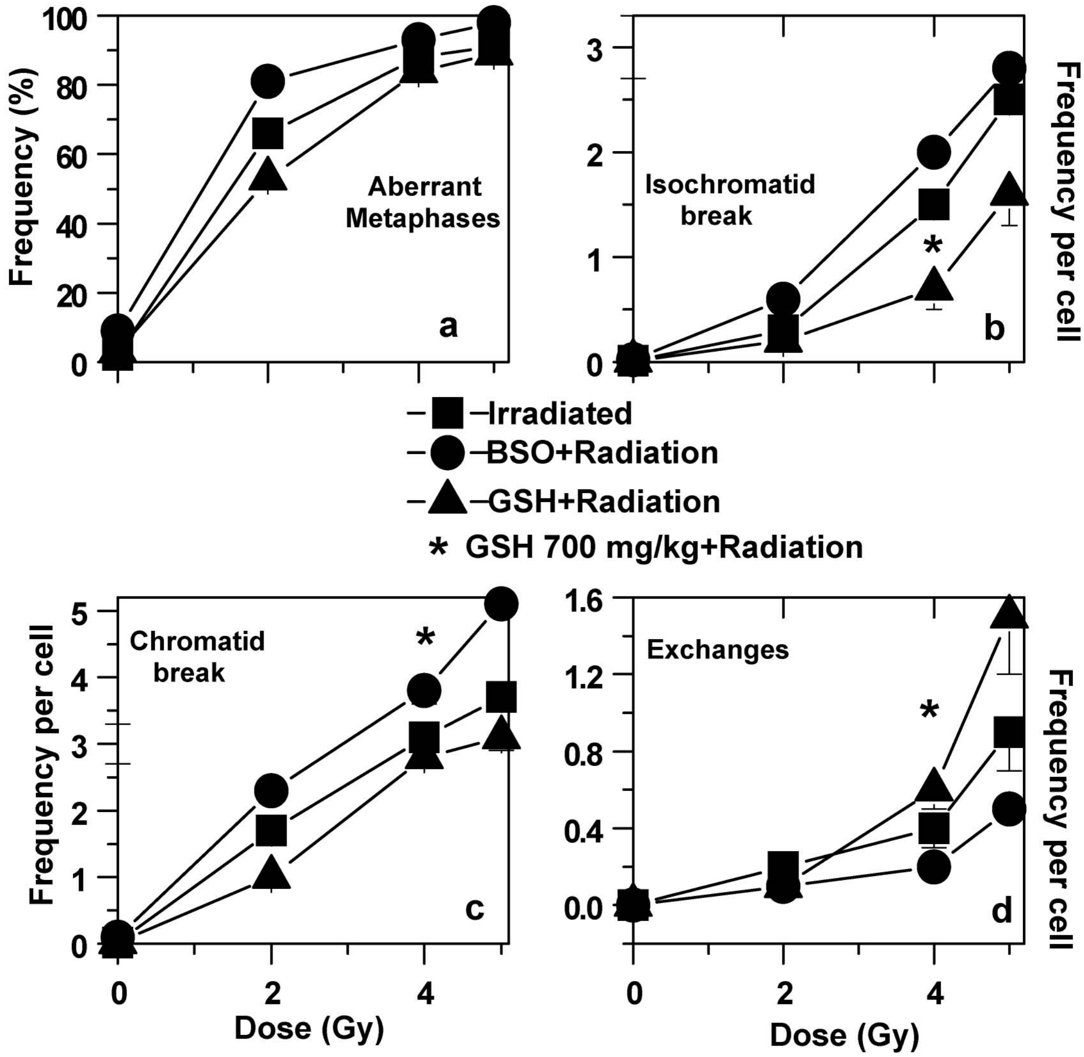

Figure 2. The effect of γ-radiation with or without BSO (200 mg kg^{-1}) or GSH (400 mg kg^{-1}) pretreatment on the frequency of (a) aberrant metaphases (b) iso-chromatid break (c) chromatid break and (d) exchanges in mouse BMCs. The star symbol shows data point from the results obtained with higher doses of GSH. The values shown are the means $\pm$ standard errors of the mean.

normally starts after 14 h of BSO-treatment, and reached pretreatment values at 72 h in mouse BMC (Lee et al. 1987). A small increase in endogenous GSH level was observed at 10 h after adding GSH exogenously. A marked increase in intracellular cysteine concentration after GSH-treatment and increase of GSH by $\sim$20% was achieved 6–8 h after GSH-treatment in Chinese hamster V79 cells by Wardman et al. (1992).

Exogenous addition of GSH has been shown to reduce the frequency of radiation-induced micronuclei (Mazur 2000) and CA in mammalian cells (Chattopadhyay et al. 1999). However, reports on the influence of GSH on radiation-induced delay in cell kinetics are scanty. There are several reports supporting the notion that cells whose division is delayed are more apt to have aberrant chromosomes than those that enter mitosis early (Boei et al. 1996, Hoffmann et al. 2002). It has also been demonstrated that the cells propagating a dicentric chromosome divide slowly (Hill & Bloom 1987, Khosland et al. 1987). It has been shown earlier that at equal levels of CA induction by bleomycin (Blem) and X-rays, the cell cycle delay is greater with X-rays than with Blem (Chatterjee & Jacob-Raman 1988). A similar observation was made by Scott and Zampetti-Bosseler (1985). They reported that the induction of G2-delay is greater with X-rays than Blem. Such comparative data between X-rays and Blem suggest that the significant cell-cycle delay caused by X-irradiation and its absence in Blem treatment, despite equivalent numbers of CA, may be due to factors additional to DNA damage in the case of X-irradiation. It is important to note that cell-membrane and cytoplasmic proteins play a role in the regulation of lymphocyte proliferation (Noelle & Lawrence 1981). Therefore, we would like to know whether the induction of CA and delay in cell cycle

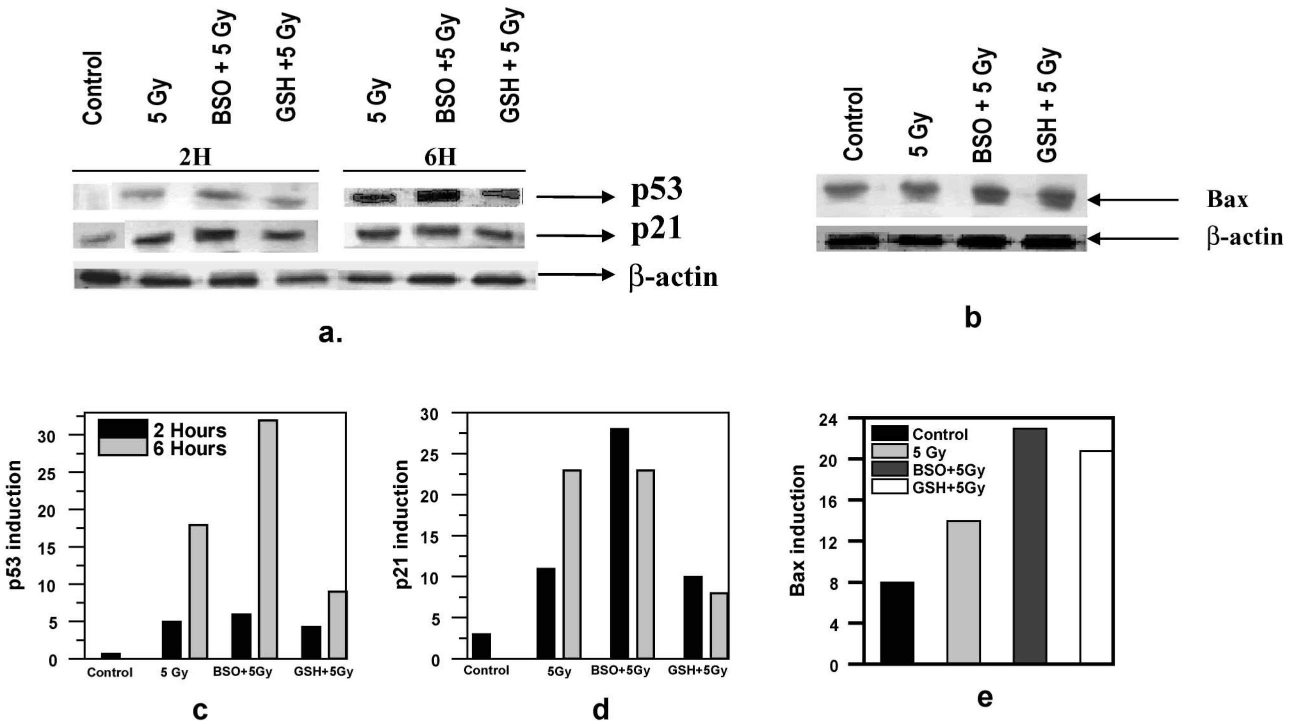

Figure 3. Immunoblotting analysis of (a) p53 and p21 proteins after 2 and 6 h of irradiation and (b) Bax protein after 24 h of irradiation with or without BSO (200 mg kg^{-1}) or GSH (400 mg kg^{-1}) in mouse BMC. The whole mouse was exposed to 5 Gy γ-rays. Histogram showing the relative induction of p53 (c), p21 (d) and Bax (e) in mouse BMC after irradiated with or without BSO or GSH.

are interlinked and whether or not the exchange aberrations play any role in the induction of cell cycle delay. Recently, it was shown that in human lymphocytes, addition of exogenous GSH removed the radiation-induced delay more convincingly in early 1st division cells than in the late 1st division cells. It was proposed that this delay-reducing effect of GSH did not corresponding to a reduction in the frequency of CA, since GSH pretreatment reduced the radiation-induced CA significantly in late-arising metaphases irradiated at Go stage but failed to reduce the delay in cell cycle (Ray & Chatterjee 2006). From the present data, it seems that CA and the delay in cell proliferation may not always be linked, since irradiation of BSO-treated cells causes more aberrations but lesser delay in the cell cycle than in BSO-untreated cells. Moreover, GSH-pretreatment reduced the radiation-induced delay in the cell cycle but increased the frequency of exchange aberrations particularly at higher radiation doses. It was also observed that the radiation-induced delay in the cell cycle is reduced more significantly by a higher concentration of GSH (700 mg kg^{-1}) whereas the frequency of exchanges increased. It is worth mentioning that at the higher concentration of GSH-pretreatment the frequency of CA induced by 4 Gy was not reduced. The explanation of this observation is not clear; however, it has been demonstrated that the induction of high GSH levels (100–200% above the normal level) provides only a small protection (Vos 1992). Therefore, it seems that the free radicals and reactive oxygen species (ROS) generated after radiation might have altered the cell membrane and cytoplasmic protein thiol groups besides damaging DNA. The presence of GSH might lead to a reduction of the damages to these targets by scavenging the free radicals and thus reducing in turn the cell cycle delays.

It has been demonstrated that cells can remain arrested in the G1 phase of the cell cycle following DNA damage caused by ionizing radiation in order to repair or recover from the induction of DNA lesions (Rudoltz et al. 1996). It is well known that nuclear phosphoprotein encoded by the tumour suppressor gene p53 is a crucial component of the cellular pathways that are invoked in response to DNA damage. Several studies demonstrated that p53 regulates the G1 checkpoint through the transcriptional upregulation of the cyclin-dependent kinase inhibitor p21/Waf1/Cip1 (Harper et al. 1993). In the present study, the level of p53 protein was raised marginally after 2 h and significantly after 6 h of irradiation. However, the level of p21 was enhanced significantly after 2 h of irradiation. The present data indicate that a significant rise in p21 in mouse BMC could be a factor responsible for radiation-induced cell cycle arrest. The presence of GSH before radiation reduced the level of both p53 and p21 proteins with respect to radiation alone and minimized the delay in cell cycle. Such reduced levels of p53 and p21 could be the additional factor causing the GSH-mediated reduction in the radiation-induced cell cycle delay besides protection of DNA damage, cell membrane and cytoplasmic proteins by scavenging radicals. On the other hand, irradiation to BSO-treated cells increased the frequency of CA and showed higher level of p53 and p21, nonetheless the cell cycle delay induced by radiation was reduced. Such a reduction in cell cycle delay could be a causative factor for increasing the frequency of radiation-induced CA in BSO-treated cells since cells would not get enough time to repair. However, there are reports that BSO can scavenge radiation-derived free radicals and affords protection against irradiation to dry barley seeds (Singh & Kesavan 1993) and in athymic mice carrying a tumor xenograft (Halperin et al. 1992). Therefore, a few molecules of BSO, either in the cytoplasm or close to cell membrane during radiation, might possibly protect the cell membrane, but not DNA, from radiation. Such BSO-mediated protection could be the reason for not increasing the delay in cell cycle in spite of having an increased frequency of CA.

The present data support our earlier observation regarding the involvement of GSH in exchange aberration formation. Exchange aberration formation is thought to arise as a consequence of illegitimate reunion (misrejoining) of free ends involving different DNA double strand breaks (dsb) (Cornforth & Bedford 1993). It was found that the repair system involved in the rejoining of DNA single strand breaks (ssb) induced oxically was different from that involved in the rejoining of hyoxically induced ssb and was clearly dependent upon GSH (Edgren et al. 1981, Revesz et al. 1984). The involvement of GSH in exchange aberration formation is supported by the observation of an increased frequency of exchange aberrations and decreased frequency of deletions in GSH/GSH-ester post-treated human lymphocytes irradiated at 4°C (Chattopadhyay et al. 1999). Recently, it was shown that combined treatment of Blem and radiation induced a high frequency of CA, particularly, exchange aberrations and interstitial deletions. This frequency was reduced when the cells were pretreated with BSO (Dutta et al. 2005). In the present study, the frequency of exchange aberrations (mostly chromatid-type) was increased, whereas that of chromatid breaks reduced in GSH-pretreated irradiated cells. This could be due to an enhancement in rejoining (both restitution and illegitimate reunion) of radiation-induced DNA dsb under the influence of increased endogenous GSH.

It seems that GSH or BSO pretreatment improves the cell kinetics of irradiated cells in spite of having higher frequency of exchanges and CA, respectively. Such aberration-loaded cells may die apoptotically since the cells with exchange aberrations or unstable aberrations die apoptotically (Bassi et al. 2003). The present observed higher expression of Bax in irradiated GSH or BSO-pretreated cells are consistent with such a phenomenon since Bax has a proapoptotic function (Bedner et al. 2000, Kobayashi et al. 2002).

In conclusion, our results indicate that the induction of CA and delays in cell kinetics may not always be interlinked and the level of endogenous GSH can exert its influence on these parameters. Exogenous addition of GSH protects against CA uniformly at lower doses of radiation but not at higher doses. Both GSH and BSO-pretreated cells showed a reduced delay in cell kinetics after irradiation and may die apoptotically, since they have either more exchanges or CA.

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References

Akerboom TPS, Sies H. 1981. Assay of glutathione, glutathione disulfide and glutathione mixed disulfides in biological samples. Methods in Enzymology 77:373–382.

Bassi L, Carloni M, Meschini R, Fonti E, Palitti F. 2003. X-irradiated human lymphocytes with unstable aberrations and their preferential elimination by p53/surviving-dependent apoptosis. International Journal of Radiation Biology 79:1–12.

Bedner E, Li X, Kunicki J, Darzynkiewicz Z. 2000. Translocation of Bax to mitochondria during apoptosis measured by laser scanning cytometry. Cytometry 41:83–88.

Boei JJWA, Vermeulen S, Natarajan AT. 1996. Detection of chromosomal aberrations by fluorescence *in situ* hybridization in the first three postirradiation divisions of human lymphocytes. Mutation Research 349:127–135.

Chatterjee A, Jacob-Raman M. 1988. A comparison of aberration distribution and cell cycle progression in cells treated with bleomycin with those exposed to X-rays. Mutation Research 202:51–58.

Chatterjee A, Jacob-Raman M. 1986. Modifying effect of reduced glutathione on X-ray-induced chromosome aberrations and cell cycle delay. Mutation Research 175:73–82.

Chattopadhyay A, Deb S, Chatterjee A. 1999. Modulation of the clastogenic activity of γ-irradiation in buthionine sulfoximine mediated glutathione depleted mammalian cells. International Journal of Radiation Biology 75:1283–1291.

Clark EP, Epp ER, Biaglow JE, Morse-Gaudio M, Zacho E. 1984. Glutathione depletion, radiosensitization and misonidazole potentiation in hypoxic Chinese hamster ovary cells by buthionine sulfoximine. Radiation Research 98:379–380.

Cornforth MN, Bedford JS. 1993. Ionizing radiation damage and its early development in chromosomes, In: Lett JT, Sinclair WK, editors. Advances in radiation biology, Vol. 17: DNA damage caused by radiation. London: Academic Press. pp 423–496.

Deb S, Chatterjee A. 1998. Influence of buthionine sulfoximine and glutathione on arecoline induced chromosomal damage and sister chromatid exchange in mouse bone marrow *in vivo*. Mutagenesis 13:243 – 248.

Dev Giri S, Chatterjee A. 1998. Modulation of Mitomycin C induced sister chromatid exchanges and cell cycle delay by buthionine sulfoximine and reduced glutathione in mouse bone marrow cells *in vivo*. Mutation Research 413:227 – 234.

Dutta A, Chakraborty A, Saha A, Ray S, Chatterjee A. 2005. Interaction of radiation and bleomycin-induced lesions and influence of glutathione level on the interaction. Mutagenesis 20:339 – 345.

Edgren M, Revesz L, Larson A. 1981. Induction and repair of single strand DNA breaks after X-irradiation of human fibroblast deficient in glutathione. International Journal of Radiation Biology 40:335 – 340.

Fukumoto S, Koyama H, Hosoi M, Yamakawa K, Tanaka S, Morii H, Nishizawa Y. 1999. Distinct role of cAMP and cGMP in the cell cycle control of vascular smooth muscle cells: cGMP delays cell cycle transition through suppression of cyclin D1 and cyclin-dependent kinase 4 activation. Circulation Research 85:985 – 991.

Halperin EC, Brizel DD, Honore G, Sontag MR, Griffith OW, Bigner DD, Friedman HS. 1992. The radiation dose-response relationship in a human slioma xenograft and an evaluation of the influence of glutathione depletion by buthionine sulfoximine. International Journal of Radiation Biology 24:103 – 109.

Harper JW, Adami G, Wel N, Keyomarsi K, Elledge S. 1993. The p21 cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75:805 – 816.

Hill A, Bloom K. 1987. Genetic manipulation of centromere function. Molecular Cell Biology 9:1368 – 1370.

Hoffmann GR, Sayer AM, Littlefield LG. 2002. Higher frequency of chromosome aberrations in late-arising first-division metaphases than in early-arising metaphases after exposure of human lymphocytes to X-rays in Go. International Journal of Radiation Biology 78:765 – 772.

Kawasaki S. 1977. Protective effect of various thiol compounds on radiation-induced mitotic delay in cultured mammalian cells (L-5). International Journal of Radiation Biology 32:577 – 581.

Khosland D, Rutledge L, Fitzgerald-Hayes M, Hartwell LH. 1987. A genetic analysis of dicentric minichromosomes in *Saccharomyces cerevisiae*. Cell 48:801 – 812.

King MC, Raposo G, Lemmon MA. 2004. Inhibition of nuclear import and cell-cycle progression by mutated forms of the dynamin-like GTPase MxB. Procedings of National Academy of Sciences USA 101:8957 – 8962.

Kobayashi T, Hidehiko S, Morikawa J, Ueno S, Katayama N, Zhang WEI, Shiku H. 2002. Bax-induction alone is sufficient to activate apoptosis cascade in wild-type Bax-bearing K562 cells and the initiation of apoptosis requires simultaneous caspase activation. International Journal of Oncology 20:723 – 728.

Lebedeva LI, Akhmamet'eva EM. 1996. Possible mechanisms of the emergence of chromosome aberrations. VIII. Cytogenetic analysis of the dynamics of repair and occurrence of potential chromosome damage in bone marrow cells of gamma-irradiated mice. Genetika 32:804 – 809.

Lee FYF, Allunis-Turner MJ, Siemann DW. 1987. Depeltion of tumor versus normal tissue glutathione by buthionine sulfoximine. British Journal of Cancer 56:33 – 38.

Lucke-Huhle C, Blakely EA, Chang PY, Tobias CA. 1979. Drastic G2 arrest in mammalian cells after irradiation with heavy-ion beams. Radiation Research 79:97 – 112.

Maisin JR, Albert C, Henry A. 1993. Reduction of short-term radiation lethality by biological response modifiers given alone or in association with other chemical protectors. Radiation Research 135:332 – 337.

Mazur L. 2000. Radioprotective effects of the thiols GSH and WR-2721 against X-ray induction of micronuclei in erythroblasts. Mutation Research 468:27 – 33.

Meister A. 1983. Selective modification of glutathione metabolism. Science 220:472 – 477.

Myers DK. 1970. Some aspects of radiation effects on cell membranes. Advance Biology Medical Physics 13:219 – 234.

Noelle RJ, Lawrence DA. 1981. Modulation of T-cell function II. Chemical basis for the involvement of cell surface thiol-reactive sites in control of T-cell proliferation. Cell Immunology 60:453 – 469.

Ray S, Chatterjee A. 2006. Influence of endogenous glutathione level on X-ray induced cell cycle delay in human lymphocytes. Cell Proliferation 39:37 – 47.

Revesz L, Edgren M, Nishidai T. 1984. Mechanisms of inherent radioprotection in mammalian cells. In: Sugahara T, Ueno Y, editors. Modification of radiosensitivity in cancer treatment. San Diego: Academic Press. pp 13 – 29.

Rudoltz MS, Kao G, Blank KR, Muschel RJ, McKenna WG. 1996. Molecular biology of the cell cycle: Potential for therapeutic applications in radiation oncology. Seminal Radiation Oncology 6:284 – 294.

Scott D, Zampetti-Bosseler F. 1985. Relationship between chromosome damage, cell cycle delay and cell killing induced by bleomycin on X-rays. Mutation Research 151:83 – 88.

Singh SP, Kesavan PC. 1993. Post-irradiation modification of O_2, N_2 and N_2O-mediated damage in dry barley seeds by glutathione and cysteine: Probable radiation chemical events. International Journal of Radiation Biology 63:483 – 491.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. 1985. Measurement of protein using bicinchonic acid. Analytical Biochemistry 150:76 – 85.

Vos O. 1992. Role of endogenous thiols in protection. Advance Space Research 12:201 – 207.

Wardman P, Madeleine F, Dennis MA, Stratford MRL, White J. 1992. Extracellular: Intracellular and subcellular concentration gradients of thiols. International Journal of Radiation Oncology Biology and Physics 22:751 – 754.

Warenius HM, Jones M, Gorman T, McLeish R, Seabra L, Barraclough R, Rudland P. 2000. Combined RAF1 protein expression and p53 mutational status provides a strong predictor of cellular radiosensitivity. British Journal of Cancer 83:1084 – 1095.

Yi X, Ding L, Jin Y, Ni C, Wang W. 1994. The toxic effects, GSH-depletion and radiosensitivity by BSO on retinoblastoma. International Journal of Radiation Oncology Biology and Physics 29:393 – 396.

Yu CK, Sinclair WK. 1970. Protection by cysteamine against mitotic delay and chromosomal aberrations induced by X-rays in synchronized Chinese hamster cells. Radiation Research 43:357 – 371.