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An Alternative Mechanism for Radioprotection by Dimethyl Sulfoxide; Possible Facilitation of DNA Double-strand Break Repair

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Dimethyl sulfoxide/Indirect action/NHEJ/Oxidative stress.

The radioprotective effects of dimethyl sulfoxide (DMSO) have been known for many years, and the suppression of hydroxyl (OH) radicals induced by ionizing radiation has been thought to be the main cause of this effect. However, the DMSO concentration used was very high, and might be toxic, in earlier studies. In the present study, we administered a lower, non-toxic concentration (0.5%, i.e., 64 mM) of DMSO before irradiation and examined its radioprotective effects. Colony formation assay and micronucleus assay showed significant radioprotective effects in CHO, but not in xrs5, which is defective in the repair function of DNA double-strand breaks. The levels of phosphorylated H2AX and the formation of 53BP1 foci 15 minutes after irradiation, which might reflect initial DNA double-strand breaks, in DMSO-treated CHO cells were similar to those in non-treated cells, suggesting that the radioprotective effects were not attributable to the suppression of general indirect action in the lower concentration of DMSO. On the other hand, 2 hours after irradiation, the average number of 53BP1 foci, which might reflect residual DNA double-strand breaks, was significantly decreased in DMSO-treated CHO cells compared to non-treated cells. The results indicated that low concentration of DMSO exerts radioprotective effects through the facilitation of DNA double-strand break repair rather than through the suppression of indirect action.

INTRODUCTION

Dimethyl sulfoxide (DMSO) has been widely used as a radical scavenger due to its specific reactivity with hydroxyl (OH) radicals. In the radiation biology field, DMSO has been used as a radioprotective agent because it is regarded as a suppressor of indirect action during irradiation with lower LET radiation. According to the studies by Roots and Okada,[1,2] diffused OH radicals generated by ionizing radiation within a DNA area react with a radical scavenger such as alcohol, and then DNA strand breaks induced during irradiation are reduced. However, this idea relies on several assumptions. For example, the distribution of OH radicals has been thought to distribute into the folded DNA of chromatin, although the precise nature of mammalian chromatin is not yet fully understood. Also, cells in these assays were treated with a high concentration of alcohol or DMSO for a short period (several minutes), because the radical scavengers should remain in the DNA areas during irradiation. In this case, other biological effects have been disregarded; therefore, the mechanism of the biological effects by DMSO, except for the suppression of indirect effects, should be examined.

We have been investigating the biological characteristics of radicals in irradiated cells, and DMSO was used as a radical scavenger.[3–5] In these studies, we confirmed that 2-hour treatment with 1.28 M (10%) DMSO could suppress lethal effects, such as chromosome aberration, in irradiated cells, but the concentration of DMSO was very toxic. On the other hand, we have also found that the bystander signal between irradiated and non-irradiated cells was suppressed by treatment of irradiated cells with non-toxic 64 mM (0.5%) DMSO for 1 hour,[6,7] therefore suggesting that DMSO can suppress signal transduction, such as bystander signals in irradiated cells. As other groups also reported pleiotropic effects of DMSO, such as apoptosis,[8,9] cell cycle,[9,10] and differentiation,[11] we should have a wider view on the radioprotective effect by DMSO and reconsider its mechanism.

Here, we used 0.5% DMSO to identify the non-toxic bio-

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logical effects on living cells. The results implied that the radioprotective effects by DMSO might be caused through the facilitation of DNA double-strand breaks repair rather than through the suppression of indirect action during X-irradiation.

MATERIAL AND METHODS

Cell culture

Chinese hamster ovary (CHO) cells and xrs5 cells (Ku80 deficient) were kindly supplied by Dr Tom K. Hei, (Columbia University, New York, USA). Cells were cultured in MEM alpha medium (Invitrogen Ltd, California, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone Laboratories, Inc., Logan, UT, USA). Cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. The vector containing human Ku80 gene was kindly provided by Dr. Keiji Suzuki (Nagasaki University, Nagasaki, Japan), and the plasmid was introduced into xrs5 cells by FuGENE HD transfection reagent (Roche Diagnostics K.K., Tokyo, Japan), and the cells were cultured in MEM alpha containing 400 μg/ml of G418 and 10% FBS. Mouse cell lines, derived from normal CB09 mouse (CB09) and *scid* mouse (SD01) were kindly provided by Dr. Seiji Kodama (Osaka Prefecture University), and these cells were cultured in MEM alpha medium supplemented with 10% FBS.

X-irradiation

Cells were irradiated with X-rays from an X-ray generator (Softex, Tokyo, Japan) at 150 kVp and 5 mA with a 0.1-mm copper filter. The dose rate of X-ray was 0.492 Gy/min.

DCFH test

Intracellular oxidative stress levels were examined by DCFH test as described previously.[6,12] Briefly, cells were treated with 1 μM DCFH-DA (Invitrogen) in PBS plus solution for 30 min. Cells were then suspended in PBS solution and the fluorescent intensity of the 2', 7' - dichlorofluorescein (Invitrogen) was measured with a fluorescence spectrophotometer F2000 (Hitachi, Tokyo, Japan). The excitation and emission wavelengths were 503 nm and 524 nm, respectively. Cells suspended in a tube, after DCFH-DA treatment, were irradiated with X-rays and fluorescence intensities were measured immediately after irradiation. When cells were treated with DMSO (Sigma-Aldrich Japan, Tokyo, Japan), cells were treated with 64 mM (0.5%) DMSO in normal medium for 30 min and then the medium was changed to PBS solution containing DCFH and DMSO for further incubation for 30 min.

Colony formation assay

To investigate cell survival after X-irradiation, cells were harvested and suspended in medium, and diluted cells were inoculated into 100 mm dishes for colony formation. Ten days later, colonies were fixed with ethanol and stained with 3% Giemsa solution. When cells were treated with DMSO, 64 mM (0.5%) DMSO was administered for 1 hour before and during X-irradiation.

Micronucleus assay

To investigate the induction of micronuclei by direct X-irradiation, exponentially growing cells in 6-well plates were irradiated with X-rays. Samples were prepared as described previously.[11,12] Immediately after irradiation, cells were treated with 2 μg/ml cytochalasin B for 24 hours. When cells were treated with DMSO for 1 hour before and during irradiation, DMSO was removed at the same time as adding cytochalasin B. They were then harvested and treated with 3 ml hypotonic (0.1 M) KCl for 20 min, and fixed with 3 ml methanol-acetic acid (5:1). The cell suspensions were centrifuged at 1,200 rpm for 5 min, the supernatant removed and cells resuspended in 4 ml methanol-acetic acid solution and incubated on ice for 5 min. After further centrifugation, the supernatant was removed and 0.5–1 ml methanol -acetic acid solution was added. Cells were resuspended and a sample was placed onto slides and stained with 7.5% Giemsa for 20 min. Micronuclei per 1000 binucleated cells were counted.

Immunofluorescent staining of Phoshphorylated H2AX and 53BP1

To estimate the induction of DNA double-strand breaks immediately after irradiation, cells on 22 × 22 mm cover slips in 35 mm dishes were irradiated with X-rays.[13–15] Fifteen minutes after irradiation, the cells were fixed with 4% formaldehyde in PBS, permeabilized for 10 min on ice in 0.5% Triton X-100 in PBS, and washed thoroughly with PBS. The cover slips were then incubated with anti-phosphorylated histone H2AX at serine 139 antibody (Upstate Biotechnology Inc., NY, USA) or anti-53BP1 antibody (Bethyl Laboratories, TX, USA) in TBS-DT (20 mM Tris-HCl, 137 mM NaCl, pH 7.6, containing 50 mg/ml skim milk and 0.1% Tween-20) for 2 hours at 37°C. The primary antibodies were washed with PBS, and Alexa Fluor 488-labeled anti-mouse IgG and Alexa Fluor 594-labeled anti-rabbit IgG secondary antibodies (Invitrogen) were added. The cover slips were incubated for 1 hour at 37°C, washed with PBS, and sealed onto glass slides with 0.05 ml PBS containing 10% glycerol (Wako, Osaka, Japan) and 2 μg/ml DAPI (4',6-diamidino-2-phenylindole; Invitrogen, CA, USA). The cells were examined using both an Olympus fluorescence microscope (Olympus, Tokyo, Japan) and Keyence fluorescence microscope (Keyence, Osaka, Japan), and the green intensity of the phospho-H2AX signal on digitized images was analyzed using Dynamic Cell Count (Keyence) or Adobe Photoshop version 7.0 (Adobe Systems Inc., CA, USA). The total pixels of the high intensity for green phosphorylated H2AX signals 15 min after 0.5 Gy-irradiation in cell

populations were determined by the Keyence software "Dynamic Cell Count" (Fig. 4). For the 53BP1 focus, the numbers of red foci for 53BP1 were counted on digitized images of Adobe Photoshop or Image J. The average foci in CHO cells were determined in 600 cells from the three independent studies. In the time course analysis of 53BP1 foci (Fig. 5 and Table 2), cells were treated with 0.5% DMSO 1 hour before and during X-irradiation, and the medium were changed into fresh medium immediately after irradiation. Cells were fixed 15 min (0.25 hours), 1 hour, 2 hours, 6 hours, and 24 hours after irradiation, and 53BP1 imaging were prepared as described above. One hundred cells were analyzed in each group.

Statistical analysis

Statistical analysis was performed using Student's *t* test.

RESULTS

Reduced level of oxidative stress by DMSO treatment

First we examined the relationship between DMSO concentration and the DCFH fluorescence value indicating oxidative stress was examined. Results revealed no reduced levels of DCFH at 0.5% DMSO concentration under non-irradiated conditions. Moreover, morphological changes and cellular detachment related to the toxic effect were not observed in 0.5% DMSO treatment for 1 hour (data not shown). Therefore, 0.5% DMSO for 1 hour is thought to be non-toxic for CHO cells, and we used this concentration in further experiments. When cells were irradiated with 1 Gy of X-rays, oxidative stress was approximately 2.5 times higher than in non-irradiated cells. In the 0.5% DMSO treatment, oxidative stress was not reduced under non-irradiated condition but was reduced in 1 Gy-irradiated cells (Fig. 1), suggesting that this DMSO treatment can suppress OH radicals induced by X-irradiation, but had little effects on metabolically oxidative radicals in non-irradiation.

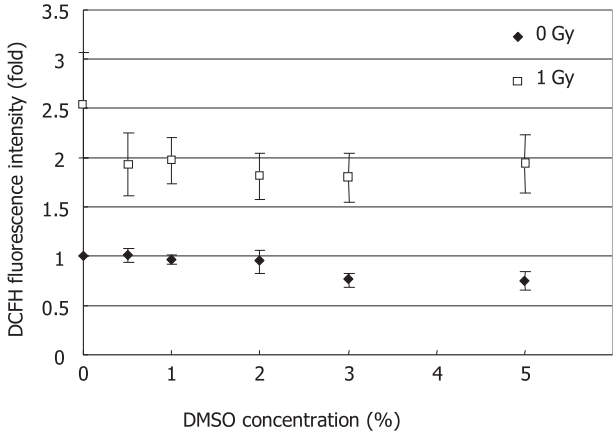

Fig. 1. Effects of DMSO on intracellular oxidative stresses. Oxidative stress levels were measured by DCFH assay. Intensity of DCFH fluorescence is expressed as a relative value against that of cells without irradiation and DMSO treatment. Closed and open symbols indicate non-irradiated (0 Gy) and 1 Gy-irradiated conditions, respectively. Each plot presents as an average of three independent analyses. Error bars indicate standard deviations.

Reduced level of DNA double-strand breaks by DMSO treatment

In order to measure the effects of DMSO in the formation of DNA double-strand breaks immediately after irradiation, we examined the level of 53BP1 foci 15 min after irradiation. As shown in Fig. 2a, 53BP1 foci 15 min after irradiation were clearly observed in the nucleus. The treatments with higher concentrations of DMSO (2 and 5%) reduced the number of foci in both of CHO and xrs5, although little effects were observed in 1% concentration (Fig. 2b). These results suggest that DMSO at higher concentrations can suppress the induction of DNA double-strand breaks in both of CHO and xrs5.

Radioprotective effect by DMSO treatment

Radioprotective effects by 0.5% DMSO were observed in

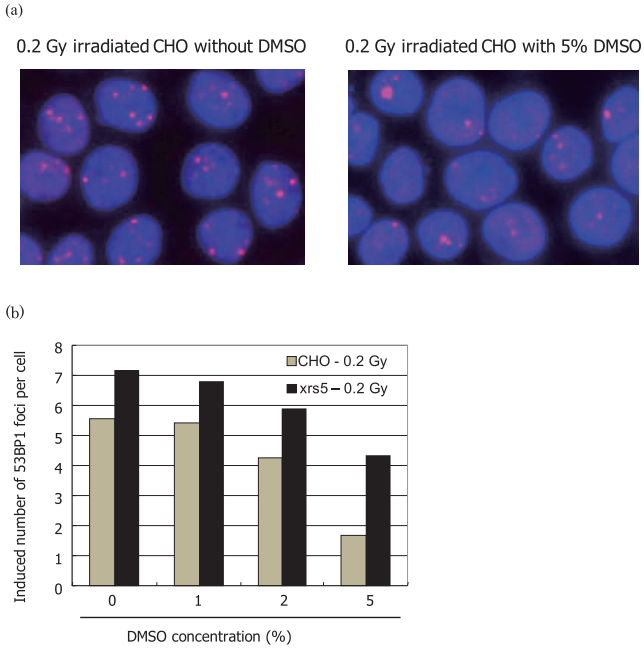

Fig. 2. Effects of DMSO on the formation of 53BP1 foci. (a) Fluorescence images of 53BP1 (red) and DAPI indicating nuclei (blue) of CHO cells. Left and right panels are showing the cells fixed 15 min after 0.2 Gy irradiation without and with 5% DMSO, respectively. Cells were treated with DMSO for 1 hour before irradiation. (b) Relationship between DMSO concentration and induced number of 53BP1 foci in CHO (gray) and xrs5 (black). The average number of 53BP1 foci per cell was obtained from 200 cells counting in two independent experiments.

CHO cells in both of colony formation and micronucleus assay (Fig. 3a left and 3b left). However, these protective effects were not observed in repair-deficient xrs5 cells (Fig. 3a middle and Fig. 3b right). These results suggest that radioprotective effects by 0.5% DMSO treatment may not be caused by the general suppression of indirect action during X-irradiation. Also, radioprotective effects by 0.5% DMSO were observed in human Ku80 complemented xrs5 (xrs5-Ku80 cells) in colony formation assay (Fig. 3a right), suggesting that Ku80-dependent repair process might be involved in these protective effects. Significant difference in surviving fraction was observed in 6 Gy irradiated conditions between non-treated cells and 0.5% DMSO treated cells ($p < 0.05$ by student t test). Ten percent survival dose (D_{10} dose) in xrs5 cells were 1.4 Gy and 1.5 Gy in non-treated cells and 0.5% DMSO treatments, respectively. On the other hand, D_{10} dose in xrs5-Ku80 were 4.2 Gy and 5.0 Gy in control and 0.5% DMSO treatments, respectively. As shown in Table 1, the suppression of X-ray-induced micronuclei by 0.5% DMSO treatment was observed in repair-proficient CHO, xrs5-Ku80 and CB09, but little in repair-deficient xrs5 and SD01. Significant differences were observed between DMSO-treated and non-treated cells in irradiated CHO and CB09. Reduced percentages of cell with micronuclei by DMSO-treatments were also observed in xrs5-Ku80 cells, although significant differences were not observed.

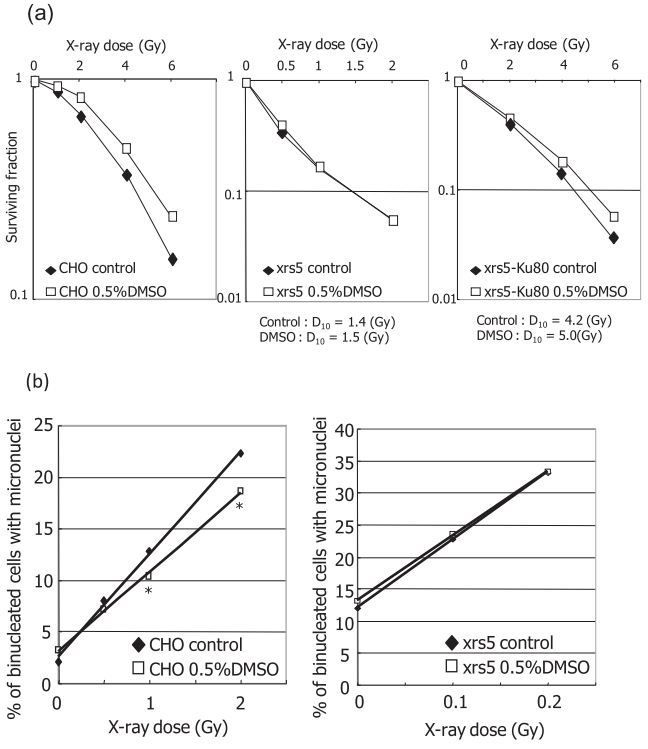

Fig. 3. (a) Effects of 0.5% DMSO on surviving fractions after X-irradiation. Left, middle and right graphs correspond to CHO, xrs5 and xrs5 complemented by human Ku80 (xrs5-Ku80), respectively. Closed and open symbols indicate non-treated and DMSO-treated conditions, respectively. Each plot presents average of the data from three independent analyses. Significant differences were observed in the 6 Gy-irradiated condition between control and DMSO-treated cells in both of CHO and xrs5-Ku80 ($p < 0.05$ by student t test). Ten percent survival doses (D_{10} value) in xrs5 (middle) and xrs5-Ku80 (right) are indicated under the graph. (b) Effects of 0.5% DMSO on the induction of micronuclei after X-irradiation. Left and right graphs correspond to CHO and xrs5, respectively. Closed and open symbols indicate non-treated and DMSO-treated conditions, respectively. Each plot presents average data from three independent analyzes. Significant difference was observed in 1 and 2 Gy-irradiated conditions between control and DMSO-treated CHO cells (* $p < 0.05$ by student t test). The equations of micronucleus inductions in control and DMSO-treated CHO cells are $y = 9.986x + 2.56$ ($R^2 = 0.9976$) and $y = 7.6571\ x + 3.1$ ($R^2 = 0.9978$), respectively, and equations for control and DMSO-treated xrs5 are $y = 106\ x + 12.067$ ($R^2 = 0.9999$) and $y = 101\ x + 13.233$ ($R^2 = 0.9995$), respectively.

Phosphorylated H2AX level immediately after irradiation

In order to quantify the level of DNA double-strand

Table 1. Summary of micronucleus assay after X-irradiation with or without DMSO

Cell line	Genotype	X-ray dose (Gy)	Percentage of cells with micronuclei[1]	
			without DMSO	with 0.5% DMSO
CHO	Normal (hamster)	0	2.4	2.9
		0.5	8.7	7.4
		1	13.2	11.2
		2	24.7	21.0
xrs5	Ku80 deficient	0	12.0	13.1
		0.1	22.8	23.6
		0.2	33.2	33.3
xrs5-ku80	xrs5 complemented by human Ku80	0	4.3	4.9
		1	14.8	13.4
		2	25.7	23.1
CB09	Normal (mouse)	0	1.5	1.2
		0.5	14.6	11.9
		1	28.8	21.9
SD01	DNAPKcs deficent	0	14.3	11.5
		0.5	38.1	38.9

1. Percentage of cells with micronuclei was obtained from 1000 or 2000 binucleated cells counted.

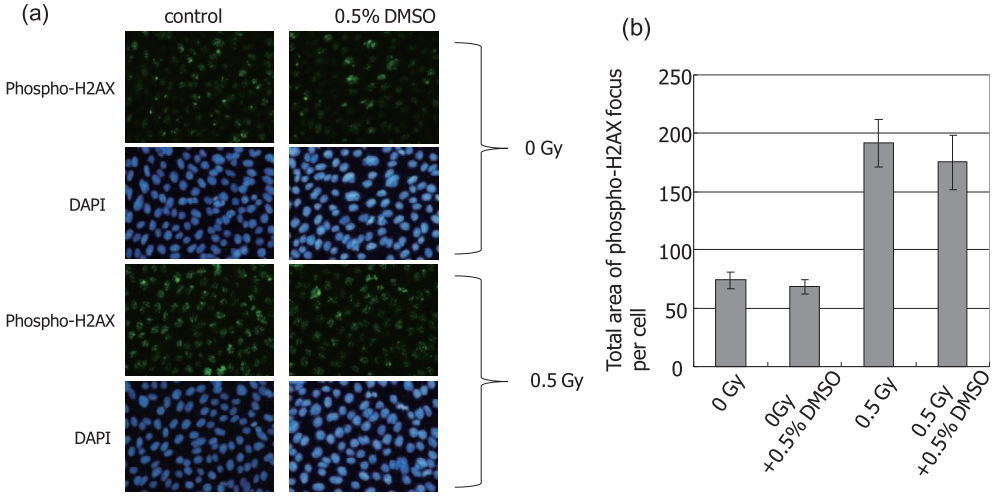

Fig. 4. (a) Fluorescence images of phosphorylated H2AX (green) and DAPI indicating nuclei (blue). Upper and lower panels indicate non-irradiated CHO cells and 0.5 Gy-irradiated CHO cells 15 min after irradiation, respectively. (b) The increased levels of fluorescent intensities for phosphorylated H2AX in 0.5 Gy-irradiated cells 15 min after irradiation, and the suppressive effect by DMSO treatment on fluorescence levels are summarized. The average of total green fluorescence values per cell was obtained from three independent analyses as shown in Materials and Methods. Error bars indicate standard deviation.

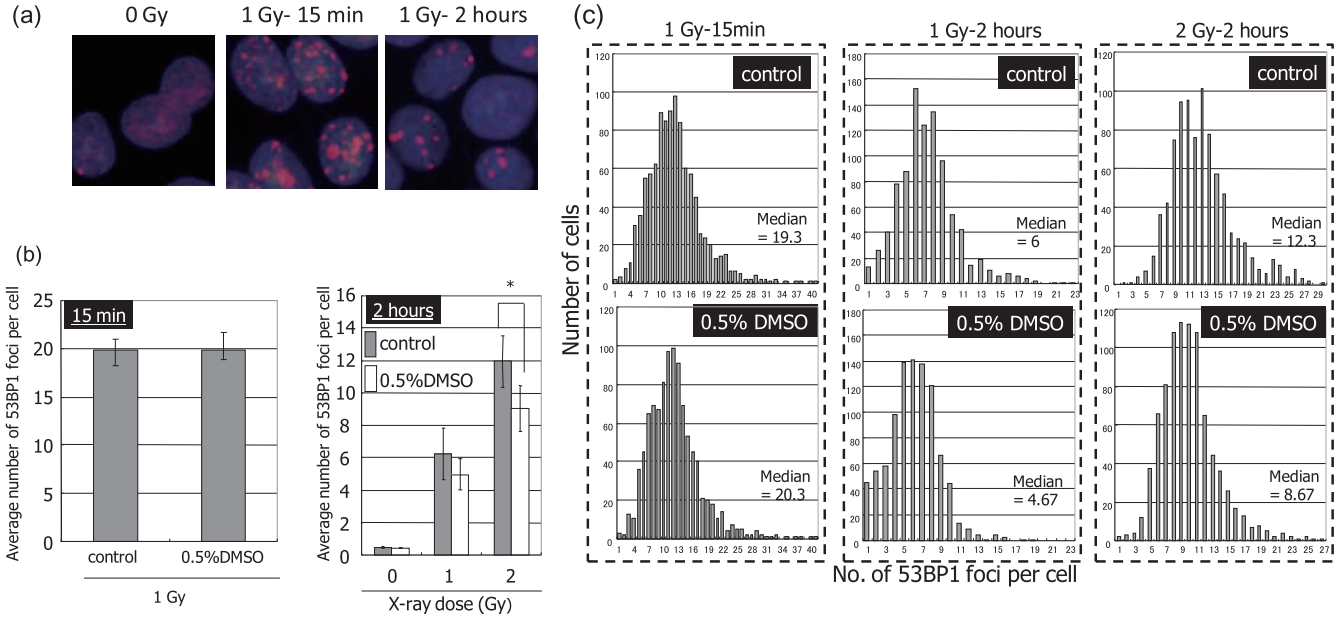

Fig. 5. (a) Fluorescence images of 53BP1 (red) on DAPI staining nucleus (blue). Non-irradiated (0 Gy) cells, and the cells 15 minutes and 2 hours after 1 Gy irradiation are shown in left, middle and right panels, respectively. (b) Average number of 53BP1 foci 15 minutes after 1 Gy irradiation in 0.5% DMSO-treated cells and non-treated cells are shown in the left graph, and dose dependency of the average number of 53BP1 foci 2 hours after irradiation is shown in both 0.5% DMSO-treated cells (open bar) and non-treated cells (closed bar). Data obtained from three independent analyzes. Significant difference was observed in 2 Gy-irradiated cells between non-treatment and DMSO-treatment (*). (c) Distributions of the number of 53BP1 foci per cell. Upper and lower panels indicate non-treated (control) and 0.5% DMSO-treated cells, respectively. The left graph is data 15 minutes after 1 Gy irradiation, and middle and right panels are 2 hours after 1 and 2 Gy irradiation, respectively. Median values are indicated in each graph.

breaks immediately after irradiation, we examined the levels of phosphorylated H2AX in cells with or without 0.5% DMSO (Fig. 4a). The results showed higher levels of green intensity for phosphorylated H2AX in irradiated cells compared to non-irradiated cells, but no significant differences were observed between DMSO-treated cells and non-treated cells (Fig. 4b). Therefore, we concluded that 0.5% DMSO treatment did not reduce the number of initial DNA double-strand breaks.

Analysis of the number of 53BP1 foci in DMSO-treated cells

In addition to phosphorylated H2AX, 53BP1 focus formation was applied to measure the levels of DNA double-strand breaks. As shown in Fig. 5a, 53BP1 foci were observed on nuclei after X-irradiation. The numbers of foci decreased with time after irradiation in CHO, as shown in Table 2. Interestingly, the average number of foci 15 min (0.25 hours) after irradiation was similar between non-treated cells and 0.5% DMSO-treated cells (Fig. 5b left), but the number of foci 2 hours after irradiation in 0.5% DMSO-treated cells was less than in non-treated cells (Fig. 5b right). The distributions of foci in each cell are summarized in Fig. 5c. As shown in the left panel of Fig. 5c, the median number of foci in 0.5% DMSO-treated cells was similar to that in non-treated cells 15 min after irradiation (19.3 vs 20.3). On the other hand, the median number for 53BP1 foci 2 hours after 1 Gy or 2 Gy irradiation was less in DMSO-treated cells than in non-treated cells (6 vs 4.67 for 1 Gy, 12.3 vs 8.67 for 2 Gy, the right panel of Fig. 5c). These results suggest that initial DNA double-strand breaks were not suppressed by 0.5% DMSO, but that DNA double-strand breaks remaining 1 or 2 hours after irradiation were decreased. The time course analyses of 53BP1 foci were also performed in xrs5 and xrs5-Ku80 as shown in Table 2. In xrs5, little effects were observed by the DMSO-treatments 1 and 2 hours after irradiation, although the numbers of foci of xrs5-Ku80 were reduced by the DMSO-treatments. These results suggest that Ku80-dependent pathway may be involved in the radioprotective effect by 0.5% DMSO.

Table 2. Summary of 53BP1 foci detection after X-irradiation with or without DMSO

Cell line (X-ray dose)	Time after X-irradiation (h)	Average number of 53BP1 foci per cells[a]		Percentage reduction in the number of foci (%)[d]
		without DMSO[b]	with 0.5% DMSO[c]	
CHO (1 Gy)	0.25	19.90	19.92	–0.1
	1	11.68	10.00	14.4
	2	6.25	4.99	20.2
	6	1.65	1.36	17.6
	24	0.51	0.47	7.8
xrs5 (0.5 Gy)	0.25	16.16	15.47	4.3
	1	11.47	11.47	0
	2	9.25	9.70	–4.9
xrs5-Ku80 (0.5 Gy)	0.25	14.33	14.14	1.3
	1	9.02	7.87	12.7
	2	5.66	4.94	12.7

a. Average number of foci were obtained from the analysis of 100 cells.
b. Cells were not treated with DMSO.
c. Cells were treated with 0.5% DMSO only 1 h before and during irradiation.
d. The percentage was calculated by the equation "d = (b–c)/b × 100 (%)".

DISCUSSION

In the past quarter century, a higher concentration of DMSO has been applied and the side effects has been disregarded in many researches using DMSO for radioprotective action.[4,16–18] In the present study, we used a non-toxic concentration of DMSO (0.5%) and analyzed the radioprotective effects, demonstrating that indirect action was not suppressed in xrs5. This result is strong evidence showing that the suppression of indirect action is not the general cause of radioprotective action by a lower concentration of DMSO. Additionally, we demonstrated little radioprotective effects by 0.5% DMSO in both Ku80-deficient xrs5 cells and DNA-PKcs-deficient mouse SD01 cells (Table 1). In agreement with this, Miyazaki *et al*. reported that XRCC4-deficient mouse cells showed weak radioprotective effects by DMSO compared to control XRCC4-proficient cells.[19] Collectively, these results indicate that radioprotective effects by DMSO in non-homologous end joining (NHEJ) pathway defective cells are weaker than that in repair-proficient cells.

To examine the possibility that the radioprotective effects by DMSO in CHO cells are caused by modification of DNA double-strand break repair rather than by suppressive effect of indirect action, we analyzed phosphorylated H2AX and 53BP1 as markers of DNA double-strand breaks. Phosphorylated H2AX is a widely used DNA double-strand break marker.[20] More precisely, we determined the relative fluorescence intensities of cell populations (Fig. 4), as we have shown this method useful for biodosimetory analyses of wide purpose.[21] It may be admitted, however, that fluorescence can be detected not only at the site of DNA double-strand breaks but also at other sites where chromatin remodeling was occurring in physiological conditions.[22] Therefore, this method is useful to examine the relative differences in irradiation effects, but it was insufficient to detect small changes in the frequency of DNA double strand breaks. To overcome this problem, we employed 53BP1 foci which

have also been widely used as a marker of DNA double-strand break as reported previously.[23] Although we cannot completely exclude a possibility that some 53BP1 foci do not reflect DNA double-strand breaks,[24] use of these two markers would greatly reduce the ambiguity and reinforce the reliability of results. If OH radicals induced by X-irradiation around DNA had been scavenged by DMSO in irradiated cells, the number of DNA double-strand breaks should have been reduced in DMSO-treated cells compared to non-treated cells. However, our results showed no significant reduction of either phosphorylated H2AX or 53BP1 by DMSO treatment 15 min after irradiation (Fig. 4 and 5). Therefore, we concluded that the suppression of indirect action did not contribute to the radioprotective effects at the DMSO concentration of 0.5%. On the other hand, 0.5% DMSO treatment decreased the number of 53BP1 foci 2 hours after irradiation. The extent of decrease was smaller in xrs5 cells than in CHO and xrs5-Ku80 cells. As is well known, the majority of DNA double-strand breaks are repaired by the NHEJ pathway within several hours after irradiation. Taken together with the finding of a lower radioprotective effect in NHEJ-deficient cell lines, radioprotective effects by DMSO should be related to the NHEJ pathway, for example, repair facilitation.

As shown in Fig. 2b, the suppressive effects of 53BP1 foci formation by DMSO were dependent on its concentration in both of CHO and xrs5. This suggests that the radioprotective effects by DMSO at higher concentration are caused by the suppression of the initial DNA double-strand breaks generated by indirect actions. Further studies are needed to clarify the relationship between DMSO concentrations and the induction and repair of DNA double-strand breaks.

What is the mechanism of facilitation of DNA double-strand break repair by low concentration of DMSO? One possibility is that OH radicals directly inhibit the NHEJ, and the depletion of OH radicals by DMSO may be a mechanism of NHEJ activation. Another possibility is that DMSO reacts with chromatin proteins, and the conformation change of chromatin may affect NHEJ ability. Goodarzi *et al*. reported that the repair activities for DNA double-strand breaks are associated with heterochromatin.[25] Therefore, DMSO may affect the conformation of heterochromatin. In both of these mechanisms, extensive suppression of oxidative stress is not needed, but specific action on NHEJ proteins or chromatin area may be important. Therefore, the DCFH value is not always reduced significantly, as was seen in the 0.5% DMSO condition (Fig. 1). Thus, we should consider the specific action of DMSO on the ability of DNA repair or chromatin remodeling for future studies.

As the conclusion, we demonstrated that 0.5% DMSO exerted radioprotective effects through the facilitation of DNA double-strand break repair rather than through the suppression of indirect action. Radioprotective effects of DMSO have been known for many years and is generally attributed to the suppression of DNA damages induced by indirect action of radiation. However, considering the current results, further studies are necessary to truly understand the effects of DMSO in modifying radiosensitivity.

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