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Yeast DEL Assay Detects Protection against Radiation-Induced Cytotoxicity and Genotoxicity: Adaptation of a Microtiter Plate Version

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Abstract

The DEL assay in yeast detects DNA deletions that are inducible by many carcinogens. Here we use the colorimetric agent MTS to adapt the yeast DEL assay for microwell plate measurement of ionizing radiation-induced cell killing and DNA deletions. Using the microwell-based DEL assay, cell killing and genotoxic DNA deletions both increased with radiation dose between 0 and 2000 Gy. We used the microwell-based DEL assay to assess the effectiveness of varying concentrations of five different radioprotectors, N-acetyl-L-cysteine, L-ascorbic acid, DMSO, Tempol and Amifostine, and one radiosensitizer, 5-bromo-2-deoxyuridine. The microwell format of the DEL assay was able to successfully detect protection against and sensitization to both radiation-induced cytotoxicity and genotoxicity. Such radioprotection and sensitization detected by the micro-well-based DEL assay was validated and compared with similar measurements made using the traditional agar-based assay format. The yeast DEL assay in microwell format is an effective tool for rapidly detecting chemical protectors and sensitizers to ionizing radiation and is automatable for chemical high-throughput screening purposes.

INTRODUCTION

Ionizing radiation exposure in humans produces an array of different DNA damages that may lead to carcinogenesis if misrepaired (1). Humans regularly receive ionizing radiation exposure from a variety of sources including environmental (2), medical (3) and occupational exposures (4). A threat of significantly greater radiation exposure exists from a radiological accident or emergency situation (5). Whereas the risk of radiation-induced cancer from low-dose exposure is still uncertain (6) and controversial (7-11), the cancer risk resulting from exposures greater than ~10 cSv is well established.

Much focus has been directed on the development and use of nontoxic radioprotectors that have the potential to reduce both the acute and chronic epidemiological risks associated with radiation exposure. Many plant extracts and antioxidants have well-described and characterized radioprotective properties [for reviews, see refs. (12) and (13)]. Since approximately two-thirds of damage by low-LET radiation is caused indirectly through the radiolytic production of reactive oxygen species (ROS), the scavenging of ROS by antioxidants offers a potent mechanism for protection against γ and X rays (14). However, high-LET radiations such as α particles, accelerated neutrons and heavy ions cause DNA damage predominately via direct action; thus antioxidant protection against high-LET

radiation is substantially less efficient (15). Pharmacological agents including immunomodulators, cytokines, protease inhibitors, endotoxins and antiapoptosis drugs have been reported to provide effective protection against radiation damage *in vivo* (16-19) and may offer stronger protection against high-LET radiation damage than antioxidants. Cells also have inducible biological protection against radiation (20), and pharmacological activation of this pathway may offer an additional approach to radiation protection.

Multiple methods for rapidly assessing the efficacy of radioprotective compounds and assays amenable for screening of new radioprotective compounds have been reported. A semi-automatable microwell-based assay using the tetrazolium-based agent MTT was first reported to quantify radioprotection *in vitro* by measuring cellular proliferation after radiation exposure. This assay takes between 3–14 days depending on the cell growth rate and is able to quantify the protective and sensitive effects of antioxidant and 5-bromo-2-deoxyuridine (BrdU) treatment against radiation-induced cytotoxicity (21). A method capable of rapidly evaluating radioprotection against γ -H2AX focus formation has also been reported; five previously established radioprotectors were used to validate the method by their capacity to reduce γ -H2AX focus formation *in vitro* measured by flow cytometry (22). A high-throughput method that can uniquely identify antioxidant compounds that protect against radiation-induced ROS has also been described (23). Thus far, no method has been reported that can rapidly characterize protection against radiation-induced genotoxicity.

The yeast DEL assay is an efficient *in vitro* system for detecting DNA deletion events (24). The yeast RS112 strain has a 6-kb internal disruption at the *his3* locus, and deletion of this disruption and subsequent restoration to wild-type *his3* is scored as a DNA deletion event. A deletion frequency is then used in the calculation of the DEL score: a ratio of cells growing on –His medium to the number of cells plated on –His and adjusted for the overall plating efficiency: $(\text{DEL } 5 \text{ [(colonies on –His)/((cells plated on –His)*(colonies on +13/cells plated on +13))]} \times 10,000)$. A DEL event has previously been established as a measurement of genotoxicity and has been suggested to be a marker for genomic instability and carcinogenesis. An observed DEL event, reversion to a functional *HIS3* gene, is a consequence of a homologous recombination and an excision of an internal disruption segment. It is deemed as an indicator of a successful DNA repair activity in response to a spectrum of various DNA lesions within this construct (25,26). Of 50 EPA-listed carcinogens, 47 induced DNA deletion events using the yeast DEL assay, and for 60 compounds of known carcinogenic activity, the DEL assay positively correlates 92% with animal carcinogenicity data (27). The correlation between carcinogen exposure and DEL event induction is so strong that the yeast DEL assay has recently been proposed as a single test system for evaluating suspected carcinogenic risk (28).

Ionizing radiation has previously been described as a potent inducer of yeast deletion events using this model (25,29,30). Here we use the yeast DEL assay for microwell-based detection of radiation-induced DNA deletion events. We also use the DEL assay for rapid characterization of radioprotectors based upon their ability to protect against DNA deletion events in yeast. We validate this method using six previously established radioprotective and radiosensitive compounds. We propose that the microwell-based yeast DEL assay is uniquely capable of simultaneously measuring radio-protection against both radiation-induced genotoxicity and cytotoxicity and is amenable for high-throughput screening purposes.

MATERIALS AND METHODS

Yeast Strains, Medium and Reagents

The diploid *S. cerevisiae* strain RS112 (MATa/MAT α ura3-52/ura3-52 leu2-3,112/leu2- Δ 98 trp5-27/TRP5 arg4-3/ARG4 ade2-40/ade2-101 ilv1-92/ILV1 HIS3::pRS6/his3 Δ 200 LYS2/lys2-801) was used to measure DNA intrachromosomal recombination events (DEL events) at the his3 locus. Synthetic complete (SC or +13) medium was prepared as yeast nitrogen base 0.67%, glucose 2%, agar 2% plus the following amino acids and bases per 900 ml of distilled water: 60 mg each of adenine sulfate, L-isoleucine, L-leucine, L-lysineHCl, L-tyrosine, 45 mg each of L-arginine-HCl, L-histidine-HCl, L-methionine, uracil, 90 mg of L-tryptophan. SC medium lacking histidine (–his) was made as above but without addition of histidine. SC medium lacking leucine (–leu) was made as above but without leucine, and the following was added per 900 ml of distilled water after autoclaving: 18 mg uracil, 36 mg adenine sulfate, and 18 mg L-histidine. For liquid medium preparation, agar was not added.

The following compounds were purchased from Sigma: L-ascorbic acid (CAS No. A-0278), DMSO (CAS No. D2650), and BrdU (CAS No. B5002). N-acetyl-L-cysteine was purchased from Alfa Aesar (CAS No. 616-91-1), Tempol from Calbiochem (CAS No. 581500), and Amifostine (WR-1065) from the NCI Chemical Carcinogen Repository (CAS No. 345308/4). MTS tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] was purchased from Promega (CAS No. G358B).

γ Irradiation

Yeast cells were irradiated with 2000 Gy in a Mark I irradiator (J. L. Shepherd and Associates, Glendale, CA) with a cesium-137 γ -ray source. A dose rate of ~33.3 Gy/min was used.

DEL Assay

The yeast DEL assay was used to score radioprotection and sensitization using agar plates. A single clone of RS112 was inoculated in ~7 ml –leu medium at 30°C for 4 h prior to irradiation; for experiments with radioprotectors and radiosensitizers, compounds were added to individual inoculation medium 2 h prior to irradiation with the exception of BrdU, which was added 4 h prior. After irradiation yeast cells were plated 200,000 per –his plate and 1,000 per +13 plate in duplicate for 2000 Gy-exposed samples and 100,000 per –his plate and 100 per +13 plate in duplicate for sham-exposed samples. Plates were incubated at 30°C for 48 h and then colonies were counted. Cytotoxicity (i.e. survival) was calculated by dividing the number of colonies counted on the +13 plates by the number of cells plated and the plating efficiency obtained for parallel measurements made using unirradiated controls. The number of colonies scored on –his plates was used to calculate the number of DEL events per 10,000 surviving yeast as described above. Each compound treatment experiment was performed in triplicate simultaneously, and untreated-irradiated control samples were always run concurrently in parallel (also in triplicate).

The yeast DEL assay was adapted for microwell measurement of radiation-induced toxicity. Similarly, a single RS112 clone was inoculated in ~6 ml –leu medium for 16 h prior to 2000 Gy irradiation. Compounds were added to individual inoculation medium 2 h prior to irradiation with the exception of BrdU, which was added 6 h prior. After irradiation, 100,000 yeast cells were placed into each of 12 microwells: six microwells containing 100 μ l of +13 liquid medium and six microwells containing 100 μ l of –his liquid medium. Then 18 μ l of MTS reagent was added to each well. For each experiment, untreated/unirradiated controls, untreated-irradiated, and compound-treated/unirradiated controls were run in

parallel. Ninety-six-well plates were incubated stationary for ~16 h at 30°C, after which colorimetric readings were taken at 490 nm using a Molecular Devices SpectraMax M5 microplate reader (Sunnyvale, CA). Cytotoxicity was determined by measuring yeast proliferation in +13 medium relative to proliferation in +13 medium of control treated yeast cells. Genotoxicity (DEL events) was determined as described previously (27) by dividing the colorimetric value of yeast measured in –his medium-containing wells by that measured in +13-containing wells.

RESULTS

Quantification of Radioprotection and Sensitization with DEL Assay

The DEL assay was used to measure protection against and sensitization to radiation-induced genotoxicity and cytotoxicity for six different compound treatments using the agar plate format. Yeast exposure to 2000 Gy γ rays produced an average of 84–201 DEL events per 10,000 surviving cells in six separate experiments; this is a significant induction over unirradiated control yeast, which exhibited fewer than 1 DEL event per 10,000 clones (Table 1). The surviving fraction and number of DEL events were scored for irradiated yeast treated with 1 mM *N*-acetyl-L-cysteine (NAC), 1 mM L-ascorbic acid (vitamin C), 1 mM Tempol, 1% DMSO, 50 μ M 5-bromo-2-deoxyuridine (BrdU), and 10 mM WR-1065; untreated cells were irradiated in parallel and compared with each compound-treatment experiment. The radioprotectors NAC, vitamin C, DMSO and WR-1065 each provided protection of varying significance against radiation-induced DEL events and cell killing at the concentrations used compared to control experiments (Table 1). Tempol protected against genotoxicity but sensitized yeast to cytotoxicity. BrdU sensitized yeast to γ -ray-induced DEL and cell killing.

Microwell-Based Measurement of Radiation DEL Response

We adapted the DEL assay for microwell plate measurement of radiation-induced cytotoxicity and genotoxicity. Yeast cells were irradiated with 0, 50, 150, 250, 500, 1000 and 2000 Gy, and proliferation was measured 16 h later in microwells containing both +13 and –his liquid medium using the MTS colorimetric reagent. Proliferation of cells in either medium increased the relative 490-nm absorbance from 0 to 16 h. Yeast proliferation in +13 medium diminished with increased radiation dose, indicative of radiation cytotoxicity (Fig. 1A). Cells that undergo DNA deletion events and revert to wild-type *his3* are able to grow in medium lacking histidine. Absolute yeast proliferation in –his medium increased with between 0 and 250 Gy and was fairly level between 250 and 2000 Gy (Fig. 1A), but growth in –his medium relative to proliferation in +13 medium increased incrementally across the entire dose range (Fig. 1B).

Microwell-Based Measurement of Radioprotection and Radiosensitization with the DEL Assay

The yeast microwell-based DEL assay was used with the five radioprotectors and one radiosensitizer to evaluate the assay's ability to detect radioprotection and radiosensitization. Yeast samples were cultured with various concentrations of NAC, vitamin C, Tempol, WR-1065, DMSO and BrdU and irradiated as above. Proliferation in microwells that contained +13 medium and –his medium was measured with MTS to score cytotoxicity and genotoxicity resulting from the radiation exposure.

The microwell format of the yeast DEL assay accurately detected chemical protection and sensitization to radiation-induced cytotoxicity and genotoxicity. One and 5 mM NAC, 0.1, 0.5 and 1 mM ascorbic acid, 10 mM WR-1065, and 0.1 and 1% DMSO each protected against the cytotoxicity of 2000 Gy as measured by greater yeast proliferation in +13

medium compared to proliferation of untreated yeast exposed to 2000 Gy (Fig. 2A). Concentrations of 0.02, 0.05, 0.1 and 1 mM Tempol and 0.1 mM BrdU caused slight radiosensitization to 2000 Gy by the same standard; 0.2 mM BrdU, which caused only slight cytotoxicity in unirradiated yeast, resulted in extreme radiosensitization when combined with 2000 Gy (Fig. 2A). Similarly, 1 and 5 mM NAC, 0.1, 0.5 and 1 mM ascorbic acid, 0.1 and 1 mM Tempol, and 0.1 and 1% DMSO each protected against radiation-induced DNA deletions, and 0.02 to 0.2 mM BrdU provided sensitization to radiation-induced deletions as measured by yeast proliferation in *-his* medium relative to proliferation in *+13* medium (Fig. 2B). A concentration of 10 mM WR-1065 did not significantly protect yeast against radiation-induced DNA deletions (Fig. 2B). Consistently across multiple experiments, WR-1065 was by itself genotoxic to unirradiated yeast.

DISCUSSION

Here the yeast DEL assay has been used in microwell-based format to rapidly determine the protective and sensitizing effects of chemicals against radiation-induced cytotoxicity and genotoxicity. The assay was used with six radioprotectors and radiosensitizers, and both protection against and sensitization to radiation and DNA deletion events were detectable by this assay.

The yeast DEL assay is a powerful method for detecting the genotoxicity of carcinogens, including radiation (28). Recently, the yeast DEL assay was adapted to microwell format and was shown to be capable of simultaneously assessing the cytotoxicity and genotoxicity of 13 different chemical compounds (27). Here we used the DEL assay in microwell format to rapidly detect chemical protection and sensitization to the cytotoxicity and genotoxicity induced in cells by radiation. Reduced cellular proliferation after radiation exposure has been a standard measurement of cytotoxicity by multiple microwell-based proliferation assays with various mammalian cell types (21,31-33). We build upon this colorimetric proliferation model as the basis for measuring radiation-induced genotoxicity in yeast. All proliferating cells of the strain RS112 will grow in *+13* complete medium, but only cells that have undergone a 6-kb deletion of genomic DNA and restoration to wild-type *his3* will grow in *-his* medium. The relative frequency of growth in *-his* medium then can be used to derive a relative DEL event score and thus establish the extent of genotoxicity and its fluctuation with different chemical entities. A measure of genotoxicity, a calculated DEL score, can be considered separately from cytotoxicity because it assesses the recombination frequency only in the fractions that survive irradiation. The ratio of yeast growth in *-his* medium to that in *+13* medium and adjusted to survival has been used as a standardized measurement of DEL genotoxicity in both agar plate assays (24,29) and liquid microwell assays (27). Here the microwell plate format of the DEL assay is capable of detecting both increased cytotoxicity and genotoxicity with radiation doses between 0 and 2000 Gy (Fig. 1). This response correlates well with similar dose responses observed in previous studies in which the DEL assay was used to measure radiation cell killing and DNA deletion events using agar plates (25,29,30). The DEL assay can accurately distinguish a purely genotoxic compound from a purely cytotoxic one because a DEL score is a ratio of irradiated cells proliferating in a selective medium to nonirradiated cells in selective medium and adjusted with a survival coefficient derived from cells in complete medium. Seeding all treated and control wells with the same cell density also allows for a direct comparison between the wells. Yeast cocultured with various concentrations of radioprotectors and radiosensitizers exhibited differences in radiotoxicity measurable 16 h after 2000 Gy exposure using the microwell-based DEL assay. NAC, ascorbic acid and DMSO each protected against both radiation cytotoxicity and genotoxicity in yeast (Fig. 2); such results are consistent with previous studies in which these agents protected from radiation cell killing and genotoxic mutation induction at the *hprt* and *CD59* loci *in vitro* (34-37). Tempol and WR-1065 offered

mixed results (Fig. 2A and B). Whereas Tempol has been shown to protect against clonogenic cell killing and mutation induction at the *xprt* and *hprt* loci in mammalian cells (38,39), in the yeast system used here Tempol offered protection against radiation-induced DNA deletions but no protection against cell killing; in fact, it behaved as a radiosensitizer. The observed lack of protection observed against cell killing by Tempol may be due to the fact the concentrations used were 1/50 and 1/500 of that which provided cytotoxic protection in mammalian cell experiments. The radioprotective effect of WR-1065 in yeast is also interesting; at 10 mM, it protected well against cytotoxicity, but no significant protection was observed against DEL events. This result is likely due to the fact 10 mM WR-1065 was by itself genotoxic to yeast cells. In previous reports, WR-1065 has been postulated to function through separate mechanisms at different concentrations to protect against radiation-induced genotoxicity and cytotoxicity (40-42). BrdU sensitized yeast to radiation, increasing both cytotoxic and genotoxic responses to 2000 Gy (Fig. 2); these results are consistent with those of others (43,44).

A comparison can be made between radioprotection and sensitization measured using the microwell- and agar-based yeast DEL methods. Overall, for the six concentrations of chemicals tested for protection and sensitization using both assay formats, the results correlated well (Table 2). NAC at 1 mM, 1 mM ascorbic acid, and 1% DMSO each provided radioprotection against cell killing and DNA deletions using both assay formats; 1 mM Tempol protected against radiation-induced DEL events at the expense of increased radio-cytotoxicity similarly under both assay formats. Conversely, 10 mM WR-1065 protected against radiation-induced cell killing and DEL events using the agar-based format; this was not fully correlated by microwell-based measurements in which the same concentration of WR-1065 protected against cell killing but offered nonsignificant protection against DEL events. BrdU at 50 μ M sensitized yeast cells to radiation-induced cell killing and deletions using both formats. Overall, a positive qualitative correlation was observed between the two DEL assay formats for 12 out of 12 of the paired cytotoxic and genotoxic measurements (Table 2). Still, radioprotection and radiosensitization differed quantitatively between assay formats.

A perfect correlation between the two DEL assay formats should not necessarily be expected. Two reasons may account for the differences in radioprotection observed between the two assay formats. First, both yeast (25) and mammalian cells (45) exhibit delayed effects to radiation; ROS have been implicated in such delayed effects (46-48), and scavenging these ROS with antioxidant administration after radiation exposure has been shown to protect against these delayed effects (34-36). In the microwell-based format, the antioxidants are present before, during and after radiation exposure; in the agar-based format, these radioprotectors are present only before and during radiation exposure and are not present to scavenge ROS after irradiation, thus possibly accounting for some of the quantitative differences in radioprotection between these two assay formats. Second, because the two assay formats measure two different end points, proliferation and clonogenicity, one would not expect the levels of relative protection to be the same for these two end points. Proliferation assays have been demonstrated to correlate imperfectly with a clonogenic response (32), and this likely is likely to be another reason for the difference between the two assay formats.

A comparison can be made between microwell DEL assay format and other microwell plate assays, notably the MTT assay, which has been used to detect radioprotection and sensitization. The MTT assay has frequently been used to quantify cellular proliferation responses to radiation (21) and toxic chemical (49) treatments. Thus far these tetrazolium-based assays have been used only to measure cytotoxicity as indicated by reduced cellular proliferation after radiation treatment. Here we used a similar tetrazolium agent (MTS)

together with the yeast DEL assay. Whereas previous methods scored only cytotoxicity to radiation, this is the first example of a microwell-based assay capable of measuring both ionizing radiation-induced cytotoxicity and genotoxicity simultaneously *in vitro*. MTT assessment of radio-cytotoxicity in mammalian cells takes between 3–14 days depending on the growth rate of the cell line used (21), yet radiation-induced genotoxicity and cytotoxicity can be assessed with the microwell DEL assay only 16–18 h after exposure.

Here a microwell-based format of the yeast DEL assay was used to measure radiation damage in yeast. The DEL assay is unique in that it can rapidly and simultaneously measure cyto- and genotoxicity. It is possible that the mechanism that confers cytotoxic protection is different and possibly independent from that which confers genotoxic protection. In our subsequent studies we plan to screen a library of yeast mutants deficient in various repair mechanisms against compounds that show either genotoxic or cytotoxic properties or both to potentially identify and differentiate the above-mentioned mechanisms. Genotoxic protection against radiation-induced DNA deletion events in yeast may serve as a unique model for protection against radiation carcinogenesis because the DEL assay has been demonstrated to be strongly correlated with the cancer-causing activity of chemicals (28). Six different compounds were used to evaluate the ability of the assay to detect radioprotectors and radiosensitizers. NAC, ascorbic acid, DMSO, Tempol and WR-1065 were observed to protect against DNA deletion events in yeast, each to different degrees. The yeast DEL assay in microwell format is capable of measuring radioprotection and is suitable for detecting radioprotectors in high-throughput screening applications.

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FIG. 1.

Panel A: Yeast proliferation 16 h after exposure to 2000 Gy is measured in microwells containing +13 and –his medium using the MTS colorimetric reagent. Increased relative absorbance from 0 to 16 h correlated with cell proliferation. Yeast proliferation in +13 medium decreased markedly with increased dose, whereas yeast growth in –his medium, indicative of deletion of 6 kb genomic DNA and reversion to wild-type *his3*, increased slightly with dose up to 250 Gy before leveling off. Panel B: The ratio of yeast proliferation in –his medium to that in +13 medium, indicative of relative DEL induction, increased incrementally across the entire dose range. The experiment was carried out using six repeats for each treatment group, and the results are presented as means $\pm$ SD.

FIG. 2.

Panel A: Yeast proliferation of samples irradiated with 2000 Gy and cocultured with different concentrations of five radioprotectors and one radiosensitizer measured in +13 medium containing microwells using MTS colorimetric reagent. Increased relative absorbance from 0 to 16 h correlated with cell proliferation. Protection and sensitization to radiation-induced cytotoxicity was observed. Panel B: The ratio of yeast proliferation measured in –his medium to that measured in +13 medium for yeast cocultured with five radioprotectors and one radiosensitizer and exposed to 2000 Gy. Results are from six individual experiments each carried out using at least six repeats for each treatment group and normalized to the untreated controls; the results are presented as means $\pm$ SD.

TABLE 1
Effects of Treatment of Irradiated Yeast with Various Radioprotectors and Readiosensitizers

	Control			With compound treatment		
	DEL	σ	Survival	DEL	σ	Survival
NAC (1 mM)	146.8	6.1	10.4%	123.8 ^{**}	3.8	14.4% [*]
Vitamin C (1 mM)	136.1	12.4	13.2%	101.3 [^]	6.2	16.7% [^]
Tempol (1 mM)	200.8	13.7	8.4%	163.5 [^]	9.6	6.6% ^{**}
WR-1065 (10 mM)	83.5	13.1	4.0%	42.0 ^{**}	4.0	9.4% ^{**}
DMSO (1%)	148.2	11.4	9.8%	115.8 [^]	5.3	16.6% ^{**}
BrdU (50 μ M)	112.3	2.6	12.3%	158.1 [*]	3.7	8.5% [*]

Notes. Results are presented as the average of three individual experiments for each measurement, and control experiments were always run in parallel with compound-treated experiments. Unirradiated control yeast cells exhibited an average DEL induction of 0.85 ± 0.27 averaged across all experiments. The DEL induction measured in unirradiated compound-treated cells did not differ significantly from control cells for any experiment (data not shown). DEL is the number of DNA deletion events per 10,000 surviving yeast and σ is the SEM. Significance calculated using a Student's *t* test:

[^] *P* 0.10
^{*} *P* < 0.05
^{**} *P* < 0.01.

TABLE 2

Effects of Radiosensitizers and Radioprotectors Determined by Microwell and Agar Plates Methods

	Microwell method		Agar plate method	
	DEL change	Proliferation change	DEL change	Survival change
NAC (1 mM)	-32.9% **	91.7% **	-15.6% **	39.2% *
Vitamin C (1 mM)	-31.6% **	46.2% **	-25.6% ^	26.1% ^
Tempol (1 mM)	-37.2% **	-20.4% **	-18.6% ^	-21.4% **
WR-1065 (10 mM)	-9.9%	45.9 **	-49.7% **	135.7% **
DMSO (1%)	-24.0% **	21.2% **	-21.9% ^	68.7% **
BrDU (50 μ M)	34.5% **	-27.0% **	40.8% *	-30.5% *

Notes. Values are presented as percentage difference between compound-treated and untreated control measurements each performed and irradiated in parallel. A comparison can be made between the radioprotection and radiosensitization measured by using both methods. Significance calculated using a Student's *t* test:

^ $P = 0.10$

* $P < 0.05$

** $P < 0.01$.