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Radioprotection against Lethal Damage Caused by Chronic Irradiation with Radionuclides *In Vitro*

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To examine the capacity of chemical protectors to mitigate damage caused by chronic irradiation by incorporated radionuclides *in vitro*, cells must be maintained in the presence of the protector during the course of the irradiation. Such long exposures to chemical protectors at concentrations high enough to afford protection usually results in extreme chemotoxicity. To overcome this problem, experimental conditions were developed to allow Chinese hamster V79 cells to be maintained in 5% DMSO for prolonged periods (up to 72 h) with no observable chemotoxicity. Under these conditions, the capacity of DMSO to protect against damage to V79 cells caused by unbound ^{32}P and $^{3}H_2O$ and DNA-incorporated ^{131}IdU, [^{3}H]dThd and ^{125}IdU was examined. The dose modification factors for ^{32}P, $^{3}H_2O$, ^{131}IdU, [^{3}H]dThd and ^{125}IdU were 2.6 ± 0.5, 2.3 ± 0.3, 1.0 ± 0.1, 1.16 ± 0.07 and 1.07 ± 0.02, respectively. These results show that 5% DMSO is capable of protecting cultured V79 cells against lethal damage caused by β particles emitted by unbound ^{32}P and $^{3}H_2O$, whereas little or no protection is afforded against damage caused by β particles emitted by DNA-incorporated ^{131}I and ^{3}H or low-energy Auger electrons emitted by DNA-incorporated ^{125}I. © 1998 by Radiation Research Society

INTRODUCTION

Many radionuclides used in medical diagnosis such as ^{123}I, ^{111}In and ^{201}Tl decay via electron capture and/or internal conversion. These radionuclides emit numerous low-energy Auger electrons, resulting in highly localized energy deposition in the immediate vicinity of the decay site (*1*, *2*). The radiotoxicity of Auger electron emitters depends strongly on the subcellular distribution of the radionuclide (*3–7*). When the prolific Auger electron emitter ^{125}I is localized in the cytoplasm of the cells, its radiotoxicity is similar to that of radiations having low linear energy transfer (LET) such as external X rays (*6*, *8*, *9*). In contrast, when the same radionuclide is incorporated into the DNA via ^{125}I-iododeoxyuridine (^{125}IdU), its relative biological effectiveness (RBE) is about the same as that of 5.3 MeV α particles emitted by ^{210}Po (*10*, *11*).

Ionizing radiation imparts damage to biological tissue by two mechanisms: direct and indirect. Direct effects are the result of energy deposited directly in critical molecular structures within the cell. Indirect effects are due to interactions between the critical molecules and the variety of free radicals ($OH^{\bullet}$, $H^{\bullet}$, etc.) produced in the radiolysis of water (*12*). The biological damage caused by high-LET radiations such as α particles has been attributed primarily to direct effects (*13*), whereas the biological effects of low-LET radiations (e.g. β particles, X rays) is caused primarily by indirect effects (*12*).

Over the last several years we have reported on the capacity of a variety of chemical agents to provide protection against damage caused by chronic irradiation by incorporated radionuclides *in vivo* (*6*, *7*, *14–19*). These studies showed that radical scavengers such as dimethyl sulfoxide (DMSO) (*19*) and vitamin C (*15*, *16*) provided substantial and equal protection *in vivo* against the damage caused by internal radionuclides (similar to that caused by low-LET radiation) and damage caused by DNA-incorporated Auger electron emitters (similar to that caused by high-LET radiation). In contrast, no protection was provided against the high-LET-type damage caused by α-particle emitters (*16*, *19*). These and other studies with the chemical protectors cysteamine (MEA) (*6*, *7*), *S*-(2-aminoethyl)isothiouronium bromide hydrobromide (AET) (*17*), soybean oil (*18*) and vitamin A (*18*) have provided strong evidence that the mechanism by which Auger electron emitters impart high-LET-type damage is largely indirect in nature. In contrast to these findings, Hofer and Bao (*20*) have suggested that indirect effects do not play a major role in the biological action of Auger electrons. They showed that when cultured Chinese hamster ovary (CHO) cells containing ^{125}IdU were frozen for accumulation of decays, both high- and low-LET-type effects were imparted depending on the length of time the cells were allowed to progress through the cell cycle prior to being frozen at −196°C in culture medium containing DMSO. When MEA was added prior to freezing, protection was afforded against the low-LET-type effects while no protection was provided against the high-LET-type effects of ^{125}I. Based on these findings

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obtained under frozen *in vitro* conditions, Hofer and Bao suggested that the mechanism by which Auger electrons impart high-LET-type biological damage does not appear to be indirect in nature. This implication contradicts our earlier conclusions based on *in vivo* data obtained using the same radioprotector, MEA (*6*), as well as more recent data with AET (*17*) and other radioprotectors (*19*).

Unlike our *in vivo* studies, Hofer and Bao (*20*) carried out their *in vitro* radioprotection experiments using frozen cells, where radicals and protectors were not free to migrate. Accordingly, we have been interested in investigating the capacity of chemical agents to provide protection against damage caused by chronic irradiation by incorporated radionuclides *in vitro* in a liquid water environment, where radicals and protectors are free to migrate. Our early efforts were hampered by the extreme chemotoxicity of standard radioprotectors such as DMSO, cysteamine, WR-2721 and WR-1065 when cultured Chinese hamster cells were chronically exposed to these agents for a 1-week colony-forming period at 37°C. The maximum nontoxic concentrations for DMSO, cysteamine, WR-2721 and WR-1065 were only 1% (v/v), 1 μg/ml, 0.2 μg/ml and 0.08 μg/ml, respectively (unpublished data). These concentrations were not high enough to afford any chemical protection against the effects of internal emitters. To circumvent this problem, we have established cell culture conditions that have allowed us to elevate the concentration of the chemical protector DMSO to levels that are sufficient to provide radioprotection, yet are not chemotoxic. Dimethyl sulfoxide is a well-known radioprotector whose protective action is the result of free radical scavenging (*21–23*). Many researchers have observed the radioprotective property of DMSO using different experimental end points (*19, 21, 24–27*). As a radical scavenger, the ability of DMSO to protect against the direct action of high-LET α particles is expected to be minimal (*19, 27*). Hence DMSO is well suited for studying the mechanisms of the action of radiation. In this work, the capacity of DMSO to protect against lethal damage to cultured Chinese hamster V79 cells in a liquid water environment caused by chronic irradiation by unbound ^{32}P and $^{3}H_2O$ and DNA-incorporated ^{131}I, ^{3}H and ^{125}I is reported.

MATERIALS AND METHODS

Radiochemicals

Tritiated water ($^{3}H_2O$), tritiated thymidine ([^{3}H]dThd) and ^{32}P orthophosphate were obtained from New England Nuclear (Boston, MA) as aqueous solutions at concentrations of 925, 37 and 370 MBq/ml, respectively. ^{125}IdU, dissolved in water, was obtained from ICN Radiochemicals (Irvine, CA) at a concentration of 37 MBq/ml, and ^{131}IdU was synthesized and purified by high-performance liquid chromatography (HPLC) in our laboratory according to procedures reported previously (*18*). All radiochemical solutions were sterile with no carrier added.

The activities of ^{32}P and ^{3}H were ascertained with a Beckman LS5500 liquid scintillation counter and an Ecoscint (National Diagnostics, Manville, NJ) scintillation cocktail with detection efficiencies of 1.0 and 0.5, respectively. The ^{125}I activity was quantified with a Packard (Meriden, CT) automatic gamma counter equipped with a 3-inch sodium iodide well crystal (efficiency = 0.5); ^{131}I activity was quantified with a Canberra HpGe well detector (photopeak efficiency = 0.812).

Cell Culture

Chinese hamster V79 lung fibroblasts (kindly provided by A. I. Kassis, Harvard Medical School) were used in these studies; clonogenic survival was the biological end point. The cells were cultured in minimum essential medium (MEM) supplemented with 2 m*M* L-glutamine, 10% fetal bovine serum, 50 U/ml penicillin and 50 μg/ml streptomycin. The pH of the culture medium was adjusted to 7.0 with $NaHCO_3$. Media and supplements were from Gibco (Grand Island, NY). Cells were maintained in Falcon 75-cm^2 sterile tissue culture flasks at 37°C and 5% CO_2 and 95% air and were subcultured twice weekly.

Rationale for Design of Protocol

As stated earlier, the primary purpose of the studies presented here was to determine whether DMSO could provide protection for cultured V79 cells against damage caused by chronic irradiation from radionuclides. However, protection against damage imparted by a chronic irradiation regimen necessarily requires the protector to be present during most if not all of the irradiation period. For clonogenic survival assays with cultured cells containing intracellular radioactivity (*11, 28*), this ideally requires the radioprotector to be present during all phases of the experiment including the 0.25–18-h period of cellular uptake of radioactivity and the 7-day colony-forming period. However, chemical radioprotectors are highly chemotoxic, and consequently cultured cells in 37°C environments can usually withstand chronic exposures to only extremely low concentrations of these chemicals. Therefore, it was necessary to find a liquid environment whereby cells could withstand high enough concentrations of chemical protectors to afford radioprotection under chronic irradiation conditions.

Survival of V79 Cells after Chronic Hypothermia and Exposure to DMSO

Chinese hamster V79 cells growing as monolayers in 75-cm^2 flasks were washed with 10 ml of phosphate-buffered saline, trypsinized and suspended at 4×10^5 cells/ml in calcium-free MEM. Aliquots of 1 ml were placed in sterile 17 × 100-mm Falcon polypropylene round-bottom culture tubes and placed on a rocker-roller for 4 h at 37°C in an atmosphere of 95% air and 5% CO_2. After this conditioning period, an additional 1 ml of MEM was added and all but two tubes were transferred onto a rocker-roller in refrigerated environments with temperatures ranging from 3°C to 10.5°C and maintained there for 72 h. The remaining two tubes were immediately serially diluted (three 10× dilutions) and 1 ml of the final dilution (~200 cells) was seeded into triplicate 25-cm^2 Falcon tissue culture flasks containing 4 ml of MEM. These flasks were placed in an incubator at 37°C, 95% air and 5% CO_2 for 1 week for colony formation. Meanwhile, after the refrigerated tubes had been chilled for 72 h, the tubes were removed and processed similarly for colony formation. The resulting colonies were washed with 0.9% saline, fixed with methanol, stained with crystal violet and scored. The fraction of cells surviving 72 h in the refrigerated environment compared to unrefrigerated controls is shown in Fig. 1. It is apparent that V79 cells are able to withstand prolonged periods of exposure to temperatures as low as 10.5°C; however, when the temperature was maintained below this level, the surviving fraction dropped precipitously. It was also noted that cell division was suspended when the cells were maintained at 10.5°C. Therefore, 10.5°C was selected as the optimal temperature for studies involving chronic exposure from radionuclides.

Additional studies were carried out to ascertain the chemotoxicity of the chemical protector DMSO at 10.5°C and 37°C. As described above, 1 ml of calcium-free MEM with 4×10^5 cells/ml was placed in culture tubes and conditioned on a rocker-roller. After conditioning, the cells were pooled, serially diluted to 200 cells/ml, seeded into flasks containing 4 ml MEM with various concentrations of DMSO (Sigma Chemical Co., St. Louis, MO), and placed in an incubator at 37°C, 95% air and 5% CO_2. After 1 week, the colonies were stained and scored, and the surviving fraction compared to controls maintained in MEM without DMSO

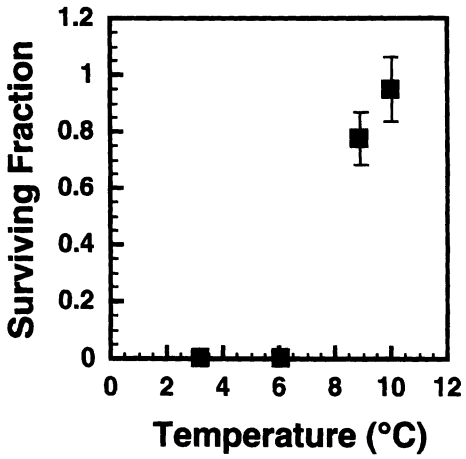

FIG. 1. Survival of Chinese hamster V79 cells after a 3-day exposure to various temperatures. Error bars represent the standard deviation of the mean.

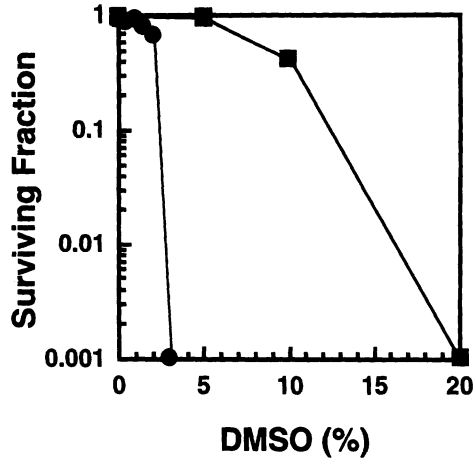

FIG. 2. Chemotoxicity of DMSO when V79 cells are exposed to the chemical at 10.5°C for 72 h (■) or 37°C for 1 week (●). Standard deviations for individual data points are of the order of the dimensions of the symbols.

was calculated. Figure 2 shows that at 37°C, the V79 cells can withstand chronic exposure to DMSO (with no chemotoxicity) only when the concentration in the culture medium is under 1% (v/v). For the studies at 10.5°C, the tubes containing conditioned cells were transferred to ice and 1 ml of MEM containing various concentrations of DMSO was added. The tubes were capped tightly and immediately transferred to a rocker-roller at 10.5°C. After 72 h, the cells were centrifuged at 2000 rpm at 4°C, washed three times with 10 ml of cold MEM, resuspended in 2 ml MEM, serially diluted and seeded into flasks containing MEM (0% DMSO) for colony formation. As shown in Fig. 2, there was no chemotoxicity when the V79 cells were maintained at 10.5°C for 72 h in culture medium containing 5% DMSO.

Survival of V79 Cells after Exposure to Unbound ^{32}P

Culture tubes containing 1 ml of conditioned V79 cells (4×10^5 cells/ml) were transferred to an ice bath and 1 ml of calcium-free MEM containing ^{32}P orthophosphate (0–2.5 MBq/ml) and 0% or 10% DMSO was added. Thus, for each concentration of ^{32}P, two tubes were prepared with final DMSO concentrations of 0% and 5%, respectively. The tubes were then capped tightly and placed on a rocker-roller at 10.5°C. After 72 h, the tubes were removed from the refrigerated environment and aliquots of the cell suspension were taken to determine cellular uptake using well-established procedures (*11*, *29*). Briefly, 100 µl of cell suspension was overlaid on 300 µl of calf serum in a 400-µl microcentrifuge tube and the tube was centrifuged for 1 min. The tubes, prepared in triplicate, were immediately frozen in liquid nitrogen and the tip containing the pellet was cut off and counted for radioactivity. An additional 100 µl of cell suspension was used to determine the cell concentration. Finally, using these data, the activity per cell was calculated. The culture tubes containing the remaining cell suspension were centrifuged at 2000 rpm, 4°C for 10 min, and the pellet was washed three times with cold MEM. After the cells were resuspended in 2 ml MEM, they were serially diluted, seeded for colony formation and placed in an incubator at 37°C, 95% air and 5% CO_2. The resulting colonies after 1 week of incubation were stained and scored. The cellular uptake was 2.6 mBq/cell for the highest ^{32}P concentration and did not depend on the presence of DMSO. It can be shown using standard procedures (*30*) that the cellular absorbed dose delivered by this intracellular ^{32}P activity was less than 2% of the total absorbed dose. Therefore, the unbound ^{32}P is the only significant source of radiation in the ^{32}P studies.

Survival of V79 Cells after Exposure to DNA-Incorporated ^{131}IdU, [^3H]dThd or ^{125}IdU

Our past studies on the radiotoxicity of ^{125}IdU in proliferating V79 cells showed that the mean intracellular ^{125}I activity in a population of cells increases linearly in time when incubated in the presence of ^{125}IdU at 37°C (*11*). In the present work involving radioprotection by DMSO, one would ideally like to have 5% DMSO present while the cells are incorporating ^{131}IdU, [^3H]dThd or ^{125}IdU. However, as noted in Fig. 2, this concentration of DMSO is highly chemotoxic at 37°C. Therefore, the cells were incubated at 37°C, 95% air and 5% CO_2 for 12 h in culture medium containing ^{131}IdU, [^3H]dThd or ^{125}IdU without DMSO. This was the minimum time deemed necessary to ensure that most cells incorporated the radioactivity in view of the 9-h cell cycle time (*31*) of these cells and the fact that thymidine and its analog iododeoxyuridine are taken up by cells only during S phase.

One milliliter of MEM containing various concentrations of either ^{131}IdU, [^3H]dThd or ^{125}IdU was added to culture tubes containing 1 ml of conditioned V79 cells (4×10^5 cells/ml). The tubes were then returned to the rocker-roller at 37°C, 95% air and 5% CO_2. After 12 h, the tubes were removed and centrifuged at 2000 rpm, 4°C for 10 min. The pellet was washed three times with 10 ml of MEM, resuspended in 2 ml of ice-cold MEM containing either 0% or 5% DMSO, and placed on a rocker-roller at 10.5°C. Parallel controls were strictly maintained for each experiment. After 72 h at 10.5°C, the cells were washed three times with 10 ml of MEM, resuspended in 2 ml of MEM, serially diluted, seeded into 25-cm^2 flasks and placed in an incubator at 37°C, 95% air and 5% CO_2. Aliquots were taken from each tube before and after the 72-h rolling period, and the mean activity per cell was determined. The flasks were removed from the incubator after 1 week and the resulting colonies were stained and scored. The surviving fraction compared to parallel controls was determined in each case.

Survival of V79 Cells after Exposure to ^3H_2O

To culture tubes containing 1 ml of conditioned V79 cells (4×10^5 cells/ml) 0.9 ml of calcium-free MEM containing different concentrations of ^3H_2O (0–100 MBq/ml) was added. The tubes were then capped loosely and returned to the rocker-roller at 37°C, 95% air and 5% CO_2. After 12 h, an additional 0.0 or 0.1 ml of DMSO was added while vortexing, the caps were snapped on tightly, and the tubes were transferred to a rocker-roller at 10.5°C. After 72 h, the tubes were removed from the cooled environment and centrifuged at 2000 rpm at 4°C for 10 min, and the pellet was washed three times with cold MEM. After the cells were resuspended in 2 ml MEM, they were serially diluted, seeded for colony formation and placed in an incubator at 37°C, 95% air and 5% CO_2. Parallel controls were strictly maintained for each experiment. The resulting colonies after 1 week of incubation were stained and scored.

RESULTS

Survival after Exposure to Unbound ^{32}P or $^{3}H_2O$

Figure 3 shows the survival of V79 cells after a 72-h exposure at 10.5°C to various concentrations (0–1.5 MBq/ml) of unbound ^{32}P in the absence and presence of 5% DMSO. The data for four experiments are shown, each fitted by least squares to the relationship

$$S = 1 - (1 - e^{-C/C_0})^n, \quad (1)$$

where S is the surviving fraction, C is the activity concentration, and C_0 and n are analogous to the D_0 and n in the single-hit multitarget model (*32*), respectively. Since the absorbed dose received by the cells is directly proportional to the concentration of ^{32}P in the culture medium, the dose modification factor (DMF), or the degree of protection provided by DMSO against the lethal effects of ^{32}P β-particle irradiation, is given by the ratio of the C_0's in the presence and absence of DMSO:

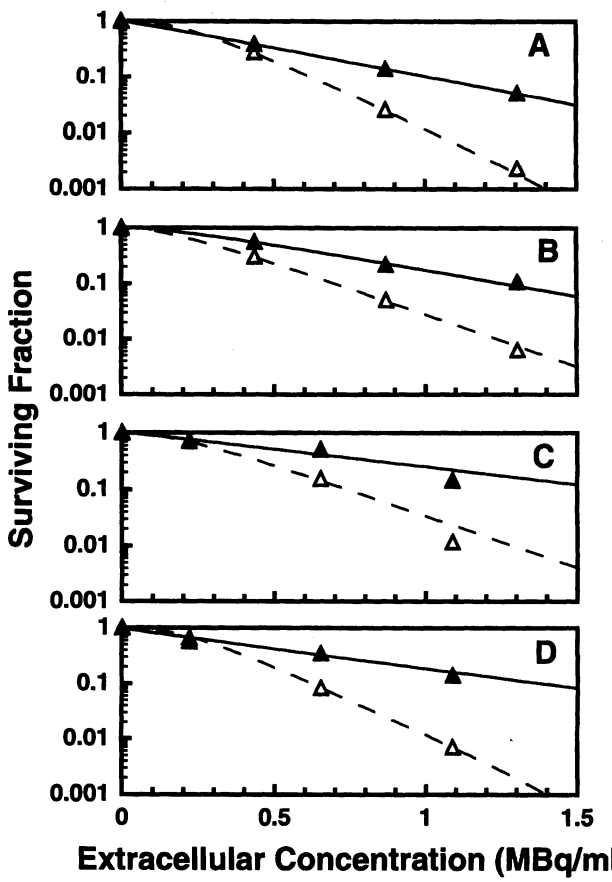

FIG. 3. Survival of V79 cells after a 72-h exposure at 10.5°C to various concentrations of ^{32}P in the culture medium in the absence (△) and presence (▲) of 5% DMSO. The data from four independent experiments are shown in panels A, B, C and D, respectively. Least-squares fits of the data are shown. Standard deviations for individual data points are of the order of the dimensions of the symbols.

$$\text{DMF} = \frac{C_0(\text{with DMSO})}{C_0(\text{without DMSO})}. \quad (2)$$

When the DMF was calculated for each individual ^{32}P experiment (Table I), the mean value for the four experiments was DMF (^{32}P, 5% DMSO) = 2.6 ± 0.5.

Figure 4 shows the survival of V79 cells after exposure to $^{3}H_2O$ in the absence and presence of 5% DMSO. The results for three independent experiments are shown. Each data set was fitted by least squares to a single-component exponential function (i.e. $n = 1$), the mean lethal concentration of $^{3}H_2O$ was determined, and the DMF was calculated. Table I gives the mean lethal concentrations and DMFs for each individual experiment. The mean DMF for the three experiments was DMF ($^{3}H_2O$, 5% DMSO) = 2.3 ± 0.3.

Survival after Exposure to DNA-Incorporated ^{131}IdU, [^{3}H]dThd or ^{125}IdU

Figures 5–7 show the survival of V79 cells as a function of ^{131}IdU, [^{3}H]dThd and ^{125}IdU activity incorporated into the cell, respectively. The activity was incorporated into the cells during a 12-h uptake period at 37°C, after which the cells were washed free of extracellular activity, maintained at 10.5°C for 72 h and plated for colony formation. The data for each experiment were fitted to the relationship

$$S = 1 - (1 - e^{-A/A_0})^n, \quad (3)$$

where A is the mean activity per cell at the end of the 12-h uptake period, and A_0 and n are analogous to the D_0 and n in the single-hit multitarget model (*32*), respectively. The DMF is simply the ratio of the values of A_0 in the presence and absence of DMSO. A value of $n = 1$ was used for the fit to the data for [^{3}H]dThd and ^{125}IdU. As shown in Table II, the mean DMFs for protection by DMSO against the lethal effects of DNA-incorporated ^{131}IdU, [^{3}H]dThd and ^{125}IdU are 1.0 ± 0.1, 1.16 ± 0.07 and 1.07 ± 0.02, respectively.

DISCUSSION

Typical cell survival experiments *in vitro* using DMSO as a radioprotector involve acute radiation exposures in the presence of DMSO concentrations as high as 15–20% (v/v) (*24*). The cells are usually washed free of the radioprotector immediately after the irradiation and plated for colony formation. When cells are irradiated by incorporated radionuclides, the radiation dose is delivered chronically at 37°C while the radioactivity is taken up by the cells during the colony-forming period (*11*). To examine the capacity of radioprotectors to protect against effects caused by chronic irradiation by incorporated radionuclides, the chemical agent should be present throughout the irradiation period. However, chronic exposure of cultured cells to chemical protectors at 37°C leads to extreme chemotoxicity, particularly when levels sufficient to

TABLE I
Dose Modification Factors for Unbound Radionuclides

Radiochemical	Experiment number	5% DMSO		0% DMSO		DMF
		n	C_0 (MBq/ml)	n	C_0 (MBq/ml)	
^{32}P	1	1.14	0.417	3.55	0.176	2.36
^{32}P	2	1.70	0.449	2.15	0.232	1.94
^{32}P	3	1.10	0.682	2.38	0.237	2.88
^{32}P	4	0.90	0.629	1.91	0.208	3.03
						2.6 ± 0.5
$^{3}H_2O$	1	1	25.3	1	9.63	2.63
$^{3}H_2O$	2	1	22.1	1	10.9	2.03
$^{3}H_2O$	3	1	28.5	1	12.0	2.36
						2.3 ± 0.3

afford protection are used. The results show (Figs. 1 and 2) that this problem can be overcome by maintaining the cells at 10.5°C. Under these conditions, the V79 cells did not divide, and no chemotoxicity was observed for either control or treated cells when the DMSO concentration was maintained at or below 5%. While higher concentrations of DMSO are desirable for maximum protection (*24*), these conditions were the best we were able to achieve.

When cells were chronically irradiated by unbound ^{32}P in the presence of 5% DMSO, a DMF of 2.6 ± 0.5 was observed. Given that the radionuclide ^{32}P emits low-LET β particles with a mean energy of 695 keV (*33*), the observed protection is within the expected range (*24*). Similarly, DMSO yielded a DMF of 2.3 ± 0.3 when the V79 cells were maintained in culture medium containing $^{3}H_2O$ and thus were irradiated by low-energy β particles emitted by ^{3}H. In contrast, when the ^{3}H was incorporated into the DNA with [^{3}H]dThd, a DMF of only 1.16 ± 0.07 was observed. Considering the experimental errors involved, one can conclude that only slight protection was observed for DNA-incorporated ^{3}H. This radionuclide emits β particles with a mean energy of 5.7 keV (*33*) with a corresponding LET in water of 3.3 keV/μm (*34*). Interestingly, the RBE for cell killing for $^{3}H_2O$ is only about 1.3 (*35*), whereas the RBE of [^{3}H]dThd for cell

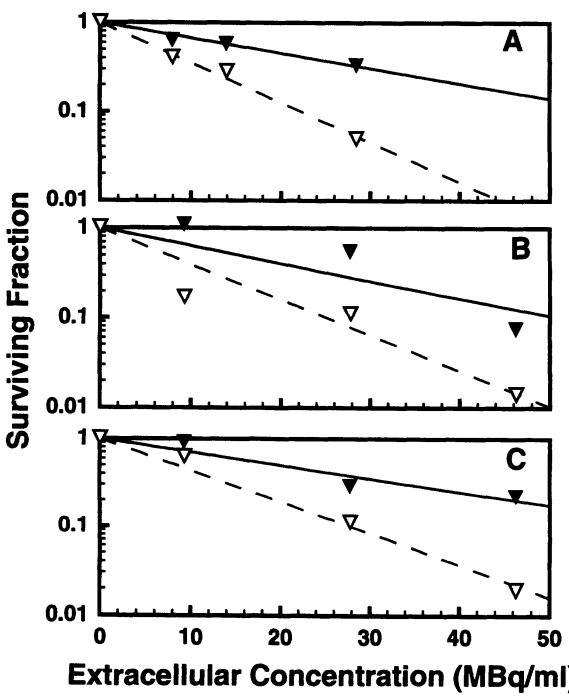

FIG. 4. Survival of V79 cells after a 12-h exposure at 37°C followed by a 72-h exposure at 10.5°C to various concentrations of $^{3}H_2O$ in the culture medium in the absence (▽) and presence (▼) of 5% DMSO. The data from three independent experiments are shown in panels A, B and C, respectively. Least-squares fits of the data are shown. Standard deviations for individual data points are of the order of the dimensions of the symbols.

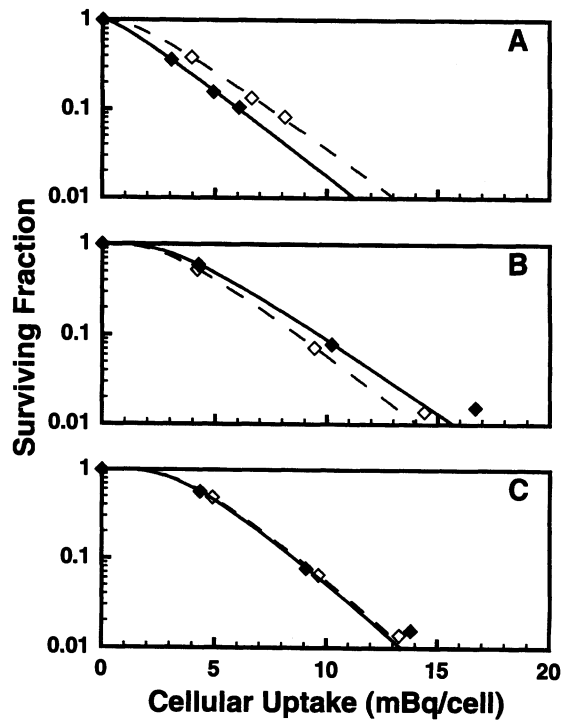

FIG. 5. Survival of V79 cells as a function of intracellular activity of ^{131}IdU in the absence (◇) and presence (◆) of 5% DMSO in the culture medium. The data from three independent experiments are shown in panels A–C along with the corresponding least-squares fits. Standard deviations for individual data points are of the order of the dimensions of the symbols.

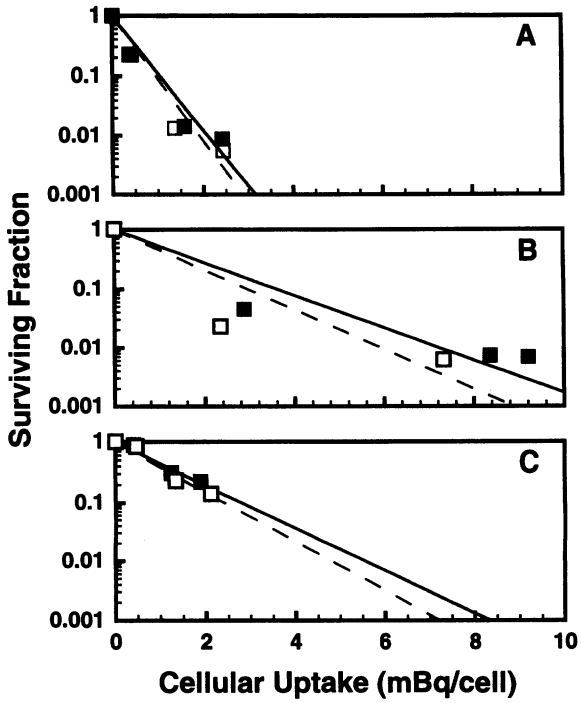

FIG. 6. Survival of V79 cells as a function of intracellular activity of [^{3}H]dThd in the absence (□) and presence (■) of 5% DMSO in the culture medium. The data from three independent experiments are shown in panels A–C. Least-squares fits of the data are shown. Standard deviations for individual data points are of the order of the dimensions of the symbols.

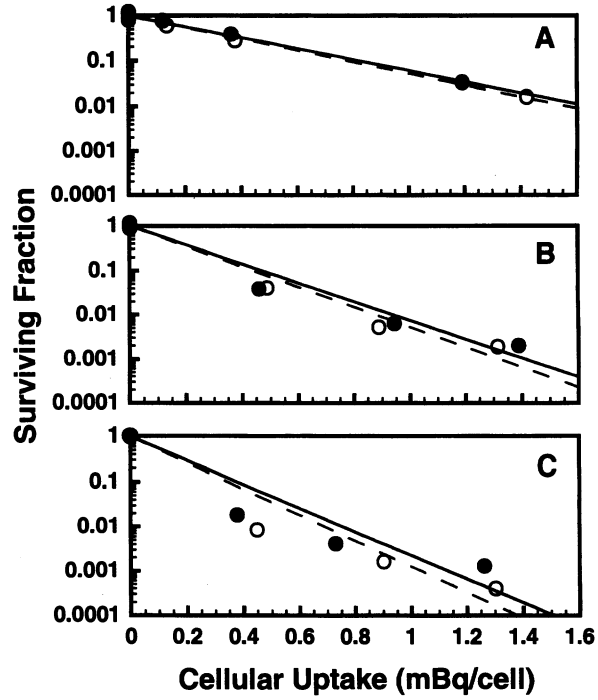

FIG. 7. Survival of V79 cells as a function of intracellular activity of ^{125}IdU in the absence (○) and presence (●) of 5% DMSO in the culture medium. The data from three independent experiments are shown in panels A–C. Least-squares fits of the data are shown. Standard deviations for individual data points are of the order of the dimensions of the symbols.

killing *in vitro* has been shown to be about 3 (*35*). A similar absence of protection was observed (DMF = 1.0 ± 0.1) against DNA-incorporated ^{131}I, which emits β particles with mean energy of about 191 keV (LET in water of about 0.3 keV/μm). Finally, when the V79 cells were exposed to ^{125}IdU, a DMF of 1.07 ± 0.02 was observed, indicating that no significant protection was afforded by 5% DMSO in this case either. The radionuclide ^{125}I is a prolific Auger electron emitter which is known to impart high-LET-type damage similar to that caused by α particles (80–100 keV/μm) when incorporated into the DNA via IdU, and has an RBE of 9 (*11*). Hence the results suggest that DMSO at the concentration employed (5%) is able to protect against damage caused by chronic irradiation by low-LET ^{32}P and medium-LET ^{3}H β particles when the emitter is not bound to DNA. However, the DMSO is unable to protect against lethal damage caused by the DNA-incorporated radionuclides. It is also interesting to note that the high-LET-type damage caused by ^{125}IdU, the relatively lower-LET-type damage caused by [^{3}H]dThd, and the low-LET-type damage caused by ^{131}IdU are

TABLE II
Dose Modification Factors for DNA-Incorporated Radionuclides

Radiochemical	Experiment number	5% DMSO		0% DMSO		DMF
		n	A_0 (mBq/cell)	n	A_0 (mBq/cell)	
^{131}IdU	1	1.52	2.25	2.28	2.41	0.93
^{131}IdU	2	4.20	2.61	4.19	2.35	1.11
^{131}IdU	3	6.20	2.10	6.38	2.13	0.99
						1.0 ± 0.1
[^{3}H]dThd	1	1	0.455	1	0.415	1.09
[^{3}H]dThd	2	1	1.56	1	1.28	1.22
[^{3}H]dThd	3	1	1.20	1	1.04	1.16
						1.16 ± 0.07
^{125}IdU	1	1	0.355	1	0.339	1.05
^{125}IdU	2	1	0.203	1	0.190	1.07
^{125}IdU	3	1	0.163	1	0.149	1.09
						1.07 ± 0.02

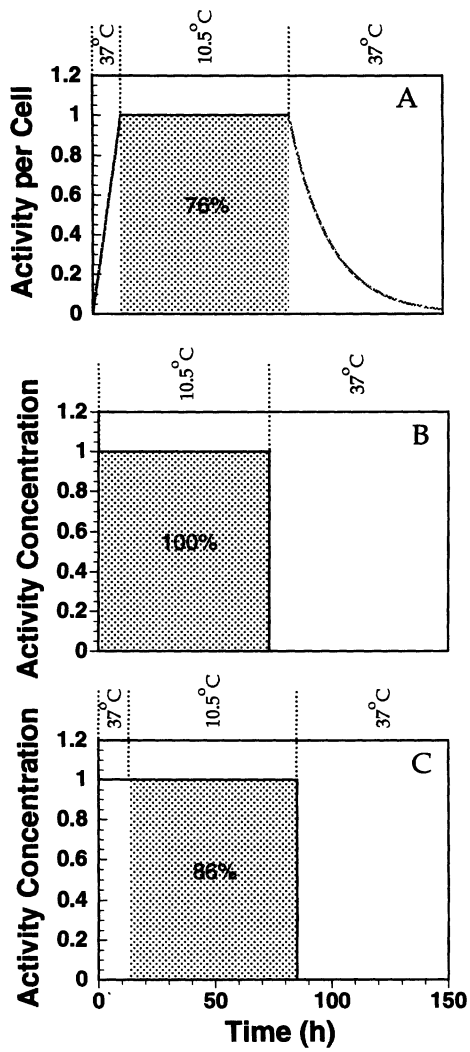

FIG. 8. Changes in temperature and the presence of DMSO (shaded regions) during exposure to the activity of nonincorporated radionuclides (^{32}P, $^{3}H_2O$) or intracellular radionuclides (^{131}IdU, [^{3}H]dThd, ^{125}IdU). The units of activity are arbitrary. Panel A: Irradiation with DNA-incorporated ^{131}IdU, [^{3}H]dThd and ^{125}IdU. Intracellular activity of ^{131}IdU, [^{3}H]dThd or ^{125}IdU as a function of time. The area under the curve is proportional to the cumulated decays in the V79 cell nucleus. The period of 0–12 h represents the uptake of the radiochemical at 37°C, after which the cells were washed free of extracellular activity. The shaded region represents the 72-h period where the cells were maintained at 10.5°C in culture medium containing 0% or 5% DMSO. Finally, the curved region corresponds to the 1-week colony-forming period at 37°C where the cellular activity has an effective half-time of ~12 h (*11*). As denoted by the shaded region, 76% of the intracellular decays occur when the cells were maintained at 10.5°C with 0% or 5% DMSO. Panel B: Irradiation with unbound ^{32}P. The concentration of ^{32}P in the culture medium containing the V79 cells was held constant over 72 h at a temperature of 10.5°C. Samples were maintained in 0% or 5% DMSO by volume during the 72-h period. The cells were subsequently washed, plated for colony formation and maintained at 37°C for 1 week. As denoted by the shaded region, 100% of the irradiation occurred during the 72-h period at 10.5°C with 0% or 5% DMSO. Panel C: Irradiation with unbound $^{3}H_2O$. The concentration of $^{3}H_2O$ in the culture medium was held constant for 12 h at 37°C and was maintained for an additional 72 h at 10.5°C in the presence or absence of 5% DMSO. The cells were subsequently washed, plated for colony formation and maintained at 37°C for 1 week. As denoted by the shaded region, 86% of the irradiation occurred during the 72-h period at 10.5°C with 0% or 5% DMSO.

equally unaffected in these experiments. Given that DMSO can protect against the indirect effects of ^{32}P and $^{3}H_2O$, and cannot protect against the high-LET-type effects of ^{125}IdU or the effects of [^{3}H]dThd and ^{131}IdU, which are presumably of an indirect nature, one can postulate that 5% DMSO cannot provide protection *in vitro* against damage caused by DNA-incorporated radionuclides regardless of whether the damage is caused by direct or indirect effects. This may suggest that, under our experimental conditions, DMSO cannot scavenge within a few angstroms of the DNA atoms.

These *in vitro* results for DMSO are somewhat different from those obtained for DMSO *in vivo* (*19*). When spermatogonial cell killing in the mouse testis was used as the biological end point, substantial protection was observed against the high-LET-type damage caused by DNA-incorporated ^{125}IdU as well as the low-LET-type effects of cytoplasmically localized ^{125}I with DMF values of 3.1 ± 1.0 and 4.4 ± 1.0, respectively (*19*). The observed differences between the *in vitro* and *in vivo* results may be due to the substantial differences in radiosensitivity and repair mechanisms between the V79 and spermatogonial cells (*36*). Furthermore, Vos and Kaalen (*24*) have shown that the protection afforded by DMSO against damage caused by exposure to acute γ rays *in vitro* is linearly dependent on the concentration, requiring as much as 15% for maximum protection. In our experiments involving chronic irradiation by incorporated radionuclides, a maximum DMSO concentration of only 5% could be used to avoid chemotoxicity. Hence it is possible that concentrations greater than 5% are required to protect against biological damage caused by DNA-incorporated radionuclides *in vitro*.

In the studies with DNA-incorporated radionuclides, DMSO was not present during the 37°C uptake period when radioactivity was being incorporated into the cells or during the 37°C colony-forming period. In fact, about 24% of the total intracellular ^{131}I, ^{3}H and ^{125}I decays occurred at 37°C and in the absence of DMSO (Fig. 8A). In contrast, the experiments involving irradiation of V79 cells by unbound ^{32}P were carried out at 10.5°C and DMSO was present throughout the irradiation period (Fig. 8B). Therefore, temperature differences during a portion of the irradiation and the absence of DMSO during these periods could be partly responsible for the absence of protection observed in the cases of the DNA-incorporated radionuclides compared to the substantial protection observed for unbound ^{32}P and $^{3}H_2O$. However, this premise is largely negated by our studies with $^{3}H_2O$, where 14% of the irradiation was carried out at 37°C in the absence of DMSO (Fig. 8C) and, despite this, a DMF of 2.3 was obtained. Therefore, it is unlikely that temperature or absence of DMSO during a portion of the irradiation period had a major impact on the degree of protection observed for ^{131}IdU, [^{3}H]dThd and ^{125}IdU.

CONCLUSIONS

This work introduces an experimental procedure which enables examination of the capacity of chemical agents to

protect against the biological effects of chronic exposure of cultured cells to incorporated radionuclides in a liquid environment. DMSO (5% v/v) provided substantial protection against the lethal effects caused by chronic exposure of cultured Chinese hamster V79 cells to unbound ^{32}P and $^{3}H_2O$. Little or no protection against the lethal effects of chronic exposure to DNA-incorporated ^{131}I, ^{125}I and ^{3}H was observed. These results suggest that 5% DMSO is not capable of protecting against lethal damage caused by DNA-incorporated radionuclides *in vitro*.

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