Radiation Protection by Cysteamine against the Lethal Effects of Intracellularly Localized Auger Electron, α- and β-Particle Emitting Radionuclides

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The mechanisms by which DNA-incorporated radionuclides impart lethal damage to mammalian cells were investigated by examining the capacity of cysteamine (MEA) to protect against lethal damage to V79 cells caused by unbound tritium (^3H_2O), DNA-incorporated $^{131/125}$I-iododeoxyuridine (IdU) and the α-particle emitter ^{210}Po-citrate. Radiolabeled cells were maintained at 10.5°C for 72 h in the absence or presence of MEA (0.65–2.6 mM) and the surviving fraction was determined. Protection against lethal damage caused by ^3H_2O, ^{131}IdU or ^{125}IdU and ^{210}Po-citrate depended on the concentration of MEA with maximum protection at 1.3–1.9 mM. The dose modification factors obtained at 1.3 mM for the radiochemicals were 2.5 ± 0.3, 1.8 ± 0.2, 1.7 ± 0.1 and 1.4 ± 0.1, respectively. MEA provides more protection against indirect than direct effects of ionizing radiation, and indirect effects play a role in the radiotoxicity of Auger electron emitters incorporated into the DNA of mammalian cells.

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It is well known that the radiotoxicity of Auger electron emitters, which decay by electron capture and/or internal conversion, depends on their subcellular distribution. For example, when the prolific Auger electron emitter ^{125}I is localized in the cytoplasm of the cell, its radiotoxicity is similar to that of radiations of low linear energy transfer (LET) such as external x-rays (1–4). In contrast, when the same radionuclide is incorporated into the DNA via ^{125}I-iododeoxyuridine (^{125}IdU), its biological effects are as lethal as the high-LET 5.3 MeV α-particles emitted by ^{210}Po (5, 6).

The exact mechanism by which DNA-bound Auger electron emitters exert high-LET type responses in biological systems has been the subject of considerable interest and sustained debate. The capacity of different chemical agents to mitigate the biological effects of radionuclides may provide insights into their mechanism of radiation action. During the past few years, the capacity of a variety of chemical agents to provide protection against damage caused by incorporated radionuclides in vivo has been reported (4, 7–12). Several different radionuclides have been investigated, including the Auger electron emitter ^{125}I, the β-particle emitter ^{131}I and the α-particle emitter ^{210}Po. Hydroxyl radical scavengers such as dimethyl sulfoxide (DMSO) and vitamin C have afforded considerable protection in vivo against low-LET type damage caused by cytoplasmically localized ^{125}I- or ^{131}I-labeled *N*,*N*,*N′*-trimethyl-*N′*-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine (H$^{125/131}$IPDM) and high-LET type damage caused by DNA-incorporated ^{125}IdU (8, 9, 12). No protection has been observed for these scavengers against the high-LET effects caused by the 5.3 MeV α-particles emitted by ^{210}Po (9, 12). These and other studies with the above radiochemicals and chemical protectors, namely, cysteamine (2-mercaptoethylamine, MEA) (4, 7), *S*-(2-aminoethyl) isothiouronium bromide hydrobromide (AET) (10), soybean oil (11) and vitamin A (11), and different radionuclides have provided strong evidence that the mechanism by which Auger electron emitters impart high-LET type damage in vivo is largely indirect in nature. The indirect mechanism of damage caused by DNA-incorporated Auger emitters was first hypothesized by Rao et al. (7) after considering published theoretical calculations (13) and the mouse testes data for MEA (4, 7).

Attempts have been made to correlate in vivo experimental findings with in vitro radioprotection studies to

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confirm the mechanism of action of Auger emitters. It has been shown that although a non-toxic concentration of DMSO (5% v/v, 0.64 M) provided a substantial protection against lethal effects caused by chronic exposure of cultured Chinese hamster V79 cells to unbound ^{32}P and tritiated water ($^{3}H_2O$) at 10.5°C, no significant protection was observed against the lethal effects of chronic exposure to DNA-incorporated ^{131}IdU, ^{125}IdU and tritiated thymidine ([^{3}H]dThd) (14). These results appear to be in contradiction with in vivo studies showing that DMSO provides substantial protection against cell killing by DNA-incorporated ^{125}IdU and cytoplasmically localized H^{125}IPDM (12). Subsequently, it was demonstrated that chemotoxic concentrations of DMSO (10%, 1.28 M) provide marked protection against DNA double strand breaks and death of V79 cells caused by ^{125}IdU at 0.3°C (15, 16). In a very recent communication, it was shown that that protection by DMSO against the lethal effects of DNA-incorporated ^{131}IdU and ^{125}IdU is concentration dependent, with maximum protection provided by 10% DMSO (17). No protection was afforded against 5.3 MeV α-particles emitted by ^{210}Po-citrate. These studies with DMSO are in agreement with earlier in vivo studies (12) and indicate that indirect effects are largely responsible for lethal damage imparted by DNA-bound Auger electron emitters.

It has also been hypothesized that indirect effects are not the primary mechanism for the lethal action of Auger electron emitters incorporated into the DNA of mammalian cells (18). The authors reached this conclusion based on experiments in which synchronized Chinese hamster ovary (CHO) cells were labeled with ^{125}IdU and frozen in cryoprotective medium with 25 mM MEA at different times following radiolabeling. Radioprotection by MEA was observed for cells frozen 30 min after labeling which showed the characteristic shouldered dose–response of low-LET radiation (i.e. indirect effects). In contrast, no protective effect was noted for cells frozen 5 h post-labeling, which is indicative of a high-LET type radiation response (i.e. direct effects). It is well known that MEA and other sulfhydryl compounds act in part by scavenging free radicals and thereby protect against indirect effects of ionizing radiation. However, there is ample evidence that MEA also mitigates direct effects of ionizing radiation (4, 7, 19, 20).

In view of this controversy, the present work evaluates the concentration dependence of the radioprotective action of MEA against lethal damage caused by different radionuclides to understand better the mechanism of the radiobiological effect of Auger electron emitters incorporated into the DNA of mammalian cells. Cultured Chinese hamster V79 cells were used as the experimental model and cell survival served as the biological endpoint. Several radiochemicals were studied, including the β-particle emitter $^{3}H_2O$, IdU labeled with either the prolific Auger electron emitter ^{125}I or the β-particle emitter ^{131}I, and the α-particle emitter ^{210}Po-citrate.

MATERIAL AND METHODS

Radiochemicals and quantification of radioactivity

The radiochemical tritiated water ($^{3}H_2O$, 370 MBq/ml) was purchased from NEN Life Science Products (Boston, MA, USA). Radiolabeled iododeoxyuridine (^{131}IdU and ^{125}IdU) and ^{210}Po-citrate were prepared as per previously published methods (6, 11). The radiochemicals ^{131}IdU, ^{125}IdU and ^{210}Po-citrate are incorporated into the V79 cells, whereas $^{3}H_2O$ diffuses freely in the cells (6, 14). The ^{3}H and ^{210}Po activity was measured with an automatic liquid scintillation counter, whereas the ^{125}I and ^{131}I activity was quantitated with a NaI well-detector (14).

Cell line

Chinese hamster V79 lung fibroblasts (kindly provided by AI Kassis, Harvard Medical School, Boston, MA, USA) were used in these studies, with clonogenic survival serving as the biological endpoint. Cell culture media and standard incubation conditions (37°C, 5% CO_2, 95% air, 100% humidity) were the same as reported previously (14, 17). Temperatures were maintained at 10.5°C when MEA was present to minimize its chemotoxicity (14).

Chemotoxicity of MEA

The chemotoxicity of the chemical protector MEA was determined at 10.5°C. Chinese hamster V79 cells, growing as monolayers in 75 cm^2 flasks, were washed with 10 ml of phosphate-buffered saline, trypsinized with 0.05% trypsin–0.53 mM EDTA, and suspended at 4×10^5 cells/ml in MEMB (calcium-free MEM with same supplements as MEMA). Aliquots of 1 ml were transferred into sterile 12× 75 mm Falcon polypropylene round-bottomed culture tubes and placed on a rocker–roller for 3–4 h in standard incubation conditions. After this conditioning period, an additional 1 ml of MEMB was added and all tubes were returned to the rocker–roller. Following a 12 h incubation period, the cells were washed three times with wash MEMA (MEMA prepared with calf serum), and finally suspended in 2 ml ice-cold MEMA containing 0.65–2.6 mM of MEA (Sigma Chemical Co., St. Louis, MO, USA). The tubes were capped tightly, transferred to the rocker–roller at 10.5°C for 72 h, centrifuged at 2000 rpm and 4°C for 10 min, and washed three times with cold wash MEMA. After resuspending in 2 ml MEMA, the cells were serially diluted (three 10× dilutions), seeded into 60× 15 mm culture dishes containing 4 ml of MEMA, and left in standard incubation conditions. After 1 week the resulting colonies were stained and scored (6). The surviving fraction compared with controls (0 mM MEA) was determined.

Lethality of ^3H_2O as a function of MEA concentration

Two sets of culture tubes (five per set) containing 1 ml of V79 cells (4×10^5 cells/ml) were conditioned as above. Then, 0.75 ml of MEMB containing 55.5 MBq of ^3H_2O was added to first set of five tubes and 0.75 ml of MEMB without any radioactivity was added to the second set of five tubes. All tubes were returned to the rocker–roller for 12 h, after which an additional 0.25 ml of MEMB containing different amounts of MEA was added to each set of tubes while vortexing to achieve final MEA concentrations of 0, 0.65, 1.3, 1.9 and 2.6 mM. The caps were then snapped on tightly and the tubes were transferred to a rocker–roller at 10.5°C. After 72 h, the cells were washed and seeded for colony formation, and the resulting colonies were scored. To correct for chemotoxicity, the cell survival fraction SF was calculated according to eq. 1:

$$SF = N(^3H_2O + \text{mM MEA})/N(\text{mM MEA}) \quad [1]$$

where N is the number of colonies corresponding to the treatment regimen described within the parentheses. The highest survival fraction was observed for 1.3 mM MEA.

Lethality of ^3H_2O in the presence of 1.3 mM MEA

In separate experiments, 0.99 ml of MEMB containing different activities of ^3H_2O (0–92.5 MBq) was aliquoted into two sets of culture tubes (five tubes per set) containing 1 ml of conditioned V79 cells (4×10^5 cells/ml). The tubes were then capped loosely and returned to the rocker–roller in standard incubation conditions. After 12 h, an additional 0.1 ml of MEMB with MEA (in the first set of tubes) or without MEA (in the second set) was added while vortexing and the caps were snapped on tightly and transferred to a rocker–roller at 10.5°C. Thus, for each concentration of ^3H_2O, two tubes were made with final MEA concentrations of 0 and 1.3 mM, respectively. After 72 h, the tubes were processed as above to determine the survival fractions. The survival fractions were calculated according to eqs 2 and 3:

$$SF(^3H_2O, \text{0 mM MEA}) = N(^3H_2O + \text{0 mM MEA})/N(\text{0 mM MEA}) \quad [2]$$

$$SF(^3H_2O, \text{1.3 mM MEA}) = N(^3H_2O + \text{1.3 mM MEA})/N(\text{1.3 mM MEA}) \quad [3]$$

Lethality of ^{210}Po, ^{131}IdU and ^{125}IdU as a function of MEA concentration

The protocols for the intracellularly bound radiochemicals were essentially the same as described for ^3H_2O, with a minor modification. Following a 30 min (for ^{210}Po) or 12 h (for ^3H_2O, ^{131}IdU and ^{125}IdU) exposure to a single concentration of the radiochemical in MEMB, the cells were washed three times with wash MEMA and resuspended in 2 ml MEMA containing different concentrations of MEA (0–2.6 mM). The caps were snapped on tightly and all tubes were transferred to the rocker–roller at 10.5°C. After 72 h at 10.5°C, the cells were washed and seeded for colony formation. Aliquots were taken from each tube before serial dilution and the mean radioactivity per cell was determined as described previously (6, 14). The surviving fraction compared with parallel MEA controls was determined using eq. 1.

Lethality of ^{210}Po, ^{131}IdU and ^{125}IdU in the presence of 1.3 mM MEA

For the experiments with 1.3 mM MEA, conditioned V79 cells were radiolabeled as described above using different concentrations of radioactivity in MEMB. After washing the cells free of extracellular radioactivity, the cells were resuspended in MEMA containing 1.3 mM MEA and kept at 10.5°C for 72 h. Parallel controls were maintained for each treatment. After this period, the cells were processed for colony formation. The survival fractions for the radiolabeled cells exposed to 0 mM and 1.3 mM MEA were calculated using eqs 2 and 3.

RESULTS

Chemotoxicity of MEA

The chemotoxicity of MEA as a function of its concentration in the culture medium was determined by averaging the data for MEA alone (no radiochemical) from all experiments. The survival fraction for cells treated with different concentrations of MEA at 10.5°C compared with cells not treated with MEA was 0.91 ± 0.03, 0.84 ± 0.02, 0.65 ± 0.05 and 0.58 ± 0.03 for 0.65, 1.3, 1.9 and 2.6 mM MEA, respectively. As observed previously, no toxicity was associated with maintaining the cells at 10.5°C without any chemical treatment (14, 17).

Dose modification factors as a function of MEA concentration

Preliminary studies were carried out a single concentration of radioactivity in the culture medium to determine the optimum concentration of MEA to achieve maximum radioprotection. The approximate mean lethal concentration C_0 (for ^3H_2O) and cellular uptake A_0 of radioactivity (^{210}Po-citrate, ^{125}IdU, ^{131}IdU) corresponding to each concentration of MEA were determined by assuming that the survival fraction is exponentially dependent on these variables, as per eqs 4 and 5:

$$SF(^3H_2O) = \exp(-C/C_0) \quad [4]$$

$$SF(^{210}Po\text{-citrate or } ^{125/131}IdU) = \exp(-A/A_0). \quad [5]$$

Since the absorbed dose received by the cells is directly proportional to the concentration of ^3H_2O in the culture medium or mean cellular uptake of ^{210}Po-citrate, ^{131}IdU or ^{125}IdU, the dose modification factor (DMF), or degree of protection provided by MEA against the lethal effects of

radionuclides, is given by the ratio of the C_0s or A_0s in the presence and absence of MEA:

$$\mathrm{DMF}(^3\mathrm{H_2O}) = \frac{C_0(\text{with MEA})}{C_0(\text{without MEA})},$$

$$\mathrm{DMF}(^{125/131}\mathrm{IdU}) = \frac{A_0(\text{with MEA})}{A_0(\text{without MEA})}. \quad [6]$$

Figure 1 shows that MEA protects V79 cells from damage caused by incorporated ^{210}Po-citrate, DNA-bound ^{131}IdU or ^{125}IdU, and unbound ^3H_2O, in a concentration-dependent manner. Little or no protection was afforded by 0.65 mM MEA against damage caused by DNA-bound ^{125}IdU or ^{210}Po-citrate, whereas some protection was observed in the case of ^3H_2O and ^{131}IdU. MEA-mediated protection was optimum at 1.3 mM for ^3H_2O and at 1.9 mM for ^{210}Po-citrate, ^{125}IdU and ^{131}IdU. However, the steeply falling DMF for ^3H_2O at 1.9 mM and the substantial chemotoxicity observed at this concentration warranted its exclusion for further studies. A marked decline in the DMF was observed for all four radiochemicals at 2.6 mM MEA. The DMF values shown in Fig. 1 were derived from a limited set of data collected with the purpose of determining the optimal MEA concentration (1.3 mM). Accordingly, the radioprotective efficacy of this MEA concentration was investigated in more detail, as described below.

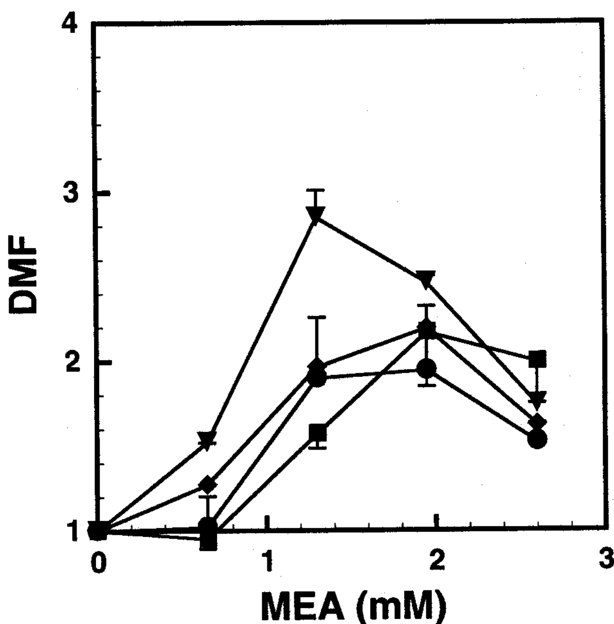

Fig. 1. Dose modification factors (DMF) as a function of MEA concentration. V79 cells were exposed to ^3H_2O (▼), ^{131}IdU (◆) or ^{125}IdU (●) for 12 h and to ^{210}Po (■) for 30 min at 37°C and subsequently treated with different concentrations of MEA at 10.5°C for 72 h. Each point indicates mean ± SD for two separate experiments.

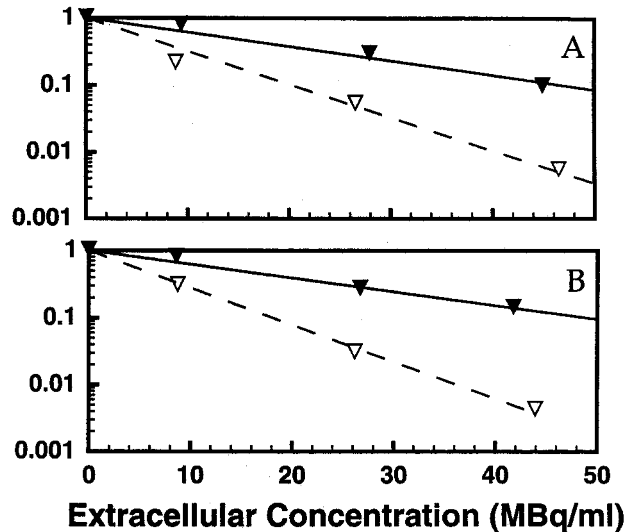

Fig. 2. Survival of V79 cells following a 12 h exposure at 37°C followed by a 72 h exposure at 10.5°C to various concentrations of ^3H_2O in the culture medium in the absence (▽) and presence (▼) of 1.3 mM MEA. The data for two independent experiments are shown in (A) and (B). Least squares fits of the data to a monoexponential function are shown. SDs for individual data points are of the order of the dimensions of the symbols.

Dose modification by 1.3 mM MEA

The radioprotective efficacy of 1.3 mM MEA was investigated by exposing V79 cells to different concentrations of unbound ^3H_2O and different cellular uptakes of DNA-bound ^{131}IdU and ^{125}IdU. The capacity of 1.3 mM MEA to protect against the direct effects of ^{210}Po α-particles was also investigated. Figure 2 shows the survival of V79 cells following a 72 h exposure at 10.5°C to various concentrations (0–46.3 MBq/ml) of ^3H_2O in the absence and presence of 1.3 mM MEA. The data were least squares fitted to eq. 4 to obtain the mean lethal concentration C_0 for ^3H_2O. The DMF values for each experiment were calculated using eq. 6 and are presented in Table 1. The mean DMF value for the two experiments was DMF (^3H_2O, 1.3 mM MEA) = 2.5 ± 0.3.

Figures 3–5 illustrate the survival of V79 cells as a function of the mean cellular uptake of radioactivity following exposure to ^{131}IdU, ^{125}IdU and ^{210}Po, respectively. Survival curves are presented for data acquired in the absence and presence of 1.3 mM MEA. The data for each experiment were fitted by least squares methods to eq. 5. The DMF is simply given by the ratio of the A_0 values in the presence and absence of MEA (eq. 6). The DMF values for each experiment are given in Table 1. The mean DMF values for protection by 1.3 mM MEA against the lethal effects of ^{131}IdU, ^{125}IdU and ^{210}Po are 1.8 ± 0.2, 1.7 ± 0.1 and 1.4 ± 0.1, respectively.

Table 1

Dose modification factors (DMF) in the presence of 1.3 mM 2-mercaptoethylamine (MEA)

Radiochemical	Exp. no.	0 mM MEA	1.3 mM MEA	DMF
		C_0 (MBq/ml)	C_0 (MBq/ml)	
^3H_2O	1	8.8	20.5	2.3
	2	7.9	21.3	2.7
				2.5 ± 0.3
		A_0 (mBq/cell)	A_0 (mBq/cell)	
^{210}Po-citrate	1	0.022	0.03	1.4
	2	0.029	0.046	1.6
	3	0.037	0.049	1.3
				1.4 ± 0.1
^{131}IdU	1	4.79	8.43	1.8
	2	3.01	6.06	2.0
	3	2.87	4.91	1.7
				1.8 ± 0.2
^{125}IdU	1	0.124	0.202	1.6
	2	0.09	0.161	1.8
				1.7 ± 0.1

DISCUSSION

The aminothiol MEA and its derivatives are well-studied exogenous thiols of biological significance. The sulfhydryl group of MEA is involved in various physiological processes related to the redox cycles of a functional cell, and consequently a wealth of literature is available on its radioprotective (21), anticarcinogenic (22) and other related properties. Although it is well known that relatively high concentrations of MEA provide substantial radioprotection against the effects of acute external beams of ionizing radiations both in vivo (23, 24) and in vitro (25), it has also been reported that low concentrations of MEA and AET afford significant radioprotection against biological damage caused by chronic irradiation of tissue-incorporated radionuclides in vivo (4, 7, 10). In these studies where spermhead survival was used as the biological endpoint, almost the same DMF was observed for DNA-incorporated ^{125}IdU (DMF = 3.6) and cytoplasmically localized H^{131}IPDM (DMF = 3.8) and H^{125}IPDM (DMF = 3.8). In contrast, a significantly lower value was observed for ^{210}Po-citrate (DMF = 2.6) (4). Thus, although the lethal effects of DNA-incorporated ^{125}IdU have been reported to be similar to or greater than those caused by 5.3 MeV α-particles (5, 6), MEA provided a substantially higher degree of protection against the former (4). Similar results were obtained with another aminothiol AET where DMF values of 4.0, 3.4 and 2.4 were obtained for ^{125}IdU, H^{125}IPDM and ^{210}Po-citrate, respectively (10). MEA provided more dramatic protection against induction of spermhead abnormalities, where DMF values of 14 and 10 were achieved for ^{125}IdU and ^{210}Po-citrate, respectively (7). The above studies suggested that MEA provides better

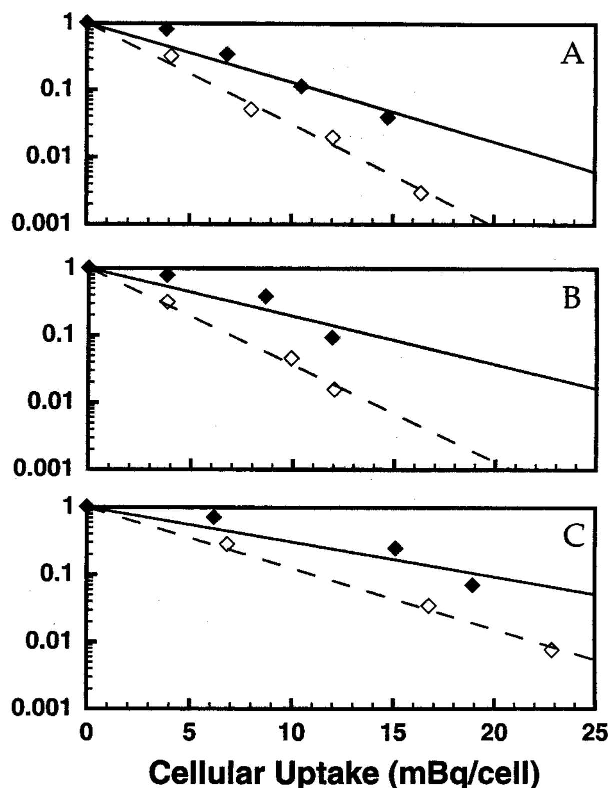

Fig. 3. Survival of V79 cells as a function of intracellular activity of ^{131}IdU in the absence (◇) and presence (◆) of 1.3 mM MEA in the culture medium. The data for three independent experiments are shown in (A), (B) and (C), along with the corresponding least squares fits to a monoexponential function. SDs for individual data points are of the order of the dimensions of the symbols.

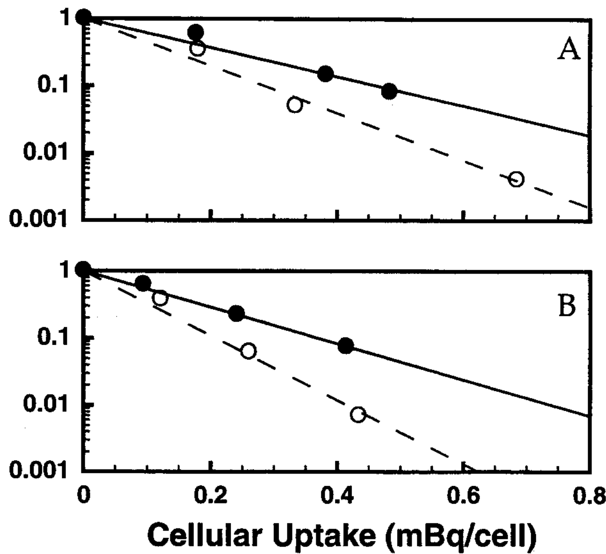

Fig. 4. Survival of V79 cells as a function of intracellular activity of ^{125}IdU in the absence (○) and presence (●) of 1.3 mM MEA in the culture medium. The data for two independent experiments are shown in (A) and (B). Least squares fits of the data to a monoexponential function are shown. SDs for individual data points are of the order of the dimensions of the symbols.

protection against indirect effects (H^{131}IPDM) of radiation exposure than direct effects (^{210}Po-citrate), and the mechanism by which DNA-incorporated Auger electron emitters (^{125}IdU) exert damage in mammalian cells is largely indirect in nature (4, 7, 10).

It has also been suggested that indirect effects do not play a major role in the lethal action of Auger electron emitters incorporated into the DNA of mammalian cells (18). When CHO cells were synchronized, pulse labeled with ^{125}IdU and frozen at -196°C for decay accumulation, both high- and low-LET type effects were imparted depending on the length of time for which the cells were allowed to progress through their cell cycle prior to freezing in culture medium containing 0.98 M (7.6%) DMSO (18). When MEA (25 mM) was added before 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, the authors suggested that the mechanism by which DNA-incorporated Auger electron emitters impart high-LET type biological damage is direct in nature. These experiments were carried out under frozen conditions, which may impact on the action of protectors (18). This may explain the different results obtained in the testes and cell culture models.

Therefore, it is of interest to study the capacity of MEA to mitigate damage caused by chronic irradiation by incorporated radionuclides in vitro in a liquid water environment. However, in vitro experiments typically use MEA ($\sim$75 mM) as a radioprotector against the effects of acute radiation exposures at 37°C. The high chemotoxicity of MEA requires that the cells be washed free of the radioprotector immediately following the irradiation (26). When cells are irradiated by radionuclides, the radiation dose is delivered over a prolonged period and therefore the chemical agent should be present during the extended irradiation time. Such a long exposure of cells to MEA can lead to severe toxicity. To minimize the chemotoxicity of MEA in the present study, previously reported techniques were used wherein the cells were maintained at 10.5°C throughout most of the exposure period (14, 17). Under these conditions, the V79 cells were not proliferating, no cryotoxicity was observed and only moderate MEA chemotoxicity resulted, even at the highest MEA concentration studied.

The data presented in Fig. 1 clearly demonstrate a marked dependence of the DMF on MEA concentration in the culture medium. The DMFs for the various radiochemicals were found to rise rapidly as the MEA concentration was increased. It has also been similarly reported that an increasing degree of protection for cultured human kidney cells was provided against the effects of acute irradiation with 200 kVp x-rays as the MEA concentration was increased from 3.5 to 14 mM (27). However, in the

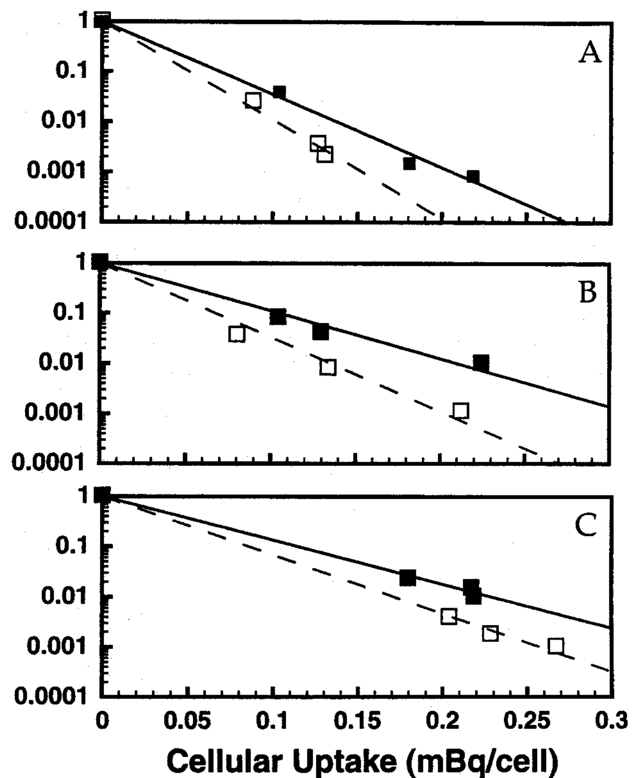

Fig. 5. Survival of V79 cells as a function of intracellular activity of ^{210}Po in the absence (□) and presence (■) of 1.3 mM MEA. The data for three independent experiments are shown in (A), (B) and (C). Least squares fits of the data to a monoexponential function are shown. SDs for individual data points are of the order of the dimensions of the symbols.

present study, the DMF dropped precipitously at 2.6 mM MEA for all radionuclides except $^{3}H_2O$, for which a drop in DMF was noticed at concentrations above 1.3 mM. This drop parallels the observed chemotoxicity profile of MEA at the same concentrations. A significant drop in radioprotection by MEA above 1 mM was also observed when V79 cells were acutely irradiated with a 4 Gy γ-radiation dose (Roberts et al. 1995, unpublished study). A similar pattern of concentration-dependent radiation protection was observed for DMSO over the range of 0.64–1.6 M (5–12.5%) using the same radiochemicals and experimental conditions as in the present study (17). This finding is in line with another report where maximum radioprotection against γ-irradiation was noticed at a DMSO concentration of about 2 M (28). Similarly, Vos & Kaalen also found a marked decline in DMF at high concentrations of DMSO (27). These results indicate that there appears to be a narrow concentration range for optimum protection with chemical radioprotectors. It is plausible that exceeding the optimum concentration results in unacceptable levels of chemotoxicity to the V79 cells that may adversely affect interpretation of the data.

Figures 2–5 and Table 1 show a direct comparison of the radioprotection provided by 1.3 mM MEA against lethal damage to V79 cells caused by $^{3}H_2O$, ^{131}IdU, ^{125}IdU and ^{210}Po-citrate. MEA provided the highest degree of protection against lethal damage caused by $^{3}H_2O$ ($DMF = 2.5 \pm 0.3$). This radiochemical emits β-particles with a mean energy of 5.7 keV, freely diffuses into the cells and does not incorporate into DNA. Somewhat less protection was afforded against the effects of ^{131}IdU (1.8 ± 0.2) and the high-LET type effects of ^{125}IdU (1.7 ± 0.1). ^{131}I emits low-LET β-particles (mean energy 191 keV), whereas ^{125}I emits a dense shower of about 25 low-energy Auger electrons per decay. The least protection ($DMF = 1.4 \pm 0.1$) was observed for the high-LET 5.3 MeV α-particles emitted by intracellular ^{210}Po. Therefore, based on the DMF values, it can be concluded that MEA provides substantial and maximum protection against the low-LET type damage caused by low energy β-particles emitted by unbound $^{3}H_2O$, moderate and equal protection against low-LET-type damage caused by DNA-incorporated ^{131}IdU and high-LET-type effects of DNA-incorporated ^{125}IdU, and somewhat lower protection against high-LET-type damage induced by α-particles. These results corroborate earlier studies in the mouse testes model (4). It is interesting to note that 10% DMSO provided substantial protection against effects caused by $^{3}H_2O$, ^{125}IdU and ^{131}IdU; however, no protection was observed against effects caused by ^{210}Po under the same experimental conditions (17). Similar observations were made in the mouse testes model (12).

The DMF values shown in Table 1 for 1.3 mM MEA are somewhat higher for unbound $^{3}H_2O$ than for DNA-incorporated ^{125}IdU and ^{131}IdU. This was also observed in experiments with DMSO (17), which led us to suggest that a portion of the lethal damage caused by radionuclides incorporated into the DNA of mammalian cells may be due to direct effects, a premise supported by other data sets (29, 30). These data suggest that direct effects from ^{125}I decays in the DNA may be responsible for DNA damage within a few base pairs of the decay site; however, the consequence of this damage appears to be small relative to the overall damage responsible for cell death (7, 12, 16, 17).

In conclusion, the present experimental findings with MEA provide additional evidence that indirect effects play a role in the lethal damage to mammalian cells from DNA-incorporated Auger electron emitters both in vivo (4, 9–12) and in vitro (14–16). Furthermore, the present results also support earlier findings that the radiotoxicity of β-particle emitters in mammalian cell suspension cultures and the degree to which their lethal effects can be mitigated with chemical modifiers appear to be dependent on their subcellular distribution (17).

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