Radioprotection Against Biological Effects of Internal Radionuclides In Vivo by S-(2-Aminoethyl)isothiouronium Bromide Hydrobromide (AET)

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Radionuclides employed in diagnostic and therapeutic nuclear medicine impart radiation energy to tissue over an extended period of time, which depends on the physical half-life and the biological properties of the radiochemical employed. It is therefore important to examine the capacity of chemical radioprotectors to mitigate damage caused by chronic irradiation by incorporated radionuclides. **Methods:** Spermatogenesis in mouse testis is used as the experimental model, and spermatogonial cell survival as measured by testicular spermhead count is the biological end point. The capacity of S-(2-aminoethyl)isothiouronium bromide hydrobromide (AET) to mitigate radiation damage caused by chronic irradiation by the radiochemicals ^{125}IUdR, H^{125}IPDM and ^{210}Po-citrate, is investigated. **Results:** The radioprotection provided by AET is substantial and similar for both of the radioiodinated compounds with dose modification factors (DMF) of 4.0 ± 1.2 for ^{125}IUdR and 3.4 ± 0.4 for H^{125}IPDM. In contrast, the damage caused by ^{210}Po alpha particles is protected against to a lesser degree (DMF = 2.4 ± 0.5). **Conclusion:** The present radioprotection data for AET, in conjunction with our earlier findings for the chemical protectors cysteamine and vitamin C in the same experimental model, suggest that such compounds may be clinically useful as mitigating agents against biological damage caused by incorporated radionuclides. The observed DMFs for AET also support our earlier premise that the mechanism by which DNA-incorporated Auger emitters impart biological damage is primarily radical mediated, and hence indirect in nature.

Key Words: radioprotection; AET; internal radionuclides; Auger electrons; spermatogenesis

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The capacity of chemical protectors to mitigate radiation damage has been well studied with acute external beams of radiation (*1*). However, very little is known about the radioprotective effects of various chemicals against radiation damage from incorporated radionuclides. In contrast to acute irradiation with external beams, incorporated radionuclides deliver the radiation dose over relatively long periods of time which depend on their physical half-lives and biological clearance patterns. Therefore, it is of interest to examine the capacity of chemical protectors to mitigate the biological effects of chronic irradiation by incorporated radionuclides.

Of particular interest to nuclear medicine and radiation research are those radionuclides which decay by internal conversion and/or electron capture (e.g., ^{67}Ga, ^{99m}Tc, ^{111}In, ^{123}I and ^{201}Tl). These radionuclides emit numerous electrons referred to as Auger electrons. Most of these electrons are of very low energy, and therefore have ranges of subcellular dimensions (*2,3*). The collective action of these low-energy electrons leads to highly localized energy deposition and formation of radical species in the immediate vicinity of the decay site (~10 nm radius) (*2,4,5*). Because of the highly localized nature of the energy deposition, the biological effects of Auger emitters are dependent on subcellular distribution of the radionuclide (*6–14*). In fact, when the Auger emitter ^{125}I is incorporated into DNA in the cell nucleus using the pyrimidine analogs 5-(^{125}I)-iodo-2′-deoxyuridine (^{125}IUdR) or iododeoxycytidine-125 (^{125}IdC), its biological effects are as lethal as those of 5.3-MeV alpha particles emitted in the decay of ^{210}Po (*11,12,15*). On the other hand, cytoplasmically localized ^{125}I and ^{123}I are only as effective as sparsely ionizing beta particles of low linear-energy-transfer (low-LET) from ^{131}I and external 120-kVp x-rays, in killing the spermatogonial cells in mouse testes (*12*). For densely ionizing high-LET radiations such as alpha particles, the primary mechanism of radiation action is generally believed to be of a direct nature (direct deposition of energy in the primary radiosensitive targets), whereas for low-LET radiations, indirect action (radical mediated) is considered to be more important. In view of the observed high-LET-type effects of DNA-incorporated ^{125}I, the question whether biological damage caused by Auger cascades is primarily direct or

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indirect is of considerable interest and debate. Experiments that examine the capacity of various chemical radioprotectors to protect against damage from Auger cascades are likely to shed some light on the mechanisms involved.

It has been generally observed that cysteamine (MEA) provides more protection against damage caused by low-LET radiations compared to high-LET radiations (*16*). This suggests that MEA may provide better protection against indirect effects of radiation exposure rather than direct effects. This notion was substantiated by our recent in vivo experimental results (*14,17*) with MEA as a radioprotector using spermatogenesis in mouse testis as the experimental model. These data showed similar dose-modification factors (DMF—defined as the ratio of the radiation absorbed doses required to achieve 37% survival in the presence and absence of the radioprotector) for cytoplasmically localized H^{131}IPDM (N,N,N'-trimethyl-N'-(2-hydroxyl-3-methyl-5-iodobenzyl)-1,3-propanediamine) and H^{125}IPDM, and a significantly lower DMF value for the alpha emitter ^{210}Po. Although the biological effects of DNA-incorporated ^{125}IUdR are similar to those of 5.3-MeV alpha particles (*15*), cysteamine provided substantially higher protection against the effects of ^{125}IUdR than against ^{210}Po-citrate (*14,17*). These results for MEA led us to postulate that the mechanism by which Auger electrons cause biological damage has a substantial indirect component.

In this work, the radioprotective capacity of S-(2-aminoethyl)isothiouronium bromide hydrobromide (AET) is investigated using our well established mouse testis model with spermatogonial cell survival as measured by testicular spermhead count serving as the biological end point. This radioprotector was chosen because of its affinity to DNA (*18*). To delineate radioprotection against damage caused by Auger electrons, the radiochemicals chosen are DNA-incorporating ^{125}IUdR, cytoplasmically localizing H^{125}IPDM and alpha emitter ^{210}Po. The results are discussed in the context of our earlier findings with MEA (*14,17*) and vitamin C (*19,20*).

MATERIALS AND METHODS

Experimental Model

The process of spermatogenesis in mice is similar to that in humans except for the time scale and radiosensitivity (*21*). In spite of its apparent complexity, this process is tractable because it progresses through a sequence of well-defined stages. The spermatogonial cells of stages A_1–A_4, In and B are the most radiosensitive cells in the mouse testis, whereas the stem and postgonial cells are relatively radioresistant. This differential sensitivity of the testicular cells facilitates the investigation of the effects of radiation at very low doses. When mouse testes are irradiated with external x-rays or internal radionuclides, the initial damage to the spermatogonia results in a reduced testicular spermhead population after the time necessary for spermatogonia to become spermatids of stages 12–16 (*22*). This time is about 4–5 wk in mice (*22*). The mouse testis model is highly radiosensitive and has been used extensively to study the biological effects of incorporated radionuclides (*12,13,15,23–25*). In addition, this model has been shown to be helpful in assessing the radioprotective capacity of various chemicals against damage from incorporated radionuclides (*14,17,19,20,26*).

Radiochemicals

Iodine-125-UdR (specific activity = 74 TBq/mmole) was obtained from ICN Radiochemicals (Irvine, CA). Iodine-125 was obtained from New England Nuclear (N. Billerica, MA) and labeled to stable HIPDM by the exchange reaction described by Lui et al.(*27*). Polonium-210 was separated from a silver matrix obtained from NRD Inc. (Grand Island, NY) as reported elsewhere (*28*). The radiochemical ^{210}Po-citrate was prepared as described earlier (*15*). Nonradioactive AET was obtained from Sigma Chemical Company (St. Louis, MO).

The radiolabeling of AET with ^{35}S was performed using the methods described by Bradford et al. (*29*), with some minor modifications. Equimolar quantities of 2-bromoethylammonium bromide and ^{35}S-thiourea obtained from Amersham at a specific activity of 11 GBq/mmole, were refluxed gently in anhydrous isopropanol (100 μl) in a Wheaton screw-cap microvial for 1 hr. After cooling, the precipitate of ^{35}S-AET was isolated by centrifugation and crystallized from a mixture of ethanol, methanol and isopropanol. The final product was filtered and dried in vacuo. The reaction yield was found to be 60%. A radiochemical purity of 99% was obtained with specific activity of 2.15 MBq/mg.

Experimental Procedures

Experimental protocols have been presented elsewhere in detail (*12–14,19,23*). Briefly, Male Swiss Webster mice aged 8–9 wk, and weighing about 30 g, were obtained from Taconic Farms (Germantown, NY). All radiochemicals and the radioprotector AET were injected intratesticularly. This mode of administration was chosen because it requires very small amounts of radioactivity and allows one to calculate the absorbed dose to the testis reliably without complications of whole-body irradiation inherent in intravenous or intraperitoneal injections (*23,30*). Furthermore, because the testicular self-absorbed dose from photon radiations is very small, the absorbed dose to the small testis (0.1 g) is predominately from particulate radiations in this mode of administration, thereby allowing one to selectively examine the effects of Auger electrons (*23*). The mice were anesthetized under ether and the radioprotector and the radiochemical injected intratesticularly (standard 3-μl volume) into the right testis using a 27-gauge microsyringe. The radioprotector AET was dissolved in 0.9% saline and injected (1.5 μg in 3 μl) 4 hr prior to the radiochemical. This amount of AET was found to be the maximum nontoxic amount that can be administered intratesticularly (see section entitled *Spermhead Survival Assay* and Fig. 3). This mode of AET administration was chosen over other modes to ensure that all testes received the same initial amount of the radioprotector. Control mice were administered 3 μl of 0.9% saline 4 hr prior to the radiochemical injection. All mice were sacrificed with an overdose of ether.

Testicular Clearance of Radiochemicals in the Presence of AET

Testicular clearance of all three radiochemicals ^{125}IUdR, H^{125}IPDM and ^{210}Po-citrate were reported earlier (*15,31*). In order to verify that the clearance was not altered by the presence of the radioprotector, AET (1.5 μg) was injected similarly into the right testes of several mice followed by the radiochemical injection 4 hr later. Mice in groups of four were sacrificed under ether at various times postadministration of the radiocompounds and

the injected testes were removed. In the case of ^{210}Po-citrate, the testes were digested in 10 ml of Fluorosol scintillation cocktail (National Diagnostics, Manville, NJ) 50°C, cooled to room temperature and counted in an automatic liquid scintillation counter (5.3-MeV alpha particle). The clearance of the radioiodinated chemicals was similarly followed by assaying the ^{125}I activity in the excised testis with a NaI scintillation well detector. The fraction of the activity retained in the testes was calculated by taking the ratio of the testicular counts to the appropriate radioactive standard sample counts. In order to establish the clearance kinetics of the radioprotector, an independent experiment was performed where ^{35}S-labeled AET was injected similarly (37 kBq/3 μl) and assayed with the liquid scintillation counter after digesting the injected testes in Fluorosol, as described above.

Optimal Day for Spermhead Survival Assay

The optimal day for spermhead survival assay is the day postinjection when the spermhead population reaches a minimum, and it must be determined experimentally for each radiochemical (*13*). About 150 animals were injected with either ^{125}IUdR (0.24 MBq), H^{125}IPDM (0.24 MBq) or ^{210}Po-citrate (0.05 kBq), and were sacrificed in groups of four at different times postadministration. The injected testes were removed and placed in 1 ml of deionized water, homogenized for 15 sec, and sonicated for 30 sec. The spermheads, which are resistant to sonication were counted using a hemocytometer under a light microscope at 400× magnification. Control mice injected with 3 μl of 0.9% saline had an average of 2.8×10^7 spermheads per testis. The experiment was repeated in the presence of AET to ensure that it did not have any effect on the optimal day. The optimal day for ^{125}IUdR and H^{125}IPDM with and without AET was determined to be Day 29 postinjection, whereas in the case of ^{210}Po-citrate, it was Day 36 (*14,15*). These experiments showed that the presence of AET did not alter the optimal day.

Spermhead Survival Assay

In order to establish a dose-effect relationship for each radiochemical, 40 mice in groups of four were injected with different concentrations of the radiocompounds in a standard 3-μl injection volume. The injected mice were sacrificed on the optimal day. The injected testes were removed and processed for spermhead counting as described above (*13,14*). Untreated animals, as well as mice injected with 0.9% saline served as controls. To investigate the protective effects of AET, the above experiments were repeated in the presence of a similarly injected nontoxic amount (see below) of the chemical. Mice injected with AET alone served as controls in these experiments.

In order to determine the maximum amount of AET that could be administered without resulting in any chemotoxicity, mice (groups of four) were intratesticularly injected with 3 μl of 0.9% saline containing various amounts of AET (0–6 μg). On an optimal day postinjection, the animals were sacrificed and the testicular spermhead count compared to controls.

Subcellular Distribution of the Radiochemicals and Radioprotector

Subcellular distributions of the radiochemicals in the testicular cells were reported earlier (*31*). These studies were repeated in the presence of AET to ensure that the added radioprotector did not alter the distribution. Briefly, the injected testes were removed after 24 hr, washed and placed in phosphate-buffered saline (PBS) (1 ml/testis). The tunica albuginea were carefully removed and the remaining testicular tissue transferred to a Potter-Elvehgem tissue grinder and homogenized with a Teflon™ pestle with four to five strokes. The tissue homogenate was filtered through a double layer of 120-μm nylon mesh and centrifuged at 4°C, with 2000 rpm for 12 min. The pellet containing the cells was separated carefully from the supernatant and the activity in the cell pellet and supernatant was determined. The cell pellet was resuspended in 2 ml of PBS, and 100 μl of NP-40 was added. The mixture was homogenized with 4 to 5 strokes of the pestle, placed in an ice bath for 12 min and an equal volume of 20% Ficoll in 0.9% saline was added. The suspension was left in an ice bath for 5 min and centrifuged at 2000 rpm for 15 min. The pellet containing the cell nuclei was separated from the supernatant, and the cell nuclei and supernatant were assayed for radioactivity. The fraction of activity in the cell nuclei that was bound to DNA was obtained using well established procedures (*32*). The nuclear pellet was washed once with 2 ml of ice cold sucrose buffer (0.25 *M*, 3 m*M* $CaCl_2$, 50 m*M* Tris EDTA, at pH 7.0) and suspended in an additional 1 ml of the same buffer. The DNA in the nuclei was precipitated by adding 2 ml of ice-cold 6 *M* guanidine-HCl and gently mixing with a glass rod. An equal volume of cold ethanol was added while mixing and the entire contents were transferred on to a Gelman Type A-E filter. The tube, rod and filter were washed three times with 1 ml cold 1:1 guanidine-HCl and ethanol mixture. The activity on the filter was assayed and considered to be the DNA-bound fraction.

To determine the subcellular distribution of AET in the testicular cells, additional animals were injected intratesticularly (~37 kBq/3 μl) with ^{35}S-AET and sacrificed after 1 day. The testes were removed and processed for subcellular distribution using the procedures described above.

RESULTS

Testicular Clearance

The testicular clearance of the radiochemicals ^{125}IUdR, H^{125}IPDM and ^{210}Po-citrate were reported earlier (*14,15*). The clearance of ^{125}IUdR and ^{210}Po-citrate from the testis was of a two-component exponential nature, whereas H^{125}IPDM cleared with three components (Fig. 1). Least-squares fits to the data are given elsewhere (*14,15*). The biological clearance shown in Figure 1 reveals that the presence of AET had no effect on the clearance patterns. Accordingly, the average absorbed doses to the testes per unit activity administered were the same as reported in our previous communications: 0.30 Gy/MBq, 2.82 Gy/MBq and 2980 Gy/MBq for ^{125}IUdR (*15*), H^{125}IPDM (*14*) and ^{210}Po-citrate (*15*), respectively.

The biological clearance of intratesticularly administered ^{35}S-labeled AET is shown in Figure 2. A least-squares fit of the data to a two-component exponential function yielded the following relationship:

$$f(t) = 0.025e^{-0.693t/103} + 0.975e^{-0.693t/0.29}, \qquad \text{Eq. 1}$$

where f(t) is the fraction of activity retained in the organ at time t (in hours).

Chemotoxicity of AET

Figure 3 shows the dependence of spermhead survival on the amount of AET injected into the testis. Aminothiols have been shown to be toxic to spermatogonial cells when administered intraperitoneally (*33*). However, in our ex-

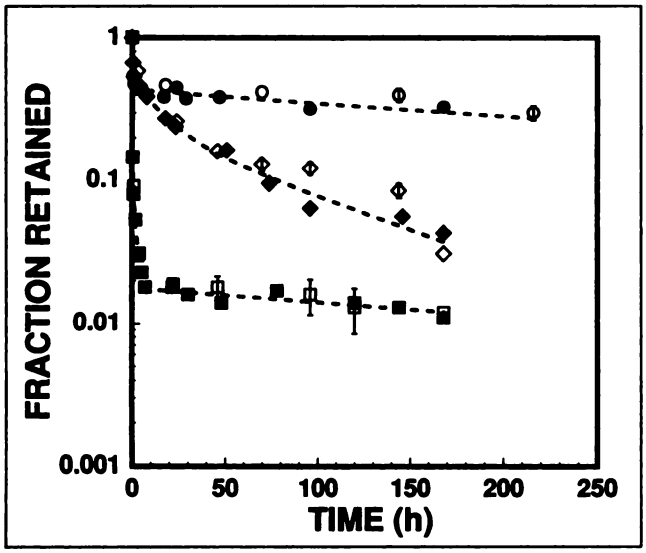

FIGURE 1. Biological clearance of radiochemicals from mouse testes following intratesticular injection. The fraction of initially administered activity retained in the organ as a function of time is presented: ^{125}IUdR alone (■) (*15*), ^{125}IUdR + AET (□), H^{125}IPDM alone (◆) (*14*), H^{125}IPDM + AET (◇), ^{210}Po-citrate alone (●) (*15*), ^{210}Po-citrate + AET (○). The error bars indicate the standard deviation of the mean.

periments, intratesticular injection of small amounts of AET (<1.5 μg) were found to be nontoxic in that the spermhead count was the same as the saline-injected controls. Accordingly, the maximum nontoxic amount of AET (1.5 μg) was administered in the radioprotection experiments.

Optimal Day for Spermhead Survival Assay

Figure 4 shows the spermhead survival fraction as a function of time postinjection for H^{125}IPDM in the presence and absence of AET. It can be seen clearly that the optimal day postinjection to sacrifice the animals for the spermhead count is not changed by the presence of AET. Furthermore, the data clearly show that AET affords substantial radioprotection against irradiation by H^{125}IPDM.

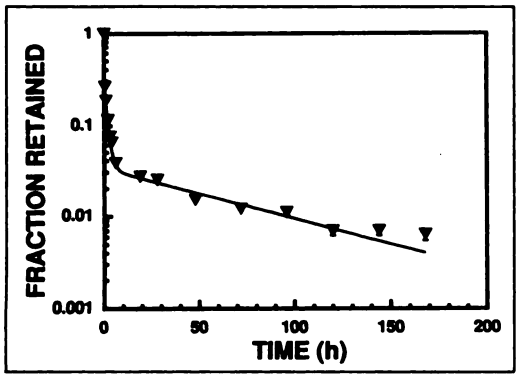

FIGURE 2. Testicular clearance of intratesticularly injected ^{35}S-AET as a function of time postadministration. The data represent the average of two experiments.

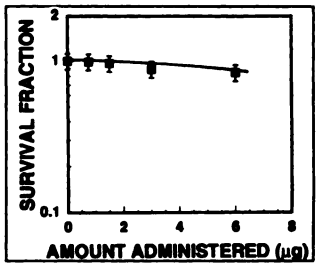

FIGURE 3. Dependence of spermhead survival on the amount of AET injected. Note that the maximum nontoxic amount is approximately 1.5 μg. Error bars represent the standard error of the mean.

Similar curves (data not shown) were also obtained for ^{125}IUdR and ^{210}Po-citrate.

Subcellular Distribution of the Radiochemicals and Radioprotector

Essentially all of the cellular activity was found in the cytoplasm for H^{125}IPDM (*14*), whereas all of the ^{125}IUdR was localized in the DNA of the cell nucleus (*15*). In the case of ^{210}Po-citrate, 20% of the intracellular activity was in the cell nucleus (*15*). The presence of AET in the testis had no effect on the subcellular distribution of the radiochemicals.

The intratesticular mode of administration of radiolabeled AET resulted in substantial cellular uptake (i.e., ~40% of the organ activity in the cells) as given in Table 1. The percent of testicular activity localized in the cell nuclei was about 8.6% and 1.2% was found in the nuclear DNA. Interestingly, the fraction of testicular activity bound to DNA was the same for cysteamine (Table 1).

Dose Response Relationships

The spermhead survival as a function of average absorbed dose to the testis from ^{125}IUdR in the absence and presence of AET is shown in Figure 5. The results for H^{125}IPDM and ^{210}Po-citrate are shown in Figures 6 and 7,

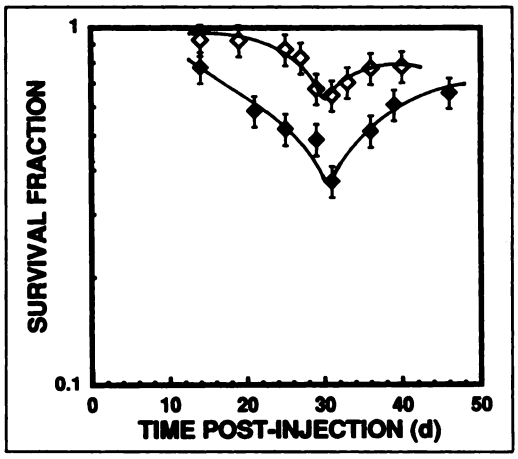

FIGURE 4. Spermhead survival fraction as a function of time following intratesticular injection of 0.24 MBq of H^{125}IPDM in the absence (◆) and presence (◇) of similarly injected AET (1.5 μg) 4 hr earlier. The elevated curve for AET + H^{125}IPDM shows clear evidence of radioprotection. Similar curves were obtained for the radiochemicals ^{125}IUdR and ^{210}Po-citrate. Standard errors are indicated.

TABLE 1
Subcellular Distribution of AET and MEA in Mouse Testes

Radioprotector	% Testicular activity in cells	% Testicular activity in nuclei	% Testicular activity in DNA
AET	39	8.6	1.2
MEA (*38*)	25	2.5	1.2

respectively. The data show a two-component exponential response in all cases with the exception of AET + $H^{125}IPDM$ which resulted in a single-component response. For the Swiss Webster mice used in these experiments, the two-component nature of the survival curve has been a recurring feature for external x-rays as well as internal radionuclides (*13–15,23,24,34*). This is due to differential radiosensitivity of the spermatogonial cell subpopulations (*30*). Similar two-component curves were also observed by others when different strains of mice were irradiated with external beams of radiation (*35,36*). It should also be noted that the dose-response relationships obtained for a variety of radiochemicals over the past several years have fit a distinct pattern. Intratesticular injection of emitters of low-LET radiations such as the pure gamma emitter ^{7}Be (*12*) and the beta emitters ^{131}I (*12,19*) and ^{114m}In (*13*), yields D_{37} values essentially the same as that obtained for acute external x-rays (i.e., RBE ~1). Similarly, radiochemicals that localize the Auger emitters ^{123}I (*12*) and ^{125}I (*14*) in the cytoplasm of the testicular cells also give RBE ~1 compared to x-rays. These data attest to the appropriateness of the use of acute external x-rays as the reference radiation.

A least-squares fit of the survival data resulted in the following dose-response relationships:

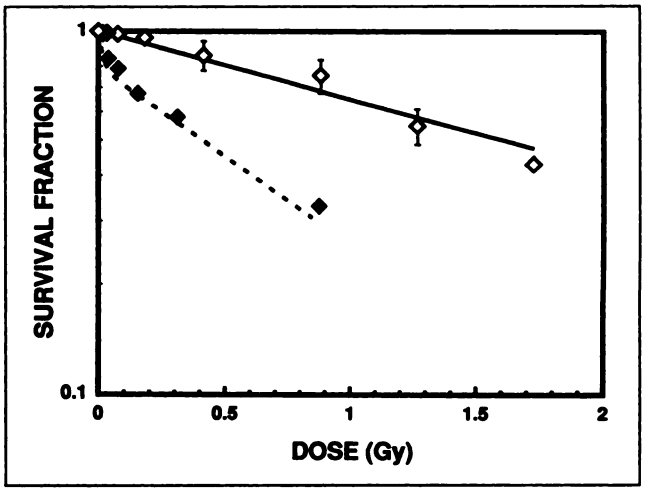

FIGURE 6. Spermhead survival fraction versus average absorbed dose to mouse testis from intratesticularly injected $H^{125}IPDM$ (2.82 Gy/MBq): $H^{125}IPDM$ alone (dashed line, (*14*)), $H^{125}IPDM$ + 1.5 μg AET (◇, solid line). Contemporaneous controls injected with $H^{125}IPDM$ alone are indicated (◆). AET clearly affords radioprotection. Data shown are the average of two experiments in both cases. Error bars represent the standard error.

$$S(^{125}IUdR) = 0.46e^{-D/0.0041} + 0.54e^{-D/0.221}, \quad \text{Eq. 2A}$$

$$S(AET + {}^{125}IUdR) = 0.31e^{-D/0.0191} + 0.69e^{-D/0.545}, \quad \text{Eq. 2B}$$

$$S(H^{125}IPDM) = 0.21e^{-D/0.018} + 0.79e^{-D/0.892}, \quad \text{Eq. 3A}$$

$$S(AET + H^{125}IPDM) = 1.0e^{-D/2.32}, \quad \text{Eq. 3B}$$

$$S(^{210}Po\text{-citrate}) = 0.30e^{-D/0.0020} + 0.70e^{-D/0.156}, \quad \text{Eq. 4A}$$

$$S(AET + {}^{210}Po\text{-citrate}) = 0.18e^{-D/0.0051} + 0.82e^{-D/0.296}, \quad \text{Eq. 4B}$$

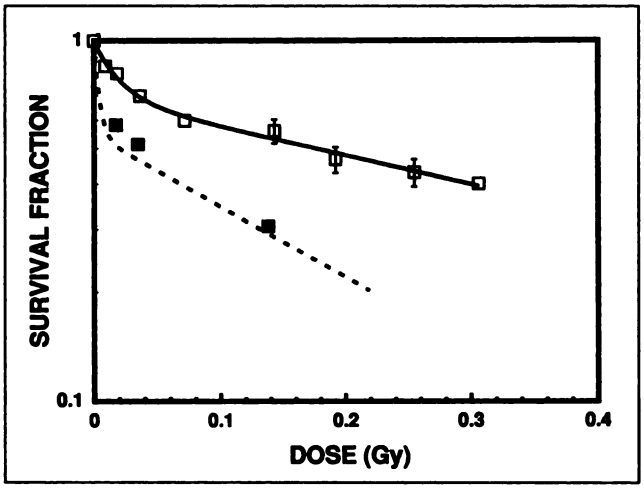

FIGURE 5. Spermhead survival as a function of average testicular absorbed dose from intratesticularly administered $^{125}IUdR$ (0.30 Gy/MBq) with and without AET: $^{125}IUdR$ alone (dashed line, (*15*)), $^{125}IUdR$ + 1.5 μg AET (□, solid line). Data represent the average of two separate experiments in each case. Contemporaneous controls injected with $^{125}IUdR$ alone are indicated (■). Standard error bars are given.

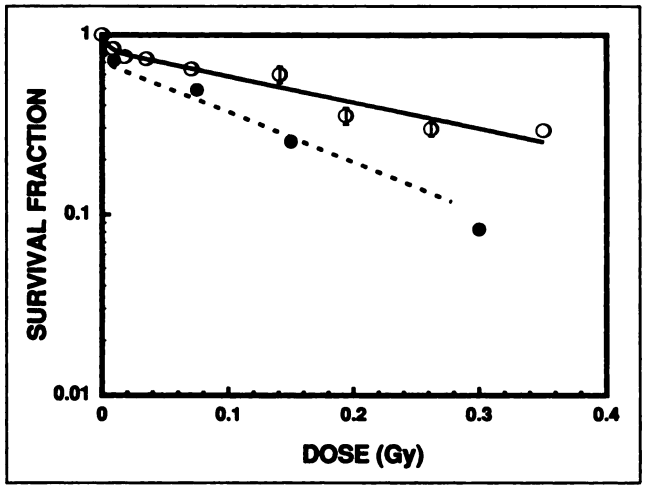

FIGURE 7. Fraction of surviving spermheads as a function of the testicular absorbed dose from intratesticularly administered ^{210}Po-citrate (2980 Gy/MBq): ^{210}Po-citrate alone (dashed line, (*15*)), ^{210}Po-citrate + 1.5 μg AET (○, solid line). Contemporaneous controls injected with ^{210}Po-citrate alone are indicated (●). The presented data are average of two experiments. Standard error bars are shown.

TABLE 2
Dose Modification Factors for Vitamin C, Cysteamine and AET

Radiochemical/ Radioprotector	D_{37} (Gy)	DMF	Reference
$H^{131}IPDM$	0.62 ± 0.06	—	(17)
$H^{125}IPDM$	0.68 ± 0.06	—	(14)
$^{125}IUdR$	0.085 ± 0.021	—	(14,15)
^{210}Po-citrate	0.10 ± 0.01	—	(15)
$H^{131}IPDM$ + Vitamin C	1.34 ± 0.14	2.2 ± 0.3	(19)
$^{125}IUdR$ + Vitamin C	0.192 ± 0.029	2.3 ± 0.6	(20)
^{210}Po-citrate + Vitamin C	0.090 ± 0.015	0.9 ± 0.17	(20)
$H^{131}IPDM$ + Cysteamine	2.37 ± 0.48	3.8 ± 0.8	(17)
$H^{125}IPDM$ + Cysteamine	2.60 ± 0.32	3.8 ± 0.6	(14)
$^{125}IUdR$ + Cysteamine	0.31 ± 0.06	3.6 ± 1.1	(14)
^{210}Po-citrate + Cysteamine	0.264 ± 0.056	2.6 ± 0.6	(17)
$H^{125}IPDM$ + AET	2.31 ± 0.14	3.4 ± 0.4	This work
$^{125}IUdR$ + AET	0.341 ± 0.052	4.0 ± 1.2	This work
^{210}Po-citrate + AET	0.236 ± 0.039	2.4 ± 0.5	This work

where S is the spermhead survival fraction and D is the testicular absorbed dose (Gy). Equations 2A, 3A and 4A are taken from our earlier work (*14,15*) and are included here for comparison. These dose-response relationships in the absence of the protector were verified in each case with several contemporaneous control groups. It is interesting to note that the presence of AET in the case of $H^{125}IPDM$ has changed the typical two-component exponential response into a single-exponential response. This may have been due to more efficient protection of the most radiosensitive spermatogonial cells relative to the less sensitive spermatogonial cell populations (*30,37*).

The mean lethal dose (D_{37}) is 0.085 ± 0.021 Gy for $^{125}IUdR$ (*15*) alone, and 0.341 ± 0.052 Gy in the presence of AET. Similarly, the D_{37} values for $H^{125}IPDM$ are 0.68 ± 0.06 Gy (*14*) and 2.31 ± 0.14 Gy in the absence and presence of AET, respectively. The higher biological effectiveness of DNA-incorporated $^{125}IUdR$ compared to cytoplasmically localized $H^{125}IPDM$ is primarily due to the highly localized energy deposition by Auger cascades in close proximity to the DNA in the cell nucleus. In the case of cytoplasmically localized $H^{125}IPDM$, only the low-LET conversion electrons with energies sufficient to penetrate into the cell nucleus can irradiate the nuclear DNA (*14*). For ^{210}Po-citrate, the observed D_{37} values are 0.10 ± 0.01 Gy (*15*) and 0.236 ± 0.039 Gy in the absence and presence of AET, respectively.

DISCUSSION

The effectiveness of a radioprotector is usually expressed in terms of the dose modification factor (DMF), which is defined as the ratio of absorbed doses at a chosen biological end point in the presence and absence of the radioprotector. A DMF of greater than one indicates radioprotection. Based on the D_{37} values obtained in these experiments with AET, the DMF values can be calculated as 4.0 ± 1.2 ($^{125}IUdR$), 3.4 ± 0.4 ($H^{125}IPDM$) and 2.4 ± 0.5 (^{210}Po-citrate). These DMF values are similar to our earlier results obtained using the radioprotector cysteamine in the same experimental model (*14,17*). For discussion purposes, Table 2 summarizes our past and present results for different radioprotectors and radiochemicals. The similarity of DMF values for cysteamine and AET (Table 2) is not surprising in view of the fact that these compounds belong to the family of aminothiols. The mechanisms of radioprotection by these compounds are likely to be very similar. Furthermore, the subcellular distribution studies of radiolabeled cysteamine (*38*) and AET, given in Table 1, indicate that both compounds localize in the cell nucleus and bind to the DNA to a similar extent.

Despite the very different subcellular distribution and biological effects of $^{125}IUdR$ and $H^{125}IPDM$, the DMF values observed were very similar when AET was employed as the protective agent. This similar degree of protection against the effects of $H^{125}IPDM$ and $^{125}IUdR$ was found for all of the radioprotectors (Table 2). In contrast to the observed protection against the biological effects of the ^{125}I compounds, the DMF values for AET (2.4 ± 0.5) and MEA (2.5 ± 0.6) against the effects of ^{210}Po-citrate are significantly lower, yet similar in magnitude. On the other hand vitamin C did not provide any protection at all against the damage caused by alpha particles (DMF = 0.9 ± 0.2), while significant and similar protection was observed against the effects of the radioiodinated chemicals. The fact that the biological effects of $^{125}IUdR$ are high-LET-type and similar to those of ^{210}Po-citrate (Table 2, D_{37} values), and yet differ substantially in the presence of radioprotectors (Table 2, DMF values), strengthens our earlier conclusion (*14*) that the mechanism by which DNA-incorporated Auger emitters cause biological damage is primarily indirect rather than direct in nature. Similar studies with the radioprotector DMSO, a well-known radical scavenger, may provide conclusive evidence for the mechanism by which damage is caused by Auger electron cascades.

Wright et al. (*4*) used Monte Carlo techniques to calculate the extent of direct physical and indirect chemical

interactions resulting from ^{125}I Auger cascades and ^{210}Po alpha particle tracks in liquid water. When ^{125}I decays are simulated on the surface of DNA, the number of direct and indirect interactions scored are nearly twice the number of such interactions encountered with the passage of a 5.3-MeV alpha particle through DNA. In both situations, the majority of interactions are from OH · radicals. These findings were recently substantiated by similar calculations of Pomplun et al. (*5,39*). These considerations provide further support for our conclusion that the damage caused by DNA-incorporated Auger emitters is primarily mediated by free radicals and therefore indirect in nature.

The mechanisms of radioprotection of thiol compounds are several and complex. It has been suggested that they include free radical scavenging, H-atom donation, creation of hypoxia around the decay site, etc. (*1*). Aminothiols are also known to provide radioprotection by alteration of the chromatin structure (*40*). For efficient radioprotection, it may be necessary that the thiol compounds bind to DNA in the cell nucleus where the radiosensitive targets are presumably located (*41*). Smoluk et al. (*42*) have found that MEA indeed binds to the negatively charged phosphates on the DNA backbone under physiological conditions. The present subcellular distribution data, given in Table 1, show that MEA and AET do localize in the cell nucleus and bind to the DNA to some extent. These results are in reasonable agreement with those of Maisin et al. (*18*) who employed whole-body autoradiography to ascertain the penetration of ^{3}H-AET into the nucleus and cytoplasm of cells.

Whatever may be the mechanism of the damage caused by Auger emitters and the protective mechanisms of AET, the present findings clearly show that substantial radioprotection can be achieved with small and nontoxic quantities of radioprotectors against chronic irradiation by tissue incorporated radionuclides in a model that is relevant to humans. This implies that chemical protectors such as AET and MEA may be useful in protecting against the effects of incorporated radionuclides. In this context, the results of Jackson et al. (*43*) show that many radionuclides localize in the testes and sperm and cause deleterious effects are of particular relevance. In view of the increasing use of radionuclides in therapy and diagnosis, the results presented in this work are significant and argue well for further research on chemical radioprotection against tissue incorporated radionuclides.

CONCLUSION

The radioprotection provided by nontoxic amounts of AET against low-LET-type radiation damage (H^{125}IPDM) and high-LET-type effects of Auger electrons (^{125}IUdR) is found to be significant in an experimental model that is relevant to humans. However, the protection provided by AET against high-LET alpha particles from ^{210}Po-citrate is substantially less than that observed against the radioiodinated chemicals. This finding, and the fact that the biological effects of DNA-bound ^{125}I are mitigated as well as the low-LET-type effects caused by cytoplasmically localized ^{125}I, supports the conclusion that the mechanism of damage caused by Auger cascades is primarily indirect in nature. Furthermore, biokinetic data on ^{35}S-labeled AET shows that the chemical is retained in organs over a protracted period of time following intraperitoneal administration (unpublished data), implying that such chemicals may find a role in protection against chronic irradiation by incorporated radionuclides.

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