Published in final edited form as:

Radiat Res. 1991 January ; 125(1): 89–97.

Induction of Sperm Head Abnormalities by Incorporated Radionuclides: Dependence on Subcellular Distribution, Type of Radiation, Dose Rate, and Presence of Radioprotectors

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Abstract

In contrast to the biological effects caused by exposure to external beams of radiation, the effects of tissue-incorporated radionuclides are highly dependent on the type of radiation emitted and on their distribution at the macroscopic, microscopic, and subcellular levels, which are in turn determined by the chemical nature of the radionuclides administered. Induction of abnormalities of sperm heads in mice is investigated in this work after the injection of a variety of radiochemicals including α emitters. When the initial slopes of the dose–response curves are used to compare the relative biological effectiveness (RBE) of different radiocompounds, the α particles emitted in the decay of ^{210}Po are more effective than Auger electrons emitted by ^{125}I incorporated in the DNA of the spermatogonial cells, and both emissions are more effective than X rays. It is also shown that the Auger emitters (^{125}I , ^{111}In) distributed in the cell nucleus are more efficient in producing abnormalities than the same radionuclides localized in the cytoplasm. These findings are consistent with our earlier observations, where spermatogonial cell survival is assayed as a function of the testicular absorbed dose. Further, chronic irradiation of testis with γ rays from intratesticularly administered ^7Be is about three times more effective in causing abnormalities than a single acute exposure to 120-kVp X rays. The resulting RBE values correlate well with our data on sperm head survival with the same radiocompounds. Finally, the radioprotector cysteamine, when administered in small, nontoxic amounts, significantly reduces the incidence of sperm abnormalities from α -particle radiation as well as emissions from ^{125}I incorporated into DNA, the dose reduction factors being 10 and 14, respectively.

INTRODUCTION

The response of testicular cells in the mammalian spermatogenic process to ionizing radiation is well known. Oakberg, Meistrich, and others extensively investigated the lethal effects of external irradiation of mouse testes by X and γ rays on the various subpopulations of murine testicular cells ((1–3) and references cited therein). The A₁–A₄ In, and B types of spermatogonia are the most radiosensitive subpopulation of cells with an LD₅₀ value of about 40 cGy for mice (3).

The high radiosensitivity of spermatogonial cells makes the mouse testis an effective *in vivo* model to study the biological effects of low doses from tissue-incorporated radionuclides. This is particularly important in view of the widespread use of radionuclides in medicine (e.g., ^{99m}Tc , ^{111}In , ^{201}Tl , ^{131}I), and their presence in the environment from both manmade (e.g., ^{55}Fe , ^{137}Cs) and natural sources (e.g., ^{40}K , ^{222}Rn). These radionuclides emit one or more of a variety of radiations including α , β , and γ rays, as well as characteristic X rays and Auger electrons. Although the effectiveness of producing biological damage by external beams of electrons, and α , γ , and soft X rays is generally well understood, the effects of such radiations when emitted by incorporated radionuclides may be different because they depend not only on the nature and energy of the radiations, but also on the biokinetics and distribution of the radionuclides in the tissue at both the macroscopic and subcellular levels. The most striking differences are observed with radionuclides decaying by electron capture or internal conversion. By virtue of inner atomic shell ionization in these processes, the decaying radionuclides emit a shower of low-energy Auger electrons with ranges of subcellular dimensions (4). Using spermatogonial cell survival as the end point, Rao *et al.* (5–7) have shown that Auger emitters such as ^{111}In , ^{201}Tl , and ^{55}Fe are more effective than their β -emitting counterparts (^{114m}In , ^{204}Tl , ^{59}Fe) in reducing the sperm population, when similarly localized in the testis. The magnitude of this difference depends on the subcellular distribution of the radionuclide (5, 8). Furthermore, Rao *et al.* (9) demonstrated that the radiotoxicity of Auger emitters, when bound to the DNA in the cell nucleus, can be as high as that of incorporated α emitters.

The *in vivo* results described above, and similar results obtained *in vitro* (10, 11), point to the need for careful assessment of the possible deleterious effects of minute quantities of incorporated radionuclides in order to establish guidelines for human exposure. Wyrobek (12) observed that a variety of gross sperm head abnormalities result from exposure of mouse testis to low doses of X rays. Following this lead, we have investigated the induction of abnormalities in the shape of murine sperm for various incorporated radiochemicals including the α emitter ^{210}Po -citrate, the γ emitter ^7Be -chloride, and the Auger emitters ^{111}In -oxine, ^{111}In -citrate, and ^{125}I -labeled iododeoxyuridine ($^{125}\text{IUdR}$) and *N,N,N*'-trimethyl-*N*'-[2-hydroxy-3-methyl-5-iodobenzyl]-1,3-propanediamine ($\text{H}^{125}\text{IPDM}$), and compared their effects to those caused by selective testicular exposure to X rays. In addition, the influence of the radioprotector cysteamine (MEA) in mitigating the radiation damage from incorporated radionuclides is examined.

Our results suggest that the induction of sperm head abnormalities is a very sensitive assay for establishing the differential response of tissue to ionizing radiations, in particular those emitted by incorporated radionuclides. In fact, this assay is much more sensitive than the spermatogonial cell survival assay described in our previous correspondences (5–7, 9, 13). The results of this work also point out the variable response of mammalian tissue to incorporated radionuclides, the response depending substantially not only on the type of radiation emitted but also on the chemical nature of the radiocompound. Finally, it is demonstrated that chronic irradiation of the testis with γ rays from testicularly localized ^7Be is more effective in producing sperm head abnormalities than acute irradiation with X rays.

MATERIALS AND METHODS

Experimental approach

In this work, induction of gross abnormalities in sperm shape in mouse testes is used as the experimental end point. Details of the time scale of spermatogenesis in mice and the relative radio-sensitivities of the various subpopulations of testicular cells are described by Oakberg (14) and Meistrich *et al.* (3). The A₁–A₄, In, and B types of spermatogonia are most sensitive (LD₅₀ ~ 40 cGy), whereas their precursors (A_{1s}, A_{4l}) as well as the postgonial cells are relatively radioresistant with LD₅₀ values of 100–300 cGy and 200–60,000 cGy, respectively (3). Because of this differential radiosensitivity, irradiation of the testis with low doses from incorporated radionuclides will result in a reduced testicular spermatid count 29–36 days later, the time required for the spermatogonia to become spermatids of stages 12–16 (6–8). It takes an additional 9 days for the spermatozoa to migrate to the epididymis, a sac-like structure where the sperm are stored. Hence it is important that abnormalities in epididymal sperm are assayed 9 days after the minimum testicular sperm count is reached (15). Since this optimal day depends on the nature of the exposure, and perhaps on the cellular incorporation of the radionuclides, it must be determined experimentally in each case (6–8).

Animals

Swiss Webster mice (Taconic Farms, Germantown, NY) aged 9–10 weeks were used in these studies. In this strain, the spontaneously occurring sperm shape abnormalities appear in ~3% of the epididymal sperm, depending somewhat on the length of time the animals are acclimated following shipping. Hence control groups were established for each experiment and treated similarly to the irradiated groups. In all cases mice were sacrificed by an overdose of anesthetic (ether).

Radiochemicals

The ¹¹¹In-oxine, ¹²⁵IUdR, and ⁷Be-chloride were obtained commercially from Medi-Physics (South Plainfield, NJ), ICN Radiochemicals (Irvine, CA), and Amersham (Arlington Heights, IL), respectively. Carrier-free ¹¹¹In-chloride (Dupont Medical Products, No. Billerica, MA) in 0.05 N HCl and ²¹⁰Po (Amersham, Arlington Heights, IL) in 3 N nitric acid were used to prepare the radiocitrates. The indium citrate was prepared according to the procedure of Sastry *et al.* (16) while ²¹⁰Po-citrate was made using the recipe of Rao *et al.* (9). Finally, HIPDM, supplied by Professor Hank Kung (University of Pennsylvania, Philadelphia, PA), was labeled with ¹²⁵I by the iodine exchange reaction described by Lui *et al.* (17).

General procedures

The radiochemicals were administered intratesticularly (i.t.) in standard 3- μ l injection volumes. The rationale for the i.t. injection and the uniformity of the resulting radionuclide distribution, etc., have been discussed elsewhere (6–9). Groups of five mice were injected for each absorbed dose value. Control groups were similarly injected with either saline or

solutions containing the nonradioactive compounds at the same chemical concentration. All of the experiments were repeated to assure reproducibility.

A comprehensive description of the methods is given in Refs. (5–7). The biological clearance pattern of the injected radioactivity from the testis was established for each radiochemical. Briefly, the radiochemical was injected into the right testis of 50 mice, the mice were sacrificed in groups of five at various times postinjection, and the testes were removed and assayed for radioactivity using liquid scintillation or an NaI well counter as appropriate. In each case the amount of radiochemical injected did not influence the pattern of biological elimination.

The optimal day for assaying the epididymal sperm head abnormalities is 9 days after the minimum testicular spermatid count is reached (15, 18, 19). Therefore, the minimum testicular spermatid count (29–36 days post-injection) was first determined according to the procedures in Rao *et al.* (5–7, 18, 19). This optimal day having been established, the studies of abnormal sperm head shapes were carried out. Mice, in groups of five, were injected with predetermined quantities of the radiochemical to deliver a range of absorbed doses to the testes. All of the groups were sacrificed on the optimal day, and the epididymides were removed. For each group of mice, the epididymides were pooled in 1 ml 0.9% saline, minced with fine scissors, and pipetted vigorously, and the resulting suspension was filtered through four layers of gauze to separate the sperm from tissue fragments (12, 15, 18, 19). Slides of the sperm were prepared by adding 20 μ l of 2% eosin-Y to the suspension, smearing the suspension on the slide, air drying, and mounting a cover slip with Permount (15, 18, 19). The morphology of murine epididymal sperm has been well studied (12). The normal sperm have a hook-like head shape, characteristic of the species. A variety of abnormalities in the head was observed (12). At least 2000 sperm (normal + abnormal) were scored under a light microscope (400X) with a bright field and green filter, and the abnormal fraction of the epididymal sperm was determined. The fractions were corrected for the spontaneously occurring abnormalities such that the resulting values were representative of the radiation effects only.

Irradiation by external X rays

Selective irradiation of the testes with external beams of 120-kVp X rays were carried out with an overhead fluoroscopy unit as described elsewhere (5).

Radioprotection against incorporated radionuclides

The potential for mitigating the induction of sperm abnormalities by incorporated radionuclides was studied by injecting 3 μ l of normal saline containing a small, nontoxic amount (0.75 μ g) of cysteamine (Sigma Chemical Corp., St. Louis, MO) into the testis 4 h prior to injection of the radiochemical. The delay of 4 h allowed the radioprotector to distribute fairly uniformly throughout the testis prior to administering the radiochemical. The presence of MEA had no effect either on the testicular clearance or on the subcellular distribution (see below) of the radiocompound, nor was the optimum day for abnormal sperm assay altered by the injection of MEA.

Macroscopic and subcellular distribution of radioactivity in the testes

Following i.t. injection, the radionuclide distribution in the testis at the macroscopic level was assessed 1 day postinjection. The testes were frozen, diced, and weighed, and the activity per gram in each slice across the testis was calculated (8). Subcellular distribution (cytoplasm, nucleus, DNA) of the radioactivity in the testicular cells was obtained using the cell fractionation methods outlined in Ref. (7).

RESULTS

Biological clearance

The patterns of biological clearance for ^7Be -chloride and $\text{H}^{125}\text{IPDM}$ are shown in Fig. 1. The shape of the curve depends on the chemical properties of the compound. The radiochemical ^7Be -chloride exhibits a two-component curve with 80% of the radioactivity clearing very quickly and the remaining 20% being retained with a long biological half-life of 648 h. Similar patterns were observed for $^{125}\text{IUdR}$ and ^{210}Po -citrate (9). In contrast, a three-component curve is obtained for $\text{H}^{125}\text{IPDM}$ (Fig. 1) and for the two radioindium complexes (5). These clearance data have been fitted by the least-squares method to a multicomponent exponential expression to obtain the biological half-lives and relative fractional weights for each component. The corresponding values of effective half-lives were calculated using the well-established physical half-lives of 2.83, 60, 138.4, and 53.3 days for ^{111}In , ^{125}I , ^{210}Po , and ^7Be , respectively.

Postinjection optimal day for abnormal sperm head assay

In this work we obtained a minimum testicular sperm head count on the 29th day postinjection for both ^7Be -chloride and $\text{H}^{125}\text{IPDM}$. The postinjection day on which the sperm head count reaches a minimum is given in Ref. (5) for the radioindium complexes and X rays, while those for ^{210}Po -citrate and $^{125}\text{IUdR}$ are given in Ref. (9). In each case, the optimal day for the abnormal sperm head assay is 9 days later. These are listed in Table I for each source of radiation used in this work.

Dosimetry

Details of the methods used to calculate the mean absorbed dose to the testis are given in our earlier communications (5, 7); the basic points will be summarized here. As prescribed by the Medical Internal Radiation Dose Committee (MIRD), the mean absorbed dose $\bar{D}$ to any region of tissue from incorporated radionuclides is given by (20)

$$\bar{D} = (\tilde{A}/m) \sum_i n_i E_i \phi_i, \quad (1)$$

where m is the mass of the testis (0.1 g). The parameter $\tilde{A}$ is the cumulated activity in the testis over the period during which the spermatogonial cells, which become spermatids 29–36 days later, are irradiated. The quantities n_i , E_i and ϕ_i are the yield, energy, and absorbed fraction, respectively, for the i th radiation component of the radionuclide (5–7, 20). The

values of these radiation parameters may be found in Ref. (5) for ^{111}In and ^7Be , and Ref. (9) for $^{125}\text{IUdR}$ and ^{210}Po . Using Eq. 1, the resulting cumulated testicular absorbed doses are 0.16, 0.23, 0.3, 2.8, 2980, and 0.08 Gy per MBq of initially injected ^{111}In -citrate, ^{111}In -oxine, $^{125}\text{IUdR}$, $\text{H}^{125}\text{IPDM}$, ^{210}Po -citrate, and ^7Be -chloride, respectively. The presence of the radioprotector MEA did not influence the value obtained for $^{125}\text{IUdR}$ or ^{210}Po -citrate. Determination of the testicular dose from X rays has been described (5).

Sperm head abnormality: dose–response curves

The fraction of epididymal sperm which exhibit abnormalities, above the spontaneous value, is plotted as a function of the cumulated dose to the testis for the various radiation sources (Figs. 2–4). Dose–response curves are presented in pairs to highlight a variety of relevant factors about either the relative efficacy of the Auger-emitting radiochemicals (Figs. 2A, 2B), types of radiation (Fig. 3), or rate at which the dose was delivered (Fig. 4). In general, these curves exhibit a linear increase in the low-dose region followed by a saturation of the response at higher doses. Although the cause of this saturation has not been determined, it is plausible that as the dose is increased spermatogonial cell killing may play a dominant role in reducing the slope of the response. The slope of the linear portion of the curve (initial slope) provides a sensitive comparison of the relative efficacy of the various types of radiation insults. Magnification of the low-dose region is presented as an inset in Figs. 2, 3, and 5 for clarity. The initial slopes (percentage abnormal sperm heads induced per centigray) are given in Table I. Also given in Table I is the relative biological effectiveness (RBE) of the radiochemicals compared to X rays, based on the initial slopes of the response curves (ratio of the slopes). Finally, Figs. 5A and 5B show the dose–response curves for $^{125}\text{IUdR}$ and ^{210}Po -citrate in the presence of nontoxic quantities of the radioprotector MEA. The corresponding initial slopes are also included in Table I.

Distribution of radioactivity

A breakdown of the subcellular distribution of the radioactivity in the testicular cells is given in Table I. Distribution of the radionuclides at the macroscopic level was quite uniform.

DISCUSSION

Influence of subcellular distribution of the radiochemical: The Auger effect

The effect of subcellular localization of Auger-electron emitters on cellular response is clearly shown by the results obtained for both the radioindium complexes (Fig. 2A) and the radioiodine compounds (Fig. 2B). In the case of ^{111}In (see Table I), the oxine compound is about four times more effective (based on initial slopes) in causing sperm abnormalities than the citrate complex. This difference is due to the localization of the radiochemicals in different subcellular compartments: 93% of the ^{111}In -oxine is localized in the cell nucleus, whereas only about 33% of the radiocitrate is in the nucleus. More drastic differences are found between the ^{125}I -labeled compounds where essentially all of the cellular activity of $\text{H}^{125}\text{IPDM}$ (>95%) is in the cytoplasm of the testicular cells while all of the $^{125}\text{IUdR}$ in the cells is in the DNA. In this case, the initial slopes (Table I) of the dose–response curves (Fig. 2B) indicate that ^{125}I is about 20 times more effective when incorporated into the DNA as $^{125}\text{IUdR}$ than when cytoplasmically localized as $\text{H}^{125}\text{IPDM}$.

The fact that proximity of Auger emitters to the DNA in the cell nucleus is an important determinant of their biological effects has been well established both *in vitro* (10, 21, 22) and *in vivo* (5–7, 9, 19). This is attributed to the localized and collective action of the very low-energy Auger electrons (4, 23) that simulate the type of damage associated with high-LET radiation. Using cultured Chinese hamster V79 cells with cell survival as the end point, Kassis *et al.* (24) determined that the RBE of ^{125}I UdR compared to 250-kVp X rays is about 7 while that of cytoplasmically localized ^{125}I -dihydrorhodamine is about 1 (10). For spermatogonial cell killing in the mouse testis model, we recently obtained RBE values of 7.9, 1.0, 4.2, and 2.0 for ^{125}I UdR (9), $\text{H}^{125}\text{IPDM}$ (8) ^{111}In -oxine (5), and ^{111}In -citrate (5), respectively, when compared to the effects of 120-kVp X rays. The present results for induction of epididymal sperm abnormalities again emphasize the importance of subcellular distribution of the radionuclides in the expression of the biological effects of Auger emitters, such distribution being determined by the chemical form of the radiocompound.

High-LET response: Auger emitters vs α emitters

Although the high-LET-type radiotoxicity of DNA-incorporated Auger emitters has been well established (4, 10, 25, 26), direct experimental comparisons with the densely ionizing high-LET radiations such as α particles have become available only recently through the *in vivo* and *in vitro* work of Rao *et al.* (9) and Howell *et al.* (11). For spermatogonial cell killing (9), RBE values of 7.9 ± 2.4 and 6.7 ± 1.4 were obtained for ^{125}I UdR and ^{210}Po -citrate, respectively, indicating that the dense shower of Auger electrons from DNA-bound ^{125}I decays is as lethal as 5.3-MeV α particles emitted by ^{210}Po . Similar RBE values were obtained for ^{210}Po -citrate (11) and ^{125}I UdR (24) in cultured V79 cells. Thus, for cell killing, it appears that DNA-bound Auger emitters are at least as effective as high-LET α particles. This does not seem to be quite the case for induction of epididymal sperm abnormalities as indicated by the present work. A comparison of the dose–response curves in Fig. 3 and the RBE values in Table I reveal that ^{210}Po -citrate is about four times more effective than ^{125}I UdR in causing abnormalities in mouse sperm. The reason for this higher efficacy is not obvious. It is interesting, however, to note the following: the number of intratesticular decays required for 37% cell survival dose (which takes the testicular clearance into account) is about 1.2×10^7 and 2.6×10^9 for ^{210}Po and ^{125}I , respectively. On the other hand, due to the increased sensitivity of the abnormal sperm assay, more than an order of magnitude fewer disintegrations of ^{210}Po and ^{125}I are required to observe a significant increase in abnormalities. During the time required to deliver the dose, about 10^6 spermatogonial cells (the radiosensitive subpopulation) are irradiated by the intratesticular decays. While more than 90% of the ^{125}I decays occur in the nuclei of the testicular cells, only 30% of the ^{210}Po decays occur in the cells, with the remainder in the intertubular spaces of the testis. Furthermore, the high-LET α particles with an energy of 5.3 MeV can irradiate most of the spermatogonial cells, whereas the very low-energy Auger electrons from ^{125}I can irradiate only the cells in S phase. Hence, in view of all the above, it is possible that the probability of subtle damage to the spermatogonial cells from ^{210}Po α particles is substantially higher than for ^{125}I decays. Further studies are necessary to clarify the observed differences.

Chronic vs acute exposure

Our studies with ^7Be -chloride and 120-kVp X rays provide an interesting comparison between chronic irradiation of the testis from incorporated radionuclides and single acute irradiation of the testis with X rays. The γ emitter ^7Be is appropriate for this comparison since it emits 477-keV photons and only a single Auger electron (~ 40 eV) per decay (27). In addition, the clearance pattern of ^7Be -chloride (Fig. 1) is such that the testis is chronically irradiated (effective half-life = 430 h) at a nearly constant rate (~ 1.1 cGy/day per MBq) over the period of interest. Previously (5), we found that for spermatogonial cell *killing* ^7Be -chloride was about as effective ($\text{RBE} = 1.1$) as X rays (Table I). In the present work, the data in Fig. 4 indicate that chronic irradiation of the testis with γ rays from testicularly localized ^7Be -chloride is about three times more effective in producing sperm head abnormalities than X rays (Fig. 4 and Table I). This result is also supported by our data for $\text{H}^{125}\text{IPDM}$ where a similar RBE value of 2.5 is obtained (Table I). The $\text{H}^{125}\text{IPDM}$ is localized in the cytoplasm of the testicular cells (Table I), thereby chronically irradiating the cell nuclei in a fairly uniform fashion with the low-LET ^{125}I conversion electrons. Our results for ^7Be -chloride and $\text{H}^{125}\text{IPDM}$, taken together, indicate that chronic low-LET irradiation of the testis by incorporated radionuclides can be more effective in causing abnormalities in sperm than a single acute exposure of the organ to X rays.

The mouse spermatogenesis process is complex indeed. It is plausible that the efficacy of chronic irradiation in producing abnormalities may involve the relative radiosensitivities of the cells in the various stages in the maturation process. There are six subpopulations of spermatogonia including $\text{A}_1\text{--A}_4$, In, and B, each stage spanning ~ 1 day (28). The differential radiosensitivities of these stages has been well recognized (2, 28). Perhaps, as the spermatogonial cells pass through the relatively more sensitive stage(s) in the maturation process, the cells receive sufficient ^7Be γ radiation, albeit at a low dose rate, to produce shape abnormalities efficiently but not cell death. If the total cumulated dose from chronic irradiation was given as a single acute exposure, then one would not have numerous cells passing through the very sensitive stage(s) during such an exposure. Thus it is likely that fewer abnormalities may result. It is also possible that BeCl_2 may have played some role in causing the sperm abnormalities. No reduction in the sperm head count was observed when $0.014 \mu\text{g}$ of stable Be, corresponding to the highest ^7Be (carrier-free: $\text{sp act} = 640 \text{ MBq}/\mu\text{g}$) dose in the experiments, was injected into the testis. Furthermore, since 80% of the injected Be is cleared from the testis in a very short time (see Fig. 1), it is unlikely that the small amount of Be administered is responsible for any significant increase in the sperm abnormalities over the spontaneous value.

Radioprotection against incorporated radionuclides: Implications for primary vs secondary mechanism of radiation damage

The role of various thiol-based compounds in mitigating the adverse effects of exposure to external beams of ionizing radiation is well established (29). Among these chemical protectors are cystamine, MEA, AET, and a series of WR compounds including the well-known WR-1065 (30). The effectiveness of a radioprotector is usually expressed in terms of the dose reduction factor (DRF), which typically is the ratio of the absorbed doses in the presence and absence of the protector at any experimental end point (e.g., D_{37}). The

magnitude of the DRF may depend significantly on the end point chosen. With induction of abnormalities in sperm shape as the end point, we have investigated the effectiveness of MEA in reducing the biological damage caused by chronic irradiation of mouse testis from incorporated α - and Auger-emitting radionuclides. The dose-response curves (Fig. 5A) for $^{125}\text{IUdR}$, in the presence and absence of MEA, clearly show that the high-LET type effects of DNA-bound ^{125}I are sharply reduced ($\text{DRF} = 14$) by the presence of nontoxic quantities of MEA. From Table I we may note that the initial slopes of 0.07% abnormalities/cGy for $\text{H}^{125}\text{IPDM}$ alone, and 0.12% abnormalities/cGy for $^{125}\text{IUdR} + \text{MEA}$, are not very different in spite of the highly localized energy deposition in the vicinity of the DNA in the latter case. That is, the effect of ^{125}I decays when bound to DNA has been reduced to nearly that of ^{125}I localized in the cytoplasm. For the α emitter ^{210}Po (Fig. 5B), the DRF is 10 (Table I). It is interesting that, despite the presence of MEA, the 5.3-MeV α particles are still 24 times more effective in causing abnormalities than X rays. The relative values for the DRF are similar when spermatogonial cell killing is the end point: $\text{DRF} = 3.4$ and 2.6 for $^{125}\text{IUdR}$ and ^{210}Po -citrate, respectively (8). These *in vivo* results may shed some light on the mechanisms of radiation action (direct or indirect) associated with Auger decays of radionuclides. The direct effect refers to the damage of target molecules by direct energy deposition in the critical structures. The indirect effect is due to the variety of free radicals produced in the radiolysis of water after ionization or excitation. The action of radioprotectors is to reduce the indirect effects of ionizing radiation (29). The data suggest that the Auger effect may be due largely to the *indirect action* of radical species produced by the shower of low-energy Auger electrons emitted by the radionuclide. This is supported by recent Monte Carlo calculations of Wright *et al.* (31) which simulate the direct and indirect interactions with DNA resulting from an ^{125}I decay on the DNA.

Relationship between the RBE for spermatogonial cell killing and the RBE for induction of sperm abnormalities

Over the past several years, we have accumulated a substantial database on the lethality of Auger-electron-emitting radiochemicals in mouse testis using spermatogonial cell survival as the end point. The RBE values compared to 120-kVp X-ray exposure for various radiochemicals are given in Table I for both assays. The data for sperm abnormalities presented in this work correlate very well with the existing survival assay data (4, 5, 9, 18, 19). With the exception of the α emitter ^{210}Po , there is a linear relationship between the RBE values for induction of sperm abnormalities and cell survival (Fig. 6), given by $\text{RBE} [\text{abnormals}] = (8.8 \pm 0.7) * \text{RBE} [\text{survival}] - (5.3 \pm 3)$. This relationship is valid only for $\text{RBE} [\text{survival}] \geq 1$. When further data for $\text{RBE} < 1$ become available, it is possible that a linear-quadratic fit to the data may better describe the relationship. It may be noted that the RBE values for ^{111}In -oxine have not been corrected for a small degree of chemical toxicity observed with unlabeled oxine. Previous chemical toxicity studies with oxine (5) indicate that this is about a 25% correction in the RBE value. Nevertheless, the correlation between the two assays is very good. Furthermore, the high RBE values (Table I) obtained in this work suggest that induction of sperm abnormalities is an extremely sensitive model to ascertain the effects of very low doses ($\sim$ few cGy) from incorporated radionuclides.

It is well recognized that RBE values are highly dependent on the experimental system as well as the end points chosen. In this work, RBE values for induction of abnormalities in sperm shape are obtained by comparing the initial slopes of dose–response curves for the various radiochemicals with the initial slope of the dose–response curve with acute 120-kVp X rays. The International Commission on Radiation Units and Measurements (ICRU) defined RBE as “the ratio of absorbed dose of a reference radiation to the absorbed dose of a test radiation to produce the same level of biological effect, other conditions being equal” (32). The biological effects of incorporated radionuclides are dependent not only on the type of radiations emitted but also on temporal and spatial factors such as dose rate and subcellular distribution as demonstrated by the present results. Is it then appropriate to use 120-kVp X rays, which deliver the absorbed dose rather uniformly and acutely, as a reference radiation for the purpose of obtaining RBE values for incorporated radionuclides? If the purpose of the RBE is limited to delineate the biological effects of radiations of different quality, other conditions such as dose rate and subcellular distribution must be the same. Clearly, this is not so in our studies. Consideration of a reference radionuclide in place of X rays suffers from the same problem in that no two radiocompounds incorporated in the tissue deliver the same dose rate because of differences in physical and biological half-lives, as well as differences in subcellular distribution as shown in this work. In view of these considerations, the RBE values presented here relative to X rays are representative not only of the quality of the low-energy Auger electrons and α particles, but also of the temporal and spatial factors associated with these radiocompounds. If we choose to compare the effects of radionuclides with those of ^7Be , a pure γ emitter, which delivers a chronic dose like most of the other radiochemicals used in this work, the RBE values for abnormal induction are lower by a factor of 3. In the context of radionuclide effects, the question of radiation reference source for the purpose of obtaining RBE values and quality factors (33) is of significant interest as the use of radionuclides for medical diagnosis and therapy increases.

CONCLUSIONS

This work contains a substantial and significant amount of new data concerning the complex nature of the biological effects of incorporated radionuclides. The assay used for abnormal sperm in mice provides an extremely sensitive *in vivo* model to examine the influence of chronic exposure to low doses (~ 0.1 cGy–100 cGy) of ionizing radiation from incorporated radionuclides. The results obtained point to the following conclusions: (1) subcellular distribution of Auger-electron emitters is the primary determinant of their radiotoxicity; (2) although electrons from DNA-bound Auger emitters are as efficient as high-LET α particles in killing cells, they are about four times less efficient in producing sperm head abnormalities as indicated by the RBE values of 59 and 245 for $^{125}\text{IUdR}$ and $^{210}\text{Po-citrate}$, respectively; (3) chronic low-LET irradiation of the testis with incorporated radionuclides is more effective in producing sperm head abnormalities than a single acute irradiation with X rays; (4) the biological effects of intracellularly localized radionuclides are strongly mitigated by the presence of small, nontoxic amounts of the radioprotector MEA; and (5) the induction of sperm head abnormalities by Auger emitters may be predicted reliably from the sperm head survival assay in view of the observed correlation (Fig. 6).

The relevance of the mouse testis model to humans has been addressed by Clifton and Bremner (34) and Meistrich and Samuels (75), who compared mouse data with the limited human data available (35). It was concluded that the biological effects of radiation on mouse testis may be extrapolated to humans; the extrapolation factor, that is, the ratio of the dose to produce a given effect in the mouse to that necessary to produce the same effect in man, is between 3 and 7 for low doses. In that event, the present results are significant for risk assessment of incorporated radionuclides.

Acknowledgments

This work is supported in part by USPHS Grant CA-32877, and NJ Cancer Commission Grants 688-009 and 689-042.

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FIG. 1. Biological clearance of the radiochemicals from mouse testes after intratesticular injection. The remaining fraction of initially injected activity is shown as a function of postinjection time for ^7Be -chloride (solid triangles) and $\text{H}^{125}\text{IPDM}$ (open circles).

FIG. 2.

Dependence of induction of abnormal sperm heads on subcellular distribution of Auger-electron-emitting radionuclides in mouse testis. The percentage of sperm expressing abnormalities in head shape is shown as a function of the average absorbed dose from intratesticular decays of the radionuclide. The dose-response curves for ^{111}In -citrate (solid squares) and ^{111}In -oxine (open squares) are given in Fig. 2A. Those for $\text{H}^{125}\text{IPDM}$ (open circles) and $^{125}\text{IUdR}$ (open diamonds) are given in Fig. 2B. The low-dose portion of the dose-response curves appears as an inset. The data are fitted by least squares to obtain the initial slope. The dependence of the response on the chemical nature of the compound, which in turn determines the subcellular distribution of the radionuclide, is clearly demonstrated in both cases. Intranuclear localization of ^{111}In and ^{125}I (^{111}In -oxine, $^{125}\text{IUdR}$) produces significantly more abnormalities than when the same radionuclides are localized in the cytoplasm (^{111}In -citrate, $\text{H}^{125}\text{IPDM}$). Standard errors are shown.

FIG. 3.

Comparison of a DNA-bound Auger-electron emitter (^{125}I) with an α emitter (^{210}Po) in causing abnormalities in sperm shape. The percentage of abnormal epididymal sperm is shown as a function of the testicular absorbed dose for ^{125}I UdR (open diamonds) and ^{210}Po -citrate (inverse open triangles). Error bars represent the standard error. An enlargement of the low-dose region of the curve is illustrated in the upper right corner. The efficacy of these radiochemicals in inducing abnormalities at very low doses is indicated by the large initial slopes (see Table I). Based on the initial slopes, the α particles emitted by ^{210}Po are about four times more effective in producing sperm abnormalities than the dense shower of Auger electrons emitted by DNA-bound ^{125}I .

FIG. 4.

Relative efficacy of chronic versus acute irradiation of mouse testis with low-LET photons. Chronic irradiation is delivered to the testis by injecting ^{7}Be -chloride (solid triangles) which emits 477-keV γ rays. Selective acute irradiation of the testis is accomplished using 120-kVp X rays (open triangles). The standard errors are shown. As indicated by the dose-response curves, induction of sperm abnormalities by chronic irradiation of the testis with intratesticularly localized ^{7}Be is about three times more effective than acute X rays.

FIG. 5.

Radioprotection provided by nontoxic amounts of intratesticularly administered cysteamine (MEA) against the effect of radionuclides incorporated into mouse testis. Figure 5A shows the mitigating effect of MEA against the Auger cascade electrons from DNA-bound ^{125}I . The open and solid diamonds represent ^{125}I UDR alone and ^{125}I UDR + MEA, respectively. Similarly, Fig. 5B illustrates the protective effect of MEA against 5.3-MeV α particles from ^{210}Po : ^{210}Po -citrate alone is represented by the open inverse triangles and ^{210}Po -citrate + MEA by the solid inverse triangles. Standard errors are given. Significant radioprotection is realized for both types of radiation, the dose reduction factors, based on the initial slopes (see inset), being 14 and 10 for ^{125}I UDR and ^{210}Po -citrate, respectively.

FIG. 6.

Correlation of spermatogonial cell killing and induction of sperm shape abnormalities in mouse testes with incorporated radiochemicals. For all the radiocompounds, the relative biological effectiveness (RBE) is obtained by comparing their effects with those of acute 120-kVp X rays. For RBE values greater than 1, there is a linear relationship between the two experimental end points.

TABLE I

Radiochemical	Radionuclide	subcellular distribution ^d	Optimal assay day	postinjection ^b	Initial slope ^c	RBE abnormalities ^d	DRF abnormalities ^e	RBE survival ^f
120-kVp X rays	—	—	38	—	0.029 ± 0.002	—	—	—
⁷ Be-chloride	46% Cy, 54% N	—	38	—	0.086 ± 0.005	3.0 ± 0.3	—	1.1 (5)
H- ¹²⁵ I PDM	100% Cy	—	38	—	0.07 ± 0.002	2.5 ± 0.2	—	1.0 (8)
¹²⁵ IUdR	100% N; 100% D	—	38	—	1.7 ± 0.1	59 ± 4	—	7.9 (9)
¹¹¹ In-citrate	70% Cy, 30% N; 9% D	—	43	—	0.35 ± 0.02	12 ± 1	—	2.0 (5)
¹¹¹ In-oxine	8% Cy, 92% N; 4% D	—	43	—	1.4 ± 0.1	48 ± 4	—	5.6 (5)
²¹⁰ Po-citrate	80% Cy, 20% N; 45% D	—	45	—	7.1 ± 0.5	245 ± 23	—	6.7 (9)
¹²⁵ IUdR + MEA ^g	100% N; 100% D	—	38	—	0.12 ± 0.01	—	14 ± 1	—
²¹⁰ Po-citrate + MEA ^g	80% Cy, 20% N; 45% D	—	38	—	0.70 ± 0.04	—	10 ± 1	—

^a% Cy = percentage of activity in cytoplasm, % N = percentage of activity in nucleus, % D = percentage of activity in cell nucleus bound to DNA.

^bDays postinjection at which epididymal sperm head abnormalities are assayed.

^cInitial slope (% abnormalities/cGy) of dose-response curves for induction of abnormalities.

^dAbnormal sperm head assay: Relative biological effectiveness compared to 120-kVp X rays based on initial slopes of dose-response curves for induction of abnormalities.

^eDose reduction factor.

^fSperm head survival assay: Relative biological effectiveness compared to 120-kVp X rays at 37% survival. Reference numbers are in parentheses.

^gMEA = cysteamine.