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IN-VIVO RADIOTOXICITY OF DNA-INCORPORATED ^{125}I COMPARED WITH THAT OF DENSELY IONISING ALPHA-PARTICLES

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Summary

When the atomic nucleus of ^{125}I decays by orbital electron capture followed by internal conversion, numerous very-low-energy electrons (Auger electrons) are emitted, so that the energy density in the immediate vicinity of the decay site is extremely high, ^{125}I incorporated into DNA was as effective as densely ionising 5.3 MeV α -particles from ^{210}Po in reducing the sperm-head population in mice. Hence the biological risks of Auger-electron emitting radionuclides widely used in biology and medicine ought to be reassessed.

INTRODUCTION

The increasing use of radionuclides in medical diagnosis and therapy, as well as their presence in the environment, necessitate investigation of the biological consequences of their radiations. Many of these radionuclides (eg, ^{55}Fe , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{125}I , ^{137}Cs - $^{137\text{m}}\text{Ba}$, ^{201}Tl) decay by electron capture (EC) or internal conversion (IC) or both, and the result is an inner atomic shell vacancy. The extremely rapid (approximately 10^{-15} s) atomic de-excitation that follows is dominated by Auger cascade transitions with the emission of numerous low-energy electrons.¹ It is estimated^{1,2} that, on average, about 20 such electrons are emitted per ^{125}I decay (table). Most of these electrons have subcellular ranges (table), with values of linear energy transfer (LET) ranging from about 10 to 25 keV/ μm . In the immediate vicinity (< 20 nm) of the decay site, the density of absorbed energy is extremely high and similar to that found along the tracks of α -particles of high LET.^{1,3,4} Hence, radionuclides decaying by EC and IC may be expected to be highly radiotoxic when they are incorporated into the radiosensitive DNA of cells.

Because iododeoxyuridine (IUdR) is an analogue of thymidine (TdR), it is possible to incorporate ^{125}I , in the form of $^{125}\text{IUdR}$, into the DNA of proliferating cells.⁵ In-vitro

studies of the radiobiological effects of ^{125}I thus incorporated on different mammalian cell lines⁵⁻⁷ have clearly shown that the Auger electron showers from ^{125}I decays can cause cytotoxic effects much like high LET radiations. Similar results^{8,9} are reported for other DNA-bound Auger emitters (^{123}I , ^{77}Br). In contrast, studies with $^3\text{HTdR}$ and $^{131}\text{IUdR}$ show that the β -emitters ^3H and ^{131}I are much less radiotoxic even when they are similarly incorporated into the DNA.^{5,6}

The high radiotoxicities of DNA-bound Auger-emitters observed in vitro prompted us to develop a very sensitive in-vivo model to determine the effects of tissue-incorporated radionuclides at low absorbed doses. By examining the effect on spermatogenesis of radionuclides distributed in the testis in mice, several Auger-emitters have been found to have relative biological effectiveness (RBE) considerably higher than 1 when compared with their β -emitting counterparts.¹⁰⁻¹² The importance of these in-vivo results has been noted.^{13,14} Despite these findings, there are no experimental data directly comparing the toxicity of Auger-emitters with that of high-LET radiations such as α -particles. This paucity of data, and the increasing concern over the effects of tissue-incorporated Auger-emitting and α -emitting radionuclides, have prompted the present study, which compares directly the in-vivo toxicity of DNA-incorporated $^{125}\text{IUdR}$ with the α -emitter ^{210}Po . ^{210}Po , a radon daughter, with a half-life of 138 days, emits 5.3 MeV α -particles which have a track-averaged LET of about 100 keV/ μm and a range in tissue of approximately 50 μm .¹⁵

The differentiated spermatogonial cells (types A_1 - A_4 , In, and B) in mouse testis are highly sensitive to ionising radiation. The complete process of spermatogenesis in mouse¹⁶⁻¹⁸ is similar to that in man but for the time scale—about 5 weeks for mouse and 10 weeks for man. Furthermore, human spermatogonial cells are at least as radiosensitive as those in mouse.¹⁶ Thus, spermatogenesis in mice is an experimental model relevant to man. Compared with the differentiated spermatogonia, their precursors and the postgonial cells (spermatocytes, spermatids, and Spermatozoa) are relatively radioresistant. Hence, any initial radiation insult to the testis will manifest itself as a reduced testicular sperm count after the time required for the differentiated spermatogonial cells to become spermatozoa.

METHODS

Our experimental procedures and protocols have been described in detail elsewhere.¹⁰ Briefly, 3 μl of solutions containing different amounts of either $^{125}\text{IUdR}$ or ^{210}Po -citrate were injected into the right testes of Swiss Webster mice (8-9 weeks of age, weight 30 g) anaesthetised under ether. This mode of administration requires very small amounts of radioactivity and enables a clear delineation of the effects of low-energy electrons without the complications of whole-body irradiation inherent in intravenous or intraperitoneal modes of injection.¹⁰

$^{125}\text{IUdR}$, dissolved in water, was obtained from ICN Radiochemicals (Irvine, California). Carrier-free ^{210}Po in 3 mol/l nitric acid was obtained commercially (Amersham, Arlington Height, Illinois). The radiochemical ^{210}Po -citrate was prepared by mixing the stock ^{210}Po solution with 1 mol/l sodium citrate (pH 4.7) in the ratio 1:9.

Testicular Clearance of the Radiochemicals

To determine the pattern of biological elimination of the radiochemicals from the testes, groups of 5 animals were killed under ether at various times after the initial injection, and the intratesticular activity determined—that of radioiodine with a NaI scintillation well detector and that of ^{210}Po by digesting the testis in 10 ml 'Fluorosol' scintillation cocktail (National Diagnostics, Manville, New Jersey) before counting in an automatic liquid scintillation counter. The biological clearance did not depend on the amount of radioactivity injected.

Determination of Survival of Spermatogonial Cells

The survival of spermatogonial cells was determined by the sperm-head survival assay.¹⁰ The best time for this assay is when the testicular sperm-head population reaches a minimum after the initial radiation insult. This is determined experimentally. About 50 animals were injected with about 0.24 MBq of $^{125}\text{IUdR}$ or about 0.16 kBq of ^{210}Po -citrate, and killed at different post-injection times. The injected testes were removed, placed in 1 ml of deionised water, homogenised for 15 s, and sonicated for 30 s. The sperm-heads, which are resistant to sonication, were counted under a microscope in a haemocytometer; at least 200 were counted. The post-injection time required to achieve the minimum sperm count was 29 days for $^{125}\text{IUdR}$ and 36 days for ^{210}Po -citrate.

Thus on days 29 and 36 after injection of the radiochemicals animals were killed, and the injected testes removed and processed for sperm-head counting. Untouched mice and those injected with normal saline served as controls.

To verify that there were no chemotoxic effects the mice were injected with non-radioactive IUdR and with citrate solution alone (without polonium) in amounts corresponding to the highest doses. To determine macroscopic radionuclide distribution in the testes several injected testes were removed one day post-injection, frozen with 'CRYOkwik' (International Equipment Company, Needham Heights, Massachusetts), and sliced into ten sections. Each slice was weighed and the activity it contained assayed. The radioactivity per gram of tissue was essentially the same in all sections, thereby indicating fairly uniform distribution of the radioactive material.

Sperm-head survival fraction (S) was determined as a function of the average absorbed dose (D) to the testis. The absorbed dose to the testis was calculated, according to Medical Internal Radiation Dose (MIRD) procedures,^{12,19} from the biological and physical half-lives and the radiation data for the radionuclides (table and ref 20).

RESULTS

98% of the injected activity of $^{125}\text{IUdR}$ was quickly eliminated from the testis, the biological half-life being 0.18 h. The remainder was cleared with a long half-life (308 h). For Po, however, a large fraction (about 40%) of the injected activity was retained, the biological half-life being 570 h (fig 1). Least-squares fitting of the survival data (fig 2) yielded mean lethal doses (D_{37}) of 8.5 (SEM 2.1) cGy and 10 (1) cGy for $^{125}\text{IUdR}$ and ^{210}Po , respectively. When the mouse testes were selectively irradiated with 60 kVp and 120

kVp external X-rays, the 37% survival dose was 67 cGy.²³ Therefore the RBE values for the DNA-incorporated Auger-emitter ¹²⁵IuDR and the α -emitter ²¹⁰Po are 7.9 (2.4) and 6.7 (1.4), respectively. Within the experimental uncertainties, both radiochemicals are equally effective in causing biological damage in the testis.

DISCUSSION

The two-component nature of the survival curves observed in these experiments (fig 2) is not an artifact of our protocols.^{10,11,21} Such survival curves were also observed when the radiochemicals were injected intraperitoneally, thereby introducing the activity into the testis by a different pathway. Furthermore, irradiation of the testis with external X-rays (60 kVp or 120 kVp) also yielded similar two-component curves.^{22,23} Perhaps, the differential radiosensitivities of the spermatogonial cell subpopulations A₁–A₄, In, and B may be responsible for the observed behaviour.^{11,24}

The RBE values we obtained are in reasonable agreement with results of in-vitro tests for ¹²⁵IuDR⁷ and the α -emitter ²¹¹At.²⁵ Our study, which directly compares the effects of an Auger-emitter with an α -emitter, gives clear in-vivo evidence of the extreme toxicity and high-LET nature of DNA-incorporated Auger-emitters in an experimental model that is relevant to man.

The importance of our findings to medical dosimetry and radiation protection, in general, should be noted. The International Commission on Radiological Protection (ICRP) assigns a quality factor of 20 for α -particles.²⁶ Hence, our RBE of 6.7 for spermatogonial cell killing with 5.3 MeV ²¹⁰Po α -particles is not surprising and is in keeping with published results for cell inactivation with high-LET radiations.^{25,27,28} However, photons and electrons, irrespective of their localised patterns of energy deposition, are treated as low-LET radiations. Accordingly, a value of 1 is used for the RBE for ¹²⁵I, an emitter of photons and seemingly harmless low-energy electrons. Thus, the risk associated with DNA-bound ¹²⁵I is severely underestimated because of the assumptions used in the dosimetry procedures recommended by MIRD and IGRU Committees.^{19,29} They tacitly assume that the radionuclide and its deposited energy are uniformly distributed in the organ. This assumption is reasonably valid for radionuclides emitting penetrating photons of macroscopic mean free paths, and energetic electrons of low LET with ranges in tissue much larger than cell dimensions. These assumptions may not be tenable for Auger-emitters such as ¹²⁵I. For example, a single ¹²⁵I decay in the nucleus of a spermatogonial cell (9 μ m cell diameter, 5 μ m nucleus diameter) deposits 10.5 keV in the radiosensitive cell nucleus; this corresponds to a dose of 2.6 cGy;²² averaging this energy over the entire cell results in a six-fold reduction in the dose. The differences are of course even greater when the dose is calculated over molecular dimensions. Indeed, the conventional dosimetric approach trivialises the importance of deposition of small amounts of energy in extremely minute volumes^{3,11,14,30} by averaging the deposited energy over the entire organ. The highly localised nature of the deposition of energy is ignored. That this is particularly true when the Auger-electron emitter is bound to DNA in the cell nucleus is demonstrated by our in-vivo experiments.

In as much as the absorbed dose is used for risk assessment, the above considerations point to the need for some modifications in the current dosimetric approaches, particularly for tissue-incorporated Auger-emitters. The in-vitro and in-vivo evidence available presents a compelling case for biophysically meaningful dosimetry at subcellular instead of organ level.^{10–12,30} Knowledge of the cellular localisation and subcellular distribution of the Auger-emitter is essential. These, in turn, are governed by the chemical form of the radiolabelled agent. These conclusions are amply supported by radiobiological studies both in vitro⁸ and in our mouse testes model^{12,23} and have been endorsed by a recent forum on microdosimetry of radiopharmaceuticals.³¹ It is possible that as more experimental data are collected on the relation between the subcellular distribution and the biological effects, each radiochemical may be assigned a quality factor based on the radionuclide Auger-electron spectrum. Until such predictions can be made with reasonable accuracy, radiation protection guidelines must rely on experimental data obtained at low doses in radiosensitive models.

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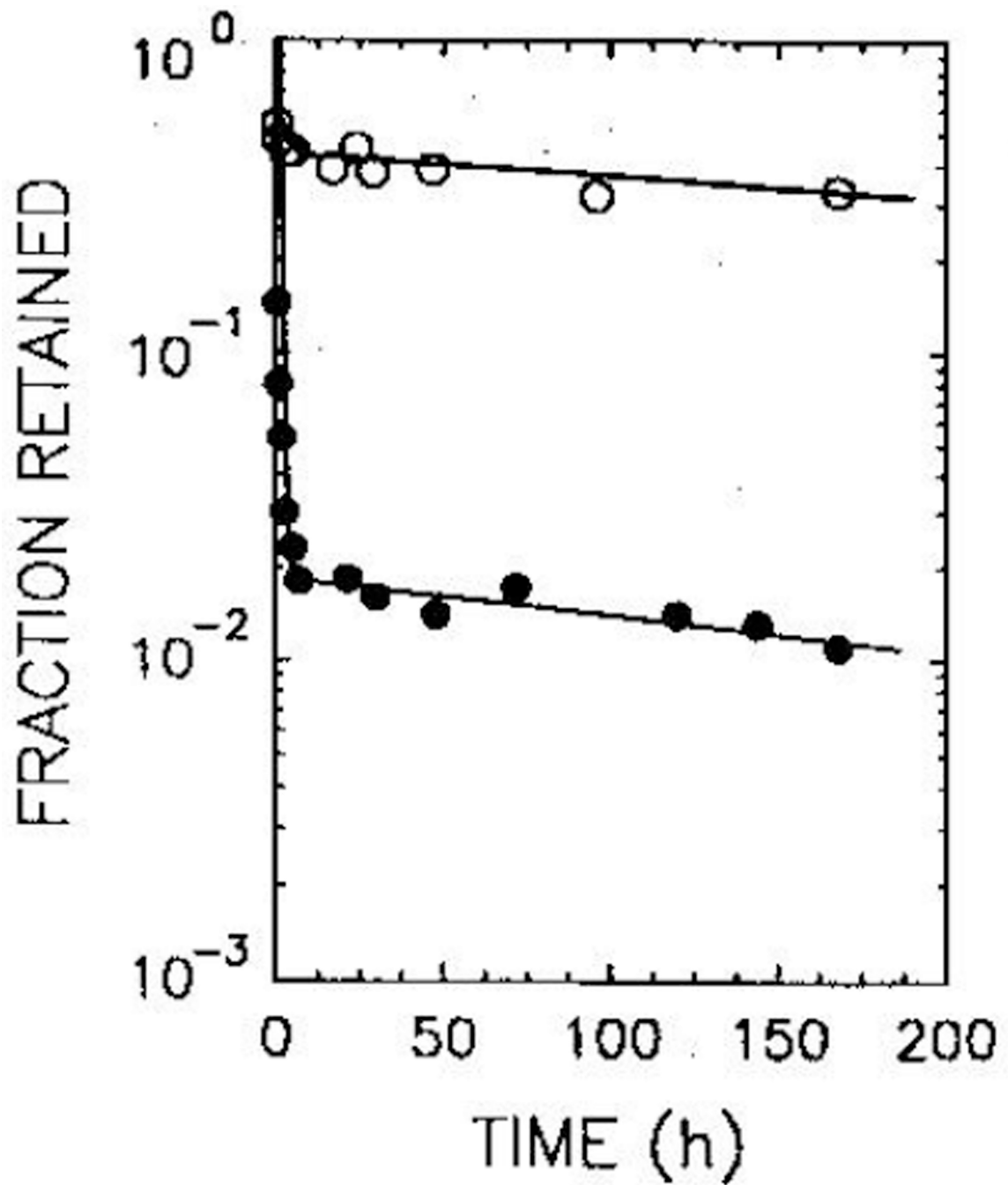


Fig 1. Fraction of injected radiochemical retained by the testis as a function of post-injection time for ^{210}Po -citrate (open circles) and $^{125}\text{IUdR}$ (closed circles). Data represent the average of 3 and 4 separate experiments for $^{125}\text{IUdR}$ and ^{210}Po -citrate, respectively.

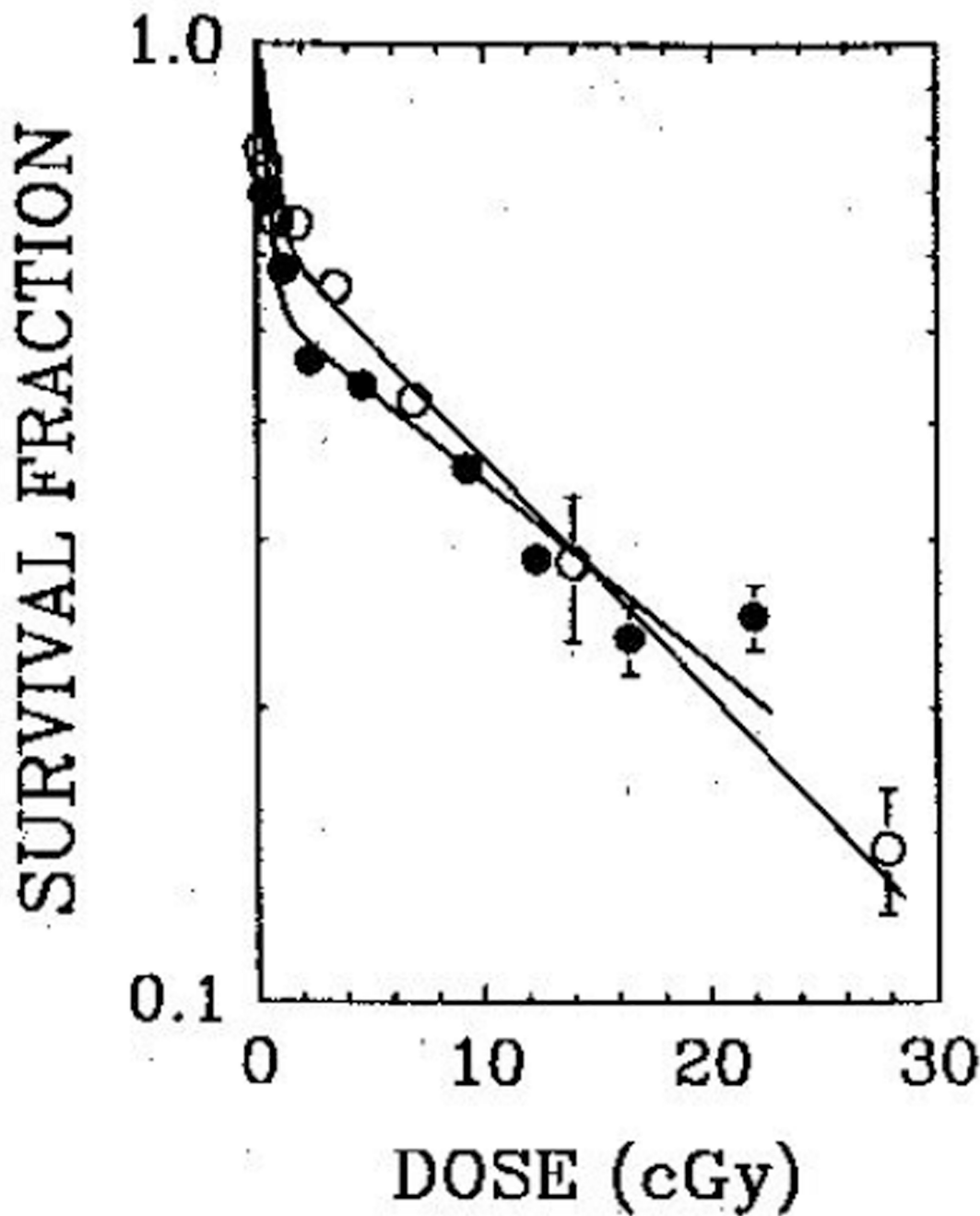


Fig 2.

Fraction of surviving sperm-heads as a function of the testicular absorbed dose from ^{210}Po -citrate (open circles) and $^{125}\text{IUdR}$ (closed circles)

Data shown are the average of two separate series for $^{125}\text{IUdR}$ and three for ^{210}Po -citrate.

Representative error bars (standard deviations) are indicated. The data are least-squares fitted to a two-component exponential function which give the following expressions for the survival fractions S as a function of the absorbed D :

$$S(^{125}\text{IUdR}) = 0.46 e^{-D/0.41} + 0.54 e^{-D/22.1},$$

$$S(^{210}\text{Po})=0.30 e^{-D/0.20}+0.70 e^{-D/15.6}.$$

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TABLEAVERAGE AUGER-ELECTRON SPECTRUM PER ^{125}I DECAY

Average energy ¹ (keV)	LET ³² (keV/ μm)	Yield per decay ¹	Range (μm) in unit density matter ³²
24.3	1.1	0.20	14.0
3.27	4.8	1.58	0.42
0.670	12.7	0.24	0.037
0.475	14.9	3.17	0.023
0.258	18.7	0.13	0.012
0.210	19.9	0.29	0.010
0.154	21.5	0.35	0.0075
0.110	23.0	0.82	0.0055
0.065	24.9	4.66	0.004
0.048	25.7	0.31	0.003
0.027	26.7	6.14	0.0015
0.016	27.3	1.36	< 0.001

LET = linear energy transfer.