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Estrogen Receptor-Mediated Cytotoxicity Using Iodine-125

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Auger effects from ^{125}I decay are singularly damaging if localized in DNA as the thymidine analogue ^{125}I-iododeoxyuridine ($^{125}IUdR$). Recent experience with steroid sex hormones extends these observations by demonstrating cytotoxicity in sites other than the DNA backbone. We have compared the cytotoxicity in human MCF-7 breast cancer cells of $^{125}IUdR$, ^{125}I-iodotamoxifen, a nonsteroidal antiestrogen that is translocated from the cytoplasm to the nucleus of receptor containing cells, and ^{125}I-iodoantipyrine, a biological indicator of the body water space. Cytotoxicity is critically dependent upon subcellular localization.

Key words: iodine-125, iododeoxyuridine, iodoantipyrine, iodotamoxifen, estrogen receptor-mediated cytotoxicity

The potential therapeutic role of unsealed sources of radiation has yet to be fully realized because of major biological and physical constraints. However, considerable evidence has accumulated in recent years indicating that Auger effects within radiosensitive targets are singularly damaging [1, 2]. This has been demonstrated by incorporating the thymidine analogue ^{125}I-labeled 5-iododeoxyuridine ($^{125}IUdR$) into DNA. Although the intracellular localization of the radionuclide is well known [3, 4], the extent to which these effects depend upon specific nuclear or molecular localization is not clear, especially in view of the fact that DNA intercalating agents are also markedly cytotoxic [5, 6].

Alternatives to DNA precursors and intercalating agents as carriers for Auger-emitting radionuclides are small molecules that enter the cell and are bound to nuclear receptors as specific hormones or antihormones [7]. Halogenated estrogens with varying degrees of receptor affinity have been synthesized [8–11]. Tamoxifen (TAM) is a nonsteroidal antiestrogen that competes with 17-β-estradiol for the estrogen receptor and is translocated to the nucleus [12].

We have compared the cytotoxicity of $^{125}IUdR$, a thymidine analogue incorporated into the DNA backbone, ^{125}I-iodotamoxifen, an antiestrogen that is translocated from the cytoplasm to the nucleus, and ^{125}I-antipyrine, a biological marker that distributes itself within the body waterspace [13], and report that cytotoxicity is critically dependent upon subcellular localization.

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MATERIALS AND METHODS

The MCF-7 cell line of human breast cancer, which was derived from a malignant pleural effusion, contains biologically active estrogen receptor and is well suited to study the effects of subcellular localization in the expression of cytotoxicity from ^{125}I decay [14]. The specific estrogen receptor level for our cells is 60 fmol/mg protein [7]. MCF-7 cells were grown at 37°C in 5% CO_2–95% air. The media used were Earle's balanced salt solution (EBSS), Eagle's minimal essential medium supplemented with 2 mM L-glutamine, 10 μg/ml streptomycin, 1% nonessential amino acids, and 4% NCTC 109 (Microbiological Associates)—complete MEM, complete MEM with 10% dextran charcoal filtered fetal bovine serum and 60 μg/ml bovine insulin–complete CF-MEM, or complete CF-MEM without Ca^{++} or Mg^{++}—spinner MEM.

^{125}I-iodoantipyrine (^{125}IAP, 1–3 Ci/mmol) and carrier-free ^{125}I-iododeoxyuridine ($^{125}IUdR$, > 2,000 Ci/mmol) were obtained from New England Nuclear Corp. The synthesis of carrier-free ^{125}I-iodotamoxifen ($^{125}ITAM$) is described elsewhere [7, 15] and was followed with only slight modification. The reaction product was extracted from the reaction mixture with ethyl acetate and washed twice with a saturated solution of NaCl, and the volume was reduced with a stream of dry nitrogen. The labeled product was isolated by high-performance liquid chromatography (Waters Associates) using a microbondapak C-18 column. Elution was performed isocratically with ethanol/0.005 M potassium phosphate at a flow rate of 1.0 ml/min. The column effluent was monitored dynamically with a Na(T1) crystal. ^{125}TAM had a retention time (RT) of 10 min.

Details of experimental procedures for $^{125}ITAM$ uptake and cellular efflux with time, $^{125}IUdR$, and ^{125}IAP cellular uptake and clonogenic survival are given elsewhere [16].

Whole-cell and nuclear uptakes of $^{125}ITAM$ were determined as a function of increasing $^{125}ITAM$ concentration. Monolayer MCF-7 cells, which had been maintained for up to 2 weeks in CF-MEM, were trypsinized, washed twice in phosphate-buffered saline (PBS), passed through a 21-gauge needle four times to disrupt cell clumps, and incubated at 37°C in 12 × 75 mm glass tubes. After 4 h, the cells were centrifuged for 10 min at 1,000g and resuspended in graded concentrations of $^{125}ITAM$. Whole-cell and nuclear $^{125}ITAM$ concentrations were determined after a 5-h exposure at 37°C.

Whole-cell uptake of $^{125}ITAM$ was assessed by layering 100-μl samples of the radiopharmaceutical containing single cell suspension over 300 μl Plageman's oil (ρ = 1.040 gm/ml) in 500 μl Eppendorf microcentrifuge tubes, centrifuging at 12,000 rpm for 15 sec, freezing in liquid nitrogen, excising the tip containing the cell pellet, and counting radioactivity by gamma counting (efficiency 77%).

Nuclear concentrations of $^{125}ITAM$ were determined using a modification of an established method [17]. All procedures were performed at 4°C. Cell suspensions were washed twice with ice-cold PBS and the cell pellets resuspended in a cell disruption buffer (20 mM Tris-HCl, 0.25 M sucrose, 1 mM $MgCl_2$, and 1% Triton X-100, pH 7.8). After 5 min, the cell suspensions were vortexed for 10 sec and the resulting crude nuclear preparations were washed twice with PBS and centrifuged through 1.8 M sucrose to obtain a greater than 98% pure nuclear pellet as determined by phase microscopy. The resulting nuclei were resuspended in 1 ml PBS, counted with a hemacytometer, collected on a 0.45-μm Amicon mixed ester filter, washed twice with 10 ml PBS, and radioactivity determined by gamma counting.

RESULTS

Cellular uptake of ^{125}IUdR and ^{125}IAP is directly proportional to media concentration (Fig. 1). Cellular accumulation, however, is quite different. ^{125}IUdR is much more avidly accumulated. It is incorporated directly into the DNA backbone as a thymidine analogue whereas ^{125}IAP is considered to distribute itself in accordance with the body water space at least on a macroscopic level.

The uptake of ^{125}ITAM is both time- and temperature-dependent (Fig. 2). Na^{125}I is effectively excluded by MCF-7 cells and remains in the extracellular space. When ^{125}ITAM is withdrawn from the culture media, there is prompt efflux. ^{125}ITAM is presumed to be distributed among three compartments: free ^{125}ITAM, nonspecifically protein-bound, or specifically bound ^{125}ITAM [18]. The rapid efflux observed after withdrawal of ^{125}ITAM probably represents depletion of unbound intracellular ^{125}ITAM. Most of the retained activity at 50 min is associated with either specific or nonspecific intracellular binding sites.

^{125}ITAM is differentially cytotoxic to cells containing estrogen receptors [7]. Cytotoxicity is presumed to result from translocation of specifically bound ^{125}ITAM from the cytoplasm to the nucleus or from direct charging of specific nuclear receptors [19]. In an attempt to assess this hypothesis, nuclear-associated radioactivity ($^{125}ITAM_{nuc}$) was distinguished from whole-cell radioactivity ($^{125}ITAM_{wc}$) in determinations of cytotoxicity. Whole cell- and nuclear-associated radioactivity increase linearly with concentration of ^{125}ITAM (Fig. 3).

When clonogenic survival of MCF-7 cells is expressed as a function of the concentration of ^{125}IUdR, ^{125}ITAM, and ^{125}IAP in the culture media, the doses to reduce survival to 0.37 are 8×10^{-4}, 2.3 and 68 μCi/ml respectively (Fig. 4). Not surprisingly, ^{125}IUdR is the most toxic of the three agents by several orders of magnitude. By comparison with the other agents, ^{125}IUdR is exclusively associated with DNA. Only a small fraction of the whole-cell ^{125}ITAM and ^{125}IAP disintegrations will occur in close association with the genome.

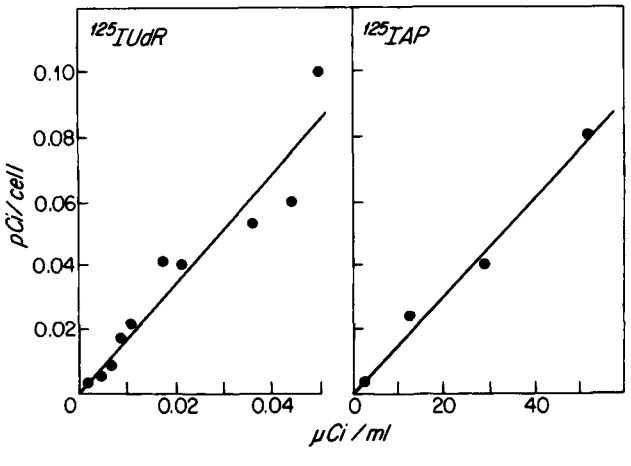

Fig. 1. Cellular uptake of ^{125}IUdR and ^{125}IAP as a function of concentration in culture media after 50-hr incubations.

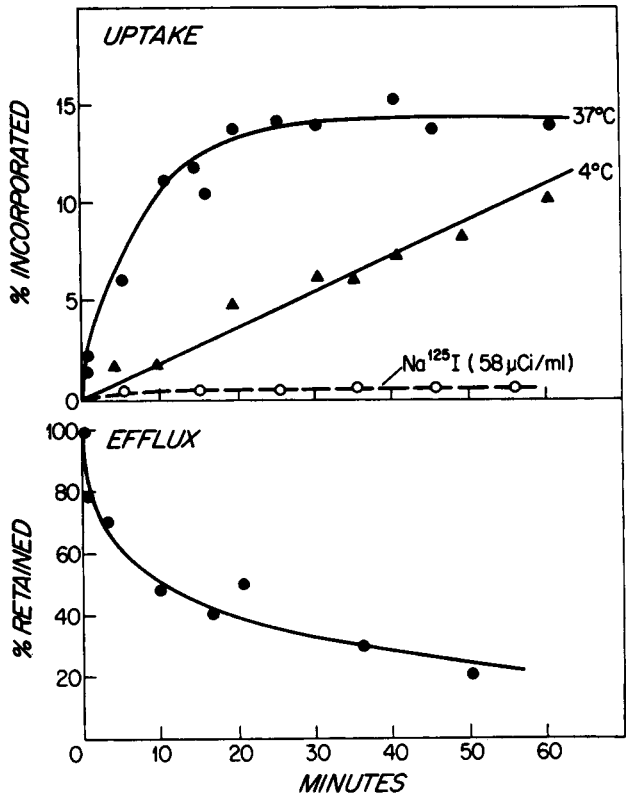

Fig. 2. Cellular uptake and efflux of ^{125}ITAM. There is no uptake of $Na^{125}I$.

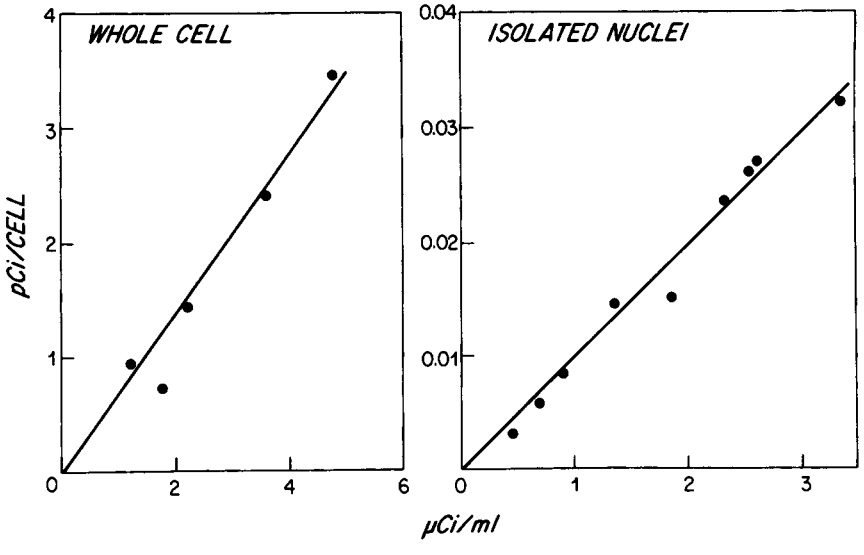

Fig. 3. Whole-cell and nuclear-associated radioactivity in MCF-7 cells after incubation with graded doses of ^{125}ITAM.

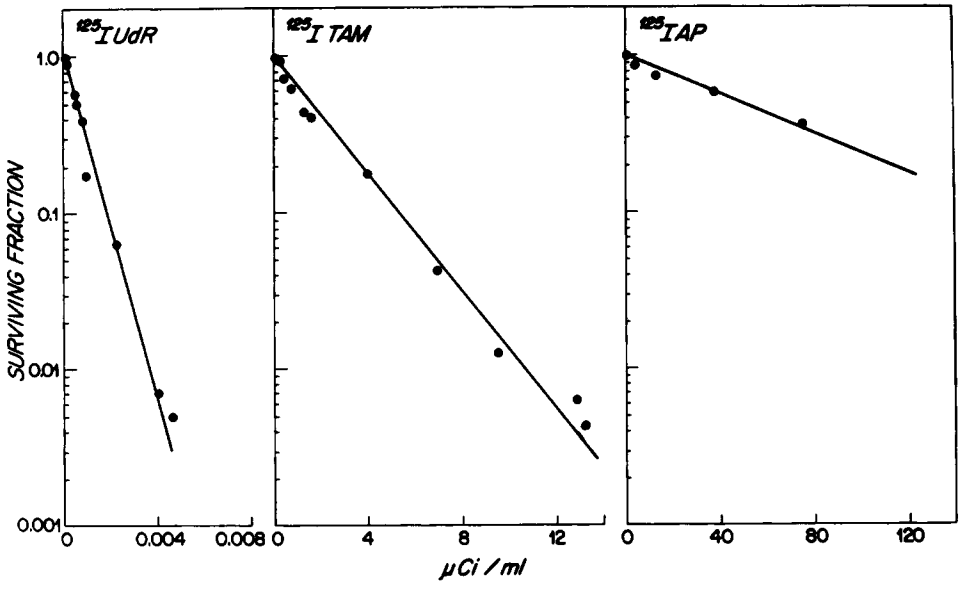

Fig. 4. Clonogenic surivival of MCF-7 cells after exposure to graded media concentrations of ^{125}IUdR, ^{125}ITAM, ^{125}IAP.

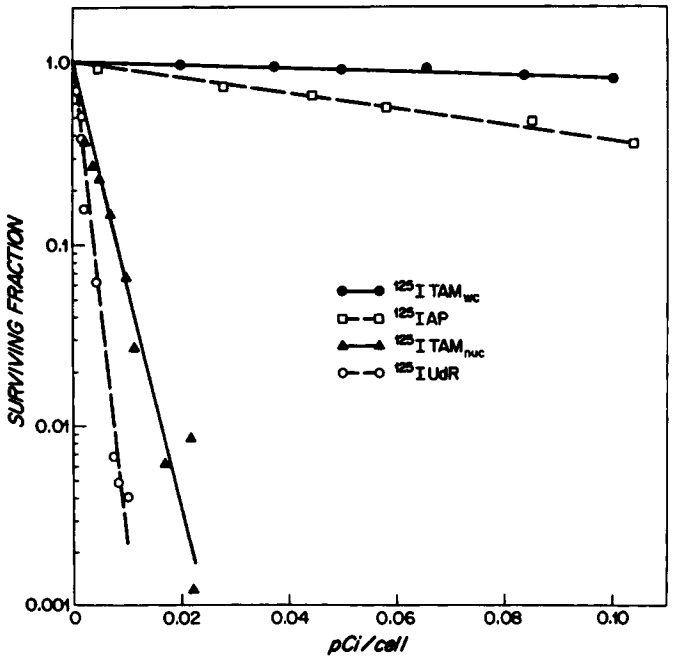

Fig. 5. Clonogenic surivival of MCF-7 cells exposed to ^{125}IUdR, ^{125}ITAM, or ^{125}IAP. ^{125}ITAM cytotoxicity is differentiated between that associated with the whole cell ($^{125}ITAM_{wc}$) or the nucleus ($^{125}ITAM_{nuc}$).

A more realistic appraisal of the cytotoxicity is obtained if clonogenic survival is expressed as a function of radioactivity per cell or, preferably, within the cell nucleus (Fig. 5). This is convincingly demonstrated in the case of ^{125}ITAM where cytotoxicity is separated into $^{125}ITAM_{nuc}$ and $^{125}ITAM_{wc}$ components. When expressed as a function of ^{125}ITAM concentration within the cell nucleus, $^{125}ITAM_{nuc}$ is just about as toxic as ^{125}IUdR localized within the DNA backbone. The fact that the survival curves for ^{125}IUdR and $^{125}ITAM_{nuc}$ cannot be superimposed probably reflects contributions of intranuclear ^{125}ITAM that is nonspecifically bound as well as differences in genome associations between ^{125}ITAM and ^{125}IUdR.

DISCUSSION

Biological consequences of ^{125}I decay can be grossly underestimated. ^{125}ITAM might appear to be minimally cytotoxic compared with ^{125}IUdR when clonogenic survival is expressed only as a function of concentration in the culture media. However, when survival is expressed on the basis of whole-cell or nuclear-associated radioactivity, striking differences are immediately apparent. The cytotoxicity of ^{125}ITAM, when only nuclear observations are evaluated, is not too dissimilar from that of ^{125}IUdR, which is exclusively localized within DNA. Although ^{125}IAP appears to be minimally cytotoxic, significant cytotoxicity might become apparent if one could evaluate only the nuclear antipyrine space.

If the full potential of unsealed sources of radiation is to be realized in the treatment of cancer, the problem of localizing internal emitters within the sensitive targets of tumor cells must be solved. Although precise mechanisms have yet to be clarified, an Auger emitter within the genetic apparatus is extremely radiotoxic, with as little as a single disintegration being lethal in some microorganisms [20]. Moreover, recently available evidence suggests that the extreme lethality is confined to a very small volume, probably on the order of molecular dimensions [21]. These facts highlight the advantages and limitations of using the Auger effect for cancer therapy. A favorable feature is that damage is confined to the cell in which decay occurs; a disadvantage is that the biochemical specificities are very great. Not only must the radioactivity be directed specifically to certain cells, but it must also be very closely approximated to their genetic structures.

ACKNOWLEDGMENTS

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