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DNA AND CHROMOSOME BREAKS
INDUCED BY ^{125}I -ESTROGEN IN CHO CELLS

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Running Head: Estrogen-Directed Radiotherapy

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Abstract

The effects of the Auger electron-emitting isotope I-123, covalently bound to estrogen, on DNA single- and double-strand breakage and on chromosome breakage was determined in estrogen positive Chinese hamster ovary (CHO-ER) cells. Exposure to the ^{123}I -estrogen induced both single- and double-strand breaks with a ratio of single- to double-strand breaks of 2.2. The corresponding ratio with ^{60}Co gamma rays was 15.6. The dose-response was biphasic suggesting that either receptor sites are saturated at high doses, or that there is a nonrandom distribution of breaks induced by the ^{123}I -estrogen. The ^{123}I -estrogen treatment induced chromosome aberrations with an efficiency of about 1 aberration for each 1000 disintegrations per cell. This corresponds to the mean lethal dose of ^{123}I -estrogen for these cells suggesting that the lethal event induced by the Auger electron emitter bound to estrogen is a chromosome aberration. Most of the chromosome-type aberrations were dicentrics and rings, suggesting that ^{123}I -estrogen-induced chromosome breaks are rejoined. The F-ratio, the ratio of dicentrics to centric rings, was 5.8 ± 1.7 , which is similar to that seen with high LET radiations. Our results suggest that I-123 bound to estrogen is an efficient clastogenic agent, that the cytotoxic damage produced by I-123 bound to estrogen is very like high LET-induced damage, and the I-123 in the estrogen-receptor-DNA complex is probably in close proximity to the sugar-phosphate backbone of the DNA.

INTRODUCTION

The decay of Auger electron-emitting isotopes, such as I-125 and I-123, produces a shower of low energy electrons. The effective range of these electrons is very short, about 10-40 nm, and results in an extremely dense sphere of ionizations within a small volume (1-3). The volume affected is less than the dimensions of the nucleus of a single cell. An important ramification of this short effective range of Auger electrons is that the decay must take place near the DNA to cause any appreciable radiotoxicity (4). If the Auger electron cascade occurs predominantly in the cytoplasm or outside the cell, the cytotoxicity is reduced by several orders of magnitude or becomes insignificant (1,5). These properties of Auger electron-emitting isotopes has lead to the suggestion that procedures which can specifically direct an Auger electron-emitter to a site in or near the DNA of tumor cells could have significant cancer therapeutic potential. One approach to nuclear targeting is to use a DNA binding protein such as the estrogen receptor. Bronzert *et al.* (6) demonstrated that Auger electron emitting isotopes covalently bound to estrogen can specifically kill estrogen receptor (ER)-containing cells. They (6) targeted the Auger electron emitting isotope I-125, as 16α - ^{125}I -iodoestradiol, to the ER-containing human mammary tumor cell line MCF-7.

The 60 day half-life of I-125 and the relatively short biologic residence time of estrogens in cells limits the effectiveness of ^{125}I -estrogen. While some toxicity is found in cells exposed under *in vitro* conditions and maintained in culture (7), most toxicity is seen only after treated cells are frozen and stored for long periods (6). Iodine-123, another Auger electron-emitter, is a more practical nuclide for producing radiotoxicity over short times because of its shorter half life of 13.2 h. The energy released per decay of I-123 is less than that seen with I-125 (8), but

dose-dependent, unlabeled estradiol-inhibitable killing of ER-positive, but not ER-negative cells *in vitro* can be shown for both non-steroidal and steroidal ^{123}I -iodoestrogens (9,10).

The dense deposition of energy in a small volume seen with the Auger electron-emitting isotopes suggests that the biological effects of these isotopes should be similar to high LET radiations. Studies on Auger electron emitters incorporated into DNA, as IUdR for example, support this hypothesis (4,11). The nature of the estrogen-receptor complex bound to DNA, however, suggests that the radionuclide covalently bound to estrogen may be more distant from the DNA than is the case for radioisotopes incorporated into DNA, and the DNA may be insulated from the radioisotope by the estrogen-receptor protein complex (12). To better understand the cytotoxicity seen with estrogens labeled with Auger electron-emitting radioisotopes, we examined the effects of ^{123}I -estrogen treatment on DNA single- and double-strand break frequencies, and on chromosome break frequencies in ER-positive mammalian cells. Our studies suggest that I-123 bound to estrogen is highly effective in producing DNA and chromosome breaks, and acts much like high LET radiation. Thus the I-123 in the estrogen-receptor complex is probably in close proximity to the DNA, and the DNA is not shielded to any significant degree from the radioisotope.

MATERIALS AND METHODS

Synthesis of 17α - ^{123}I -iodovinyl- 11β -methoxyestradiol

The E and Z isomers of 17α - ^{123}I -iodovinyl- 11β -methoxyestradiol (E or Z- ^{123}I -VME2) were prepared by the halodestannylation reaction similar to that reported previously (9,10). In brief, 20-100 μg of the E or Z- 17α -tributylstannylvinyl- 11β -methoxyestradiol in ethanol was

added to the vial containing the carrier-free Na^{123}I (Nordion International, Kanata, Ontario) in 0.1 M NaOH, followed by 100 μl of a 1:10 mixture of H_2O_2 :acetic acid which had been preincubated at room temperature for 1 h. The reactants were mixed in the closed vial and incubated for 15-20 min at room temperature. The reaction mixture was taken up in a gas tight syringe, the radioactivity assayed in a Capintec dosimeter, and the contents injected into an Econosil (Alltech, Deerfield, IL) 10 μm C18 reverse phase HPLC cartridge (4.6 x 100 mm) equilibrated in 40% acetonitrile/water. The column was eluted with 40% acetonitrile/water for 30 min followed by a gradient to 100 % acetonitrile over 30 min (Z isomer) or by the 40-100% acetonitrile gradient directly (E-isomer) at 1 ml per min. The peak fractions were combined and evaporated to dryness in a Rotovap evaporator (Buchi Industries) and the ^{123}I -VME2 taken up in 50% or 100 % ethanol to maintain sterility.

Determination of Specific Activity

The specific activities of the ^{123}I -labeled estrogens were determined using sedimentation analysis in sucrose gradients, comparing the specific binding of the ^{123}I -labeled estrogen to the estrogen receptor of immature rat uteri with that of ^3H -E2 of known specific activity (13). To obtain the specific binding to ER, the rat uterine cytosol were incubated with saturating concentrations of the labeled estrogens (5-10 nM for the ^{123}I -labeled estrogen) alone or in the presence of a 100 fold-excess unlabeled estradiol to prevent binding to ER. The mixture was sedimented in low salt sucrose density gradients, under which conditions the receptor complex sediments at about 8S, to separate specific from nonspecific binding. In some cases the amount of specifically bound labeled estrogen was confirmed by antibody shift on high salt gradients (9,10).

For that purpose the ^{123}I -labeled and tritiated estrogens were incubated with ER of rat uterine cytosol alone or in the presence of the anti-ER antibody, H222. The mixtures were then analyzed by sedimentation in high salt (0.4 M KCl) sucrose gradients.

Cell Culture and Exposure to ^{123}I -VME2

The Chinese hamster ovary (CHO) cell line CHO-ER, which contains Cd/Zn-inducible estrogen receptor and expresses large amounts of ER (14), was used for these experiments. The cells were maintained in DMEM/F12 medium supplemented with 10% FBS, 1.5 mg/ml NaHCO_3 , 10 units/ml penicillin, and 10 $\mu\text{g}/\text{ml}$ streptomycin. The medium was also supplemented with 40 μM Cd^{+2} and 50 μM Zn^{+2} to effect stimulation of the production of ER. Single-cell suspensions of exponentially-growing CHO-ER cells were diluted to $1-4 \times 10^6$ cells/ml and incubated with varying concentrations of ^{123}I -labeled estrogen, in the absence or presence of excess unlabeled estrogen. The ethanol concentration was less than 3%. Control incubations contained either medium with similar concentrations of ethanol or estradiol. The cells were incubated for 30-60 min at 37°C with occasional mixing. Ten ml of medium was then added to dilute the ^{123}I -VME2 and, after mixing, the tubes were centrifuged for 5 min at $500 \times g$ to collect the cells. The cells were resuspended in 1 ml of medium and cell number was determined. Cells were either immediately cultured for 24 h or viably frozen for 1-2 weeks to accumulate decays. Cells were viably frozen in medium supplemented with 10% glycerol to -135°C at a constant freezing rate.

To assess the amount of ^{123}I -VME2 bound to the estrogen receptor, the radioactivity associated with cells was assayed. Cells were diluted to 5×10^4 cells/ml to allow dissociation of weakly bound ligand, and after 30-60 min, triplicate 1 ml aliquots centrifuged in an Eppendorf

microfuge for 1 min at 7,000 x g at room temperature. The supernatants were decanted, the tubes inverted to drain and the bottoms of the tubes cut off and counted for radioactivity. The activities were converted into disintegrations per cell after taking into account the physical half-life of I-123 (13.2 h) and the biological half-life of the estrogen in the cells. The biological half-life was calculated to be about 8 h. This was determined by measuring the initial number of molecules per cell, and the number of molecules per cell remaining after 18-20 h in culture (corrected for decay). Based on these numbers, a 2-point exponential decay curve was fit to the data to obtain the biological half life. The number of decays per cell were then calculated from the following equation: $N = N_0(C/D)(1 - e^{-Dt})$, where $C = \lambda A$ (ie. $\ln 2/13.2$ h), $D = \lambda A + \lambda B$ (where $\lambda B = \ln 2/\text{biologic half life}$), N_0 = initial molecules per cell, and T = time in hours.

Measurement of DNA Single- and Double-Strand Break Frequencies

DNA single- and double-strand break frequencies were determined by filter elution analysis (15,16) as described Schwartz *et al.* (17). Cells were subcultured for 24-72 h in complete medium supplemented with 0.02 $\mu\text{Ci/ml}$ ^{14}C -thymidine into 100-mm plastic Petri dishes to label growing cells. When cultures were 25-50% confluent, the radioactive medium was washed off, cells were cultured a further 2-4 h in ^{14}C -free medium, and then samples were prepared for treatment. Cells were incubated with ^{125}I -VME2, washed as described above, and then viably frozen for 1 week before break frequencies were determined. Cells were thawed and approximately $1-5 \times 10^5$ cells were washed onto 20-mm polycarbonate filters (2 μm pore size) with ice-cold saline. The frequency of DNA single-strand breaks was estimated by DNA alkaline elution procedures (16,17). The filters were washed twice with saline and the cells lysed on the

filters with a 15-min treatment with 5 ml of a lysis solution containing 0.04 M Na₂-EDTA, 2mM NaCl, and 0.2% SDS (pH 10). After lysis, the filters were rinsed with 0.20 M Na₂EDTA (pH 10). Elution was carried out in the dark with 25 ml of an alkaline eluting solution (0.02 M Na₄EDTA, 2% tetrapropylammonium hydroxide, 0.1% SDS, pH 12.2) at a flow rate of 2 ml/h. Each sample was run in triplicate. Fractions were collected every 90 min for 12-18 h. After elution, the filters were treated with 1.0 N HCl for 1 h at 60 °C and then the radioactivity in each fraction and on the filters measured by liquid scintillation spectrometry. The fraction of DNA remaining on the filter 12 h after elution was used to compare samples.

The frequency of DNA double-strand breaks was estimated using the neutral elution assay (15,17). Cells were washed onto polycarbonate filters and then lysed with a 15-min treatment with 5 ml of a lysis solution containing 0.05 M Tris, 0.05 M glycine, 0.025 M Na₂-EDTA, 2% SDS, and 0.5 mg/ml proteinase K (pH 9.6). After lysis, the lysis solution was slowly pumped off and 25 ml of the neutral eluting solution added (0.05 M Tris, 0.05 M glycine, 0.025 M Na₂-EDTA, 2% SDS, pH 9.6). Each sample was run in triplicate. Fractions were collected every 90 min for 12-18 h and processed as described above for the alkaline elution assay.

Cytogenetic Analyses

Following treatment with the ¹²³I-labeled estrogen, cells were cultured for 24 h and then cells were arrested in metaphase with a 2-h treatment with 2×10^{-7} M colcemid. Mitotic cells were removed by mitotic shake-off, incubated in 0.075 M KCl for 20 min, and fixed in 3:1 methanol:acetic acid (18). Fixed cell suspensions were dropped onto slides, air-dried, and stained in a 5% Giemsa solution for later cytogenetic analysis. Chromosomes were analyzed for

chromatid deletions, isochromatid breaks and exchanges, dicentrics, rings, interstitial deletions, and terminal deletions, as described in Savage (19). Fifty cells per dose point were analyzed. In one set of experiments, cells were first synchronized by mitotic shake-off before being exposed and either cultured for a further 24 h, or viably frozen for 1 week and then thawed and cultured for 24 h.

RESULTS

DNA single- and double-strand break frequencies were estimated by filter elution. The results are presented in figure 1. Data were plotted as the percentage of DNA remaining on filters 12 h after elution. All curves were at least biphasic and there was a lot of scatter at the higher doses (>5000 dpc). Linear regression analysis was done on the initial dose points (<5000 dpc) for both the neutral and alkaline elution data. The calculated slopes were 0.0172 ($r^2=57.5\%$, $p=0.03$) for the alkaline elution data and 0.0096 ($r^2=94.1\%$, $p=.001$) for the neutral elution data. Using these initial slopes, a dose to reduce the percentage of DNA remaining on filters to 50% was estimated to be 2470 dpc for single strand break induction and 5318 dpc for double-strand break induction. This yields a single-to-double strand break ratio of 2.2. For comparison, the ratio of single-to-double strand breaks induced by ^{60}Co gamma rays in these same cells (Figure 2) was 15.6.

CHO-ER cells have a near-tetraploid karyotype and show a high level of dicentric chromosomes. The frequency of these dicentric chromosomes averaged about 0.2/cell. Presumably, these reflect telomeric associations, as there was no evidence for their developing from chromosome breaks; there were no associated acentric fragments as would have been

expected if the dicentrics developed from the recombination between chromosome breaks. The background frequency of chromosome breaks in these cells is low, about 0.02 acentric fragments/cell, while the background frequency of chromatid breaks is 0.18/cell. The frequency of chromatid breaks is higher than that seen in CHO-K1 cells which show background aberration frequencies of around 0.02/cell (20). The higher levels of aberrations in CHO-ER cells may be due to the Cd/Zn treatment.

Chromosome breakage was analyzed initially in asynchronous cells exposed and then cultured for 24 h. The results for both chromatid- and chromosome-type aberrations are shown in figure 3. The dose-response curves for chromosome aberration induction were biphasic with a break around 10,000 to 20,000 dpc. This break in the curve is likely to be an artifact. At these dose levels, the cytogenetic damage is high with more than 15 aberrations/cell. Accurate scoring of aberration frequencies greater than 15/cell is difficult. In fact, there was a dose-dependent increase in the frequency of cells containing too many aberrations to score (Figure 4). These were not included in the calculations of aberration frequency. It is also likely that the more heavily damaged cells are delayed in the cell cycle and thus are not scored at the 24 h time point (20).

Chromatid-type aberrations are break and recombination events that involve just one of the chromatids in a metaphase chromosome. They develop from chromosome breakage that occurs in S phase or G2. The predominant chromatid-type aberration found in the [123I]-exposed cells was a chromatid deletion (Figure 5), which represents an unrejoined chromatid break. Chromatid exchanges were much less frequent. Chromosome-type aberrations develop from breaks and recombination events that occur prior to DNA replication. Most of the chromosome-type aberrations seen were dicentrics, rings, and interstitial deletions, all

recombination events (Figure 5). The frequency of terminal deletions, which represent unrejoined chromosome breaks was low, suggesting that most ^{123}I -estrogen-induced chromosome breaks are rejoined. At a cytogenetic level, there was no evidence for any non-specific breakage. All chromosomes appeared equally susceptible to breakage. This suggests that there is a broad distribution of estrogen-responsive elements throughout the CHO genome.

About 90% of the damaged cells had both chromatid- and chromosome-type aberrations, and about 80% of all aberrations were chromatid-type. The 24 h time point was chosen to produce cells that had been irradiated in G1. The CHO-ER cells have a cell doubling time of about 16 h and a normal distribution of cells in the cell cycle (unpublished observation). In an asynchronous culture, about 45% of the cells are in G1, 45% are in S, and about 10% are in G2/M. Thus analysis of cells 24 h after treatment should result in an analysis of cells that were treated primarily in G1. Finding predominantly chromatid-type aberrations 24 h after treatment may reflect a delay in cell cycle progression in exposed cells, which results in our analysis of cells exposed in S or G2, not just G1. Alternatively, it could be due to continued association of the $[^{123}\text{I}]$ -estrogen with the DNA into S phase and G2. The biological half-life of the estrogen receptor bound to DNA is about 8 h (9,10). To examine this question, cells were first synchronized by mitotic shake-off (20) to produce populations of cells that were greater than 95% G1. These cells were then exposed to the ^{123}I -estrogen. Cells were harvested 24 h later and aberration frequencies were determined. About one-third of the cells had only chromatid-type aberrations. Another third had only chromosome-type aberrations, while the remaining damaged cells showed both chromatid- and chromosome-type aberrations. Almost 60% of the aberrations were chromosome-type (Figure 6). The increased levels of cells showing just chromosome-type

damage and the increased proportion of chromosome-type aberrations in these G1-synchronized cells suggests that cell cycle delays do play a role in defining the types of aberrations seen in asynchronous cultures.

The observation of both chromatid- and chromosome-type aberrations in these G1-synchronized cells also suggests that significant numbers of decays are occurring in S and G2. Freezing down G1-treated cells and then thawing and analyzing aberration frequency 1 week later shows a much different aberration pattern. Here, nearly 75% of the cells show only chromosome-type aberrations, and the chromatid-type aberrations seen are predominately isochromatid-chromatid exchanges (Figure 5), which are thought to reflect interactions between chromatid and chromosome breaks. Ninety-seven percent of the aberrations were chromosome-type (Figure 6). Thus the ^{123}I -estrogen-receptor complex remains associated with the DNA through S phase and G2, and these treatments produce significant cell cycle delays that affect aberration frequencies and types.

DISCUSSION

By limiting radiotoxicity just to the targeted cells, the use of I-123 bound to estrogen could improve therapeutic outcome in the treatment of ER-positive tumors. The dense deposition of energy released in the decay of Auger electron-emitting isotopes suggests that these isotopes should act like high LET radiations. When incorporated into DNA, Auger electron emitting isotopes act like high LET radiations (4,11,21-28). An additional consequence of the short range of the low energy electrons and the limited area of energy deposition, is that the position of the isotope relative to the DNA molecule can have an important impact on DNA damage and toxicity.

The position of I-123 bound in an estrogen-estrogen receptor complex relative to the DNA backbone is not well established. The estrogen receptor is concentrated in the nucleus (29,30), and when associated with estrogen in a high affinity complex, binds tightly to distinct sites in the DNA of responsive cells, the estrogen response elements. These elements are key to the estrogen-induced regulation of specific gene expression (31). Detailed studies of the structure of the DNA binding domain of the estrogen receptor indicate that it is located in the major groove of the DNA helix (12). The radionuclide in ^{123}I -labeled estrogen, which is bound to the 66 kDa receptor protein that binds to the DNA, may be somewhat distant from the DNA, and the Auger electrons emitted quite possibly may also be substantially insulated by the protein. Our results suggest, however, that decay of I-123 bound to the estrogen receptor complex, is highly efficient in producing DNA and chromosome damage, and therefore the I-123 must be in close proximity to the DNA sugar-phosphate backbone, and the DNA is probably not shielded to any great extent.

One measure of radiation quality is the relative efficiency with which DNA single- or double-strand breaks are produced. With increasing LET, the ratio of DNA single- to double-strand breaks should decrease as the probability of an energy deposition resulting in a DNA double-strand break increases. We found that the ratio of single- to double-strand breaks is 15.6 for ^{60}Co γ rays and 2.2 for ^{123}I -estrogen. Surprisingly the ratio of single- to double-strand breaks has been reported to be about 10 for ^{125}I -IUdR (32). The decay of I-125 generally involves two valence cascades resulting in the emission of an average of about 14 low energy Auger and Coster-Kronig electrons per decay, while I-123 has only 1/6th the likelihood of undergoing the second cascade and thus fewer electrons are emitted per decay (8). I-125 should therefore be more efficient in producing breaks than I-123. The ratio of 2.2 for ^{123}I -estrogen-induced single-

to double-strand breaks suggests that the isotope in the receptor-DNA complex may actually be positioned closer to the DNA backbone than what is found in ^{125}I -IUDR.

The dose response for DNA breakage was biphasic and there was a lot of scatter at doses of greater than 5000 dpc. Presumably, the initial slopes reflect receptor-specific damage, induced by the interaction of receptor-estrogen complexes with the DNA. It is not clear why there is a break in the curve around 5,000 dpc. It is probably not due to damage induced by nonspecific binding of ^{123}I -estrogen. Firstly, the number of estrogen receptors in CHO-ER cells is at least an order of magnitude greater than 5,000 (14). The biphasic induction curve is also probably not due to some unusual property of CHO-ER cells. Linear break induction kinetics are seen in CHO-ER cells exposed to ^{60}Co γ rays (Figure 2). DNA elution is based on measurements of average DNA sizes (15,16). As the size of the average DNA molecule gets smaller, which occurs when more breaks are induced, the percentage of DNA remaining on filters decreases. The relationship between DNA strand break frequency and elution rate assumes a random distribution of damage. The shallower slope on the break induction curves at higher doses may reflect a nonrandom distribution of breaks induced by the ^{123}I -estrogen.

Auger electron-emitting isotopes, either covalently or noncovalently bound to DNA have been shown to be effective clastogenic agents (23, 33-35). The ^{123}I -estrogen treatment was effective in producing chromosome breaks in the CHO-ER cells. In asynchronous cultures, a 24 h treatment produced 0.00086 ± 0.00023 chromatid-type aberration per disintegration and 0.00017 ± 0.00002 chromosome-type aberration per disintegration for an overall efficiency of about 1 chromosome aberration for every 1000 disintegrations. The mean lethal dose of ^{123}I -estrogen in CHO-ER cells, which corresponds to the dose where an average of one lethal event has been

produced in the exposed population of cells, is between 800-1000 disintegrations per cell (9,10).

It is generally accepted that the primary lethal lesion induced by ionizing radiation is a lethal chromosome mutation and that there is a linear relationship between cytotoxicity and chromosome mutation induction that is independent of LET or whether the radiation is from an external source or from an internal emitter (23, 36-45). Our studies on ^{123}I -estrogen support these earlier reports, and suggest that the major lethal event induced by ^{123}I -estrogen in the CHO-ER cells is a chromosome aberration. As mentioned above, the mean lethal dose for ^{123}I -estrogen in CHO-ER cells is between 800 and 1000 disintegrations per cell (9,10), a dose which results in about 1 aberration/cell. When the relationship between chromosome aberration frequency and cytotoxicity is compared in CHO-ER cells (Figure 7), there is a highly significant linear relationship between the log of survival and chromosome aberration frequency ($r^2=93.5\%$, $p=0.03$), further supporting the hypothesis that chromosome mutations are the primary lethal event induced by ^{123}I -estrogen treatment. In figure 7, the mean lethal dose also corresponded to an aberration frequency/cell of about 1. Thus the cytotoxic event induced in CHO-ER cells by ^{123}I -estrogen is a chromosome aberration.

Most chromosome aberrations result from the interaction between two chromosome breaks. These breaks can be on different chromosomes and yield an interchromosome exchange event such as a dicentric, or the breaks can be on the same chromosome and produce an intrachromosome exchange event such as a centric ring. If chromosome breaks are randomly produced and have equal probabilities of interaction, then the ratio of interchromosome to intrachromosome exchanges (F-ratio) should be about 86 for diploid human cells (46). Experimentally, this F-ratio is much smaller because the probability of intrachromosome

interactions are greater than the probability of interchromosome interactions (46). Radiation track structure also influences the F-ratio because the distance between breaks defines their probability of interaction (47). An analysis of the ratio of interchromosome exchanges to intrachromosome exchanges can therefore provide information on the nature of the distribution of energy depositions. The range of dicentric:centric ring ratios found following x ray or γ ray exposure is between 10-20 (46,47). Our own studies on γ -irradiated CHO cells yields a ratio of about 12 (unpublished observations). With high LET radiations, the ratio is smaller because breaks are produced closer together which makes the likelihood of an intrachromosome exchange greater (47). With α particles, the ratio of dicentrics:centric rings is about 4.8 for CHO cells (unpublished observations). With the ^{123}I -estrogen treatment, the ratio of dicentrics:centric rings was 5.8 ± 1.7 , similar to α particles and consistent with the dense depositions of energy seen with Auger electron-emitting isotopes.

In conclusion, these studies demonstrate that ^{123}I -estrogen is an efficient clastogenic agent producing DNA and chromosome damage similar to that seen with high LET radiations, and that the lethal lesion induced by the ^{123}I -estrogen is a chromosome aberration. These studies suggest that the I-123 in the estrogen-receptor-DNA complex is in close proximity to the sugar-phosphate backbone of the DNA.

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Figure Legends:

Figure 1. DNA elution analysis of DNA single-strand damage (alkaline) and DNA double-strand damage (neutral) induced by ^{123}I -estrogen.

Figure 2. DNA elution analysis of DNA single-strand damage (alkaline) and DNA double-strand damage (neutral) induced by ^{60}Co γ rays.

Figure 3. Chromosome aberration frequencies following treatment with ^{123}I -estrogen.

Chromatid-type breaks ($\circ$, total frequency; $\bullet$, chromatid deletions; $\diamond$, isochromatid deletions; $\blacklozenge$, chromatid exchanges). Chromosome-type breaks ($\circ$, total frequency; $\bullet$, dicentric; $\diamond$, interstitial deletions and rings; $\blacklozenge$, terminal deletions).

Figure 4. Frequency of heavily damaged cells following treatment with ^{123}I -estrogen.

Figure 5. Examples of chromosome- and chromatid-type aberrations. A, chromatid break; B, isochromatid-chromatid exchange; C, dicentric; D, acentric fragment; E, interstitial deletion.

Figure 6. Fraction of total aberrations induced by ^{123}I -estrogen in G1-synchronized cells that are either chromatid-type or chromosome-type. Cross-hatched bars, G1 cells exposed and then immediately cultured for 24 h prior to cytogenetic analysis. Filled bars, G1 cells exposed, viable frozen for 1 week, and then thawed and cultured for 24 h prior to cytogenetic analysis.

Figure 7. Comparison of cytotoxicity and chromosome aberration induction by ^{123}I -estrogen in G1-synchronized cells.