ABSTRACT

Purpose DNA ligands labelled with $^{125}\text{I}$ induce cytotoxic DNA double-strand breaks (DSB), suggesting a potential for Auger endoradiotherapy. Since the 60-day half-life of $^{125}\text{I}$ is suboptimal for therapy, we have investigated another Auger-emitter $^{124}\text{I}$, with shorter half-life (4.18 days), and the additional feature of positron-emission, enabling positron emission tomography (PET) imaging. The purpose of this study was to compare the two radionuclides on the basis of DNA DSB per decay.

Materials and methods Using a $^{124}\text{I}$- (or $^{125}\text{I}$)-labelled minor groove binding DNA ligand, we investigated DNA breakage using the plasmid DNA assay. Biodistribution of the conjugate of the labelled ligand with transferrin was investigated in nude mice bearing a K562 human lymphoma xenograft.

Results The probability of DSB per decay was 0.58 and 0.85 for $^{125}\text{I}$ and $^{124}\text{I}$, respectively, confirming the therapeutic potential of the former. The crystal structure of the ligand DNA complex shows the iodine atom deep within the minor groove, consistent with the high efficiency of induced damage. Biodistribution studies, including PET imaging, showed distinctive results for the conjugate, compared to the free ligand and transferrin, consistent with receptor-mediated delivery of the ligand.

Conclusions Conjugation of $^{124}\text{I}$-labelled DNA ligands to tumor targeting peptides provides a feasible strategy for Auger endoradiotherapy, with the advantage of monitoring tumor targeting by PET imaging.

Introduction

$^{125}\text{I}$ is the best known member of a family of radionuclides referred to as Auger emitters. The decay of these radionuclides is characterized by the emission of multiple low energy electrons (Charlton & Booz 1981), and the consequent highly focused radiochemical damage at the site of decay, which is particularly evident when the decay is closely associated with DNA (Martin & Haseltine 1981). For $^{125}\text{I}$ covalently incorporated into DNA as a labelled pyrimidine, the majority of DNA single-stranded breaks (SSB) are clustered within a few base pairs of the decaying atom (Lobachevsky & Martin 2000a). On average, each such decay induces a DNA double-strand break (DSB) (Schmidt & Hotz 1973), and the accumulation of 50–100 such events is sufficient to kill mammalian cells (Burki et al. 1973), prompting consideration of the potential of the Auger effect for cancer therapy (Adelstein et al. 1991, 2003). In this context, the requirement of cell cycle activity for incorporation of labelled DNA precursors has the potential to provide a basis for preferential uptake by tumors (Bloomer & Adelstein 1977), but this has not yet proved useful clinically.

The demonstration that decay of $^{125}\text{I}$ that is non-covalently associated with DNA can induce DNA DSB and cause consequent cytotoxicity, initially using a labelled intercalator (Martin et al. 1979; Kassis et al. 1989), and later minor groove binding ligands (Adelstein & Kassis 1996; Walicka et al. 1999; Lobachevsky & Martin 2004a; Balagurumoorthy et al. 2006; Lobachevsky et al. 2008; Balagurumoorthy et al. 2012) opened up the possibility of more sophisticated targeting of the Auger effect. In a further example, decay of $^{125}\text{I}$ incorporated into one of the strands of a DNA triplex induces breaks in the unlabelled strands (Panyutin & Neumann 1996; Sedelnikova et al. 1998), thus prompting strategies to target $^{125}\text{I}$-induced breaks to specific DNA sequences. However, delivery of triplex-forming labelled oligodeoxynucleotide to the nucleus has proved challenging.

Receptor-mediated targeting of labelled DNA ligand-protein conjugates is another possible strategy to target the Auger effect to the DNA of tumor cells, supported by demonstration of targeted delivery of DNA-binding cytotoxics (Fenton & Perry 2005) and photosensitizers (Karagiannis et al. 2006) by receptor-mediated endocytosis of the respective protein.
conjugates. This approach is an extension of the early use of specific nuclear receptors to target $^{125}$I-tamoxifen (Bloomer et al. 1980), or $^{125}$I-triiodothyronine (Sundell-Bergman & Johanson 1982) to the DNA of certain tumor cells.

In considering the use of $^{125}$I DNA ligand-protein conjugates in Auger endoradiotherapy, the 60-day half-life of $^{125}$I is a severe limitation for therapeutic applications. Clinical endoradiotherapy studies using $^{125}$I in large amounts pose considerable radiation protection problems (Welt et al. 1996). These considerations have generated an interest in $^{123}$I (Makrigiorgos et al. 1992), an Auger emitter with a much shorter physical half-life (13.2 hours), and which is also better suited to in vivo imaging of biodistribution. Although $^{123}$I is a ‘weaker’ comparing to $^{125}$I Auger emitter, the efficient induction of DSB by DNA associated decay of $^{123}$I has been reported in several studies (Lobachevsky & Martin 2005; Balagurumoorthy et al. 2008), and incorporation of $^{123}$I-iodo-2'-deoxyuridine into DNA resulted in radiotoxicity (Makrigiorgos et al. 1989, 1992). The short half-life of $^{123}$I obviates radiation protection problems, however it is actually a little too short, given the experience of many radioimmunotherapy studies which show that optimal tumor/blood ratios are achieved only 1–2 days after administration of radioimmunoconjugates (Goldenberg 2002). Other Auger electron emitting radionuclides, such as for example $^{99m}$Tc (6.0 h half-life) and $^{111}$In (2.8 d half-life), that are widely used for nuclear medicine imaging, have also been evaluated for the decay-induced DNA breakage (Sahu et al. 1995; Karamychev et al. 2000; Haefliger et al. 2005; Kotzerke et al. 2014) and the use in Auger endoradiotherapy (Tavares & Tavares 2010; Cornelissen et al. 2012). They however are less efficient in inducing DNA DSB, especially considering that chelating agents incorporating these metal radionuclides have to be conjugated to DNA ligand which imposes restrictions on positioning of the decay close to DNA. In this context, another halogen Auger emitter $^{124}$I (4.18 d half-life) incorporated into a DNA minor groove binder, represent a good opportunity. Moreover, this radionuclide is also a positron emitter, so in the context of Auger endoradiotherapy, it has the important complementary potential for assessment of tumor targeting by positron emission tomography (PET) imaging. However, this promise relies on the assumption that $^{124}$I, like the prototype Auger emitter $^{125}$I, induces DNA double-stranded breaks upon decay in the vicinity of the DNA molecule. This assumption may not be valid since $^{124}$I, like $^{123}$I, is known to have a ‘weaker’ Auger decay compared to $^{125}$I (Pomplun et al. 1996; Iimura et al. 1997). We report here for the first time, the results of a direct comparison of $^{124}$I and $^{125}$I, both on the basis of Monte Carlo simulation of the decay events, and plasmid DNA breakage experiments. For the latter, we used a $^{124}$I-labelled ligand, para-iodoHoechst, that positions the iodine atom in the DNA minor groove, as verified in the crystal structure of the ligand-DNA complex. Our results show that $^{124}$I, although somewhat less efficacious than $^{125}$I, nevertheless induces DNA double-stranded breaks with a robust efficiency. Finally, we took advantage of the availability of the stannylated precursor of the transferrin conjugate of the meta-isomer of iodoHoechst, prepared for another project (Karagiannis et al. 2006), to demonstrate PET imaging of receptor-mediated delivery of the $^{124}$I-labelled DNA ligand.

Materials and methods

Organic synthesis and preparation of para-[$^{124}$I/$^{125}$I]-iodoHoechst

The synthesis of para-iodoHoechst (1 in Figure 1) is described in detail in the Supplementary Information (available online). Briefly, the linear synthesis approach, as described for

![Figure 1. Crystal structure of the bibenzimidazole para-iodoHoechst molecule (1) bound in the minor groove of the DNA dodecamer d(CGCAAAATTTGGG). Carbon atoms are coloured black, nitrogen atoms are blue, and the iodine atom is purple. The ligand spans the 5'-ATTTGC site, with the iodine atom positioned centrally in the minor groove. The diagram also depicts the highly focused radiochemical damage associated with the decay of $^{124}$I and other Auger-emitting isotopes, and the positron emission which would provide the basis for the use of PET imaging to monitor tumor-targeting of such $^{124}$I-labelled DNA ligands. Receptor-mediated targeting of protein-DNA ligand conjugates provides a potential strategy for tumor-targeting.](image-url)
methylproamine (Martin et al. 2004), was adopted. Thus p-iodobenzaldehyde was reacted with a substituted ortho-phenylenediamine (generated in situ from the nitro-aniline) to form the second benzimidazole ring. A similar approach was used to produce the substrate required to prepare the $^{124}$I and $^{125}$I-ligands, using p-tri-methylstannylbenzaldehyde.

The synthesis of the ligand derivatives required for preparation of the amide-linked ligand-transferrin conjugates, with a succinyl moiety introduced onto the aliphatic nitrogen of the piparazine ring, is also described in detail in the Supplementary Information.

Para-[^{124}I]/[^{125}I]-iodoHoechst was prepared from the precursor para-tri-methylstannyl Hoechst (pTMSH; structure 3 in Supplementary Figure S1) by iododestannylation, carried out in 20–25 mM HCl (Sigma, St Louis, MO) containing 0.5 mM pTMSH, 3–10 μM KI (Ajax Chemicals, Auburn, NSW, Australia) 0.02–0.04 mg/ml lactoperoxidase (Sigma) and 0.03–0.05% hydrogen peroxide (Sigma). The reaction solution contained 60–80 MBq of $^{124}$I (Na$^{124}$I), 0.37 MBq/μl (ANSTO Radiopharmaceuticals and Industrials, Menai, NSW, Australia) in a total volume of 200–250 μl or 15–20 MBq of $^{125}$I (Na$^{125}$I), 3.7 MBq/μl, ANSTO Radiopharmaceuticals and Industrials) in a total volume of 40–50 μl. Following incubation for 30 min, the reaction solution was diluted to 400 μl with 10% acetonitrile (Merck, Darmstadt, Germany), 0.1% trifluoroacetic acid (TFA, Sigma) and injected onto a reverse phase high pressure liquid chromatography (HPLC) column to separate para-iodoHoechst from the precursor and the destannylation by-product (Supplementary Figure S1; R = H). Two types of columns were used: Alltech C8 Rocket 53 x 7 mm (Alltech Associates, Baulkham Hills, NSW, Australia) and Luna C8(2) 150 x 4.6 mm (Phenomenex Australia, Lane Cove, NSW, Australia). Para-iodoHoechst was eluted using 35% (Rocket column) or 25% (Luna column) of acetonitrile, 0.1% TFA at a flow rate of 0.5 ml/min. Radionuclide activity was calculated in the fraction containing radiolabelled ligand, and the solution was aliquoted in incubation tubes and dried in a vacuum concentrator.

Conjugation of iodoHoechst to transferrin

To prepare conjugates for investigation of receptor-mediated targeting, a carboxylic acid analogue of meta-tri-methylstannyl Hoechst was synthesized (1, Figure S4) as described in the Supplementary Information and used as a precursor for conjugation. At the first stage the precursor was iodinated with $^{125}$I or $^{124}$I by iododestannylation, purified on HPLC and dried in a vacuum concentrator as described in the previous section for para-iodoHoechst. The pellet of the radiolabelled intermediate (2–5 MBq of $^{125}$I or 10–40 MBq of $^{124}$I) was dissolved in 50 μl of tetrahydrofuran (Merck)/dimethylsulfoxide (DMSO, Sigma) (1:1) containing 1 mM of the ‘cold’ carboxylic acid analogue of meta-[$^{127}$I]-iodoHoechst (2, Figure S4). Then the solutions of N-hydroxysuccinimide (Sigma, 500 mM in DMSO, 3–4 μl) and N,N-dicyclohexyl carbodiimide (Sigma, 290 mM in DMSO, 5–6 μl) were added, and the reaction mixture was incubated at 60 °C for 4–6 h. The reaction resulted in formation of the N-hydroxy-succinimidy-ester of meta-iodoHoechst (3, Figure S4). Following incubation, an aliquot of the reaction mixture (15–20 μl) was added to a solution of transferrin (Sigma, 15 mg/ml) in 50 mM borate buffer at pH 8.5, and the reaction mixture was incubated for 2 h. The reaction resulted in formation of an amide linkage between aliphatic amino groups on the protein and the N-hydroxy-succinimidy-ester of meta-iodoHoechst (4, Figure S4). Following incubation, 350 μl of phosphate buffered saline (PBS) containing 0.6 mM FeCl₃ (Sigma) were added to the reaction mixture. The conjugate was separated from the reaction mixture by gel filtration chromatography on a Sephadex G-25 NAP-5 cartridge (Pharmacia Biotech, Piscataway, NJ) equilibrated with PBS.

DNA binding and X-ray crystallography

The binding affinity of para-iodoHoechst for DNA was investigated using spectrophotometric titration with 12-mer synthetic self-complementary oligodeoxynucleotide d(CGCAAATTGGCG)₂ (GeneWorks, Thebarton, SA, Australia) or calf thymus DNA (Sigma). The titration of the ligand was performed in a spectrophotometric cuvette with the absorbance spectrum in the range from 300–400 nm recorded following addition of each subsequent aliquot of DNA. Spectrophotometric measurements were performed on a Cary 300 UV-Vis spectrophotometer (Varian Australia, Mulgrave, VIC, Australia). A set of recorded spectra was used to calculate the fractions of DNA bound ligand (f). The fraction of bound ligand at various DNA concentrations was then subjected to non-linear regression analysis to calculate binding parameters Kₘ(binding dissociation constant) and β (frequency of binding sites). As an alternative approach, binding parameters were derived from non-linear regression analysis of the plasmid breakage data at different DNA concentration and ligand/plasmid ratio. Details of non-linear regression analysis are described in the online Supplementary Information (Data Analysis section).

For the X-ray crystallography studies, preparation of crystals of the complex formed between para-iodoHoechst and d(CGCAAATTGGCG)₂, and the subsequent structural analysis, generally followed the procedures used for the counterpart methylproamine complex (Martin et al. 2004). The procedures are also described in detail in the Supplementary Information (X-ray crystallography section and Table S1).

Monte Carlo simulation

The Auger-electron and conversion electron spectra were calculated using the BrIccEmis code (Lee et al. 2012) and nuclear structure data obtained from (Katakura & Wu 2008; Katakura 2011). The calculations were based on the atom in the condensed phase approximation, i.e., assuming fast neutralization. The ray tracing calculations were carried out using the Penelope 2008 code system (Salvat et al. 2008).

Plasmid/DNA-ligand incubation and quantitation of plasmid forms

We varied the conditions in the ligand-plasmid solution, such as DNA concentration A (plasmid/μm²) and molar ligand/plasmid ratio P, in order to better evaluate the contribution of
the remote component of DNA breakage and the effect of the fraction of bound ligand. To control the ligand/plasmid ratio, non-radioactive para-iodoHoechst was added to some samples as a solution in methanol and dried in a vacuum concentrator, prior to addition of the radio-labelled ligand.

Plasmid pBR322 DNA (Roche Diagnostics, Mannheim, Germany, 4361 bp, molecular weight 2.83 x 10^6 dalton, 250 μg/ml) was diluted to the required concentration (10–40 μg/ml) in 20 mM Tris, 1 mM ethylene-diamine tetraacetate (EDTA, Sigma), 100 mM NaCl (Sigma) pH 7.3 (TE), or the same buffer containing 2M DMSO (TE + DMSO). Aliquots of the DNA solution (70–80 μl) were added to incubation tubes containing iodinated ligand, and the ligand was dissolved in the DNA solution. Given that a fraction of the ligand remained adsorbed to the tube wall, in some experiments an aliquot of 90–95% of the initial volume was transferred to another tube. Tubes were incubated at 0 °C for accumulation of decay events. Aliquots of the incubation samples (typically 4 μl volume) were taken after various incubation times and mixed with 16–20 μl of the gel loading buffer (Sigma) containing 0.03% bromophenol blue, 0.03% xylene cyanol and 3% glycerol in electrophoresis buffer. One part of this volume (6–12 μl, ~50 ng of pBR322) was used for analysis by agarose gel electrophoresis, and the remaining part was used for radioactivity measurement.

Supercoiled, relaxed and linear form of plasmid were separated by electrophoresis on 0.8% agarose gel containing 0.005% of Vistra Green fluorescent dye solution (Amersham Biosciences, Piscataway, NJ) for DNA detection, in 42 mM Tris-borate-aceete/1 mM EDTA buffer for 2.5–3 h at 70 V. Gel images were obtained on a Molecular Imager FX scanner (Bio-Rad Laboratories, Hercules, CA). Fluorescence intensities of gel bands corresponding to supercoiled, relaxed and linear plasmid were calculated using image analysis software Quantity One (BioRad Laboratories). To calculate the relative fractions of supercoiled, relaxed and linear form, fluorescence intensities were multiplied by correction coefficients reflecting variation in binding of the dye to different plasmid forms. These coefficients were determined in a separate experiment in which equal amounts of supercoiled, relaxed and linear forms were analysed on the same gel. The values of the coefficients were 1.0, 0.69 and 0.70 for supercoiled, relaxed and linear form, respectively.

### Evaluation of radionuclide activity in samples

Radioactivity was counted on a Wallac 1470 Gamma Counter (Perkin Elmer Australia, Glen Waverley, VIC, Australia). For each sample analyzed by gel electrophoresis, radioactivity was normalized to the initial incubation sample volume and the start of incubation date and time. The average was calculated for each incubation sample.

Given the limited solubility of para-iodoHoechst in aqueous solutions and its ability to adsorb to the incubation tubes, a fraction of the radiolabelled ligand remained insoluble and was not recovered in the solution. At the end of incubation, the ligand-DNA solution was removed and the insoluble activity remaining in the tube was counted and normalized to the start incubation time. Based on the amount of the soluble and insoluble activity, we calculated the recovery ratio $R$, which is the fraction of the activity in solution (soluble activity) to the total activity in a sample. Values of $R$ for each sample are presented in Tables S3–6.

### Plasmid breakage analysis

The relationship between the number of radionuclide decay events per plasmid, $n$, and the fraction of supercoiled $S(n)$, relaxed $R(n)$ and linear $L(n)$ plasmid forms is described by the following expressions (Lobachevsky & Martin 2004b):

$$S(n) = \frac{e^{-\lambda n}}{1 + d_s n}$$

$$L(n) = \frac{d_s n}{1 + d_s n}$$

$$R(n) = 1 - S(n) - L(n)$$

where $s_s$ and $d_s$ are the apparent (observed) yield per plasmid per decay of SSB and DSB respectively. Values of $s_s$ and $d_s$ were obtained from the non-linear regression analysis of the experimental fractions of supercoiled $S(n)$ and linear $L(n)$ plasmid form for various numbers of accumulated decay events $n$ using expressions 1 and 2. As discussed in detail in earlier publications (Lobachevsky & Martin 2004a, 2004b), breakage of a particular (‘target’) plasmid molecule can result from two quite different mechanisms. The first of these, and the one which is the focus of attention for the current study, we refer to as internal breakage; damage arising from a decay event involving a labelled ligand bound to the target plasmid. The second mechanism or component concerns damage to the target plasmid arising from decays in labelled ligands bound to neighbouring plasmid molecules or in unbound ligands and molecules, and thus referred to as remote breakage. The two components are reflected in the following expressions for the apparent breakage yields (expressions S10–11 in the Supplementary Information):

$$s_a = sf + \sigma A \frac{R}{d}$$

$$d_a = df + \delta A \frac{R}{d}$$

where the first term represents internal breakage with $d$ and $\sigma$ being the probabilities per decay of DSB and SSB respectively; $f$ is the fraction of bound ligand (fraction of DNA-associated decay events). The second term represents remote breakage determined by the yield of DSB, $\delta$ and SSB, $A$ per unit of absorbed dose (decay/μm$^3$ for convenience); $A$ is the DNA concentration, $R$ accounts for the presence of non-soluble radioligand that contributes to the remote breakage, as described in the previous section, but not to the evaluation of activity in solution. The dimensions of $\sigma$ and $\delta$ in Equations 4–5 are breaks per plasmid per decays per μm$^3$, and $A$ is expressed as plasms per μm$^3$.

The objective of the analysis is to determine the efficiency of internal breakage, that can be calculated from the apparent yield of breaks ($s_a$ and $d_a$) by subtraction of the remote
component (σA and δA) and correcting for the fraction of DNA associated decay events (f):

\[
s = \frac{1}{f} \left( s_a - \sigma \frac{A}{R} \right)
\]

\[
d = \frac{1}{f} \left( d_a - \delta \frac{A}{R} \right)
\]

(6–7).

Non-linear regression analysis of the dynamics of plasmid forms was performed using SigmaPlot for Windows Version 11.0 software (Systat Software Inc, San Jose, CA). The regression tool of SigmaPlot was used to obtain values and standard errors of curve fitting parameters.

In vivo biodistribution studies in mice bearing K562 cell xenografts

Experimental protocols that involved animals were approved by the Peter MacCallum Cancer Centre Animal Experimentation Ethics Committee. The biodistribution of the amide linked 125I-labelled transferrin conjugate was investigated in BALB/c nu/nu mice bearing a K562 human lymphoma (100–200 mm³) subcutaneously implanted into the right flank. Mice were injected (100 μl intraperitoneal injection) with 125I-labelled transferrin (130 kBq, 0.33 mg protein), the amide linked meta-[125I]-iodoHoechst-transferrin conjugate (12 kBq, 0.5 mg protein) or meta-[124I]-iodoHoechst carboxylic acid (2 in Figure S5; 5.6 kBq, 60 μM). Mice were euthanized 24 h after injection and various tissue samples were weighed and assayed for 125I-activity on a Wallac 1470 Gamma Counter.

For PET imaging, the mice bearing xenograft tumor described above were injected intravenously with meta-[124I]-iodoHoechst-transferrin conjugate (3.3 MBq) or directly-labelled 124I-transferrin (2.6 MBq). The biodistribution of the radionuclide was investigated at various time intervals after administration by small animal PET imaging and tissue autoradiography. PET images were obtained from 15-min scans on a Phillips MOSAIC small animal PET scanner (Philips Medical Systems, Eindhoven, Netherlands), while mice were anaesthetized by inhalation of 2.5% isoflurane (Abbott Medical Systems, Eindhoven, Netherlands), while mice were on a Phillips MOSAIC small animal PET scanner (Philips radiography. PET images were obtained from 15-min scans.

The following values of binding parameters were obtained: \( K_d = 88 \pm 47 \text{nM}, \beta = 0.030 \pm 0.005 \text{bp}^{-1} \) for TE buffer and \( K_d = 220 \pm 84 \text{nM}, \beta = 0.047 \pm 0.006 \text{bp}^{-1} \) for TE + DMSO.

These values were used to calculate fraction of bound ligand and to correct the breakage probabilities for each individual sample in Tables S3–6.

Monte Carlo simulation results

The complete list of the emitted radiations (γ-rays, conversion electrons, Auger electrons and X-rays) are given in the Supplementary Information and in Lee et al. (2016). The energy spectra of the Auger and conversion electrons are shown in Figure S5 and S6 for 124I and 125I decay, respectively. These spectra were generated using 2.5 eV wide energy bins. The probability distributions of the absorbed energy for different radius water spheres are also shown in Figures S5 and S6. In the simulations, \( 10^8 \) electrons were ejected from the centre of the spheres. The results of Monte Carlo simulations are summarized in Table 1 that presents average per decay values for a range of parameters characterizing electron and electromagnetic radiations from Auger decay of 124I and 125I. There is a good agreement in terms of radiation yields between the present results and Howell’s calculations (Howell 1992) up to the N-shell. The total number of Auger electrons is about 25% higher in the later publication. Most of the extra yield in 125I can be attributed to low energy OOX transitions. Our calculations (Lee et al. 2016) show that these transitions are energetically forbidden even in a single ionized atom. While the shape of the emitted Auger and conversion electron spectrum is very similar for 124I and 125I, the average absorbed electron energy is different, due to the difference in the number of electrons per decay. The absorbed electron

Table 1. Summary of Monte Carlo simulation. Average per decay values of parameters describing Auger electron and electromagnetic radiation following decay of 124I and 125I in condensed state.

| Parameter                              | 124I  | 125I  |
|----------------------------------------|-------|-------|
| Number of Auger electrons              | 20.0  | 8.38  |
| Number of CE (conversion electrons)    | 0.93  | 0.0035|
| Auger electrons energy (keV)           | 11.35 | 4.67  |
| CE energy (keV)                        | 7.19  | 2.12  |
| X-rays energy (keV)                    | 40.13 | 12.70 |
| Gamma-rays energy (keV)                | 2.42  | 863.0 |
| Energy deposited in 1 nm sphere (eV)   | 364.3 | 155.3 |
energies for a 1 nm sphere are 155.3 eV and 364.3 eV for $^{124}I$ and $^{125}I$ decay respectively.

**Results of plasmid DNA breakage experiments**

We investigated DNA breakage induced following incubation of pBR322 plasmid with $[^{125}I]^{-}$- or $[^{124}I]^{-}$-para-iodoHoechst, as well as with $[^{125}I]^{-}$- or $[^{124}I]^{-}$-sodium iodide, in TE or in TE + DMSO buffer. Dynamics of the relaxed and linear plasmid forms for representative samples following accumulation of decay events in TE + DMSO or in TE buffer are shown in Figures 2 and 3, respectively. The properties of the representative samples are listed in Table 2. Dynamics of the change in the relative proportions of the plasmid forms are shown as a function of the accumulated decay events per plasmid molecule for the case of iodinated ligands, or per $\mu$m$^3$ for the case of freely distributed radionuclide (sodium iodide). The use of these two chemical forms of the radionuclide is a reflection of two major mechanisms of DNA breakage – internal and remote for these two cases respectively that determine the appropriate variables and units for each mechanism (decay/plasmid or decay/$\mu$m$^3$) as described in more detail previously (Lobachevsky et al. 2004; Lobachevsky & Martin 2004a). The curves shown in Figures 2 and 3 were generated by non-linear regression analysis of the experimental data. The best fit values for the apparent break yields, $s_a$ and $d_a$, obtained from the regression analysis are given in Tables 3 (for SSB) and 4 (for DSB).

The diagrams in panels A and B (Figure 2) demonstrate that the production of the linear form exceeds that of the relaxed form for incubation of plasmid with both $[^{125}I]^{-}$- and $[^{124}I]^{-}$-para-iodoHoechst. By contrast, in the case of incubation with freely distributed radionuclide, formation of the relaxed plasmid, that reflects the induction of SSB, predominates as demonstrated in panels C and D (Figure 2). Closer inspection of the results in panels C and D reveals that the yield of SSB damage is somewhat higher for $^{124}I$ decays compared to $^{125}I$ decays. For example for $^{125}I$-sodium iodide, about half the plasmid molecules are relaxed after the accumulation of about 400 decays/$\mu$m$^3$, whereas for $^{124}I$-sodium iodide, a similar amount of damage is induced with only about 100 decays/$\mu$m$^3$.

Whilst these comparisons relate to differences between the two radionuclides in relation to the minor non-radical

![Figure 2.](image-url)
mediated component of remote damage, the greater efficacy of $^{124}$I in inducing SSB also extends to the dominant, radical-mediated component of remote damage. This is evident in comparing panels A and B in Figure 3 which shows the accumulation of strand breakage for incubation in TE buffer alone, without DMSO. Whilst for $\text{para}^{[125]}\text{I}$-iodo Hoechst, linearization is predominant (panel A), for $\text{para}^{[124]}\text{I}$-iodo Hoechst there is more relaxed plasmid produced than linear (panel B).

From data such as that shown in Figures 2 and 3, the probabilities of DSB and SSB per decay were calculated according Expressions 6 and 7, thus considering the contribution of remote breakage ($r_A$ and $d_A$) and fraction of bound ligand ($f$). For this calculation, values for $r$ and $d$ were obtained from the regression analysis of the data from the experiments involving only freely distributed radionuclide (panels C and D in both Figures 2 and 3) assuming $f = 0$ in Expressions 4 and 5. The results of calculation are shown in Table 3 (for SSB/relaxation events) and Table 4 (for DSB/linearization events).

The two salient features of comparison of the values for $r$ and $d$ given in Tables 3 and 4 are the dominance of SSB over DSB induction by remote breakage and the higher efficiencies of remote breakage for $^{124}$I compared to $^{125}$I. The SSB/DSB ratio values in TE $+$ DMSO are 54 and 94, and in TE 74 and 133, for $^{125}$I and $^{124}$I, respectively. Decay of $^{124}$I is more efficient than $^{125}$I by a factor of 4.1 and 6.3 for SSB induction, and by a factor of 2.3 and 3.5 for DSB induction, in TE $+$ DMSO and TE, respectively. Examination of the results in Table 4 confirms that the contribution of remote DSB is

Table 2. Properties of representative samples. The results and data analysis for these samples are shown in Figures 2 and 3 and Tables 2 and 4. The sample ID identifies samples in the complete set of results from an extensive series of experiments (more than 80 entries in total) performed under various conditions and listed in Tables S3, S4, S5 and S6 in the Supplementary Information, available online. Apart from parallel experiments done in TE in the presence and absence of 2M DMSO, the ligand/plasmid ratio was varied over two orders of magnitude (2–400), and the DNA concentration varied within the range of 2–8 plasmids per $\mu\text{m}^3$.

| Sample ID | Radionuclide | Condition | DNA concentration (A) (plasmid/$\mu\text{m}^3$) | Fraction of bound ligand $f$ |
|-----------|--------------|-----------|--------------------------------------------|-----------------------------|
| 5D10      | $^{125}$I    | TE $+$ DMSO | 3.88                                      | 0.84                        |
| 4D14      | $^{124}$I    | TE $+$ DMSO | 5.62                                      | 0.86                        |
| 5T9       | $^{125}$I    | TE         | 3.85                                      | 0.89                        |
| 4T6       | $^{124}$I    | TE         | 5.39                                      | 0.92                        |

Figure 3. Accumulation of linear (triangles, solid line) and relaxed (squares, dashed line) forms following incubation of pBR322 plasmid DNA with $\text{para}^{[125]}\text{I}$-iodo Hoechst (panel A), $\text{para}^{[124]}\text{I}$-iodo Hoechst (panel B), sodium $^{[125]}\text{I}$ iodide (panel C) and sodium $^{[124]}\text{I}$ iodide (panel D) in Tris buffer (TE), and calculation of the yield of SSB (relaxation events) and DSB (linearization events). Symbols depict experimental values measured as described in Materials and methods, lines were produced as a result of non-linear regression analysis, details of which are explained in Materials and methods. Values of the yield of breaks calculated from non-linear regression follow: $s_A = 0.240 \pm 0.017$, $d_A = 0.776 \pm 0.025$ for $^{125}$I and $s_A = 1.36 \pm 0.19$, $d_A = 0.566 \pm 0.064$ for $^{124}$I ($s_A$ and $d_A$ denote the apparent yield per plasmid of SSB and DSB, respectively, per decay event per plasmid); $\sigma = (3.2 \pm 0.1) \times 10^{-5}$, $\delta = (4.3 \pm 0.6) \times 10^{-6}$ for $^{125}$I and $\sigma = (30.2 \pm 0.4) \times 10^{-5}$, $\delta = (1.5 \pm 0.4) \times 10^{-3}$ for $^{124}$I ($\sigma$ and $\delta$ denote the yield per plasmid of SSB and DSB respectively per decay event per $\mu\text{m}^3$).
negligible for both radionuclides in the presence of the radical scavenger. However for SSB under scavenging conditions, the contribution of the remote component is significant for $^{125}$I (~5%) and substantial (>10%) for $^{124}$I. Under non-scavenging conditions the contribution of remote breakage is still modest for DSB, but much higher for SSB, as reflected in the values of $\alpha A$ and $\delta A$ in Tables 3 and 4, respectively. For DSB induction, the contribution of remote damage is low (~0.2%) for $^{125}$I, but somewhat higher (~1.4%) for $^{124}$I decays. For para-$^{125}$I-iodoHoechst in the absence of DMSO, and at the prevailing DNA concentrations, the relative contributions of remote and internal SSB breakage are similar, but for para-$^{124}$I-iodoHoechst, the majority (~80%) of apparent damage is due to the remote mechanism.

The results and data analysis shown in Figures 2 and 3, and Tables 3 and 4, are representative of the complete set of
results detailed in the Supplementary Information. A distillation of the results obtained from samples (listed in Tables 3–6 in the Supplementary Information, online) for internal breakage is summarized in Table 5, as probabilities of SSB and DSB formation following decay of a single DNA-associated radionuclide. These results demonstrate that DNA associated decay of a 'weaker' Auger electron emitter $^{124}\text{I}$ induces a DNA DSB with a probability of 0.58 in comparison to 0.85 DSB per decay of $^{125}\text{I}$.

**PET imaging and biodistribution studies**

The biodistribution of radionuclide following injection of $^{124}\text{I}$-labelled DNA ligand-transferrin conjugate into mice bearing tumor xenograft was investigated by small animal PET imaging and autoradiography of tissue sections. In some experiments, $^{125}\text{I}$ was used instead of $^{124}\text{I}$ with sampling of tissues and measurement of $^{125}\text{I}$-activity.

PET images of mice at various time intervals following injection of meta-$^{124}\text{I}$-iodoHoechst-transferrin conjugate or directly-labelled $^{124}\text{I}$-transferrin are shown in Figure 4A, and autoradiographs of tumor, liver and muscle are shown in Figure 4B. Although the high liver uptake precluded imaging of the tumor xenograft implanted on the right flank of the mice, the outstanding feature of the results is the persistence of the tumor xenograft investigated by small animal PET imaging and autoradiography of tissue sections. In some experiments, $^{125}\text{I}$ was used instead of $^{124}\text{I}$ with sampling of tissues and measurement of $^{125}\text{I}$-activity.

The results of the uptake of radionuclide in a range of tissues 24 h after administration of meta-$^{124}\text{I}$-iodoHoechst-transferrin conjugate to tumor-bearing mice are shown in Figure 5. Directly labelled $^{125}\text{I}$-transferrin and meta-$^{125}\text{I}$-iodoHoechst were used as controls in this experiment. Analysis of tissue samples collected demonstrated for liver 5.7, 0.8 and 1.2% ID/g (injected dose per gram) for conjugate, transferrin and unconjugated ligand respectively. The corresponding figures for tumor were 1.3, 1.1 and 0.13%.

Table 5. Summary of internal breakage probabilities of $^{124}\text{I}$- and $^{125}\text{I}$-para-iodoHoechst. Values represent the average and the standard deviation for a number of samples for each experimental condition. Values of $d$ and $s$ for each sample were calculated using Expressions 6-7 as illustrated in Tables 3 and 4.

![Table 5](image)

Discussion

Given the characteristic feature of Auger decay, the emission of multiple low-range electrons, the location of the radionuclide decay relative to the DNA is critical in generating strand breaks. The crystal structure of the ligand-DNA dodecamer complex obtained in our study confirmed that para-iodo Hoechst binds to the minor groove of DNA. The consensus binding site for benzimidazole ligands is 3–4 consecutive AT base pairs (Pjura et al. 1987; Murray & Martin 1988), so the binding site in the crystal structure, AAATTT is one of a large family of sites that occur in native DNA. The general features of the structure are comparable to published structures of Hoechst ligands with the same or similar DNA dodecamers (Squire et al. 2000), and to that for Hoechst 33258 bound to the same DNA as used in this study (Spink et al. 1994).

It is known from previous studies with decay of $^{125}\text{I}$ (Kassis et al. 1999; Lobachevsky et al. 2004; Lobachevsky & Martin 2005) that the majority of the remote DNA breakage (> 95%) is radical-mediated (i.e., indirect DNA damage), while internal breakage is induced mainly by direct ionization of atoms in the DNA molecule. Accordingly, inclusion of a radical scavenger such as DMSO minimizes the contribution of remote...
breakage in plasmid breakage experiments, so the observed breakage closely reflects internal breakage. This approach was adopted for the experiments with incubation of plasmid DNA with \(^{125}\text{I}\) or \(^{124}\text{I}\)-\text{para-iodoHoechst} in TE + DMSO buffer that clearly demonstrated predominant induction of DSB by internal breakage. By contrast, the predominant induction of SSB in experiments with freely distributed decay events for both radionuclides is consistent with the sparsely ionizing nature of the radiation flux responsible for the remote component of damage, and illustrates the established requirement that Auger decay needs to be DNA-associated for efficient induction of a DSB. Comparison of the remote breakage induced by the two radionuclides demonstrated the higher efficiencies of \(^{124}\text{I}\) as compared to \(^{125}\text{I}\) for induction of both SSB and DSB, and this feature is in agreement with the high yield of positron and gamma emission components of \(^{124}\text{I}\) decay that contribute to remote breakage.

Our results of Monte Carlo simulation confirmed that decay of \(^{124}\text{I}\) in the condensed condition, that produces on average 8.38 Auger electrons, is a weaker Auger emitter compared to \(^{125}\text{I}\), decay of which generates 20.0 Auger electrons. Considering the energy deposited by the decay event in a 1 nm sphere as a parameter for the prediction of DNA damage, there is a good correlation between values of this energy for \(^{124}\text{I}\) and \(^{125}\text{I}\) obtained from Monte Carlo simulation (155.3 and 364.3 eV, respectively) and the probabilities of DSB induction (0.58 and 0.85, respectively). The observation that the ratio of energy depositions for \(^{125}\text{I}/^{124}\text{I}\) (2.35) is higher than the ratio of DSB probabilities (1.47) is consistent with the concept that for high energy deposition situations, associated with the induction of multiple DSB, would still be recorded as a single linearization event in the plasmid. Thus, the probability of linearization is expected to approach 1 as the energy deposition increases. Also, we have not considered in our analysis the potential contribution of the charge neutralization effect (Lobachevsky & Martin 2000b).

We demonstrated that use of the DNA ligand \text{para-iodoHoechst}, that positions radionuclide in close vicinity to DNA, enables induction of a DSB by decay of \(^{124}\text{I}\) with a relatively high probability (0.58 per decay). This efficiency can be regarded as being appropriate to exploit DNA-targeted \(^{124}\text{I}\) as a potential radionuclide for Auger endoradiotherapy, especially considering the additional feature, namely positron emission, that makes \(^{124}\text{I}\) suitable for PET imaging. The potential for a combined therapy and PET imaging strategy is depicted in Figure 1.

The capability of PET imaging to monitor receptor-mediated delivery of DNA-targeted \(^{124}\text{I}\) is demonstrated by the results of the proof-of-concept experiment summarized in Figure 4. We attribute the persistence of the liver image to accumulation of the labelled DNA ligand bound to nuclear DNA, with successive rounds of receptor-mediated endocytosis and intracellular degradation of the conjugate and consequent release of the labelled DNA ligand. Although this interpretation remains conjecture until nuclear uptake is demonstrated, it is consistent with high expression of transferrin receptors on liver cells (hepatocytes) that are involved in the transferrin bound iron metabolism (Gkouvatsos et al. 2012; Tandara & Salamunic 2012). Indeed, the number of transferrin receptors on liver endothelial cells was reported to be higher than that for K562 cells (Soda & Tavassoli 1984; Tavassoli et al. 1986), although this is controversial (Vogel et al. 1987).

The results of the \(^{125}\text{I}\)-biodistribution experiments are consistent with PET imaging and autoradiography observations. These results indicate good uptake into hepatocytes and splenocytes, (the latter are also known to express relatively high levels of transferrin receptors), which can be attributed to sequestration of the \(^{125}\text{I}\)-labelled ligand in the cells following injection of the conjugate, internalization and subsequent intracellular cleavage of the amide bond and nuclear localization of the \(^{125}\text{I}\)-labelled ligand. However the observation that the uptake of the conjugate relative to that of transferrin is higher in the liver than in the tumor requires consideration of other explanations for the high liver uptake of the conjugate. For example, the \(^{125}\text{I}\)-labelled ligand could be prematurely cleaved from the conjugate, and then accumulated in liver. This is consistent with relatively high uptake in the liver, compared to other tissues, following injection of the non-conjugated ligand, although overall recovery was low. Finally, the conjugate, in contrast to transferrin, could be accumulated in the liver as a part of its metabolism and elimination process, consistent with the general observation of high liver uptake of radiolabelled bioconjugates (Goldenberg 2002). It is relevant to note the low \(^{125}\text{I}\)-activity in the neck following injection of the conjugate or the ligand, compared to the directly labelled transferrin (through \(^{125}\text{I}\)-tyrosine), indicates that the labelled ligand is stable in vivo, in contrast to the directly labelled transferrin, which is known to be de-iodinated with subsequent accumulation of radiiodine in the thyroid.

In conclusion, we suggest that conjugates of \(^{124}\text{I}\)-labelled DNA ligands with tumor-targeting proteins that are internalized by receptor-mediated endocytosis have significant potential as a general therapy and PET imaging platform. The strategy invokes two levels of targeting. The specificity of receptor-mediated targeting to tumor cells, with evaluation of the efficacy of the chosen receptor system by PET imaging, and the DNA ligand targets the highly focused radiochemical damage associated with Auger emitters to the radiosensitive molecular target.

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References

Adelstein SJ, Kassis Al. 1996. Strand breaks in plasmid DNA following posi-
tional changes of Auger-electron-emitting radionuclides. Acta Oncol. 35:797–801.

Adelstein SJ, Kassis Al, Baranowska-Kortylewicz J, van den Abbeele AD, 
Mariani G, Ito S. 1991. Potential for tumor therapy with iodine-125 
labeled immunoglobulins. Int J Rad AppI Instrum B. 18:43–44.

Adelstein SJ, Kassis Al, Bodei I, Mariani G. 2003. Radiotoxicity of iodine-
125 and other auger-electron-emitting radionuclides: Background to 
therapy. Cancer Biother Radiopharm. 18:301–316.

Balagurumoorthy P, Chen K, Bash RC, Adelstein SJ, Kassis Al. 2006. 
Mechanisms underlying production of double-strand breaks in plasmid 
DNA after decay of 125I-Hoechst. Radiat Res. 166:333–344.

Balagurumoorthy P, Wang K, Adelstein SJ, Kassis Al. 2008. DNA double-
strand breaks induced by decay of 129I-labeled Hoechst 33342: Role 
of DNA topology. Int J Radiat Biol. 84:976–983.

Balagurumoorthy P, Xu X, Wang K, Adelstein SJ, Kassis Al. 2012. Effect of 
distance between decaying 125I and DNA on Auger-electron induced 
double-strand break yield. Int J Radiat Biol. 88:998–1008.

Bloomer WD, Adelstein SJ. 1977. S-125I-iodo-deoxyuridine as prototype for 
radiocytotoxicity tests with Auger emitters. Nature. 265:620–621.

Bloomer WD, McLaughlin WH, Weichselbaum RR, Tonnesen GL, Hellman 
S, Seitz DE, Hanson RN, Adelstein SJ, Rosner AL, Burstein NA, et al. 
1980. Iodine-125-labelled tamoxifen is differentially cytotoxic to cells 
containing oestrogen receptors. Int J Radiat Biol Relat Stud Phys Chem 
Med. 38:197–202.

Burki HJ, Roots R, Feinendegen LE, Bond VP. 1973. Inactivation of mam-
alian cells after disintegration of 3H 125I in cell DNA at −196 °C. 
Int J Radiat Biol Relat Stud Phys Chem Med. 24:363–375.

Charlton DE, Booz J. 1981. A Monte Carlo treatment of the decay of 
125I. Radiat Res. 87:10–23.

Cornelissen B, Waller A, Target C, Kersevans M, Svant S, Vallis KA. 2012. 
111In-BnDTPA-F3: An Auger electron-emitting radiotherapeutic agent 
that targets nucleolin. EJNMMI Res. 2:9.

Fenton C, Perry CM. 2005. Gemtuzumab ozogamicin: A review of its use 
in acute myeloid leukaemia. Drugs. 65:2405–2427.

Gkouvatos K, Papanikolaou G, Pantopoulos K. 2012. Regulation of 
iron transport and the role of transferrin. Biochim Biophys Acta. 
1820:188–202.

Goldenberg DM. 2002. Targeted therapy of cancer with radiolabeled anti-
bodies. J Nucl Med. 43:693–713.

Haeffliger P, Agorastos N, Renard A, Giambonini-Brugnoli G, Marty C, 
Cornelissen B, Waller A, Target C, Kersemans V, Smart S, Vallis KA. 2012. 
Modelling of initial events for Monte Carlo simulation of electron and photon 
transport. Math Methods Med. 2012:651475.

Kotzerke J, Punzet R, Runge R, Ferl S, Oehme L, Wunderlich G, 
Freudenberg R. 2014. 99mTc-labeled HYNIC-DAPI causes plasmid DNA 
damage with high efficiency. PLoS One. 9:e104653.

Lee BQ, Kibedi T, Stuchbery AE, Robertson KA. 2012. Atomic radiations in 
the decay of medical radioisotopes: A physics perspective. Comput 
Math Methods Med. 2012:651475.

Lee BQ, Nikiho J, Ekman J, Jonsson P, Stuchbery AE, Kibedi T. 2016. A 
sto-chastic cascade model for Auger-electron emitting radionuclides. Int J 
Radiat Biol (current issue).

Lee BQ, Nikiho J, Ekman J, Jonsson P, Stuchbery AE, Kibedi T. 2016. A 
sto-chastic cascade model for Auger-electron emitting radionuclides. Int J 
Radiat Biol (current issue).

Lozachevsy PN, Karagiannis TC, Martin RF. 2004. Plasmid DNA breakage 
by decay of DNA-associated Auger electron emitters: Approaches to 
analysis of experimental data. Radiat Res. 162:84–95.

Lozachevsy PN, Martin RF. 2000a. Iodine-125 decay in a synthetic oligo-
deoxyribonucleotide. I. Fragment size distribution and evaluation of break-
age probability. Radiat Res. 153:263–270.

Lozachevsy PN, Martin RF. 2000b. Iodine-125 decay in a synthetic 
oligo(deoxy)ribonucleotide. II. The role of auger electron irradiation 
compared to charge neutralization in DNA breakage. Radiat Res. 
153:271–278.

Lozachevsy PN, Martin RF. 2004a. An improved approach to the analysis 
of plasmid DNA breakage by decay of DNA-associated auger emitters. 
Int J Radiat Biol. 80:861–866.

Lozachevsy PN, Martin RF. 2004b. Plasmid DNA breakage by decay of 
DNA-associated auger emitters: Experiments with 125I/123I-iodoHoechst 
33258. Int J Radiat Biol. 80:915–920.

Lozachevsy PN, Martin RF. 2005. DNA breakage by decay of Auger elec-
tron emitters: Experiments with 125I-iodoHoechst 33258 and plasmid 
DNA. Radiat Res. 164:766–773.

Lozachevsy PN, White J, Leung M, Skene C, White J, Martin RF. 2008. 
Plasmid breakage by 125I-labeled DNA ligands: Effect of DNA-iodine 
atom distance on breakage efficiency. Int J Radiat Biol. 84:991–1000.

Makrigiorgos GM, Berman RM, Baranowska-Kortylewicz J, Bump E, Humm 
JL, Adelstein SJ, Kassis Al. 1992. DNA damage produced in V79 cells by 
DNA-incorporated iodine-123: A comparison with iodine-125. Radiat 
Res. 129:309–314.

Makrigiorgos GM, Kassis Al, Baranowska-Kortylewicz J, McElvany KD, 
Welch MJ, Sastry KS, Adelstein SJ. 1989. Radiotoxicity of 5-125I/5-123I-
deoxyuridine in V79 cells: A comparison with 5-129I-iodo-2'-deoxyura-
dine. Radiat Res. 118:532–544.

Martin RF, Bradley TR, Hodgson GS. 1979. Cytotoxicity of an 125I-labeled 
DNA-binding compound that induces double-stranded DNA breaks. 
Cancer Res. 39:3244–3247.

Martin RF, Broadhurst S, Reum ME, Squire CJ, Clark GR, Lozachevsy PN, 
White JM, Clark C, Sy D, Spothem-Maurizot M, et al. 2004. In vitro 
studies with methylproamine: A potent new radioprotector. Cancer 
Res. 64:1067–1070.

Martin RF, Haseltine WA. 1981. Range of radiocytotoxic damage to DNA 
with decay of iodine-125. Science. 213:896–898.

Murray V, Martin RF. 1988. Sequence specificity of 125I-labeled Hoechst 
33258 damage in six closely related DNA sequences. J Mol Biol. 
203:63–73.

Panyutin IG, Neumann RD. 1996. Sequence-specific DNA breaks produced 
by triplex-directed decay of iodine-125. Acta Oncol. 35:817–823.

Pjura PE, Grzeskowiak K, Dickerson RE. 1987. Binding of Hoechst 33258 to 
the minor groove of B-DNA. J Mol Biol. 197:257–271.

Pomplun E, Terrissol M, Demonchy M. 1996. Modelling of initial events 
and chemical behaviour of species induced in DNA units by Auger 
electrons from 125I, 123I and carbon. Acta Oncol. 35:857–862.

Sahu SK, Kassis Al, Makrigiorgos GM, Baranowska-Kortylewicz J, Adelstein 
SJ. 1995. The effects of iodinium-111 decay on pBR322 DNA. Radiat Res. 
141:193–198.

Salvat F, Fernández-Varea JM, Sempau J. PENELOPE-2008: A code system 
for Monte Carlo simulation of electron and photon transport. 
PENELOPE-2008 workshop and training course, 2008 Barcelona, Spain. 
OECD.

Schmidt A, Hotz G. 1973. The occurrence of double-strand breaks in colli-
phage T1-DNA by iodine-125 decay. Int J Radiat Biol. 24:307–313.

Sedelnikova OA, Panyutin IG, Thierry AR, Neumann RD. 1998. 
Radiotoxicity of iodine-125-labeled oligodeoxyribonucleotides in mam-
malian cells. J Nucl Med. 39:1412–1418.

Soda R, Ravazzoli M. 1984. Liver endothelium and not hepatocytes or 
Kupffer cells have transferrin receptors. Blood. 63:270–276.
Spink N, Brown DG, Skelly JV, Neidle S. 1994. Sequence-dependent effects in drug-DNA interaction: The crystal structure of Hoechst 33258 bound to the d(CGCAAAATTGCG)2 duplex. Nucleic Acids Res. 22:1607–1612.

Squire CJ, Baker LJ, Clark GR, Martin RF, White J. 2000. Structures of m-iodo Hoechst-DNA complexes in crystals with reduced solvent content: Implications for minor groove binder drug design. Nucleic Acids Res. 28:1252–1258.

Sundell-Bergman S, Johanson KJ. 1982. Impaired repair capacity of DNA strand breaks induced by 125I-triiodothyronine in Chinese hamster cells. Biochem Biophys Res Commun. 106:546–552.

Tandara L, Salamunic I. 2012. Iron metabolism: Current facts and future directions. Biochem Med (Zagreb). 22:311–328.

Tavares AA, Tavares JM. 2010. (99m)Tc Auger electrons for targeted tumour therapy: A review. Int J Radiat Biol. 86:261–270.

Tavassoli M, Kishimoto T, Soda R, Kataoka M, Harjes K. 1986. Liver endothelium mediates the uptake of iron-transferrin complex by hepatocytes. Exp Cell Res. 165:369–379.

Vogel W, Bomford A, Young S, Williams R. 1987. Heterogeneous distribution of transferrin receptors on parenchymal and nonparenchymal liver cells: Biochemical and morphological evidence. Blood. 69:264–270.

Walicka MA, Ding Y, Roy AM, Harapanhalli RS, Adelstein SJ, Kassis AI. 1999. Cytotoxicity of [125I]iodoHoechst 33342: Contribution of scavengable effects. Int J Radiat Biol. 75:1579–1587.

Welt S, Scott AM, Divgi CR, Kemeny NE, Finn RD, Daghighian F, Germain JS, Richards EC, Larson SM, Old LJ. 1996. Phase I/II study of iodine 125-labeled monoclonal antibody A33 in patients with advanced colon cancer. J Clin Oncol. 14:1787–1797.