Correlation between energy deposition and molecular damage from Auger electrons: A case study of ultra-low energy (5–18 eV) electron interactions with DNA

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Abstract

Purpose—The present study introduces a new method to establish a direct correlation between biologically related physical parameters (i.e., stopping and damaging cross sections, respectively) for an Auger-electron emitting radionuclide decaying within a target molecule (e.g., DNA), so as to evaluate the efficacy of the radionuclide at the molecular level. These parameters can be applied to the dosimetry of Auger electrons and the quantification of their biological effects, which are the main criteria to assess the therapeutic efficacy of Auger-electron emitting radionuclides.

Methods—Absorbed dose and stopping cross section for the Auger electrons of 5–18 eV emitted by ¹²⁵I within DNA were determined by developing a nanodosimetric model. The molecular damages induced by these Auger electrons were investigated by measuring damaging cross section, including that for the formation of DNA single- and double-strand breaks. Nanoscale films of pure plasmid DNA were prepared via the freeze-drying technique and subsequently irradiated with low-energy electrons at various fluences. The damaging cross sections were determined by employing a molecular survival model to the measured exposure–response curves for induction of DNA strand breaks.

Results—For a single decay of ¹²⁵I within DNA, the Auger electrons of 5–18 eV deposit the energies of 12.1 and 9.1 eV within a 4.2-nm³ volume of a hydrated or dry DNA, which results in the absorbed doses of 270 and 210 kGy, respectively. DNA bases have a major contribution to the deposited energies. Ten-electronvolt and high linear energy transfer 100-eV electrons have a similar cross section for the formation of DNA double-strand break, while 100-eV electrons are twice as efficient as 10 eV in the induction of single-strand break.

Conclusions—Ultra-low-energy electrons (<18 eV) substantially contribute to the absorbed dose and to the molecular damage from Auger-electron emitting radionuclides; hence, they should be considered in the dosimetry calculation of such radionuclides. Moreover, absorbed dose is not an appropriate physical parameter for nanodosimetry. Instead, stopping cross section, which describes the probability of energy deposition in a target molecule can be an appropriate nanodosimetric parameter. The stopping cross section is correlated with a damaging cross section

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(e.g., cross section for the double-strand break formation) to quantify the number of each specific lesion in a target molecule for each nuclear decay of a single Auger-electron emitting radionuclide.

Keywords

Auger electrons; nanodosimetry; low energy electrons; DNA damage; cross section

1. INTRODUCTION

Targeted radionuclide therapy (TRT) employs a radiolabeled molecule to selectively deliver a lethal dose of radiation to a target in a disease site, such as DNA in a cancer cell.¹ TRT is now a treatment modality for patients with lymphoma, pheochromocytoma, and neuroblastoma, and a promising technique for the treatment of neuroendocrine tumors and skeletal metastases.^{2–4} Current research efforts in TRT include the selection of both the radionuclide and a targeting vehicle, both of which determine the therapeutic efficacy of TRT.⁵ Auger-electron emitting (AE) radionuclides (e.g., ¹²⁵I, ⁶⁷Ga, ¹¹¹In, ^{99m}Tc, ¹²³I, ²⁰¹Tl) can induce extreme cellular toxicity, thereby enhancing therapeutic efficacy, in a way similar to high linear energy transfer (LET) ionizing radiation; however, their applications are limited by the precondition for decays to occur within or in close proximity to vital biomolecules (e.g., DNA).^{6–9} Despite this limitation that requires a sophisticated carrier system, AE radionuclides have been suggested as a promising therapeutic approach for treating numerous cancers, particularly for the treatment of single-cell metastatic cancers, leukemia and disseminated diseases.^{10–13}

AE radionuclides mainly decay via internal conversion and/or electron capture, which result in the emission of a cascade of electrons, including Auger, Coster-Kronig, and super Coster-Kronig electrons (generally termed Auger electrons), and characteristic x rays with energies between a few eV and 1 keV.¹⁴ Owing to the very short range of these electrons, they deposit a substantial fraction of their energy in a nanoscopic volume. This specific characteristic makes AE radionuclides an excellent choice for DNA-targeted radiotherapy.¹⁵ To assess their potential therapeutic efficacy, it is essential to determine the absorbed dose of Auger electrons and its correlation with the biological effects.²

The two main parameters for the dose calculation of AE radionuclides are radiation spectra and energy loss characteristics of the Auger electrons.¹⁶ The radiation spectra of AE radionuclides provided by International Commission on Radiological Protection (ICRP) and Medical Internal Radiation Dose (MIRD) committees are usually employed in the calculation of dose to microscopic volumes such as a cell. Such spectra ignore Auger electrons emitted from outer shells (e.g., O-shell), which have very low energies of 0–20 eV, since their contributions to the overall absorbed dose of a cell from an AE radionuclide is negligible.¹⁷ In contrast to microscopic volumes, these ultra-low energy electrons (ULEEs) substantially contribute to the absorbed dose within nanoscale volumes, which is a highly significant parameter in biomolecular targeting. Previous studies have reported that the inclusion of ULEEs in DNA dosimetry significantly increases the absorbed dose compared to those calculated based on ICRP and MIRD radiation spectra.¹⁶ Inclusion of O-shell Coster–Kronig electrons in the nanodosimetry of ¹²⁵I, for example, increases the mean

energy deposited in a cylinder of radius 1.15 nm by a factor of 3.^{16,18,19} In addition, Auger electrons and x rays with energies higher than 20 eV produce ULEEs in each ionization interaction. Therefore, calculation of absorbed doses and quantification of subsequent molecular modifications by ULEE irradiation are essential steps to accurately estimate the biological effects of any type of radiation, particularly that of Auger electrons.

ULEEs interact with matter and deposit their energy by several processes including electronic and vibrational excitations, ionization, and dissociative attachment. For electron energies higher than 12 eV, these energy losses occur mostly via direct potential scattering, whereas at lower energies a transient anion is often initially formed and then decays into the inelastic channels.²⁰ The energy loss characteristics of ULEEs have been experimentally determined in the condensed phase, under ultra-high vacuum conditions, for various molecules including DNA subunits such as cytosine, adenine, and thymine using high-resolution electron-energy loss spectroscopy (HREELS).²¹ Based on such EEL spectra, Michaud *et al.* developed a nanodosimetric model to calculate absorbed doses from Auger electrons emitted within a nanometer scale volume.²² As a first elementary example, they calculated an absorbed dose of 79 kGy in a cytosine circular shell occupying a volume of 4 nm³. The local dose was produced by 0–18 eV ¹²⁵I Auger electrons emitted from the center of the shell. This high dose resulted mainly from electronic excitation and ionization, while vibrational excitation had a negligible contribution (i.e., 3%).

The biological effects of Auger electrons have been widely studied in relation to the formation of DNA lesions, particularly double-strand breaks (DSB).²³ A single decay of ¹²⁵I has been reported to induce on average one DSB in plasmid DNA and prokaryotic cells.²⁴ In eukaryotic cells, several studies have reported different numbers, which are in the range of 0.3–2.0 DSB per ¹²⁵I decay.^{25–27} Despite the substantial contribution of ULEEs to the absorbed dose in DNA, it was previously believed that ULEEs made no contribution to the actual formation of DSB. They were considered as nontoxic for cells, because their energies either lie below the ionization threshold or are not sufficient to efficiently ionize DNA.¹⁶ By irradiating nanometer thick solid films of either plasmid DNA or synthetic oligonucleotides and precise analysis of molecular modifications, later studies have demonstrated that ULEEs do in fact induce a variety of toxic DNA lesions including base modifications, base release, single strand breaks (SSB), DSB, and cluster damages, even at electron energies less than required for ionization and electronic excitation of any organic molecules.^{28–31} ULEEs do so efficiently; since the yields for the formation of SSB and DSB by ULEEs have been reported to be comparable with those induced by high LET ionizing radiations such as soft x rays and electrons of 50–100 eV.³²

In this paper, we present the first study of the relationship between the dose absorbed by DNA from very low-energy Auger electrons and the subsequent molecular lesions. The absorbed dose and stopping cross sections (SCS) are calculated for a DNA molecule composed of the four bases (i.e., adenine, thymine, cytosine, and guanine), the deoxyribose and phosphate groups, and water, using the nanodosimetric model proposed by Michaud and co-workers.²² The radiation source consists of ULEEs emitted from ¹²⁵I. To estimate the molecular damage from ULEEs, we determined the cross section (CS) for ULEE damage to

DNA, which is referred to as a damaging CS (DmCS). The latter is compared to that of 100-eV electrons having a high LET of 48.8 eV/nm.³³

2. THEORETICAL AND EXPERIMENTAL METHODS

The models of nanodosimetry and molecular survival, and experimental procedures employed in the present study have been reported in detail in Refs. 22 and 34. Here, we provide only a brief description of the most pertinent elements.

2.A. Nanodosimetric model

Our model for nanodosimetry has been developed from the cellular dosimetric model proposed by the MIRD committee.³⁵ In the model, the mean absorbed dose $D(r_T \leftarrow r_S)$ is defined as the average energy imparted to the target region r_T from activity in the source region r_S per mass of target region:

$$D(r_T \leftarrow r_S) = A_S S(r_T \leftarrow r_S), \quad (1)$$

where A_S is the cumulated activity of the radionuclide in the source region r_S and $S(r_T \leftarrow r_S)$ is the absorbed dose in the target region r_T per a single nuclear decay in the source region r_S . In the present nanodosimetric model, which consists of 1-nm radius homogeneous sphere representing a double-stranded DNA with the ^{125}I decaying at its center, S is given as

$$S(r_T \leftarrow r_S) = \frac{1}{m_T} \sum_{E_0} N(E_0) E_0 \sum_i \sigma_i(E_0) \frac{\varepsilon_i}{E_0} n_s \\ = \frac{\Delta\phi(r_T \leftarrow r_S)}{m_T}, \quad (2)$$

where m_T is mass of the target region; $N(E_0)$ and E_0 are frequency distribution and energy of Auger electrons for a single nuclear decay, respectively, and ε_i and σ_i denote the energy of the i th excitation mode of a target molecule and the integral CS to transfer ε_i into the excitation mode i , respectively, and n_s is the surface number density of the molecules as projected at the surface of the sphere. $\phi(r_T \leftarrow r_S)$ represents the fraction of energy emitted from the source region r_S that is absorbed in the target region r_T per nuclear decay. It should be noted that ε_i and σ_i describe the stochastic energy deposition in a nanoscale volume. Therefore, MRID model, which has generally been developed for dosimetry in macroscopic volumes, can be principally employed to nanometric volumes.

Assuming a B-type DNA, which includes 20 nucleotides (10 base pairs) in each turn of the double helix (i.e., 3.4 nm), the surface number density n_s of nucleotides is $9.36 \times 10^{13} \text{ cm}^{-2}$. With an identical probability for the distribution of bases in the DNA, n_s for each base is $2.34 \times 10^{13} \text{ cm}^{-2}$, while its value for the sugar and phosphate groups is $9.36 \times 10^{13} \text{ cm}^{-2}$, ε_i and σ_i are mainly obtained from available EEL spectra; these have been measured by HREELS for the DNA bases, and tetrahydrofuran (THF) and phosphoric acid, as analogues of the sugar and phosphate groups in the DNA backbone, respectively.^{21,36,37} For electron energies where such experimental data were not available,^{38–42} The ε_i and σ_i were

extracted from theoretical data. EEL spectra have been obtained from a single electron collision regime.⁴³ In the present study, since the dimension of the target region (i.e., a sphere of 1 nm radius) is smaller than the mean free path of ULEEs,^{33,34,44} the single collision condition appears to be a reasonable assumption for the calculation of energy deposited in the target region.

2.B. Molecular survival model

Molecular survival model provides the probability of a given molecule such as DNA to keep its structural integrity after being exposed to radiation.³⁴ Auger electron interactions leading to the formation of DNA lesions, particularly DSB within condensed DNA are of crucial importance to evaluate the biological effectiveness and cellular toxicity of the electrons.^{7,24} As shown previously, absolute CS for such interactions in condensed DNA can be obtained by applying a molecular survival model to the exposure–response curves for the formation of DNA strand breaks, as measured by the irradiation of freeze-dried plasmid DNA films with electrons.³⁴ The DmCS, σ_D can be calculated from the initial slope $P'(0)$ of the exposure–response curves:

$$\sigma_D = -\frac{P'(0)}{P_0 J_0 f_1}, \quad (3)$$

where P_0 and J_0 are the percentage of intact DNA in the nonirradiated film and the surface current density of incident electron beam, respectively. f_1 is a penetration factor that corrects for the effect of film thickness on the initial slope of the exposure–response curves. This factor depends on the film thickness h and attenuation length λ of the incident electrons inducing DNA strand breaks:

$$f_1 = \frac{\lambda}{h} (1 - e^{-h/\lambda}). \quad (4)$$

Attenuation length is the length x in a given substance such that the intensity of a monoenergetic electron beam passing through x is reduced to $1/e$ of its initial value. It will be identical to the inelastic mean free path, if elastic-scattering effects are negligible. It has been estimated that the attenuation length could be smaller than the related inelastic mean free path by up to about 30%.^{45,46} For 10- and 100-eV electrons, this factor is 0.64 and 0.71, respectively, for a 10-nm thick film of freeze-dried plasmid DNA.³⁴

2.C. Experiment

Plasmid DNA [pGEM-3Zf(–), 3197 base pairs, ca. 1 968 966 amu per plasmid] was extracted from *Escherichia coli* JM109, purified with a HiSpeed plasmid Maxi kit (QIAGEN) and redissolved in TE buffer (Tris–EDTA: 10–1 mM) with pH 8. The purified plasmid DNA consisted of 97% super-coiled, 2% concatemeric, and 1% nicked circular forms. The ratio of ultraviolet absorption of DNA and any contaminating protein at 260 and 280 nm, respectively, was 1.99, which corresponds to a purity greater than 95%. The TE buffer was

separated from DNA by gel filtration with a Sephadex G-50 medium. Thus, the final solution consisted of DNA and distilled deionized (dd) H₂O.

To prepare solid films of plasmid DNA with 10-nm average thickness, 7 μ l of the DNA solutions containing 210 ng DNA was deposited onto clean tantalum (Ta) substrates (7 $\times$ 20 mm). The latter consisted of a thin layer (450 $\pm$ 50 nm) of Ta sublimated onto either a 0.4 mm thick silicon wafer or a clean borosilicate glass. Then the deposited DNA samples were first frozen at -65 $^{\circ}$ C for 10 min in a glove box and then dried under a pressure of 5–7 mTorr by a hydrocarbon-free turbomolecular pump for 2 h to form solid films. The DNA films were then placed on sample holders inside a UHV chamber equipped with an electron irradiator. The latter consists of an electron gun producing a beam adjustable in energy between 5 and 1000 eV. The spot size of the beam can be varied between 2 and 50 mm at working distances of 10 and 50 mm. In the present experiment, it was set to irradiate an area of about 0.9 cm², which was 7 times larger than the DNA sample. The chamber was evacuated for 24 h by a hydrocarbon-free turbomolecular pump to a pressure of 5×10^{-9} Torr at room temperature.

After stabilization of the beam current at 2 nA, corresponding to the current density of $9.95 \times 10^{10} \text{ e s}^{-1} \text{ cm}^{-2}$, the DNA films were individually irradiated with electrons of either 10 or 100 eV for periods between 5 and 90 s. After irradiation, the films were immediately retrieved from the apparatus and dissolved in TE buffer from their substrate with 95%–98% efficiency. The relative percentage of the different structural forms of DNA, including nicked circular and linear corresponding to the formation of SSB and DSB were obtained by agarose gel electrophoresis. The amount of each structural form of the DNA was then analyzed by ImageQuant (Molecular Dynamics, Sunnyvale, CA) software.

3. RESULTS

3.A. Absorbed dose in DNA by Auger electrons of 5–18 eV

The absorbed dose and the deposited energy by ULEEs emitted by ¹²⁵I within a volume of 4.2 nm³ of either a hydrated or a dry DNA are calculated for the energy range of 5–18 eV. Table I shows the reported energy E_0 , frequency distribution $N(E_0)$, and their product for Auger electrons energies between 5 and 18 eV from the nuclear decay of a single ¹²⁵I.^{19,47} Below 5 eV, electrons mostly deposit their energies within DNA via vibrational excitation, which has negligible contribution to the total absorbed dose of DNA by ULEEs (i.e., 3%) compared with electronic excitation and ionization processes (i.e., 39% and 58%, respectively).²² Furthermore, the reported threshold energy for the induction of DSB by ULEEs is 6–7 eV.³² Table II presents the calculated absorbed dose and deposited energy in DNA and nucleosomes by the ULEEs. The nucleosome is the basic structural and functional unit of chromatin consisting of 146 base pairs of DNA wrapped in two superhelical turns around a histone octamer with a variable length of linker DNA to give approximately 166 base pairs.⁴⁸ For a single decay of ¹²⁵I, the chosen nanometric volumes of DNA that includes approximately 4 base pairs (i.e., eight nucleotides) absorbs 271.7 and 205.1 kGy, resulting from the deposition of 93.8% and 70.8% of the electrons' energy inside the hydrated and dry DNA, respectively. In this dose calculation, hydrated DNA is assumed to contain a hydration shell of 20 water molecules per nucleotide. For dry DNA, this ratio is

2.5, which corresponds to tightly bound water molecules attached to the phosphate group that cannot be removed by vacuum desiccation.^{49,50} Assuming a nucleosome as a cylinder with diameter and height of 10 and 6 nm, respectively, the absorbed dose in the nucleosome is 2.6 kGy, which is 2 orders of magnitude smaller than that in the DNA, owing to the smaller mass of DNA relative to that of nucleosome. However, the dose of 2.6 kGy is still much higher, by 2 or 3 orders of magnitude, than those currently administered in the clinic for macroscopic volumes.

Figure 1 shows the contribution of the DNA subunits to the deposited energy in the nanometric volume of hydrated and dry DNA. The largest fraction of ULEE energy is absorbed by DNA bases, i.e., 58.7% and 44.3% for dry and hydrated DNA, respectively. Among the DNA bases, cytosine and guanine absorb a larger fraction of the electron energy than adenine and thymine. THF, i.e., the analogue of 2-deoxyribose, substantially contributes to the dose absorbed in both dry and hydrated DNA (i.e., 26.9% and 20.3%, respectively). In hydrated DNA, H₂O absorbs 28% of the total imparted energy, whereas the percentage drops to 4.8% in dry DNA. With respect to the surface density of H₂O and THF versus each base (i.e., 187.2×10^{13} and 9.36×10^{13} versus $2.34 \times 10^{13} \text{ cm}^{-2}$, respectively), the energy imparted to a single H₂O or THF molecule is smaller than that to each base. Phosphoric acid, the analogue of the phosphate group, makes less contribution than THF and the bases in the absorbed energy of ULEEs. Therefore, DNA subunits can be classified according to the following energy deposition by ULEEs as

$$\varphi_{\text{H}_2\text{O}} < \varphi_{\text{PO}_4\text{H}_3} < \varphi_{\text{THF}} < \varphi_{\text{Thymine}} < \varphi_{\text{Adenine}} < \varphi_{\text{Guanine}} < \varphi_{\text{Cytosine}}.$$

3.B. Stopping cross sections for ULEE interaction with DNA

Similar to other moving charged particles, ULEEs lose their energy and momentum via a series of inelastic interactions with molecules in a medium before being stopped. The SCS σ_S is associated with the probability of such interactions with all excitation modes of DNA subunits and is expressed by

$$\sigma_S = \sum_i \sigma_i(E_0) \varepsilon_i. \quad (5)$$

Figure 2 shows SCS of dry DNA, bases, and THF as a function of incident electron energy, resulting from both electronic excitation and ionization. For the purine bases [panels (c) and (d)], SCS increases, by almost 2 orders of magnitude, with electron energies from 5 eV to 12 and 13 eV for thymine and cytosine, respectively, then slightly decreases at higher energies. In the case of thymine, there are small increases in SCS at energies higher than 14 eV. For pyrimidine bases [panels (e) and (f)], as with the purines, SCS has an initial large increase at electron energies between 5 and 8 eV, which is followed by slight increases at higher energies. THF has two peaks in the SCS at 8–9 and 11 eV, and small increases at energies between 13 and 18 eV. Phosphoric acid and H₂O contribute to less than 5% of total SCS of DNA, so their SCS curves are not shown in Fig. 2. For complete dry DNA, the SCS

increases by an order of magnitude from 5 and 8 eV (i.e., from 8.9×10^{-16} to 9.4×10^{-15} eV cm², respectively, within a range of only 3 eV); afterwards, it increases only slightly up to 18 eV (i.e., 3.7×10^{-14} eV cm²). Sharp increases in the initial portion of SCS curves are mainly related to the electronic excitation of the molecules by ULEEs, while the slight increases at the higher energy end of the curves result from the superimposition of both electronic excitation and ionization processes. It should be noted that the SCS for DNA are derived from the summation of SCS for each DNA constituent, not for the entire DNA molecule. At electron energies higher than 20 eV, theoretical studies have shown that the total inelastic cross section of DNA differs slightly from a summation of the cross sections for the DNA components. In particular, this may arise from the difference between the excitation modes of DNA and those of individual DNA components.^{51–53}

3.C. ULEE damaging cross section for DNA

The supercoiled configuration of plasmid DNA converts to the circular or linear configuration, when a SSB or DSB is formed, respectively. Figure 3 presents exposure–response curves for the formation of the circular and linear DNA by 10- and 100-eV electrons. SSB formation first increases with irradiation time before saturating, owing to depletion of the initial targets and the penetration effect, which depends on the film thickness and attenuation length of incident electrons.³⁴ DSB formation increases linearly with irradiation time and no saturation level is observed up to 90 s of electron exposure. In both SSB and DSB cases, 100-eV electrons induce more DNA lesions as expected. Table III presents the cross sections for the induction of SSB and DSB by electrons of 10 and 100 eV in the 3200-base-pair plasmid DNA. As expected from the respective exposure–response curves, 100-eV electrons have larger CS than 10-eV electrons by factors of 2.0 and 1.3 for SSB and DSB formation, respectively. At both energies, SSBs have a higher CS than DSB.

4. DISCUSSION

Two important parameters to evaluate the efficacy of AE radionuclides for use in targeted radiotherapy are the absorbed dose and biological effectiveness of Auger electrons. Our results indicate that electrons of 5–18 eV emitted from ¹²⁵I substantially enhance both the absorbed dose and damage formation to DNA, when the decay occurs within DNA. With respect to the ULEE spectra of ¹²⁵I (Table I), the absorbed dose of these ULEEs in the 4.2-nm³ volume of a DNA molecule (i.e., containing approximately 4 base pairs) is 272 and 205 kGy for hydrated and dry DNA, respectively (Table II). Since these extremely large doses arise from absorption of only 12.1 and 9.1 eV, respectively, one may wonder if such energy depositions are sufficient to induce lethal lesions in DNA, particularly DSB.

DSB formation requires the rupture of two phosphodiester bonds; i.e., the covalent bonds between the phosphate and 2-deoxyribose groups, in opposite strands of DNA. The thermodynamic threshold energy of phosphodiester (C–O) bond dissociation is 335 kJ/mole (3.2 eV).^{30,54} Therefore, the deposited energies by ULEEs (12.1 and 9.1 eV) from ¹²⁵I are in principle sufficient to induce two strand breaks in a very small portion of DNA, leading to the formation of DSB. Despite such a simple explanation, it should be noted that ULEEs mainly interact with molecules via a resonance process that arise from basic quantum

mechanical principles. Within DNA, electron resonances result in the temporary capture of the electron into an unfilled orbital of a subunit of the molecule (i.e., in the formation of a transient anion of the subunit).^{30,55} In this case, phosphodiester bond cleavage would not depend on bond energy considerations, but rather on the availability of dissociating anionic states at the energy of the incident electron. Boudaiffa *et al.* have experimentally shown the dependency of the formation of SSB and DSB on the electron energy, as the yields for such DNA lesions had maximum values at around 10 eV.³⁰ Huels *et al.* also reported that electrons of 6–7 eV can induce DSB on a dried plasmid DNA.³²

To estimate the efficiency of ULEEs to induce DNA strand breaks, we can compare their DmCS with that of electrons of higher energies. Both experimental and theoretical studies have reported that 100-eV electrons have the highest stopping power (e.g., 287 MeV g cm⁻² in DNA) (Ref. 44) and the largest inelastic CS for their interaction with biological matter;^{56–59} hence they are highly efficient in the induction of DNA damage.³² Comparison of the CS for the formation of SSB by 100-eV electrons with those by 10 eV (Table III) indicates that electrons of 100 eV are twice as efficient as those of 10 eV (i.e., $R_{100/10} = 2.0$). This ratio is in agreement with our previous results.³⁴ The ratio of the DmCS for the induction of DSB by 100- and 10-eV electrons is 1.3. With respect to the standard errors on the CS, there is no significant difference between electrons of 100 and 10 eV in the DSB formation; hence 10-eV electrons induce DSB with high efficiency similar to 100-eV electrons. In addition, by considering the fact that 100-eV electrons produce several ionizations in DNA along their track, resulting in the generation of cations and ULEEs, it can be inferred from the similarities between the DmCS that ULEEs are the main species responsible for the induction of DSB.

Considering the length of DNA (i.e., 4 base pairs) in a 4.2-nm³ volume assumed in this study, the dose of 200 kGy could maximally induce one or two DSB and/or eight SSB, if the DmCS of ULEEs is close to unity. However, absorption of 1 Gy radiation dose from low-LET radiations in a mammalian cell nucleus has been estimated to induce 1000 SSB and 40 DSB in DNA.^{60,61} These results show that in nanometric volumes, the correlation of absorbed dose with molecular damage from radiation differs greatly from that in microscopic volumes. In addition, since interaction of radiation with matter is a stochastic process, the absorbed dose, which is a deterministic parameter, is prone to substantial statistical error, particularly at molecular levels such as DNA.⁶² It has been suggested that the uncertainty in the value of absorbed dose could be as large as the value itself.⁶³ With respect to the randomness of radiation interactions, stopping and damaging CS can be appropriate parameters to estimate the probabilities of both the energy depositions and the molecular damage from radiation. The efficacy of Auger electrons in the induction of molecular damage can be expressed as the yield for the formation of a specific molecular lesion (e.g., DNA DSB) per unit energy of radiation (i.e., eV) deposited to the target molecule r_T from the source region r_S :

$$Y(r_T \leftarrow r_S) = \sum_j \frac{\sigma_{D,j}(E_j)}{\sigma_{S,j}(E_j)}, \quad (6)$$

where E_j is the energy of Auger electrons emitted from ^{125}I . The number of damages per total radiation energy deposited to the target molecule per a single nuclear decay in the source region is given by

$$N_D(r_T \leftarrow r_S) = \Delta\phi(r_T \leftarrow r_S) Y(r_T \leftarrow r_S). \quad (7)$$

The CSs for SSB and DSB formation by 10-eV electrons presented in Table III are determined for a 3200-base pair DNA. By normalizing them to a 4-base pair DNA (i.e., the length of DNA considered for the calculation of SCSs), it is possible to calculate Y and N_D for 10-eV electrons. For the formation of SSB and DSB, Y has the values of 3.6×10^{-3} SSB/eV and 1.1×10^{-4} DSB/eV, respectively. Assuming these yields as average Y for 5–18-eV electrons emitted from ^{125}I , the number N_D for SSB and DSB induced by the ULEEs in a single nuclear decay of the AE radionuclide is 0.5 and 0.02, respectively. It should be noted that these numbers were obtained for dry DNA, which differs from cellular DNA mainly in terms of the presence of water and oxygen molecules and histone proteins. Previous studies have shown that the formation of DNA strand breaks by ULEEs is significantly enhanced in the presence of water and oxygen, while amino acids have negligible effects on the DmCS (Refs. 34, 49, and 62).

It should also be pointed out that 5–18 eV electrons are a part of the spectrum of Auger electrons emitted from ^{125}I . To have a number of SSB and DSB for the whole spectrum, such a calculation should be performed for the entire range of Auger electrons of different energies emitted by ^{125}I or by other AE radionuclides. At electron energies between 0.1 and 4.5 keV, Zhange and Tan calculated that a single electron hit to DNA produces approximately 0.25 and 0.02 SSB and DSB, respectively.⁶⁴ In this Monte Carlo simulation, DNA strand breaks have been determined by assuming an energy deposition threshold of 17.5 eV resulting in ionization. In addition to the DNA strand breaks, such ionization generates an ULEE, which is efficient to induce another DNA lesion. In nanometric volumes, therefore, ULEEs also contribute to the biological effect of high-energy electrons.

5. CONCLUSION

The present study introduces quantitative parameters to estimate the efficacy of AE radionuclides in the induction of molecular damage by a direct correlation between the parameters resulting from a nanodosimetric model and a molecular survival model. This correlation determines the number of DNA lesions, most importantly DSB per single decay of each AE radionuclide. In a nanometric volume of DNA (i.e., 4.2 nm^3), the absorbed dose of low-energy Auger electrons (i.e., 5–18 eV) is 5 orders of magnitude larger than those in macro- and microscopic volumes, while the number of molecular lesions is much less than those in the larger volumes, owing to the very limited volume of energy deposition. However, such a small number of damages could have a higher biological effect owing to the complexity of the lesions, e.g., DNA cluster damage that is difficult to repair. These results indicate that absorbed dose is no longer an appropriate physical parameter to represent energy deposition at the molecular level. Since energy deposition and subsequent molecular

modification are stochastic processes, the present study introduces both stopping and damaging CS as biologically related physical parameters, respectively, to estimate the probability of energy deposition and resultant molecular lesions in a nanometric volume. Correlation of these parameters determine the number of each DNA lesion per deposited energy, which can be used in bottom-up approaches assessing the therapeutic efficacy of AE radionuclides by measuring the number of DNA lethal lesions.

Moreover, our results show that ULEEs are as efficient as high-LET electrons in the induction of complex lesions in DNA. For example, the CS for DSB formation by 10-eV electrons is similar to that of 100-eV electrons. This finding suggests that ULEEs, even those having a very small cross section for DNA ionization contribute considerably to the biological effect of AE radionuclides. Hence they should be considered in dosimetric and radiobiological investigations related to the therapeutic properties of radionuclides.

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Fig. 1. Contribution of DNA subunits and H₂O to the energy deposited by 5–18-eV electrons emitted from ¹²⁵I within hydrated (a) and dry (b) DNA.

Fig. 2. Stopping cross sections for dry DNA (a), tetrahydrofuran (b), cytosine (c), thymine (d), guanine (e), and adenine (f) as a function of electron energies between 5 and 18 eV.

Fig. 3.

Exposure–response curves for the formation of circular (a) and linear (b) DNA, corresponding to the induction of SSB and DSB, by 10- and 100-eV electrons. Data are means $\pm$ standard error from five measurements. The dashed line in panels (a) and (b) are exponential and linear fit to the data, respectively.

Table I

Spectra of 5–18-eV Auger electrons emitted by ^{125}I . $N(E_0)$ is the absolute number denoting the frequency distribution of the Auger electron energy E_0 (eV) and $E_0 N(E_0)$ is the product of $N(E_0)$ and E_0 .

E_0 (eV)	$N(E_0)$	$E_0 N(E_0)$ (eV)
5	0	0
6	0.15	0.9
7	0.1	0.7
8	0.2	1.6
9	0.1	0.9
10	0.07	0.7
11	0.08	0.88
12	0.1	1.2
13	0.15	1.95
14	0	0
15	0.07	1.05
16	0.1	1.6
17	0.04	0.68
18	0.04	0.72

Table II

Absorbed dose, deposited energy, and ϕ -value of hydrated and dry DNA, and nucleosome resulting from 5- to 18-eV electrons emitted by a single nuclear decay of a ^{125}I within the molecules. The volumes of 4.2 and 471.2 nm³ are assumed for DNA and nucleosome, respectively.

Nanometric target	Absorbed dose (kGy)	Deposited energy (eV)	$\phi(r_T \leftarrow r_S)$
Hydrated DNA	272	12.1	0.94
Dry DNA	205	9.1	0.71
Nucleosome	2.6	12.9	1.00

Table III

Cross section for the formation of SSB and DSB by 10- and 100-eV electrons. $R_{100/10}$ denotes the ratio between the damaging cross sections at 100-eV electrons and those at 10 eV.

DNA lesion	Damaging cross section (cm ²)		$R_{100/10}$
	100-eV electrons	10-eV electrons	
SSB	$(7.5 \pm 1.6) \times 10^{-14}$	$(3.7 \pm 1.0) \times 10^{-14}$	2.0 ± 0.7
DSB	$(1.5 \pm 0.4) \times 10^{-15}$	$(1.15 \pm 0.3) \times 10^{-15}$	1.3 ± 0.5