A Single Subexcitation-Energy Electron Can Induce a Double-Strand Break in DNA Modified by Platinum Chemotherapeutic Drugs

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The sensitization of malignant cells to ionizing radiation is the clinical rationale for the use of platinum-based concurrent chemoradiotherapy (CCRT) for cancer treatment; however, the specific mechanisms of radiosensitization and their respective contributions still remain unknown ^[1]. Biological mechanisms such as inhibition of DNA repair may contribute to the efficacy of CCRT ^[2]; nevertheless, there is a dearth of information on the possible contribution of nanoscopic mechanisms to the generation of lethal DNA lesions such as double-strand breaks (DSB). The present study demonstrates that the abundant near zero-eV (0.5-eV) electrons, created by ionizing radiation during radiotherapy, induce DSB in supercoiled plasmid DNA modified by platinum anticancer drugs (Pt-drugs), but not in unmodified DNA. They do so more efficiently than other types of radiation, including soft X-rays and 10-eV electrons. The formation of DSB by 0.5-eV electrons is found to be a single-hit process. These findings reveal insights into the radiosensitization mechanism of Pt-drugs that can have implications for the development of optimal clinical protocols for platinum-based CCRT and the deployment of *in-situ* sources of subexcitation-energy electrons (e.g., Auger-electron emitting radionuclides) to efficiently enhance DSB formation in DNA modified by Pt-drugs in malignant cells.

Pt-drug molecules react with cellular components, including DNA, RNA and proteins, via ligand exchange at the platinum atom ^[3]. Binding of Pt-drugs to DNA causes a local distortion of the DNA conformation affecting the physical and chemical properties of the molecule, particularly at the site of platination ^[4]. These modifications are believed to enhance the effects of radiation on the modified DNA by perturbing processes occurring during the physical and chemical stages of the interaction of radiation with DNA ^[5]. In an aqueous solution of DNA, cisplatin enhances the formation of DNA strand breaks by the reaction of both hydroxyl radicals and hydrated electrons at the site of platination through

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the increased accessibility of these species to sugar moieties and dissociative electron transfer [6]. Additionally, in the presence of Pt-DNA adducts, low energy electrons (LEEs, i.e., electrons of 0–30 eV) have a higher cross section for DNA damage than other types of radiations [7,8]. Previously, we hypothesized that at such energies, an incident electron is captured by a DNA constituent at the platination site to form a local transient negative ion (TNI) via a resonance process, which may subsequently rupture chemical bonds via either dissociative electron attachment (DEA) or dissociative electronic excitation (DEE) [8,9]. To determine the primary mechanism responsible for the enhanced damage to DNA modified by Pt-drugs, in the present study, we investigated the contribution and efficiency of these decay channels in the induction of damage to modified DNA.

First, we studied the effect of electrons with energies below those required for the ionization and electronic excitation of biomolecules (i.e., subexcitation-energy electrons) on supercoiled plasmid DNA (i.e., pGEM-3Zf-, 3199 base-pairs) in the presence of Pt-drugs including cisplatin, carboplatin and oxaliplatin. Nanometer-scale DNA films were irradiated with electrons of 0.5 eV (Supplementary Fig. S1 and Table S1). Fig. 1 presents the respective exposure-response curves for the formation of nicked circular and linear DNA corresponding to SSB and DSB production, respectively. No DSB were observed in irradiated unmodified DNA, as expected, since the reported threshold for DSB induction is 6–7 eV [10]. In striking contrast to unmodified DNA, 0.5-eV electrons induce DSB in DNA modified by Pt-drugs.

It is well known that the primary radiation beam in radiotherapy, which consists predominantly of photons or electrons of 0.2–20 MeV interacts with biomolecules via several processes (e.g., photoelectric effect and Compton scattering) generating electrons with a wide energy distribution. Energetic electrons transfer their energy to biological matter in localized quanta, separated by mean free paths much larger than atomic dimensions. Since the energy and momentum transfer is quite small in each interaction (e.g., 24 eV loss on average in DNA), the energy deposition can be described by the first Born approximation [11]. Therefore, ionizing radiations with energies from keV to a few MeV interact with matter via the same fundamental process. To represent the direct effects of high-energy ionizing radiation on DNA, we then irradiated unmodified and platinated DNA films with 1486-eV X-rays photons. These photons interact with DNA via the photoelectric effect and generate photoelectrons with energies around 1 keV. Exposure-response curves for the formation of circular and linear DNA in the irradiated samples, as shown in Fig. 2, clearly indicate that Pt-drugs substantially enhance the induction of DSB by X-rays, but have minimal effect on SSB formation.

The yields of SSB and DSB induced by 0.5-eV electrons and 1486-eV X-rays are presented in Table 1 and compared with 10-eV electrons from our previous study [8]. The yields for SSB formation in both modified and unmodified DNA are greater for 0.5-eV electrons than for 10 eV and X-rays (P -value < 0.05). For 0.5-eV electrons, there are enhancements in the yields of SSB by factors of 1.5, 2.2 and 1.6 in the presence of cisplatin, carboplatin and oxaliplatin, respectively. Furthermore, 0.5-eV electrons are as efficient as 10 eV and X-rays in the DSB formation in modified DNA. At 10 eV, the incident electrons can induce damage to DNA via DEA and/or DEE, whereas 0.5-eV electrons can only produce DNA lesions via

DEA since their energy is well below that required for the electronic excitation of organic molecules. At such energies, electron capture usually occurs via a shape resonance, which requires a centripetal barrier (i.e., nonzero angular momentum of the electron with respect to the site of temporary attachment). However, near zero eV, s-wave attachment is also possible via the formation of nuclear excited Feshbach resonances. In this case, suitable curve crossing with a dissociative anion potential-energy curve is needed for DEA to occur. Since LEEs constitute a major portion of the secondary species produced by high-energy radiation, these results suggest that electron resonances are highly efficient in the radiosensitization of DNA by Pt-drugs and subexcitation-energies electrons play an important role in the formation of DNA damage, particularly DSB.

The exposure-response curves shown in Figs. 1 and 2 for the DSB formation in the modified DNA exhibit a linear behaviour, particularly at low fluences (Supplementary Model S.1). This linearity implies that a single-hit process is responsible for DSB induced by these radiations. Linear dose-response curves for the formation of DSB by the direct effect of photons have also been reported [12], where it is proposed that an X-ray photon of 1.5 keV ionizes a DNA constituent via the photoelectric effect to produce a SSB and the departing photoelectron interacts with the opposite strand to induce a second strand break. Our data also indicate that the interaction of only one 0.5-eV electron with DNA leads to the formation of DSB, which requires two separate opposite lesions. To estimate the contribution of single- and double-hit processes to the DSB induction in modified DNA, we employed an analytical method proposed by McMahon and Currell, with modifications [13] (Supplementary Model S.1). At 2 Gy, i.e., a typical clinical radiation dose, the total number of DSB resulting from single-hits is much greater than that from double-hits, by factors of 748, 531 and 351 in the presence of oxaliplatin, cisplatin and carboplatin, respectively (Supplementary Table S2). Since DSB are not observed at this energy in the absence of Pt-adducts, it seems reasonable to conclude that electron attachment occurs in the vicinity of these adducts and initiates a series of highly efficient events resulting in the formation of DSB.

Theoretical and experimental studies have shown that the formation of SSB in unmodified DNA by dissociative attachment of subexcitation electrons proceeds substantially by attachment to the lowest π^* orbital of a base, followed by electron transfer to the σ^* orbital of the phosphate group [14]. The transient phosphate anion results in the rupture of phosphodiester bond mostly via C-O bond cleavage, leading to the formation of a dephosphorylated C3' radical of the sugar moiety and a terminal phosphate anion [14]. The total cross section for DEA to DNA thus depends on the probability of both electron capture on the base and then anion survival versus electron autodetachment on the phosphate group. In the case of modified DNA, the high electron affinity of the platinum atom lowers the energy of TNIs and is expected to displace the TNI toward the site of platination. Lowering the energy of a TNI not only increases electron capture cross section, but also reduces the number of vibrational decay channels, which lead to an increase in the lifetime and hence the magnitude of DEA. Therefore, the presence of Pt-adducts should favour not only the dissociation of the phosphodiester bonds, but also that of the guanines-Pt (Pt-G) bonds crosslinking the DNA strands (Fig. 3a), by enhancing the probability of both electron capture and anion survival, and displacing the resonance site. Moreover, the Pt-G bonds

distort the conformation of DNA by unwinding and bending its double helix (Fig. 3a). Such distortions modify the physical and chemical stability of the DNA leading to the weakening of its chemical bonds, which could modify the range of nuclear separation over which dissociation takes place.

The interaction of a single near zero-eV electron with gas phase cisplatin $[(\text{NH}_3)_2\text{PtCl}_2]$ has been reported to break two Pt-Cl bonds simultaneously with considerable probability [15]. This might be explained by the charge delocalization process proposed by Polanyi and co-workers [16], in which the wave functions of a near zero-eV electron lie simultaneously on multiple orbitals, causing concerted bond breaking. In DNA modified by interstrand binding of cisplatin, the chlorine atoms are mainly replaced by two guanines on opposite strands. Similar to $(\text{NH}_3)_2\text{PtCl}_2$, we expect the delocalized electron density to be high in the dissociative σ^* bonds between the guanines and platinum [15]. Fig. 3b illustrates hypothetical potential-energy curves for ground and anionic states of an interstrand Pt-adduct between two guanines. Attachment of an excess electron produces a TNI that evolves along the upper, repulsive potential-energy curve. Delocalization of the electron over the two Pt-G bonds could permit both to relax simultaneously. Following the autodetachment of the electron, cleavage of the Pt-G bonds is possible provided that sufficient kinetic energy (KE) has been gained to overcome the remaining energy-barrier (EB) to molecular dissociation (Fig. 3b). The cleavage of Pt-G bonds produces guanine radicals (Fig. 3d):

where reactions (1) and (2) indicate the formation of guanine radicals from the interaction of a single LEE with cisplatin, carboplatin and oxaliplatin adducts, respectively.

Base-centered radicals are known to abstract a hydrogen atom from the 2'-deoxyribose moiety, resulting in a radical that can break the DNA backbone (Fig. 3d) [17]. Since, at low adduct levels in supercoiled DNA, Pt-drugs form mainly interstrand crosslinks between guanines in opposite strands [18], the simultaneous formation of two guanine radicals by a single LEE could induce a DSB by a single-hit process with considerable efficiency (Fig. 3e).

Conclusively, in Pt-DNA adducts, the cross section for the interaction of subexcitation-energy electrons is increased, resulting in the enhanced formation of DNA strand breaks. Such LEEs induce DSB only in Pt-modified DNA via DEA by a single-hit process.

Experimental Section

Plasmid DNA (pGEM-3Zf-) was extracted from *E-Coli* and mixed with the solutions of Pt-drugs including cisplatin, carboplatin and oxaliplatin. The platinated plasmid contained an average of two Pt-drugs per plasmid as measured by inductively coupled plasma-mass

spectroscopy. An aliquot of the pure or platinated DNA solution was deposited on either tantalum or glass substrate and freeze-dried to form solid, dry films with an average thickness of 10 nm (Supplementary Table S1). The nanoscale films were irradiated with either 0.5-eV electrons or 1.486-keV X-rays in homemade laboratory apparatuses (Supplementary Figs. S1 and S2).

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 including supercoiled, nicked circular, linear and interduplex CL in each DNA and Pt-drug-DNA sample was obtained by agarose gel electrophoresis. The amount of each structural form of the DNA was then analyzed by ImageQuant (Molecular Dynamics) software. The yields for electrons induced SSB and DSB were derived from the initial linear slopes of the respective exposure–response curves (Supplementary Information S.2).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Seiwert TY, Salama JK, Vokes EE. *Nat Clin Pract Oncol*. 2007; 4:86–100. [PubMed: 17259930]
2. Wilson GD, Bentzen SM, Harari PM. *Semin Radiat Oncol*. 2006; 16:2–9. [PubMed: 16378901]
Shewach DS, Lawrence TS. *J Clin Oncol*. 2007; 25:4043–4050. [PubMed: 17827452] Mierzwa ML, Nyati MK, Morgan MA, Lawrence TS. *Oncologist*. 2010; 15:372–381. [PubMed: 20413642]
3. Jung Y, Lippard SJ. *Chem Rev*. 2007; 107:1387–1407. [PubMed: 17455916]
4. Todd RC, Lippard SJ. *Metallomics*. 2009; 1:280–291. [PubMed: 20046924] Jamieson ER, Lippard SJ. *Chem Rev*. 1999; 99:2467–2498. [PubMed: 11749487]
5. Sanche L. *Radiation Damage in Biomolecular Systems*. Gomez, G., Fuss, M., editors. Springer; 2012. p. 3–43. Lacombe S, Sech CL. *Surf Sci*. 2009; 603:1953–1960.
6. Rezaee M, Sanche L, Hunting DJ. *Radiat Res*. 2013; 179:323–331. [PubMed: 23368416] Kuduk-Jaworska J, Chojnacki H, Janski JJ. *J Mol Modeling*. 2011; 17:2411–2421.
7. Sanche L. *Chem Phys Lett*. 2009; 474:1–6.
8. Rezaee M, Hunting DJ, Sanche L. *Int J Radiat Oncol Biol Phys*. 2013; 86:847–853.
9. Alizadeh E, Sanche L. *Chem Rev*. 2012; 112:5578–5602. [PubMed: 22724633]
10. Huels MA, Boudaïffa B, Cloutier P, Hunting D, Sanche L. *J Am Chem Soc*. 2003; 125:4467–4477. [PubMed: 12683817]
11. LaVerne JA, Pimblott SM. *Radiat Res*. 1995; 141:208–215. [PubMed: 7838960] Inokuti, M. *Atomic and molecular data for radiotherapy and radiation research*. IAEA; Vienna: 1995. p. 754. Nikjoo H, Emfietzoglou D, Watanabe R, Uehara S. *Radiat Phys Chem*. 2008; 77:1270–1279.
12. Sutherland BM, Bennett PV, Sutherland JC, Laval J. *Radiat Res*. 2002; 157:611–616. [PubMed: 12005538] Krisch RE, Flick MB, Trumbore CN. *Radiat Res*. 1991; 126:251–259. [PubMed: 1850853]
13. McMahon SJ, Currell FJ. *Radiat Res*. 2011; 175:797–805. [PubMed: 21466384]

14. Zheng Y, Cloutier P, Hunting DJ, Sanche L, Wagner JR. J Am Chem Soc. 2005; 127:16592–16598. [PubMed: 16305248] Barrios R, Skurski P, Simons J. J Phys Chem B. 2002; 106:7991–7994. Berdys J, Anusiewicz I, Simons J. J Am Chem Soc. 2004; 126:6441–6447. [PubMed: 15149241]
15. Kopyra J, Koenig-Lehmann C, Bald I, Illenberger E. Angew Chem. 2009; 121:8044–8047. Angew Chem Int Ed. 2009; 48:7904–7907.
16. Ning Z, Polanyi JC. Angew Chem Int Ed. 2013; 52:320–324. Huang K, Leung L, Lim T, Ning Z, Polanyi JC. J Am Chem Soc. 2013; 135:6220–6225. [PubMed: 23582020]
17. Dizdaroglu M, Jaruga P. Free Radic Res. 2012; 46:382–419. [PubMed: 22276778]
18. Vrana O, Boudny V, Brabec V. Nucleic Acids Res. 1996; 24:3918–3925. [PubMed: 8918792]

Figure 1.

Exposure-response curve for the formation of nicked circular (a–d) and linear (e) DNA configurations corresponding to the SSB and DSB, respectively, by 0.5-eV electrons in DNA unmodified or modified by cisplatin, carboplatin and oxaliplatin. %SSB and %DSB denote the increased percentage for the formation of SSB and DSB, respectively. ϕ indicates electron fluence. Data are means $\pm$ standard deviation (SD) from five measurements. They have been fitted by a least squares regression analysis.

Figure 2.

Exposure-response curve for the formation of nicked circular (a–d) and linear (e) DNA configurations corresponding to the SSB and DSB, respectively, by Al K α 1486-eV X-ray photons in DNA unmodified or modified by cisplatin, carboplatin and oxaliplatin. %SSB and %DSB denote the increased percentage for the formation of SSB and DSB, respectively. ϕ indicates photon fluence. Data are means $\pm$ standard deviation (SD) from five measurements. They have been fitted by a least squares regression analysis.

Figure 3.

Illustration of the possible radiosensitization effects of Pt-drugs on DNA. (a) A double helix DNA, Pt-drugs modified DNA and the formation of a TNI on Pt-DNA adduct. Formation of Pt-adducts such as interstrand crosslinks between two guanines (G), as shown here, distorts the conformation of DNA. (b) Schematic diagram showing potential-energy curves for the ground and anionic states of an interstrand Pt-adduct between two guanines. Formation of a TNI on Pt-adduct induces repulsive impulses on the bonds between platinum and guanines (Pt-G) due to the delocalization of the excess electron. When the gained kinetic energy (KE) is larger than the energy-barrier (EB), Pt-G bonds dissociate after the autodetachment (AD) of the electron. U and R denote potential energy and Pt-G distance, respectively. R_E corresponds to the equilibrium internuclear separation of the neutral ground state of the adduct; F-C region denotes transition in Frank-Condon region. (c – e) Possible mechanism for the formation of DSB by a single subexcitation-energy electron in the presence of Pt-adducts. Electron delocalization within a Pt-adduct (e.g., interstrand crosslink between two G) results in the simultaneous cleavage of two Pt-G bonds, formation of two guanine radicals ($G^\bullet$), followed by hydrogen abstraction from the backbone, cleavage of phosphodiester bonds in opposite strands, and formation of a DSB. X corresponds to NH_3 , NH_3 and $C_6H_{10}(NH_2)_2$ for cisplatin, carboplatin and oxaliplatin, respectively. dR and C denote deoxyribose and cytosine.

Table 1

Yields of DNA damage ($\times 10^{-8}/\text{Gy/bp}$) for soft X-rays, 10- and 0.5-eV electron irradiations of unmodified and Pt-modified DNA.

Irradiation	DSB Formation				SSB Formation			
	Pure DNA	Cisplatin+DNA	Carboplatin+DNA	Oxaliplatin+DNA	Pure DNA	Cisplatin+DNA	Carboplatin+DNA	Oxaliplatin+DNA
0.5-eV Electrons	0	4.4 ± 1.5	6.0 ± 2.3	7.4 ± 3.1	324.9 ± 73.8	487.4 ± 74.5	701.5 ± 44.3	531.7 ± 59.1
10-eV Electrons ^b	1.2 ± 0.2	3.0 ± 0.2	3.7 ± 0.2	2.9 ± 0.2	78.7 ± 16.2	110.1 ± 15.8	120.4 ± 21.7	114.3 ± 26.3
1.5-keV X-rays	ND ^c	4.9 ± 0.8	7.0 ± 1.0	5.4 ± 0.7	199.0 ± 25.3	204.0 ± 26.8	237.2 ± 29.7	215.3 ± 37.3

^aLET: Linear energy transfer;

^bFrom Table 1 of Ref. [8];

^cND = Not detected