Role of Secondary Low-Energy Electrons in the Concomitant Chemoradiation Therapy of Cancer

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Solid films of DNA with and without the chemotherapeutic agent cisplatin bonded to guanine were bombarded with electrons of 1, 10, 100, and 60 000 eV causing single and double strand breaks. In the presence of cisplatin this damage was increased by factors varying from 1.3 to 4.4 owing to an increase in bond dissociation triggered by the formation of transient anions. This mechanism may lie at the basis of the efficiency of concomitant cisplatin-radiation therapy.

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I. Introduction.—Among the different strategies to improve the treatment of cancer, combining chemotherapeutic drugs with radiation (i.e., chemoradiation) has met with considerable success [1,2]. In many clinical trials, it has been shown that the administration of both modalities increases the survival rate of cancer patients compared to those who received radiation alone [2]. Furthermore, the improvement of local tumor control was much more obvious when radiation was administered synchronously with the chemotherapeutic agent [1–3]. This observation has been attributed to a superadditive effect on tumor regression, which must be due to an as-yet unidentified synergic interaction between the radiation and the drug. In the case of the chemotherapeutic agent cisplatin, whose nomenclature appears in Fig. 2(a), essentially two mechanisms, related to the binding of this molecule to DNA *in vivo* have been proposed [3]. Studies in tissue cultures [4] and tumor-bearing mice [5] suggest a synergistic effect between cisplatin and radiation due to inhibition of repair of radiation-induced damage to DNA. Another possibility is that the immediate species created by the primary radiation in cells cause additional damage when cisplatin is covalently bonded to DNA [6]. Such a mechanism would necessarily require the synchronous presence of the drug and radiation in cancer cells.

The immediate species created by the high energy radiation used in radiotherapy consists essentially of ions, radicals, and secondary low-energy electrons (LEE). Most of the radiation energy flows into the motion of secondary electrons, which are created in large numbers with a most probable energy of only 9–10 eV [7]. As recently shown, the impact of LEE on DNA can inflict considerable damage causing single and double strand breaks (SSB and DSB), base deletions, and singly and multiply damaged sites [8,9]. In the present Letter, we show that SSB and DSB induced by LEE are substantially enhanced when cisplatin is covalently bonded to DNA. Furthermore, the formation of transient anions is found to play a key role in this enhancement. Comparison of the LEE results with those obtained with high energy radiation, suggests that the superadditive effect observed in tumor regression may be at least partly related to this enhancement. Understanding such basic mechanisms of the direct effects of radiation in chemosensitized DNA may have implications in the design of new chemotherapeutic and radiosensitizing drugs as well as in the development of more efficient protocols in concomitant chemoradiation therapy.

II. Experimental procedures.—Supercoiled DNA [pGem-3Zf(-), 3197 base pairs] was prepared and purified as previously described [10]. An aqueous solution of the molecule with a Tris-NH_3^+ counterion was obtained in the last step of the purification procedure, where the DNA was washed with a buffer containing Tris-EDTA. A final molar radio of 12:1 of salt to DNA was retained so as to maintain conditions which resemble those found *in vivo* in cellular DNA [11]. A solution of cis-diammineplatinum (II) dichloride (cisplatin, 98% purity, Sigma Aldrich) was mixed with the plasmid DNA solution so as to obtain different molar ratio (R), of cisplatin to plasmid molecules. The mixture was kept in darkness at 37 °C for 48 h. Under these conditions, cisplatin binds to DNA preferentially at the N7 atom of guanine [12] and produces about 90% of the interstrand adduct shown in Fig. 2(b).

Lyophylized films of pure plasmid and plasmid-cisplatin complexes were prepared on a clean tantalum foil as previously described [10]. The lyophilized DNA formed a film of an estimated thickness of 15 nm (5 monolayers: ML) with a measured diameter of 3.5 ± 0.2 mm. Such a thickness is sufficient to absorb most of the energy of 1–100 eV electrons without inducing significant charging at low doses [9]. The samples were irradiated in ultrahigh vacuum (UHV) with an electron beam of 1.5 nA and electron energies of 1, 10, and 100 ± 0.5 eV, respectively, for irradiation times of seconds up to 3 min. High energy electron irradiations were performed with the source of a transmission electron microscope (TEM) (H-7100 Hitachi) set at 60 keV and 15 μA, respectively, for irradiation periods varying from 5 to 30 s. Because of the low scattering cross section of high energy electrons and also to avoid the effect of secondary electrons produced at the

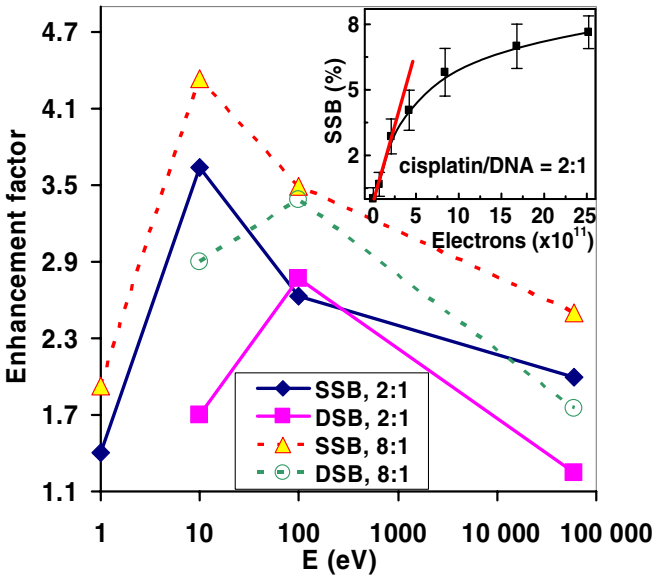

FIG. 1 (color online). Enhancement of damage arising from the bonding of 2 and 8 cisplatin molecules to DNA as a function of electron energy. The exposure response curve for production of SSB by 100 eV electrons is shown in the upper inset.

metal substrate, much thicker films of 2900 nm were used in the TEM experiments. The various DNA and cisplatin-DNA complex films were irradiated under the same experimental conditions. For each energy, a sample was also transferred to the TEM or LEE irradiation chamber without being irradiated and kept in vacuum for an identical duration. Once removed from the UHV chamber, the samples were recovered and the SSB and DSB analyzed and quantified by gel electrophoresis, as previously described [10]. Exposure response curves were obtained at each energy for SSB and DSB in DNA and cisplatin-DNA complexes of different ratios R. Data were recorded at seven different doses and each data point was the average of three experiments.

III. Results.—As an example, the upper inset of Fig. 1 shows the dependence of the yield of SSB on exposure to 100 eV electrons of films of cisplatin-plasmid complexes in a ratio R of 2:1. The yields expressed as the percentage of SSB and DSB per electron and molecule were obtained from the initial slope of such exposure response curves. They are given in Table I for different energies and $R =$ 2:1 and 8:1. For both R values, cisplatin binding to DNA increases the production of SSB and DSB, but in quite different proportions depending on electron energy. The ratios of the yields with and without the presence of cisplatin (defined as EF, the enhancement factor) are plotted as a function of electron energy in Fig. 1. The EF maximizes at 10 eV for SSB and at 100 eV for DSB. The curves follow the same trend for $R = 2$ and 8. Interestingly, the EF is the same, within experimental errors, for SSB and DSB at 100 eV. This phenomenon is persistent from $R = 1$ to 8, as determined from the curves in Fig. 2, which represent the yields of SSB (A) and DSB (B) as a function of R for 100 eV electrons. These curves show that the yield of DSB saturates beyond eight cisplatin molecules per plasmid. Comparing the first two points in Fig. 2, we find that a single cisplatin molecule bound to a plasmid composed of 3197 base pairs increases the number of SSB and DSB by a factor of 2.4.

IV. Discussion.—At 1 eV, SSB in DNA occur only via dissociative electron attachment (DEA) [13]. According to current experimental [9,13] and theoretical evidence [14], the incoming electron is first captured by a base where it forms a shape resonance. Afterwards, it transfers to the phosphate group to form another local transient anion, which dissociates by breaking the C—O bond of the chain with the captured electron remaining on the oxygen atom. Thus, the EF of 1.4 and 1.9 for SSB estimated from Table I for 1 eV electrons are due to an increase of the magnitude of the DEA process. The cisplatin molecule has a shape resonance near zero eV, which leads to DEA [15], but the absolute magnitude of the process is not known. Close to zero eV, however, DEA cross sections often reach huge values of the order of 10^{-13}–10^{-15} cm^2 owing to the $1/k$ momentum factor, which enters into the expression of the captured cross section [16]. Furthermore, in DNA this cross section could be increased by base to base electron transfer along the chain [9], which would act to draw additional electrons to the site of cisplatin. Such a cross section enhancement at the site of cisplatin binding could explain why only a few cisplatin molecules are needed to produce a considerable increase in SSB. One eV electrons

TABLE I. Yields (in 10^{-15} electron^{-1} molecule^{-1}) for the formation of SSB and DSB induced by 1, 10, 100 eV electron impact on 5 monolayer (ML) DNA films and 60 KeV electron impact on 2900 nm DNA films deposited on a tantalum substrate. The error represents the deviation of three identical measurements.

Form of damage	SSB				DSB			
Energy (eV)	1	10	100	60 000	1	10	100	60 000
Thickness		5 ML film		2900 nm		5 ML film		2900 nm
DNA	27 ± 3	33 ± 3	57 ± 5.5	3.2 ± 0.3	Not detected	10 ± 1	13 ± 2	1.0 ± 0.5
Cisplatin:DNA = 2:1	38 ± 3	120 ± 11	150 ± 15	6.5 ± 0.8	5 ± 1	17 ± 1	36 ± 4	1.3 ± 0.5
Cisplatin:DNA = 8:1	52 ± 5	143 ± 14	199 ± 18	8.1 ± 1.0	5 ± 2	29 ± 2	44 ± 4	1.9 ± 0.3

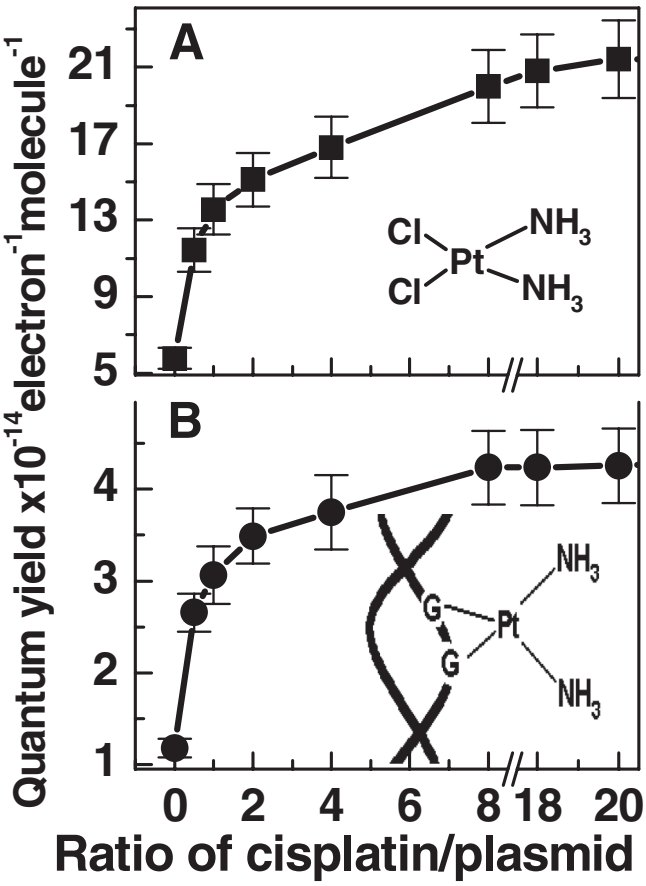

FIG. 2. Comparison of the yield of SSB (A) and DSB (B) as a function of different cisplatin to plasmid ratios. The yields were obtained from exposure curves of 5 ML cisplatin/plasmid mixture deposited on a tantalum substrate and bombarded with 100 ± 0.5 eV electrons.

do not produce DSB in unmodified DNA [13], but here in the cisplatin-DNA complex they do. Cisplatin locally modifies the topology of DNA and weakens the adjacent bonds in the backbone [12]. Combined with the high electron affinity and chemical reactivity of cisplatin, these modifications could provide energy required to break two adjacent bonds.

To date, a large number of experiments have been performed with 10 eV electrons impinging on thin films of various topological forms of DNA [8,9]. These have shown that the formation of SSB and DSB occur essentially via the formation of core-excited resonances localized on DNA subunits, which decay by DEA or into electronically excited dissociative states. The 10 eV results shown in Fig. 1 illustrate that for $R = 2$ the magnitudes of these resonant processes are considerably increased leading to a rise in SSB and DSB by factors of 3.6 and 1.7, respectively. For $R = 8$ these factors increase to 4.3 and 2.9, respectively. Here again, order-of-magnitude increases in dissociative processes induced by the formation of transient anions at the site of cisplatin binding must be invoked to account for such large EF.

As seen from Table I, the impact of 100 eV electrons on plasmid DNA induces more SSB and DSB than at 10 eV. One hundred eV electrons cause principally ionization so that this increase can be partly attributed to damage induced by the ionization process itself in addition to that induced by the liberated electrons. According to Fig. 1, these processes reduce the EF for SSB, but increase it for DSB. We can explain the decrease in EF for SSB from 10 to 100 eV by a reduction of the number of LEE near 10 eV where the EF is the largest. However, owing of the large ionization cross section at 100 eV and thermalization distances of the order of the film thickness, a single 100 eV electron can induce multiple ionizations. In this case, the probability of breaks occurring on adjacent chain sites by two secondary electrons from ionization created by a single incident electron increases and so does the EF for DSB. This discussion is also consistent with the 60 keV data. The results at 60 keV essentially represent the direct radiation effects of clinical (0.5 to 10 MeV) x rays, which mainly produce Compton electrons. The latter subsequently create LEE whose distribution maximizes at 9–10 eV [7], so that a sizable EF is retained. The lower EF at 60 keV possibly accounts for other processes and electrons having much more than 10 eV, both of which may contribute less significantly to the enhancement.

V. Conclusions.—When a cisplatin-DNA complex is irradiated by high energy particles, capture of secondary LEE at the site of cisplatin, followed by rupture of the backbone, is increased by orders of magnitude. The increase in the ionization cross section of secondary electrons, due to the presence of Pt atoms, also increases the quantity of LEE near cisplatin and therefore indirectly contributes to this huge increase in local damage. The considerable sensitivity to LEE translates into an increase of SSB and DSB by factors of 2.0 and 1.3, respectively, with only 6.25×10^{-4} cisplatin molecules per base pair (mol./b.p.) covalently bound to DNA and irradiated by 60 keV electrons. In chemotherapy, the cisplatin concentration can reach values of 4×10^{-4} cisplatin mol./b.p. in DNA assuming a uniform distribution of the drug in cancer tissues [17]. Thus, strand breaks in the DNA of cancer cells could be significantly increased in concomitant cisplatin-radiation therapy by the mechanisms proposed in this letter, when sufficient quantities of the drug are delivered or if it accumulates preferentially in the DNA of cancer cells. However, cisplatin is one of the most toxic chemotherapeutic agents [3]. So, less toxic Pt compounds, such as carboplatin [3], or other types of agents, which could be administered in larger quantities, may be more appropriate to trigger radiosensitization by LEE in concomitant chemoradiation therapy. We presently investigate LEE induced reactions in such compounds as well as in cisplatin bonded to short single DNA strands at the site shown in

Fig. 2(b). This research was financed by the Canadian Institutes of Health Research.

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